INTEGRATED MANAGEMENT OF CERCOSPORA LEAF SPOT ON SUGAR BEET

by

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ABSTRACT

Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is the most important foliar disease of sugar beets in Montana. Losses in research plots ranging from 5-15 metric tons per hectare and 0.5-1.5% lower sugar in the last 7 years. Increased levels of storage rot, sugar impurities, and loss of sugar to molasses have also contributed to losses. Current management strategies are heavily dependent upon the application of a few registered fungicides. Continued use of these fungicides is threatened by registration removal through the Food Quality Protection Act of 1996, and the development of fungicide resistance in *Cercospora beticola*. This study examined management strategies for CLS integrating host-plant resistance, a CLS prediction model, registered fungicides, and a *Bacillus mycoides* biocontrol agent, BmJ. The effects of these strategies on disease development and yield were evaluated in field trials from 2001 to 2003. Disease levels were rated 4-5 times per season to generate a disease value for each treatment quantified as the area under the disease progress curve. Yield was determined as metric tons of beets and kilograms of extractable sucrose per hectare, and percent sucrose. Results showed more resistant varieties to give equal disease control with 1-2 fewer fungicide applications than more susceptible varieties without sacrificing yield in moderate to light CLS pressure. BmJ applications (4/year) reduced disease levels below the accepted economic threshold and gave disease control equal to fungicide applications when mixed with a single spray of a half rate of tetraconazole at disease onset. BmJ was highly effective in CLS management when combined with more resistant varieties. The levels of *C. beticola* fungicide resistance to benomyl, azoxystrobin and tetraconazole isolated from research plots in 2001 and 2002 were measured by spore germination and mycelial growth assays. Total insensitivity to benomyl was observed in more than 70% of isolates and reduced sensitivity to azoxystrobin and tetraconazole up to 10 ppm was also recorded. Preliminary results indicate growing more resistant varieties of sugar beet and spraying BmJ may aide in managing fungicide resistance by reducing the number of fungicide applications necessary for CLS control.

CHAPTER 1

INTRODUCTION

Sugar Beet, *Beta vulgaris* L.

Sugar beet (*Beta vulgaris* L.) is a member of the beet genus classified in the family Chenopodiaceae. The Middle East was the origin of today's many beet species, including many weeds and four general categories of agricultural beets. The different species of wild beet arose from isolation in different regions of Turkey, Iran, Russia and the Canary Islands (Cooke and Scott, 1993). The wild beet *Beta vulgaris* ssp. *maritime* was chosen by breeders attempting to increase levels of sugar in the beet and is the wild beet ancestor of today's sugar beet. *Beta vulgaris* ssp. *maritime* was chosen due to its high level of resistance to the most destructive pathogen of then and today, Cercospora leaf spot caused by *Cercospora beticola* (Winner, 1993).

Agricultural beets other than sugar beet are the foliage beets, garden beets and mangolds. The foliage beets are grown for their leafy tops used for human food. Garden beets, including red and table beets, are grown for their roots for human food. The mangolds, otherwise known as fodder beets, are exclusively grown for livestock feed. Other wild beets include the weedy species lambsquarter, pigweed, winged pigweed, mallow, wild buckwheat and common unicorn flower (Viard et al, 2002). It is recommended that all weedy species of beet be closely managed in sugar beet growing areas because they are more competitive than sugar beets and can serve as alternative

hosts for many pests of sugar beet (Schweizer and May, 1993; Whitney and Duffus, 1986).

Sugar beet is a biennial, herbaceous, dicotyledonous crop with a large taproot consisting of 15-20% sugar (Smith, 1987). The taproot is harvested for its sugar at the end of the first growing season (Viard et al, 2002). As a biennial plant, seed production does not occur until the second year after vernalization when green flowers having five sepals but no petals are produced on a stalk 1.2 -1.5 m tall (Smith, 1987). Flowers are fertilized by out-crossing because sugar beets are usually self-incompatible (Shaw, 1914). A spherical to kidney-shaped seed is produced in an individual fruit from each flower (Artschwage, 1926). Commercial sugar beets are planted as diploids or triploids from monogerm seed (Bornscheuer et al, 1993). Sugar beets are naturally diploid having 18 chromosomes (Elliot and Weston, 1993).

Sugar beets are grown in more than forty countries in Europe, Asia, the Middle East, Northern Africa, and South and North America (Whitney and Duffus, 1986). Sugar beet production in the United States mostly occurs in the northern plains states of Minnesota, Michigan and North Dakota. Other states with major sugar beet production areas include California, Colorado, Idaho, Montana, Nebraska, Texas and Wyoming (Cattanach et al, 1991). The production of sugar beets in Montana is dependent on the availability of irrigation water. As a result, the majority of sugar beet acreage is in the river valleys where furrow irrigation is possible. The use of pivot irrigation systems in recent years has allowed the development of sugar beet growing areas away from the river valleys. Pivot irrigation will not only expand the acreage available for production,

but also aid in managing disease and pest epidemics by increasing the distances between sugar beet fields from year to year. Approximately 60,000 acres of sugar beets are currently produced each year in Montana.

Sugar beets are primarily produced as an economical source of crystalline sucrose (Schnieder et al, 2002) with approximately 80% of all sugar in the beet in the sucrose form. However, sugar beets are also used for a source of molasses (Ulber et al, 2000) and animal feed. Molasses (saccharose) is a large constituent of the remaining 20% of sugar left after sucrose is removed. It is used as a component of cattle feed (Scipioni and Martelli, 2001), a carbon source for yeast production (Atiyeh and Duvnjak, 2002), and as a precursor for chemical and pharmaceutical production (Faurie and Fries, 1999). What is not sucrose or molasses in the sugar fraction of the beet consists of sugar impurities that are difficult and expensive to process into sucrose (Bichsel, 1987). The beet pulp left over after processing and the sugar beet tops are used as feed for livestock over the winter when a highly nutritious feed is desired (Scipioni and Martelli, 2001). The beet pulp is fed to cattle or sheep in a wet or dried, pelleted form (Fiems et al, 2002). Sugar beets are also grown for the purpose of producing seed for the following year (Pospisil et al, 2000), mostly in the state of Oregon where the dry climate inhibits the development of diseases that can be carried on the seed (Hampton et al, 1998).

Cercospora leaf spot of Sugar beet

The deuteromycete fungus, *Cercospora beticola* Sacc., is the causative agent in the leaf spotting disease Cercospora leaf spot (CLS) of sugar beet. Cercospora leaf spot

occurs in sugar beet growing areas worldwide (Smith and Ruppel, 1974; Georgopoulos and Dovas, 1973) and can lead to reductions in gross sugar yield of up to 42% (Shane and Teng, 1992). Loss of sugar in the beet occurs as new leaves are grown to replace those heavily damaged by CLS (Vereijssen et al, 2003; Steinkamp, 1979). Losses are manifested as a reduction in root weight, lower sugar content, and increased impurities, leading to a loss of sugar to molasses (Smith and Martin, 1978). Smith and Ruppel (1971) determined that CLS also caused increased storage rot as a result of cercosporin and beticolin, toxins produced by *C. beticola*.

Cercospora leaf spot is a polycyclic disease dependant on relative humidity greater than 90% and temperatures above 15.5° C for disease progression (Windels et al, 1998; Shane and Teng, 1984). A full disease cycle can occur in as few as 10 days given the correct weather conditions of high relative humidity and high temperatures and result in multiple infection cycles per growing season. The lifecycle of *C. beticola*, beginning at initial infection early in the growing season, is characterized by five stages. In the first stage, conidia present on infected leaf residue from the previous year or conidia produced by overwintering stromata on leaf residue are moved by wind and rain splash to the leaf surface of newly developing beets where the conidia germinate and infect through stomata. Relative humidity above 90% and temperatures above 15.5° C are required for germination (Whitney and Duffus, 1986). Steinkamp et al. (1979) studied the *C. beticola* infection process determining that *C. beticola* infection begins by attachment of the conidia to the leaf surface and subsequent germination and entry of a germ tube through stomata during periods of leaf wetness. Rathaiah (1977) determined that hydrotropism

was important in the ability of the germ tube to locate stomata. A higher relative humidity in the stomatal opening as compared to lower ambient levels was correlated with infection through stomates. Stomata do not need to be open to facilitate fungal entry (Rathaiah, 1976). In the second stage, intercellular movement ensues with occasional attachment of the mycelia to the plant cell wall. Necrosis of individual cells occurs in a localized area with interspersion of necrotic and healthy cells. *Cercospora beticola* forms macroscopically observable necrotic lesions on the leaf surface at the site of infection after approximately 10-14 days as long as temperatures are adequately high. Lesions are small, ranging in size from 3-5 mm in diameter (Whitney and Duffus, 1986). A mature lesion is characterized by completely necrotic tissue having fungal hyphae growing throughout (Steinkamp et al, 1979; Cunningham, 1928). The center of the lesion is often a dark grey to ashen color with a fuzzy texture when conidia have been produced (Windels et al, 1998). The border of the lesion is well defined, having two general regions. Necrotic but intact cells with thickened cell walls characterize the inner region. Fungal hyphae extend into this region from the center of the lesion. The outer region contains healthy metabolizing cells surrounded by deposits of granular pectic material in the intercellular spaces. This region is often discolored by plant production of anthocyanins and does not contain hyphal growth.

Dense fungal pseudo-sclerotia growths called stromata develop on the surface of mature lesions. Stromata, having a dark brown color, give rise to hyaline conidia during periods of high relative humidity and serve as overwintering structures (Whitney and Duffus, 1986). Spore formation is the next stage in the *C. beticola* life cycle. Details of

conidiogenesis on stromata and free hyphae were described by Pons et al (1985). It was determined that conidiophores arise enteroblastically from stromatic cells. Conidia then holoblastically arise from the conidiophore (Minter et al, 1982). Delimitation of the conidium occurs by a transverse septum at the locus of conidiogenesis (Burnett, 1976). Once conidia are produced, they are spread by wind and rain splash (Whitney and Duffus, 1986). Secondary infections occur in one to three weeks after conidial development. The fourth stage in the lifecycle occurs as infected leaf residues and stromata overwinter at the soil surface. The last stage occurs when conditions are favorable for conidiogenesis; conidia are produced as described above and are ready to infect newly emerging beets.

Management of Cercospora Leaf Spot

Management of Cercospora leaf spot in sugar beet growing areas of Montana currently depends on crop rotation, host-plant resistance in sugar beet cultivars, the use of registered fungicides, scouting, and a prediction model developed by Shane and Teng (1983-1985) at the University of Minnesota (Windels et al, 1998). A description of each management tool is discussed below.

Crop Rotation

It is recommended that sugar beets be grown in 3-6 year rotations with crops such as small grains, corn, dry beans and alfalfa that do not serve as hosts for *C. beticola* and other sugar beet pests and diseases (Wilson, 2001). Two-year rotations are still used in the Sidney growing area but are not recommended. Spatial separation of at least 100 m

from the previous year's sugar beet growing area to the current year's fields is recommended to inhibit inoculum movement from leaf debris (Windels et al, 1998). A two-year rotation hinders spatial separation, especially in the river valleys near Sidney, MT where sugar beet production has historically been concentrated in the irrigated valleys. However, new production areas away from the river valleys are being developed through the use of sprinkler irrigation. Spatial separation of sugar beet production may be managed more easily in these areas.

Host-Plant Resistance to Cercospora leaf spot in Sugar Beet

Resistance to Cercospora leaf spot in sugar beet cultivars is considered quantitative and controlled by four or more genes (Smith, 1985). Studies of variety interactions with potentially different races of *C. beticola* showed this quantitative resistance to be robust. In that study, isolates of *C. beticola* from Colorado, Italy, Spain and Greece responded similarly to host resistance (Smith, 1985). Sugar beet resistance to CLS has been historically linked with lower agronomic performance (Smith and Campbell, 1996). Because CLS resistance is multigenic, incorporation into high yielding varieties has been difficult. Studies of quantitative trait loci (QTL) have aided in the progression of breeding for CLS resistance (Setiawan et al, 2000). Setiawan et al. (2000) identified four QTLs associated with CLS resistance on chromosomes III, IV, VII, and IX.

Varietal resistance shows rate reducing characteristics that slow the progression of CLS at all stages of development from spore formation to latent period (Rossi et al, 1999). Disease progression models developed by Rossi et al. (1999) showed that rate-

reducing characteristics should be selected for by breeders during the initial screening of germplasm. As the CLS resistance level increases, the ease and importance of improving rate-reducing resistance is lessened. Rossi (1999) suggested that there are no differences in the relative importance of slowing specific stages of fungal development.

Registered Fungicides

There are several fungicides currently labeled for use in controlling Cercospora leaf spot. They fall into two categories; protectant fungicides that rely on direct contact with the pathogen, and systemics that are absorbed by the plant and distributed systemically. The protectant fungicides include an organo metal, triphenyltin hydroxide (TPTH) (SuperTin®, AgriTin®), and the ethylene bis dithiocarbamates (Maneb®, Mancozeb®, Manex® and Penncozeb®). The protectant fungicides offer consistent disease control but kill only germinating spores and have no ability to affect the fungus once infection has occurred. These fungicides are active against several biochemical sites, reducing the likelihood of resistance development in the pathogen. However, isolates of C. beticola tolerant to TPTH have been detected in the Sidney growing area (Jacobsen et al, 2003) as well as in Minnesota (Bugbee, 1995).

Both protectant and systemic fungicides will persist for approximately two weeks. However, systemic fungicides have “kick-back” activity that allows the curing of infections 24-72 hours after an infection has taken place. This allows for greater flexibility in the application timing of systemics. The systemic fungicides include the benzimidazole, thiophanate methyl (Topsin-M®), the triazole, tetraconazole (Eminent®),

and the strobilurins, azoxystrobin (Quadris®) and trifloxystrobin (Gem® and Headline®). These systemic fungicides all have very specific modes of action that allow for the development of resistance. Resistance development limits the number of applications recommended in each growing season and requires that steps be taken to reduce the development of fungicide resistance such as rotating or alternating with fungicides with different modes of action (Karaoglanidis et al, 2001; Köller and Wilcox, 1999). For an overview of the modes of action of the systemic fungicides, see the following section; Fungicide Resistance in *Cercospora beticola*.

Cercospora Prediction Model

The Cercospora prediction model developed by Shane and Teng (1983-1985) is an important tool in sustainable CLS management by allowing for judicious application of registered fungicides. Windels et al. (1998) reviewed the prediction model in its current form as implemented in the sugar beet growing areas of eastern North Dakota and Minnesota. Fields are monitored for the presence of Cercospora leaf spot symptoms about the time of row closure, from late June to early July. Row closer provides a suitable microclimate (relative humidity >90%) under the leaf canopy for disease development.

The timing of the first fungicide application is usually determined by the occurrence of initial symptom development and the use of a Cercospora advisory based on the daily infection value (DIV) calculated from the prediction model. The DIVs are calculated from the number of hours per day (midnight to midnight) with relative humidity above 90% and the average temperature during that time period and expressed

as a whole number from 0-7. Weather data is recorded by remote weather stations with sensors placed in the sugar beet canopy. The DIVs for two consecutive days are added together to describe the potential for infection as a value from 0-14. The DIV calculation was developed by Shane and Teng (1984) using greenhouse studies along with published data (Wallin and Loonan, 1971). A DIV value less than 6 for a 48 hr period indicates a low likelihood of infection; a value of 6 equals a marginal chance of infection; a value greater than 6 predicts conditions good for infection. Experience in MT suggests that 48 hr. DIV between 4 and 6 allows for disease development (Jacobsen, unpublished). After the first fungicide application, the Cercospora advisory is used to determine if additional fungicide sprays are needed (Windels et al, 1998).

The implementation of the Cercospora prediction model in MT has resulted in a 1-2 spray reduction in fungicide applications since 1998. This is not only beneficial to the commercial grower by reducing the cost of inputs, but also reduces fungicide use and the selection pressure for the development of fungicide resistance in *C. beticola*.

Fungicide Resistance in *Cercospora beticola*

The development of reduced sensitivity to fungicides by several plant pathogenic fungi in the field has been well documented (e.g. Bent et al, 1971; Georgopoulos and Dovas, 1973; Ruppel, 1975; Ben-Yephet et al, 1974; Bollen and Scholten, 1971; Harding, 1972; Magie and Wilfret, 1974; Vargas, 1972; Bugbee, 1995; Albertini et al, 1999; Jones, 1981; Köller et al, 1997). Studies of fungicide resistance in *Cercospora beticola* have largely indicated reduced sensitivity to the classes of systemic fungicides used in this

study. Resistance to benzimidazole fungicides has been documented in Greece (Georgopoulus and Dovas, 1973), Texas, (Ruppel and Scott, 1975) Arizona (Ruppel, 1980), Michigan (Weiland and Halloin, 2001), Minnesota (Bugbee, 1984) and North Dakota (Weiland and Smith, 1999). *C. beticola* with reduced sensitivity to the ethylene bis dithiocarbarmate, triphenyltin hydroxide, triazole and strobilurin fungicides have also been reported (Briere et al, 2003; Karaoglanidis et al, 2000; Köeller and Wilcox, 2001; Weiland and Halloin, 2001; Bugbee, 1982). Fungicide resistance is classified as two types. Complete insensitivity is usually caused by a mutation in a single gene that results in complete failure of the fungicide to interact with the target protein in a way detrimental to its function. Fungicides such as the benzimidazoles and strobilurins that have a very specific mode of action reliant on biochemical interactions with a single protein are susceptible to the development of complete insensitivity, otherwise known as qualitative resistance. The other type, known as quantitative resistance, is determined by mutations in several genes and results in varying levels of tolerance to a fungicide (Köller, 1988; Georgopoulos, 1994). The specific number and location of mutations that confer resistance determine the level of tolerance. This type of resistance is developed in a stepwise manner usually against fungicides with multiple sites of action such as the organo metal protectant, triphenyltin hydroxide. However, resistance to the very site-specific sterol biosynthesis inhibiting fungicides such as tetraconazole has been described as quantitative as well (Karaoglanidis et al, 2002). This indicates the existence of factors other than target site alterations that can influence resistance to a fungicide.

Benzimidazole Resistance

The widespread use of the benzimidazole class of fungicides, including benomyl (Benlate®) and thiophanate methyl (Topsin M®), during the early 1970's led to the first reports of fungicide resistance in *C. beticola*, both in the United States (Ruppel and Scott, 1975) and Europe (Georgopoulos and Dovas, 1973). Because benzimidazole resistance is qualitative, a single mutation resulted in complete resistance. Benzimidazoles interfere with the binding of β-tubulin proteins as they form microtubules. The cause of resistance to the benzimidazole class of fungicides (including benomyl) was identified in *Cercospora kikuchii* (Matsumoto & Tomoyasu) M.W. Gardner to be a single mutation in the gene for the cytoplasmic protein β-tubulin, conferring complete resistance (Upchurch et al, 1991). The most common mutation is the substitution of glutamic acid by alanine, glycine, lysine, or glutamine at the 198th codon. This has been determined for other pathogens showing benzimidazole resistance (Albertini et al, 1999; Koenraadt et al, 1992; Cunha and Rizzo, 2003).

Strobilurin Resistance

The strobilurin class of fungicides is relatively new to Cercospora leaf spot management (Bartlett et al, 2002; Sauter et al, 1999; Ypema and Gold, 1999). Strobilurins are compounds developed from the natural compound strobilurin A, a secondary metabolite of the Basidiomycete *Strobilurus tenacellus*. Strobilurins have a mode of action that inhibits electron transport between cytochrome *b* and cytochrome c_1 in the mitochondrial respiratory chain. These compounds block the Q_o site of cytochrome *b* resulting in a disruption in ATP production (Anke, 1995; Bartlett et al, 2002).

Concerns have been raised over the development of resistance to these fungicides due to their highly specific activity (Jones, 1981). Studies by Briere et al. (2003) have identified *C. beticola* field isolates from Colorado, Montana, Nebraska and Wyoming that were resistant to the strobilurin azoxystrobin.

Recent studies have determined resistance to the strobilurin class of fungicides to be dependent on the ability of fungi to use an alternative oxidase (AOX) or one of two mutations in cytochrome b, G143A and F129L (Wood and Hollomon, 2003, Koller et al. 2001, Zheng et al. 2000). As summarized recently by Wood and Hollomon (2003), strobilurin efficacy in *Magnaporthe grisea* is strongest during spore germination and is reduced as the fungus enters vegetative growth, both *in planta* and *in vitro* (Avrila-Adame and Köller, 2003). AOX is less efficient in oxidation than the Q_o and Q_i sites of the mitochondrial complex III. During periods of high metabolic activity, such as spore germination and plant infection, AOX is insufficient for fungal survival when the Q_o site has been blocked by a strobilurin. Studies of AOX importance in strobilurin resistance have used salicylhydroxamic acid (SHAM) to inhibit the activity of AOX during strobilurin resistance testing (Avila-Adame and Köller 2002, 2003). By inhibiting AOX, resistance to the strobilurins becomes dependent on mutations in the cytochrome b gene.

The cytochrome b mutations G143A and F129L give different levels of strobilurin resistance (Koller et al, 2001; Zheng et al, 2000). Studies of *M. grisea*, *M. fijiense*s, and *Venturia inaequalis* indicate that G143A, a substitution of alanine for glycine at the 143 position, confers very strong resistance approaching immunity while F129L, a substitution of phenylalanine by leucine is less effective resulting in 10-100x

reductions in sensitivity (Wood and Hollomon, 2003; Avila-Adame and Köller, 2003). Practical resistance in the field is most likely determined by the G143A mutation (Avila-Köller, 2003). The combination of either the G143A or F129L mutations with AOX may lead to stable resistance in competitive fungal isolates (Wood and Hollomon, 2003).

Resistance to the Sterol Biosynthesis Inhibitors

Sterol biosynthesis inhibitors (SBIs) such as the triazoles have been used for fungal disease control in many cropping systems (Robbertse et al, 2001; Karaoglanidis et al, 2003). These fungicides inhibit the activity of a cytochrome P450 catalyzing the 14α-demythylation of lanosterol (Sanglard et al, 1998; van den Bossche et al, 1990; White, 1997). The SBI fungicide tetraconazole has been used widely for control of Cercospora leaf spot in eastern Montana over the last few years, raising concerns about the development of SBI resistance in the local *C. beticola* population. Resistance to SBI fungicides has been reported for many pathogens (Stanis and Jones, 1985; Braun and McRae, 1992; Kendall et al, 1993; Eckert et al, 1994; Romero and Sutton, 1997).

The presence of resistance to SBIs in *C. beticola* has been previously documented in a growing area in Greece where SBIs had been used for control of Cercospora leaf spot over about a 15 year period (Karaoglanidis et al, 2000). Tests of field populations in this area of Greece harboring SBI tolerant isolates showed resistance development to be a slow adaptation, indicating changes in multiple genes similar to other fungi (Köller, 1988; Georgopoulos, 1994). Indications are that these genetic changes result in reduced fitness. Isolates with reduced sensitivities were determined to be less aggressive, have reduced sporulation capacity, and lower competitive ability than sensitive isolates

(Ioannidis and Karaoglanidis, 2000; Karaoglanidis et al, 2001). Resistant isolates exposed to prolonged cold temperatures have also been shown to become more sensitive to SBI activity, indicating SBI resistance results in an increase in cold sensitivity (Karaoglanidis and Thanassoulopoulos, 2002).

The possible reasons proposed for the development of resistance to an SBI include a mutation in the binding site of the target enzyme, an increase in the amount of enzyme present in the cell, and the presence of a drug efflux protein used to rid the cell of chemicals. Mutations in the 14α-demythylase gene (*CYP51A1*) conferring SBI resistance have been reported for field strains of *Uncinula necator* and *Erysiphe graminis* (Deleye et al, 1997, 1998). The *CYP51A1* gene in these strains carried a single nucleotide mutation within the substrate recognition site that resulted in an amino acid change from phenylalanine to tyrosine. Studies by Schnabel and Jones (2001) have shown that SBI resistant field isolates of *Venturia inaequalis* (the fungus causing apple scab) from orchards with practical SBI resistance overexpress the *CYP51A1* gene. Overexpression of the *CYP51A1* gene is thought to result in higher amounts of the target enzyme, 14α-demythylase, necessitating the application of higher amounts of SBI fungicide to achieve adequate control. Evidence for the use of chemical efflux from the fungal cell to develop resistance to the SBI fungicides has been found in *Venturia inaequalis*. Palani and Lalithakumari (1999) found that laboratory mutants of *V. inaequalis* resistant to an SBI possess an energy dependent efflux mechanism. Similarly, high expression of two genes that encode drug efflux proteins of the ATP-binding cassette transporter family has been linked with resistance to an SBI (Del Sorbo et al, 1997; Nakaune et al, 1998). In light of

the evidence for multiple mechanisms of SBI-resistance development, it is recommended that strategies for the management of SBI resistance in *C. beticola* should rely on reduced applications of SBI fungicides and rotation with non-SBI fungicides during each growing season (Karaoglanidis et al, 2001).

As a result of high practical resistance to the benzimidazole fungicides in *Cercospora beticola* populations near Sidney, MT, these fungicides are no longer recommended for use in controlling Cercospora leaf spot. The loss of this class of fungicides places more dependence on the available protectant (ethylene bis dithiocarbamates and triphenyltin hydroxides) and systemic fungicides (strobilurins and triazoles) for control of CLS. Tolerance to triphenyltin hydroxide has been identified in the Sidney growing area and is of concern (Jacobsen et al, 2003). Therefore, monitoring of resistance to these fungicides as well as proper fungicide use will continue to be of great importance.

Biological Control

Biological control is the use of a beneficial organism to reduce the population and detrimental effects of another organism on a crop or ecosystem (Emmert and Handelsman, 1999). Biological control organisms are not widely used in most commercial agricultural systems for many reasons including narrow spectrum of activity, reduced efficacy and difficulty of application compared to chemicals. However, with an increasing amount of regulation and restriction being applied to chemicals because of concerns for human and environmental health, biological control is becoming an

increasingly viable and attractive mechanism of pest control (Emmert and Handelsman, 1999). Biological disease controls may also prove useful alternated with chemical fungicides for resistance management (Chapter 3).

Biological Control of Plant Disease

A wide range of microbes including bacteria (Wolff et al, 2003), yeast, filamentous fungi (Punja and Utkhede, 2003), and oomycetes (Picard et al, 2000) have been employed as biological control agents. Biocontrol agents can be effective through the production of hydrolytic enzymes and antibiotics, niche colonization and competition for host nutrients, induction of plant host defense mechanisms, and interference with pathogenicity factors (Punja and Utkhede, 2003).

The ability of fungi such as *Trichoderma* spp. to parasitize other fungi was first reported in the 1930s (Howell, 2003). Other fungi such as *Ampelomyces quisqualis* and *Coniothyrium minitans* have since been shown to parasitize pathogenic fungi and control disease in the field enough to be commercialized (Rotem et al, 1999; Budge and Whipps, 2001). Yeasts were discovered to reduce spore production and growth of plant pathogenic fungi (Avis and Bélanger, 2001; Urquhart and Punja, 2002). The oomycete *Pythium oligandrum* has been used to control other *Pythium* spp. and other fungi through parasitism (Madsen and Neergaard, 1999).

The production of antibiotic compounds effective in controlling plant pathogens has been well documented in fungi such as *Trichoderma virens* (Howell, 2003) and *Pseudozyma flocculosa* (Avis and Bélanger, 2001), as well as numerous species of bacteria (Siloh-Suh et al, 1994). Many biological control agents produce hydrolytic

enzymes that play a central role in the efficacy of the agent. *Trichoderma* spp. produce chitinases (Baek et al, 1999; Limón et al, 1999), endoglucanases (Migheli et al, 1998), and proteinases (Flores et al, 1997) that are essential factors in their disease control activity. These enzymes also play a role in disrupting the hydrolytic enzymes of pathogenic fungi, effectively reducing their infectivity (Elad and Kapat, 1999).

Niche occupation by filamentous fungi (Harman, 2000; Eparvier and Alabouvette, 1994), yeasts (Filonow, 1998) and bacteria (Wulff et al, 2003; Barden et al, 2003) has been reported. These organisms inhibit pathogens by occupying the sites of infection used by the pathogen and using the nutrients that are needed for infection (Punja and Utkhede, 2003), thereby competitively displacing the pathogen.

The ability of some biocontrol agents to induce a host plant to produce an array of defense compounds and enzymes effective in fighting off disease is a developing area of biological control research (Benhamou, 1996). Induction of systemic disease resistance has been shown by the plant growth-promoting rhizobacteria (Ryu et al, 2003; Bakker et al, 2003) and phyllosphere colonizing bacteria (Bargabus et al, 2002), yeasts (Droby et al, 2002), and has been suggested for some fungi (Bao and Lazarovits, 2001). The utility of biological control agents with the ability to induce a systemic response in the plant allows broad spectrum control of several diseases (Jetiyanon et al, 2003) and is less dependent on high populations of biological control agents for efficacy as compared to antibiosis and niche occupation.

Bacillus mycoides, Isolate BmJ

Characterization

Bacillus mycoides belongs to the gram-positive, rod-shaped *Bacilliaceae* family of bacteria. It is considered a saprophyte (Sorheim et al, 1989), although the ability of *Bacillus mycoides* to cause bacteriosis in roots of sugar beet has been reported in Europe (Stankiewicz and Krezel, 1984). All *Bacillus* spp. are able to form endospores that serve as survival structures (Priest, 1993). This feature has made them very attractive for development of biological control agents because of the relatively long shelf-life and ease of formulation and field application afforded by endospore formation.

Bacillus mycoides is related to the *Bacillus cereus* group members *B. anthracis* and *B. thuringiensis* (von Wintzingerode et al, 1997) but is considered a separate species due to morphological and genetic differences (Nakamura and Jackson, 1995). Unlike most *bacilli*, *B. mycoides* is not motile and grows in long chains forming a rhizoidal colony shape in plate cultures (Di Franco et al, 2002). Isolate BmJ was isolated from the phyllosphere of sugar beet leaves from EARC in 1994.

Induction of Systemic Acquired Resistance by BmJ

Studies by Bargabus et al. (2002, 2003) have confirmed the ability of *B. mycoides* isolate BmJ to induce a defense response in sugar beet consistent with Systemic Acquired Resistance. Pathogenesis related proteins (PR proteins) that are induced by BmJ in sugar beet include chitinase, β-1,3-glucanase and peroxidase. All are indicators of systemic acquired resistance. In addition to the production of PR proteins, BmJ induces an

oxidative burst consistent with a hypersensitive response shortly after application to the leaf surface. Production of PR proteins follows and reaches maximum levels after approximately seven days. The defense response often lasts about two weeks. In greenhouse and field experiments, the induction of systemic resistance in sugar beet by BmJ has been shown to control Cercospora leaf spot to levels similar to fungicide treatments (Bargabus et al, 2002).

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CHAPTER 2

IMPACT OF VARIETY SELECTION ON DISEASE DEVELOPMENT AND YIELD

Introduction

Cercospora Leaf Spot (CLS), caused by the fungus *Cercospora beticola* Sacc., is the major foliar disease on sugar beets (*Beta vulgaris* L.) in Montana. Losses in Montana over the last 7 years have averaged between 2 and 3 tons per acre with 0.5 and 1.5 percent lower sugar (Jacobsen et al, 2003). Losses are shown in a reduction in root weight, lower sugar content, and increased impurities leading to a loss of sugar to molasses (Smith and Martin, 1978). Smith and Ruppel (1971) determined that CLS also caused increased storage rot because of cercosporin and beticolin, toxins produced by *C. beticola*.

Cercospora leaf spot management strategies in eastern Montana currently depend on crop rotation, a few registered fungicides, scouting, a Cercospora leaf spot prediction model developed by Shane and Teng (1983-1985), and partially resistant sugar beet varieties. It is recommended that sugar beets be grown in 3 year or greater rotations with crops such as small grains, corn, dry beans and alfalfa that do not serve as hosts for *C. beticola* and other sugar beet pests and diseases (Wilson, 2001). Control of host weeds such as winged pigweed, lambsquarter, red root pigweed, mallow and wild buckwheat in these crops is also important in reducing primary inoculum (Schweizer and May, 1993; Whitney and Duffus, 1986). Two-year rotations are still used in some districts of the Sidney growing area but are not recommended. Spatial separation of at least 100 m from

the previous year's sugar beet growing area to the current years fields is recommended to inhibit inoculum movement from leaf debris (Windels et al, 1998). A two-year rotation hinders spatial separation, especially in the Yellowstone and Missouri river valleys near Sidney, MT where sugar beet production has historically been concentrated in the irrigated valleys and has adhered to a two-year rotation. However, new production areas away from the river valleys are being developed through the use of sprinkler irrigation. Spatial separation of sugar beet production may be managed more easily in these areas.

Registration of newly developed fungicides to treat pathogens of food crops is closely regulated to prevent the over exposure of the food supply to these new fungicides (Food Quality Protection Act, 1996). Major food crops are generally given priority for pesticide registration by industry because of the financial and temporal costs of the registration process. Because agrochemical companies do not consider sugarbeets a major crop, newly developed fungicides have not been readily available. Fungicides currently registered for CLS control are threatened by the development of fungicide resistance and potential revocation of their registration through the implementation of the Food Quality Protection Act (1996). For example, TPTH was reduced to no more than two applications in any crop year to reduce the residues and environmental hazards posed by this fungicide. Therefore, the development of sustainable CLS management options preserving use of available fungicides is of high importance.

The Cercospora prediction model developed by Shane and Teng (1983-1985) is an important tool in sustainable CLS management by allowing for judicious application of registered fungicides targeted for infection periods and when the economic threshold

of 3% leaf area infected is reached. In short, CLS infection periods are predicted by evaluating the presence of CLS on sugar beets and the weather conditions optimal for infection and sporulation (i.e. relative humidity >90% and nighttime temperatures >15.5˚ C) after row closure occurs in late June to early July (Shane and Teng, 1983-1985; Windels, 1998). Fungicide applications are then timed to coincide as closely as possible with the CLS infection periods predicted by the model. The southern Minnesota, Minn-Dak and American Crystal sugar cooperatives of eastern North Dakota and Minnesota, as well as the Sidney Sugars LLC (Sidney, MT growing area) and Western Sugar have adapted this model to fit their respective sugar beet growing regions. In this study, the Cercospora prediction model was used primarily to document the occurrence of infection periods throughout the growing season. It was also used as a supplement to observations of nighttime temperatures above 15.5˚ C (required for adequate CLS infection) and identification of CLS symptoms for determining the timing of the first spray in each field season.

Sugar beet hybrids with increased resistance to CLS are valuable for CLS management, especially in areas where disease levels are significant each year. However, Smith and Campbell (1996) report that there is a negative correlation between CLS resistance and yield potential in sugar beet hybrids. Studies by Rossi et al. (1999) determined CLS resistance in sugar beet to be characteristic of rate-reducing resistance. This type of host-plant resistance slows the rate of disease development. The stages of development affected are unique for each pathosystem. In sugar beet resistance to CLS, efficiency of infection is reduced, incubation period (time from conidial attachment to the

leaf to symptom development) is lengthened, and the number of conidia produced on necrotic lesions is reduced (Rossi et al, 1999). The conidiogenesis period (time from lesion appearance to conidia formation) is not affected by resistance in sugar beet. The level of CLS resistance in each variety is determined by rating the amount of disease occurring in Betaseed Nurseries in Shakopee and Rosemount, MN. The Klienwanzleber Saatzucht (KWS) scale is used to determine the amount of CLS symptoms. The KWS scale ranges from 0-9 with zero representing no disease symptoms and 9 being greater than 60% leaf area infected (Klienwanzleber Saatzucht Ag. Einbeck, 1970). More resistant sugar beet varieties have a lower KWS score. In this study, varieties with a range of KWS scores from 4 to 6.3 were tested.

In the field trials described here several integrated disease management strategies were evaluated. These strategies were use of sugar beet varieties having a range of CLS resistance, rotations of different classes of fungicides based on their respective modes of action, variation in the number of fungicide applications, and use of the biological control agent, BmJ, applied alone and integrated with half-label rate of tetraconazole (75 ml formulated product/ha). The biocontrol agent, BmJ, is a *Bacillus mycoides* strain isolated from a sugar beet leaf in the Sidney MT area in 1994. BmJ acts as an inducer of systemic resistance in sugar beet (Bargabus et al, 2002). When induced by BmJ, the sugar beet produces systemic defense compounds and proteins to fight the infection by pathogens. BmJ can give disease control comparable to fungicide programs, especially when applied in combination with reduced rates of fungicide and varieties with moderate levels of resistance (Bargabus et al, 2002; Jacobsen et al, 2003).

Materials & Methods

Fungicides and the biological control agent, BmJ were applied to sugar beet varieties with a range of CLS resistance levels to determine effects on the development of Cercospora leaf spot and subsequent yield. Variables tested were the number of fungicide applications per season (0-4 sprays); BmJ applied alone or in combination with tetraconazole at half the label rate (75 ml formulated product/ha); and the degree of CLS resistance in the cultivars used. Nine varieties were tested over the three-year period from 2001 to 2003. Each variety's level of CLS resistance was identified with a KWS score ranging from 4 to 6.3 on a 0-9 scale (lower values indicate resistance). The KWS score was assigned to each variety after controlled testing performed by the Betaseed Nurseries in Shakopee and Rosemount MN (Steen, 1998; Niehaus, 2001 and 2002).

Treatments were tested at the Eastern Agricultural Research Center in Sidney, MT (EARC) from 2001 to 2003. Experimental plots were six rows wide, 9.14 m long, with a row spacing of 61 cm. The middle four rows of each plot were treated leaving an untreated border row on each side of each plot. The list of treatments used each year is shown in Table 2.1. Assays of fungicide resistance development in the local *C. beticola* population indicated resistance to benomyl and reduced sensitivity to tetraconazole. Therefore, changes in fungicide use were made to reflect these indications. Benomyl was eliminated after 2001 and tetraconazole applications were moved from the first spray to the second spray in 2003. Treatments were applied at 14-day intervals beginning at disease onset. In addition, in 2003, BmJ was applied approximately one week prior to disease onset in selected treatments. This was done because BmJ elicits systemic induced

Table 2.1: Variety trial treatments used in each year. Fungicide spray amounts are of formulated product.

Spray Program	2001	2002	2003
untreated control	untreated control	untreated control	untreated control
1 spray	1[1]: tetraconazole (0.15 L/ha)	1: tetraconazole (0.15 L/ha)	1: trifloxystrobin (108 ml/ha)
2 sprays	1: tetraconazole (0.15 L/ha), 2: benomyl (92 g/ha)	1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha)
3 sprays	1: tetraconazole (0.15 L/ha), 2: benomyl (92 g/ha), 3: triphenyltin hydroxide (57 g/ha)	1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: triphenyltin hydroxide (57 g/ha)	1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha) -2, 3: triphenyltin hydroxide (57 g/ha)
4 sprays	1: tetraconazole (0.15 L/ha), 2: benomyl (92 g/ha), 3-4: triphenyltin hydroxide (57 g/ha)	1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: triphenyltin hydroxide (57 g/ha), 4: trifloxystrobin (110 ml/ha)	1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: triphenyltin hydroxide (57 g/ha), 4: trifloxystrobin (84 ml/ha)
BmJ + tetraconazole	1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})
BmJ + tetraconazole	-	-	BmJ (10^7 cfu ml^{-1}) prior to disease onset, 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})
BmJ alone	1-4: BmJ (10^7 cfu ml^{-1})	1-4: BmJ (10^7 cfu ml^{-1})	BmJ (10^7 cfu ml^{-1}) pre-disease onset, 1-4: BmJ (10^7 cfu ml^{-1})

1- numbers preceding fungicides/BmJ indicate order of application
- the first spray was applied at disease onset with other sprays applied at 14 day intervals thereafter.

resistance in sugar beet that takes approximately one week to reach a full defense response (Bargabus et al, 2002). Treatments were replicated six times in a randomized complete block design. All field trials in Sidney were conducted with the collaboration of the EARC. Agronomic and harvest work was performed by EARC staff. Variety trials were planted on May 4, May 3, and April 28 in 2001, 2002 and 2003, respectively.

In 2001, three varieties, Beta 2185 (Betaseed), HH 111 (Holly Hybrids), and HM 7054 (Hilleshog), were tested. These varieties have a range of resistance (KWS scores of 6.3, 5.3, and 4.1, respectively). The 2002 variety trial evaluated six varieties. Added to the study were Monarch (Seedex), HH 115 (Holly Hybrids), and Beta 3820 (Betaseed), having KWS scores of 5, 4.9, and 4.4, respectively. Six varieties were tested in the 2003 variety trial as well. However, the three varieties used in 2001 were replaced by varieties Trophy (Hilleshog), AC 927 (American Crystal) and VDH 66556 (Van der Have), having KWS scores of 4.2, 4 and 4.9, respectively. Varieties were selected to reflect the most popular varieties planted by growers in the Sidney area and those with the widest range of CLS resistance that were approved for use that had high levels of resistance.

Plots were sprayed beginning on July 17 in 2001, July 2 in 2002, and July 7 in 2003 with subsequent sprays following at 14-day intervals. An application of BmJ was applied to selected plots on June 29 in 2003 to test the impact of BmJ applied before disease onset.

Spray Formulation and Application:

Treatments were applied at roughly 247 L ha^{-1} at 207 kPa on the middle four rows of each plot using a CO_2 hand sprayer equipped with a four-nozzle boom. Four Teejet

8002 VS nozzles (Teejet Inc.) were spaced approximately 48 cm apart on the spray boom. Fungicide sprays were formulated in water and applied at label rates unless otherwise noted. The *Bacillus mycoides* biological control agent, BmJ, was formulated in water to a concentration of $1x10^7$ cfu ml^{-1}.

Preparation of BmJ:

For BmJ treatments, stock cultures were grown from original cultures of field-isolated BmJ stored at -80° C. An aliquot of 100 μL of original BmJ was added to 250 ml of a shake-flask culture of tryptic soy broth (TSB). This stock culture was fermented at room temperature for at least 48 hours before being separated into aliquots of 2 ml and stored at -10° C. Prior to spray formulation, approximately 1 ml of the stock culture was added to 250 ml of TSB. Fermentation was carried out in 1 L Nalgene bottles, fitted with glass pipettes attached to aquarium air pumps (Elite 799, Rolf C. Hagen Inc.) to aerate the culture. Contaminates were filtered from the air by cotton packed into the top of the pipette. BmJ was then diluted by 1:100 for the final spray solution of approximately 10^7 cfu per ml. Exact BmJ cell numbers were not counted because the chain forming nature of BmJ made individual cells difficult to count. Fresh BmJ cultures were used for BmJ treatments in 2001 and 2002. In 2003, BmJ sprays were formulated from dry powders of lyophilized endospores produced by Osprey Technologies, Sarasota, FL.

CLS Evaluation:

Disease levels were rated four separate times (five in 2001) throughout the season using the CLS disease scale of 0-10 developed by Shane and Teng (1983) and referred to

by them as the Spot Percentage scale. An average disease level was calculated for 100 leaves in the upper 2/3s portion of the canopy taken from each plot. New leaves were not rated, because they were not old enough to show disease symptoms. The CLS disease scale rates damage as ten degrees of percent leaf damage, ranging from 0.1% to 50% (see Appendix). These data, quantified as the area under the disease progress curve (AUDPC), were used to determine the overall disease levels for the season.

Cercospora Prediction Model:

The Cercospora prediction model developed by Shane and Teng (1983-1985) and reviewed by Windels (1998) was used to calculate infection periods. A lower threshold of relative humidity of 87 % was used in the model instead of the original 90%. This lower threshold was used in accordance with changes made by the sugar beet industry (Windels, 1998) and to compensate for a lack of sugar beet canopy around the weather sensors. When sensors are not placed in the canopy, relative humidity levels in the canopy are underestimated (Enz et al. 1996). In addition, leaf wetness often persists until noon in the canopy for several days after irrigation. Therefore, a more conservative approach to interpreting the weather conditions when using the Cercospora prediction model and its output compensated for the lack of canopy around the weather sensors. Daily infection values (DIVs) were calculated using the information in Table 2.2 according to the number of hours of $\geq$87 % relative humidity at specific temperatures. Daily infection values $\geq$4 indicated conditions were adequate for a significant infection period while lower values (1-3) indicated a lower potential for CLS infection.

Table 2.2: Daily Infection Values for Cercospora leaf spot calculated from the number of hours per day (24 hr) with relative humidity ≥ 87% and the average temperatures for this time period. Value totals for any 48 hour period less than 4 indicate low likelihood of disease development, a value of 4 marginal potential, and greater than 4 high potential.

Hours/day ≥ 87% RH	Daily Infection Values																																				
24	1	2	4	5	5	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
23	1	2	3	4	5	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
22	1	1	3	4	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
21	0	1	2	4	4	5	5	5	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
20	0	1	2	3	4	5	5	5	5	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
19	0	0	1	3	4	5	5	5	5	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
18	0	0	1	2	3	4	4	4	4	4	4	5	5	5	5	5	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
17	0	0	1	2	3	4	4	4	4	4	4	4	5	5	5	5	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
16	0	0	0	2	3	4	4	4	4	4	4	4	4	4	4	4	5	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
15	0	0	0	1	3	4	4	4	4	4	4	4	4	4	4	4	4	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
14	0	0	0	1	3	3	3	3	3	3	3	4	4	4	4	4	4	4	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
13	0	0	0	0	3	3	3	3	3	3	3	3	3	4	4	4	4	4	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
12	0	0	0	0	2	3	3	3	3	3	3	3	3	4	4	4	4	4	4	5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
11	0	0	0	0	2	3	3	3	3	3	3	3	3	3	3	3	3	3	4	5	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
10	0	0	0	0	2	2	2	2	2	2	2	3	3	3	3	3	3	3	4	4	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
9	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	4	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
8	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
7	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
6	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
°C	16					18						21						24						27						29						32			

The timing of the initial spray in each year was determined using the Cercospora prediction model, identification of CLS symptoms and observations of nighttime temperatures above 15.5 C (necessary for CLS development). Subsequent sprays were applied at two-week intervals, per standard practice, to adequately test the impact of each additional spray on CLS development. Output from the Cercospora prediction model after the initial spray was then used for post-seasonal analysis of the CLS progression.

Harvest and Processing:

Research plots were harvested on September 19, 25, and 24 in 2001, 2002, and 2003, respectively. One of the two center treated rows from each plot was harvested to determine yield in kilograms per row. Tare samples of 12-15 beets were processed at the sugar beet processing factory tare lab in Sidney (Holly Sugar in 2001, 2002; Sidney Sugars LLC in 2003) to determine percent tare, percent sugar, and percent nitrogen. Further analysis to determine sugar loss to molasses and levels of impurity in the sugar was conducted at the Holly Sugar Lab in Sheridan WY, in 2001 and 2002, and at Ag Terra Technologies Inc. Lab in Sheridan WY, in 2003. Gross yield was measured in metric tons of beets per hectare. Net yield was determined as kilograms of extractable sucrose per hectare.

Statistical Analysis:

Analysis of variance (ANOVA) was performed on data representing AUDPC disease levels, percent sucrose in the beet, gross yield in metric tons of sugar beets per hectare, and net yield in kilograms of extractable sucrose per hectare from each field trial

using the general linear model by the SAS® program (SAS Institute, 1988). A Students *t* Test (Fisher's) was used to determine the level of least significance difference (LSD) among field treatments at the P=0.05 level. Correlation procedures were used in SAS to determine impacts of AUDPC on yield and the correlation of the slope of CLS progression with the KWS value of each variety.

Results

In each of the three years, results from the variety trial have shown that varieties with KWS ratings < 4.9 have lower AUDPC values than varieties with higher KWS scores. Table 2.3 shows the disease levels (AUDPC values) for the untreated control plots. Three varieties were tested in 2001. Beta 2185 (Betaseed), HH 111 (Holly), and HM 7054 (Hilleshog) have KWS scores of 6.3, 5.3, and 4.1, respectively. The 2002 Variety Trial evaluated HH 115 (Holly), Monarch (Seedex), and Beta 3820 (Betaseed), having KWS scores of 4.9, 5, and 4.4, respectively. Three additional varieties were tested in 2003; Trophy (Hilleshog), AC 927 (American Crystal) and VDH 66556 (Van der Have), having KWS scores of 4.2, 4 and 4.9, respectively.

The AUDPC levels shown in Table 2.3 roughly match the KWS score assigned to each variety. Exceptions occurred with HH 111 (KWS=5.3), HH 115 (KWS=4.9) and Monarch (KWS=5) in 2002. These varieties have a lower published KWS value than Beta 2185 (KWS=6.3), but had higher levels of disease. Disease levels also did not match the published KWS score for Trophy (KWS=4.2) in 2003 when compared to

varieties that are more susceptible. Relatively low CLS pressure and localized nature of infections in the field in 2003 may have resulted in these anomalies.

Table 2.3: Variety trial AUDPC[1] values of untreated control plots by year for each variety.

Variety (KWS)	2001	2002	2003
Beta 2185 (6.3)	57.7	53.1	-
HH 111 (5.3)	48.0	61.6	-
HM 7054 (4.1)	35.3*	33.8*	-
HH 115 (4.9)	-	61.1	13.7*
Monarch (5)	-	55.3	25.4
Beta 3820 (4.4)	-	30.2*	2.4*
VDH 66556 (4.9)	-	-	9.5*
Trophy (4.2)	-	-	10.4*
AC 927 (4)	-	-	5.5*
$LSD_{(0.05)}$	*12.87*	*9.66*	*0.64*

1 AUDPC = area under the disease progress curve.
* significantly different (P=0.05) from the most susceptible variety in each year (Beta 2185 in 2001,2002 and Monarch in 2003).

Disease levels were moderate in 2001 and 2002, reaching the economic threshold level of 3 % leaf damage (Windels, 1998) in the untreated plots of the susceptible variety, Beta 2185 by 50 days post disease onset in 2001 and 65 days post disease onset in 2002. (Figures 2.1 and 2.2). Disease levels were much lower in 2003, with leaf damage never exceeding 1.2 % in untreated plots (Figure 2.3).

Figures 2.1 and 2.2 show the impact of host-plant resistance in slowing the rate of CLS development. In 2001, CLS levels reached 3 % leaf damage on Beta 2185 (KWS=6.3) approximately five days before CLS on HH 111 (KWS=5.3) and ten days before CLS on HM 7054 (KWS=4.1). The amount of time that a sugar beet crop sustains CLS levels above 3 % leaf damage determines the amount of economic loss due to the

disease (Windels, 1998). Varieties that are more resistant should experience less economic loss by reducing the time spent above the economic threshold.

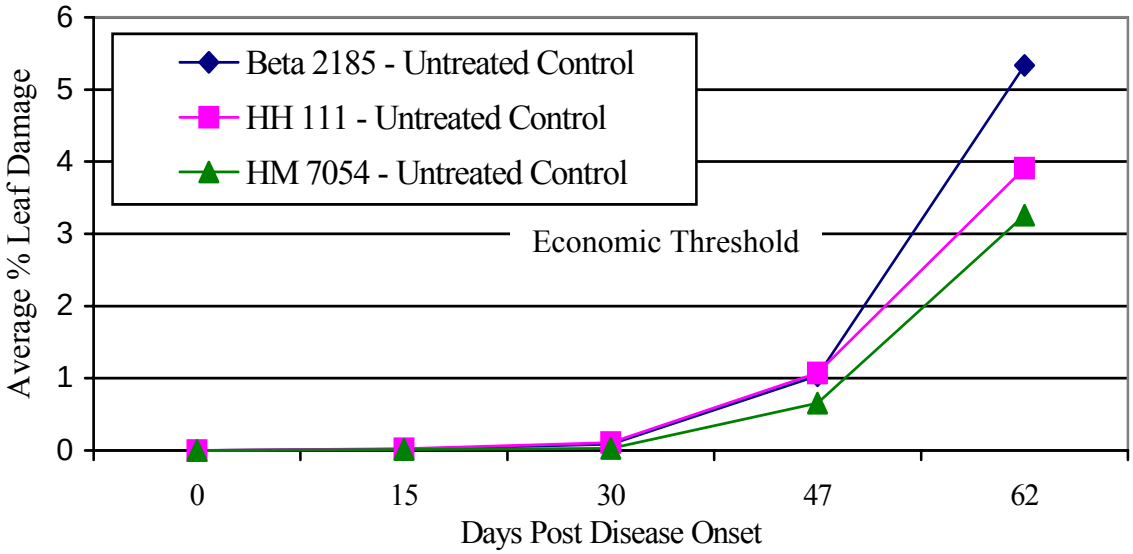

Figure 2.1: Disease progress curves for untreated control plots of each variety in the 2001 Variety trial. The onset of CLS disease occurred on July 17, 2001. Onset of disease is when first leaf spots were seen and when spray programs were started.

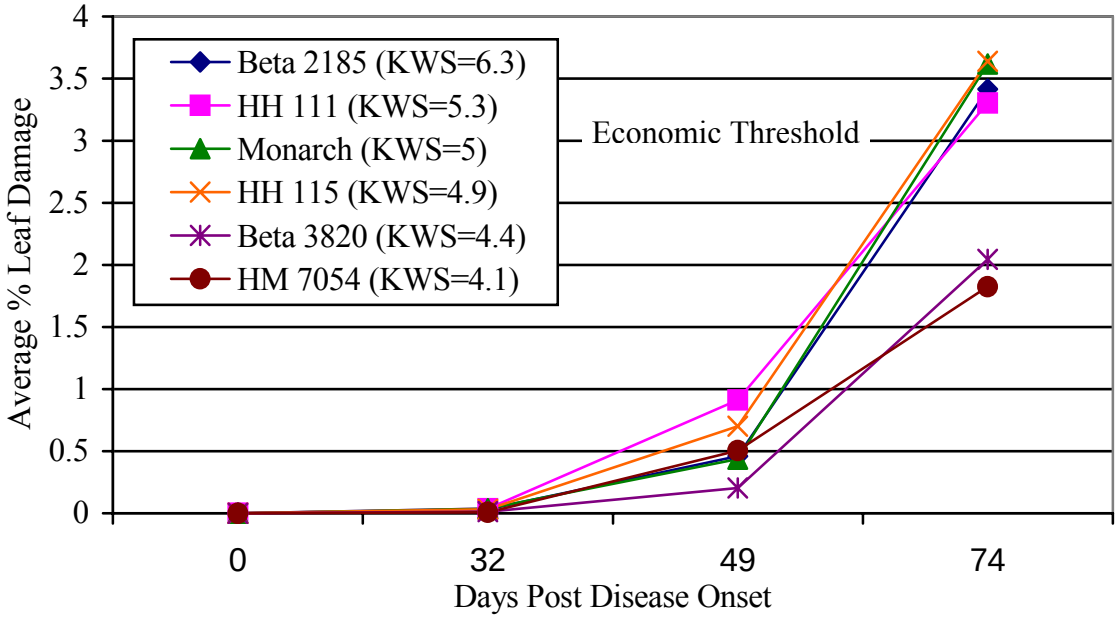

Figure 2.2: Disease progress curves for the untreated control plots of each variety in the 2002 Variety trial. The onset of CLS disease occurred on July 2, 2002. Onset of disease is when first leaf spots were seen and when spray programs were started.

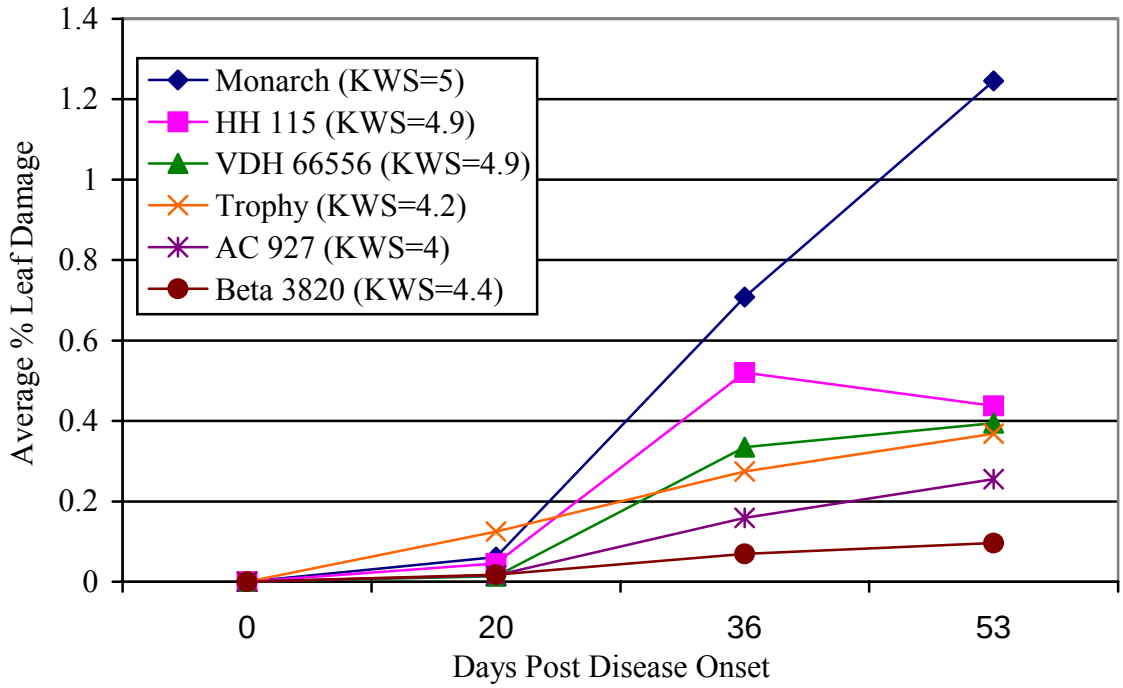

Figure 2.3: Disease progress curves for the untreated control plots of each variety in 2003. Monarch is considered the most susceptible. Disease levels were below the economic threshold of 3% leaf damage. The onset of CLS occurred on July 7, 2003. Onset of disease is when first leaf spots were seen and when spray programs were started.

The impact of weather conditions on CLS development was monitored using the Cercospora prediction model. Figures 2.4 through 2.6 summarize the CLS infection periods for 2001 through 2003 as determined by the Cercospora prediction model and indicate the timing of irrigation events, spray applications, CLS ratings and the onset of disease. The Cercospora prediction model indicated six infection periods with DIVs greater than zero in 2001 beginning on July 11. Three significant infection periods with DIVs of four or greater occurred in July (12-16, 20, and 26-28) and one occurred on August 8.

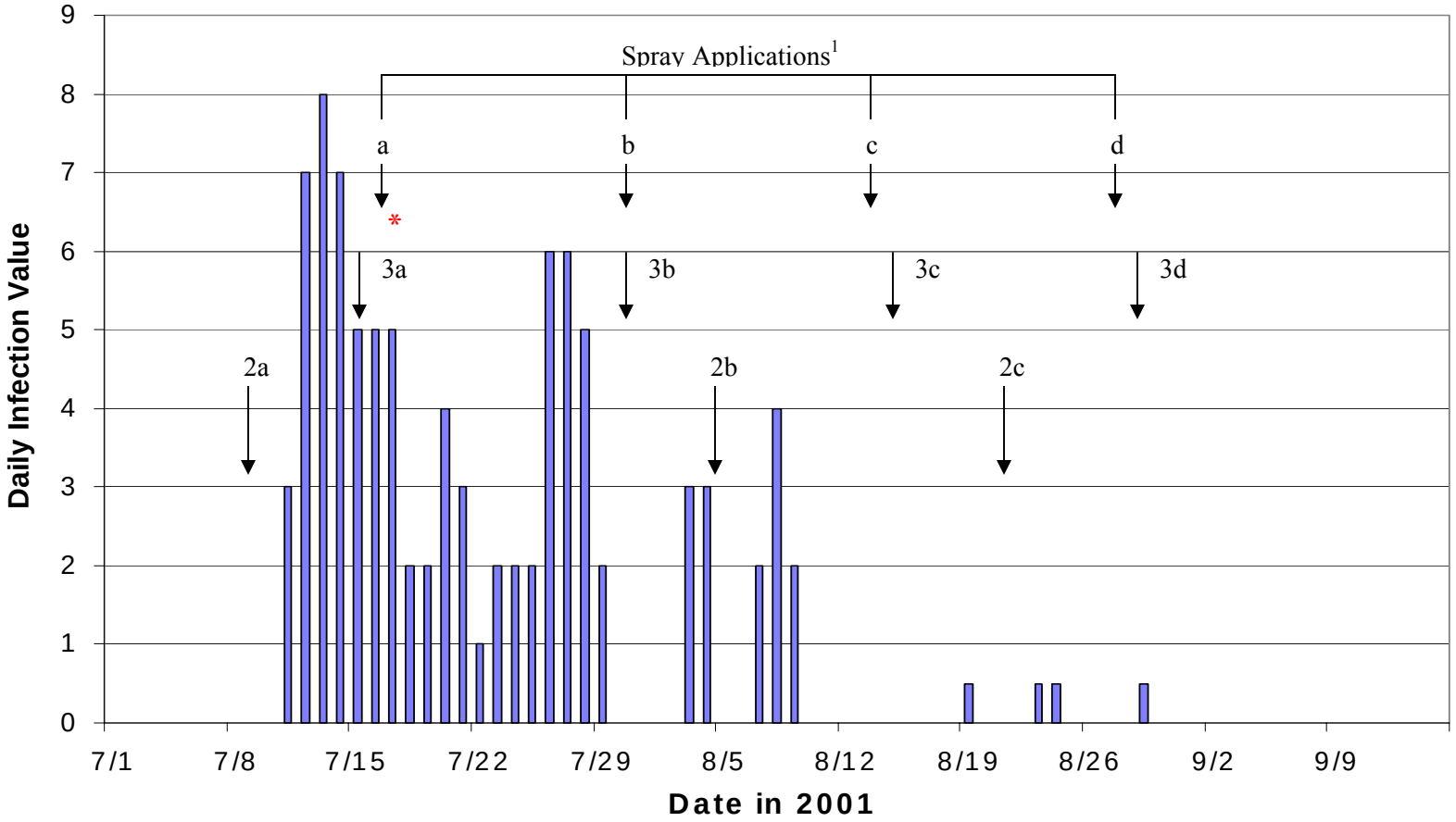

Figure 2.4: Daily infection values as indicated by the Cercospora prediction model in 2001. Arrows indicate timing of spray applications (1a-d), flood irrigation events (2a-c), CLS disease ratings (3a-e), and the onset of disease (*).

1- spray applications: a-tetraconazole (0.15 L ha^{-1}) on July 17; b-benomyl (92 g ha^{-1}) on July 31; c,d-triphenyltin hydroxide (57 g ha^{-1}) on August 14 and 28.

2- flood irrigation events: a-July 9, b-August 6, c-August 20.

3- CLS disease ratings: a-July 15, b-July 30, c-August 14, d-August 31, e-September 15.

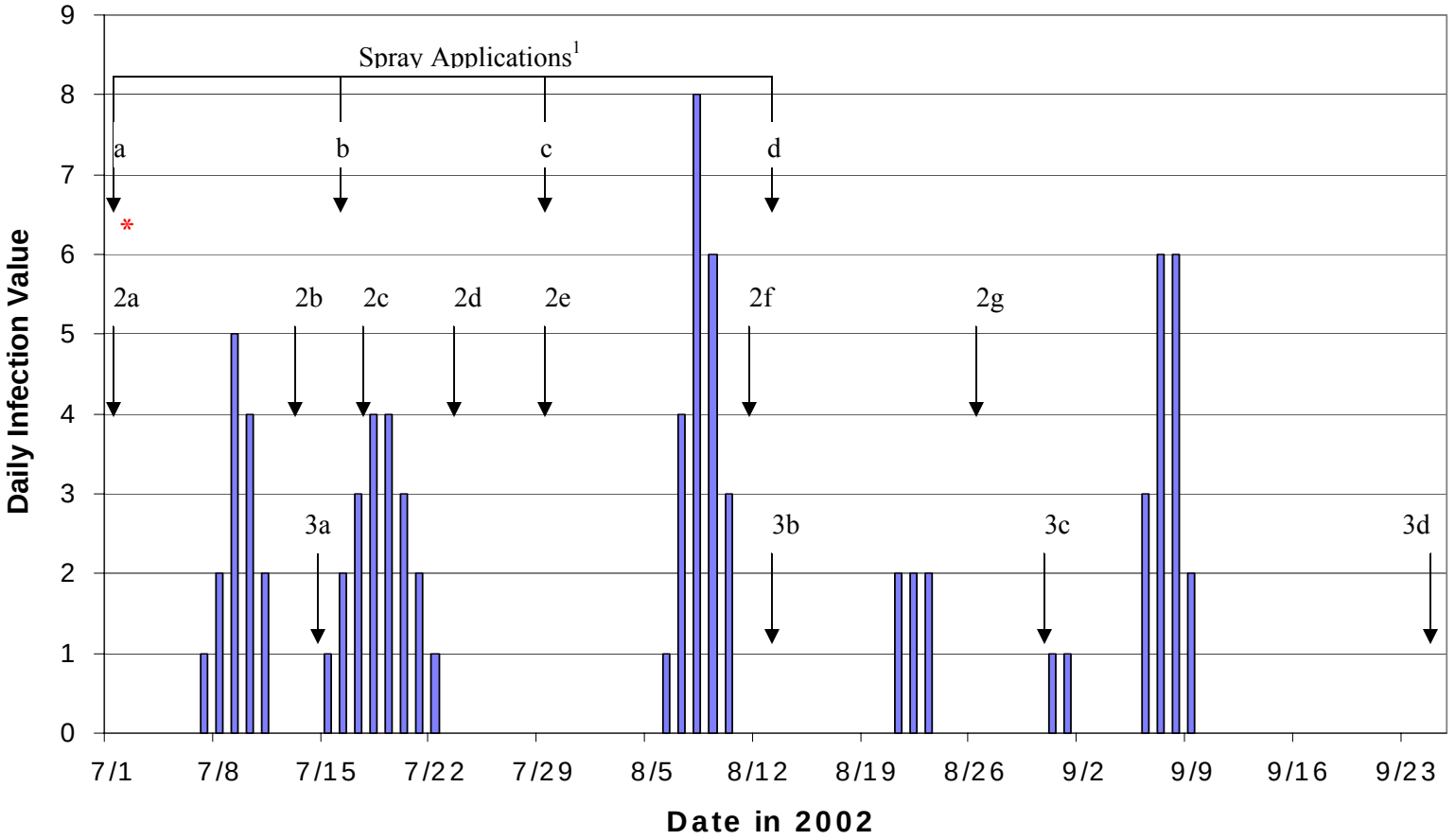

Figure 2.5: Daily infection values as indicated by the Cercospora prediction model in 2002. Arrows indicate timing of spray applications (1a-d), sprinkler irrigation events (2a-g), CLS disease ratings (3a-d) and the onset of disease (*).

1- spray applications: a-tetraconazole (0.15 L ha^{-1}) on July 2; b-trifloxystrobin (84 ml ha^{-1}) on July 16; c-triphenyltin hydroxide (57 g ha^{-1}) on July 30; d-trifloxystrobin (110 ml ha^{-1}) August 13.

2- sprinkler irrigation events: a-July 1, b-July 12, c-July 17, d-July 24, e-July 31, f-August 12, g-August 26.

3- CLS disease ratings: a-July 15, b-August 13, c-August 31, d-September 25.

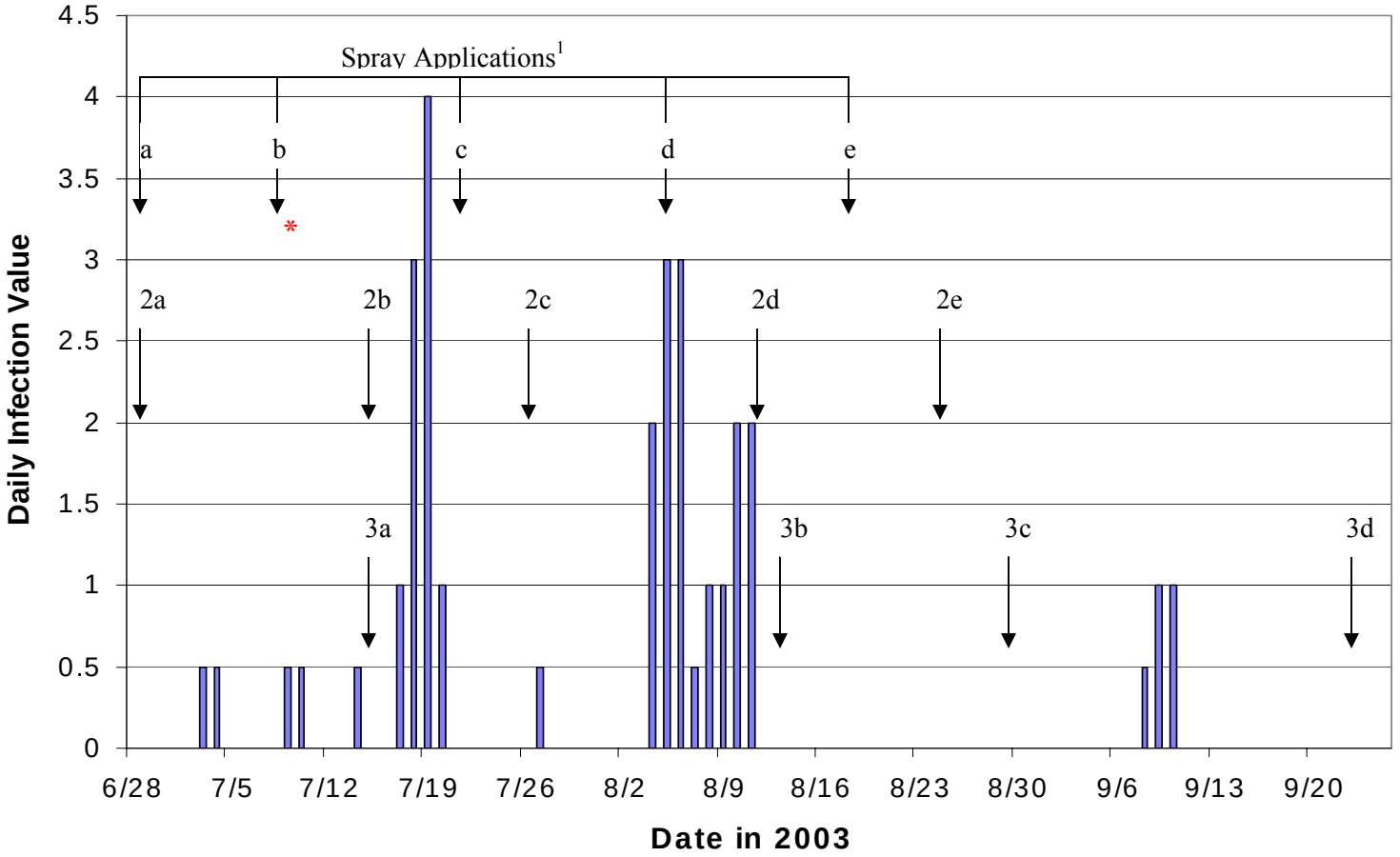

Figure 2.6: Daily infection values as indicated by the Cercospora prediction model in 2003. Arrows indicate timing of spray applications (1a-d), flood irrigation events (2a-e), CLS disease ratings (3a-d), and the onset of disease (*).
1- spray applications: a-BmJ in select treatments; b-trifloxystrobin (108 ml ha-1) on July 9; c-tetraconazole (0.15 L ha^{-1}) on July 22; d-triphenyltin hydroxide (57 g ha^{-1}) on August 5; e-trifloxystrobin (84 ml ha^{-1}) August 18.
2- flood irrigation events: a-June 30, b-July 14, c-July 27, d-August 11, e-August 25.
3- CLS disease ratings: a-July 15, b-August 20, c-September 5, d-September 22.

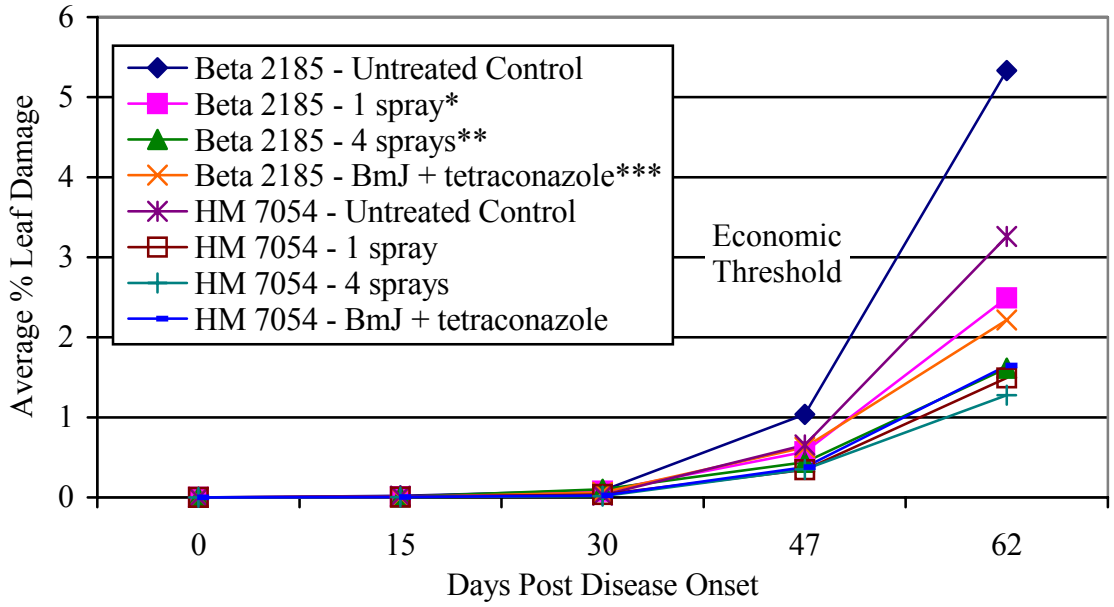

Figure 2.7: Disease progress curves for select treatments in the 2001 Variety trial. The onset of CLS occurred on July 17, 2001. * 1 spray = tetraconazole (0.15 L ha^{-1}) at disease onset. ** 4 sprays = 1: tetraconazole (0.15 L ha^{-1}), 2: benomyl (92 g ha^{-1}), 3-4: TPTH (57 g ha^{-1}). *** BmJ + tetraconazole = 1: BmJ with tetraconazole (75 ml ha^{-1}), 2-4: BmJ.

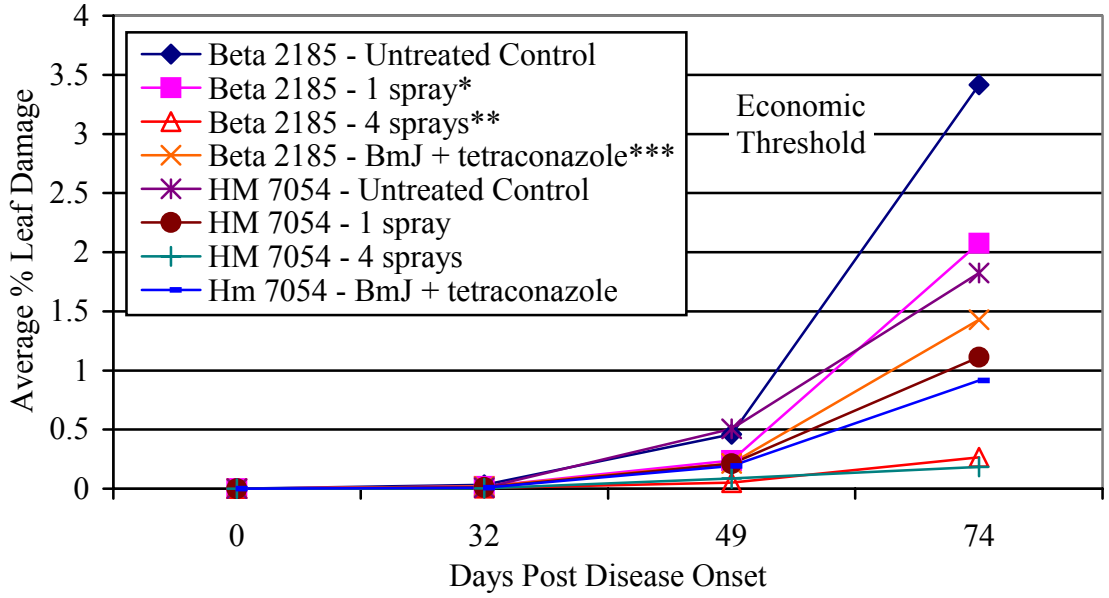

Figure 2.8: Disease progress curves for select treatments in the 2002 Variety trial. The onset of CLS occurred on July 2, 2002. * 1 spray = tetraconazole (0.15 L ha^{-1}) at disease onset. ** 4 sprays = 1: tetraconazole (0.15 L ha^{-1}), 2: trifloxystrobin (84 ml ha^{-1}), 3: TPTH (57 g ha^{-1}), 4: trifloxystrobin (110 ml ha^{-1}). *** BmJ + tetraconazole = 1: BmJ with tetraconazole (75 ml ha^{-1}), 2-4: BmJ.

In 2002, five infection periods occurred from July 8 to September 8 with DIVs greater than zero. Significant infection periods with DIVs of four or greater occurred twice in July and once each in August and September. In 2003, only three infection periods with DIVs greater than zero occurred from mid-July to early September. A single significant infection period with a DIV of four occurred on July 19.

The effects of select treatments when applied to the susceptible variety Beta 2185 (KWS=6.3) and the moderately resistant variety HM 7054 (KWS=4.1) on the CLS progress in 2001 and 2002 are shown in Figures 2.7 and 2.8, respectively. A single application of tetraconazole at 0.15 L formulated product ha^{-1} maintained disease levels below the economic threshold of 3 % leaf damage in both years. The application of BmJ with tetraconazole at half the label rate (75 mL formulated product ha^{-1}) in the first spray followed by three consecutive sprays of BmJ gave CLS control on HM 7054 comparable to the single spray and four-spray fungicide treatments on Beta 2185 in 2001. In 2002, the same treatment of BmJ with tetraconazole gave CLS control better than the single spray of tetraconazole at the full rate of 0.15 L formulated product ha^{-1} on both varieties.

Select treatment effects on the CLS progress when applied to Monarch (KWS=5) and Beta 3820 (KWS=4.4) in 2003 are shown in Figure 2.9. A single spray of trifloxystrobin on Monarch accomplished significant disease control. The integrated treatment of BmJ sprayed a week before disease onset, then a spray of BmJ with tetraconazole (75 ml ha^{-1}) at disease onset, followed by three more sprays of BmJ also gave a significant reduction in CLS on Monarch. The use of CLS spray treatments did

not reduce CLS levels on Beta 3820. The host-plant resistance in Beta 3820 was sufficient to maximize CLS control under the light disease pressure.

Results from all years (Tables 2.4 - 2.7) show that disease levels (AUDPC values) are greatly reduced with a single application of a systemic fungicide at disease onset (tetraconazole in 2001 and 2002; trifloxystrobin in 2003). Further sprays were not necessary to maintain disease levels below the economic threshold in 2001 and 2002 (Figures 2.7 and 2.8). Fungicide applications were not needed in 2003 due to low disease pressure (Figure 2.9). In all years, growing moderately resistant varieties (KWS<4.9) allowed statistically equal disease control with one or two fewer fungicide applications.

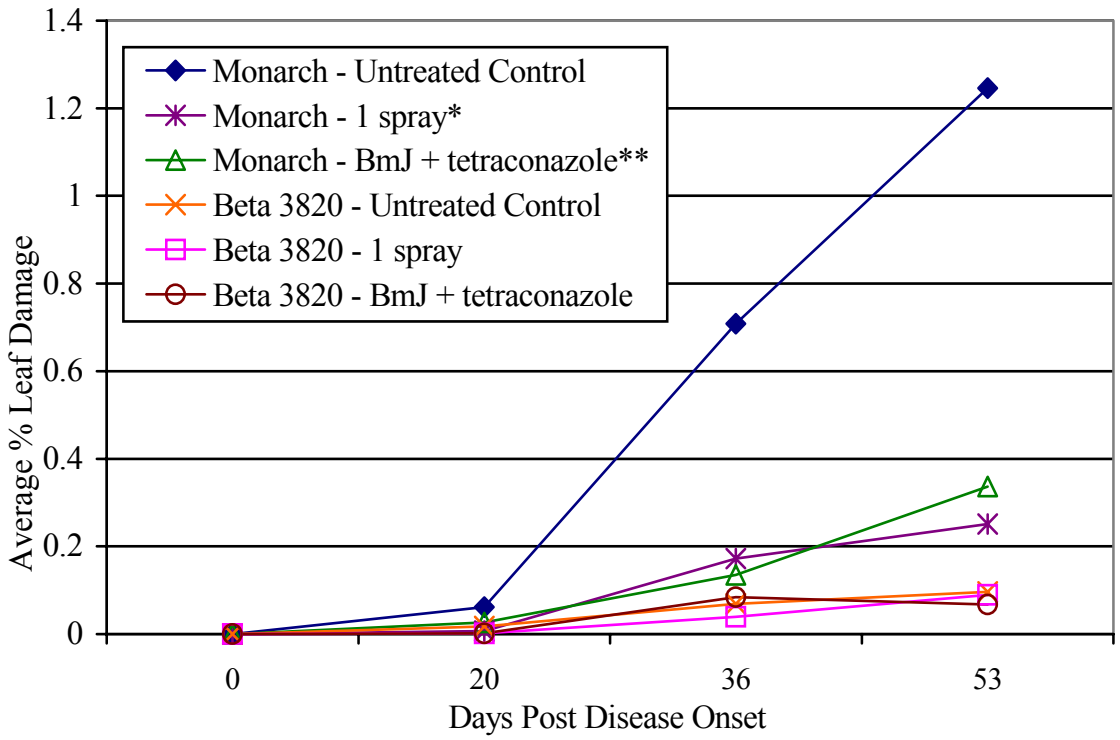

Figure 2.9: Disease progress curves for select treatments in the 2003 Variety trial. Monarch is considered the most susceptible. Disease levels are below the economic threshold of 3% leaf damage. The onset of CLS occurred on July 7, 2003. * 1 spray = trifloxystrobin (108 ml ha^{-1}). ** BmJ + tetraconazole = BmJ prior to disease onset; 1: BmJ with tetraconazole (75 ml ha^{-1}); 2-4: BmJ only.

Table 2.4: Variety trial AUDPC values for common varieties in 2001 and 2002.

Treatment[1] (beginning at disease onset and followed at 14 day intervals)	Variety (KWS) 2001 Beta 2185 (6.3)	2001 HH 111 (5.3)	2001 HM 7054 (4.1)	2002 Beta 2185 (6.3)	2002 HH 111 (5.3)	2002 HM 7054 (4.1)
1) untreated control	58.3	48.6	35.6	53.1	61.6	33.8
2) 1 spray	29.2	24.7	17.4	31.4	44.2	18.7
3) 2 sprays	30.1	30.8	16.2	13.7	25.9	15.1
4) 3 sprays	31.9	30.9	16.6	9.5	12.1	6.1
5) 4 sprays	24.9	21.5	17.7	4.6	6.0	4.2
6) 4 sprays BmJ + tetraconazole	27.7	23.8	18.9	22.8	38.3	15.7
7) 4 sprays BmJ	54.9	36.5	25.0	38.8	47.1	17.5
$LSD_{(0.05)}$		*12.87*			*9.66*	

1- In 2001, first spray = tetraconazole (0.15 L/ha); second spray = benomyl (92 g/ha); third and fourth sprays = triphenyltin hydroxide (57 g/ha). The 4 spray treatment results are an average of two identical 4 spray treatments. In 2002, first spray = tetraconazole (0.15 L/ha); second spray = trifloxystrobin (84 ml/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (110 ml/ha).

- In 2001 and 2002, the BmJ + tetraconazole treatment was a first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}).
- In 2001 and 2002, BmJ was four sprays of BmJ (10^7 cfu ml^{-1}) beginning at disease onset.

Table 2.5: Variety trial AUDPC values for common varieties in 2002 and 2003.

Treatment[1] (beginning at disease onset and followed at 14 day intervals)	Variety (KWS) 2002 Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)	2003 Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)
1) untreated control	55.3	61.1	30.2	25.4	13.7	2.4
2) 1 spray	31.6	34.3	19.7	5.1	2.5	1.4
3) 2 sprays	23.1	20.9	14.9	1.0	1.0	0.4
4) 3 sprays	11.0	8.5	4.0	0.5	0.4	0.2
5) 4 sprays	3.7	5.6	3.4	0.5	0.3	0.1
6) 4 sprays BmJ + tetraconazole	30.0	33.9	16.0	6.5	5.7	2.8
7) 4 sprays BmJ	37.2	33.0	18.0	9.2	3.7	3.2
$LSD_{(0.05)}$		*9.66*			*4.37*	

1- In 2002, first spray = tetraconazole (0.15 L/ha); second spray = trifloxystrobin (84 ml/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (110 ml/ha). In 2003, first spray = trifloxystrobin (108 ml/ha); second spray = tetraconazole (0.15 L/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (84 ml/ha).

- In 2002, BmJ + tetraconazole was a first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays of BmJ (10^7 cfu ml^{-1}). In 2003, BmJ + tetraconazole was applied in two different ways. Results are an average of these two treatments. One BmJ + tetraconazole treatment began with a spray of BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset; then the first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}).
The other BmJ + tetraconazole treatment was a first spray of BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}).

- In 2002, the BmJ treatment was four sprays of BmJ (10^7 cfu ml^{-1}) beginning at disease onset. In 2003, the BmJ treatment began with a spray of BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset followed by four sprays of BmJ (10^7 cfu ml^{-1}) beginning at disease onset.

Table 2.6: 2002 Variety trial AUDPC values for treatments on each variety.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Beta 2185 (6.3)	HH 111 (5.3)	Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)	HM 7054 (4.1)
1) Untreated Control	53.14	61.57	55.25	61.10	30.18	33.80
2) 1[1]: tetraconazole (0.15 L/ha)	31.36	44.22	31.61	34.31	19.72	18.67
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	13.68	25.90	23.07	20.90	14.88	15.07
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	9.52	12.14	10.99	8.49	4.04	6.08
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (110 ml/ha)	4.56	5.99	3.67	5.58	3.40	4.20
6) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	22.76	38.34	30.04	33.94	16.03	15.71
7) 1-4: BmJ (10^7 cfu ml^{-1})	38.76	47.11	37.19	33.00	17.96	17.51
$LSD_{(0.05)}$			*9.66*			

1 – numbers preceding fungicides/BmJ indicate order of sprays.
2 – TPTH = triphenyltin hydroxide

Table 2.7: 2003 Variety trial AUDPC values for treatments on each variety.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Monarch (5)	HH 115 (4.9)	VDH 66556 (4.9)	Beta 3820 (4.4)	Trophy (4.2)	AC 927 (4)
1) Untreated Control	25.373	13.713	9.508	2.421	10.447	5.451
2) 1[1]: trifloxystrobin (108 ml/ha)	5.102	2.524	3.667	1.443	1.455	1.639
3) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha)	1.045	1.039	0.609	0.381	0.542	0.508
4) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH[2] (57 g/ha)	0.468	0.383	0.253	0.194	0.273	0.161
5) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (84 ml/ha)	0.468	0.306	0.19	0.132	0.252	0.199
6) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	5.564	5.371	4.618	1.988	4.947	2.856
7) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1-4: BmJ (10^7 cfu ml^{-1})	9.191	3.687	8.143	3.188	4.697	3.213
8) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	7.52	6.017	9.014	3.551	5.082	4.374
$LSD_{(0.05)}$			*4.37*			

1 – numbers preceding fungicides/BmJ indicate order of sprays
2 – TPTH = triphenyltin hydroxide

The use of BmJ as a CLS control measure was effective in all years, especially when applied with tetraconazole at half the label rate. Disease levels did not reach the economic threshold in BmJ treated plots of the moderately resistant variety HM 7054 (KWS=4.1) in 2001 (Figure 2.7). In 2002, treatments of BmJ applied alone gave statistical reductions in AUDPC when compared to the untreated control. When applying BmJ with tetraconazole, AUDPC values were significantly lower than the untreated control in all varieties in 2001 and 2002, and in all but two of the most resistant varieties, Beta 3820 and AC 927, in 2003 (Tables 2.4, 2.6 and 2.7). Resistance in these varieties was sufficient in itself.

In comparing the two treatments integrating BmJ with tetraconazole in 2003, the treatment that included an application of BmJ prior to disease onset and mixed with tetraconazole at disease onset gave better CLS control than the treatment that lacked the early applications of BmJ, although this difference was not significant (Table 2.9). This observation suggests the importance of using BmJ early in CLS progression to allow the full induction of systemic resistance in the sugar beet. However, this aspect of BmJ use needs further study under more severe disease pressure. Disease pressure was most likely not high enough to adequately test BmJ efficacy in 2003, especially on more resistant varieties that already maintain lower disease levels.

The combination of BmJ treatments with more resistant varieties reduced the rate of CLS disease progress (Figures 2.4 – 2.6) and AUDPC levels as compared to similar treatments on more susceptible varieties (Tables 2.4 and 2.5). This suggests that BmJ is best utilized when combined with sugar beet varieties with a KWS score less than five.

Yields of moderately resistant varieties, measured as kilograms of extractable sucrose per hectare, were consistently similar to yields of the susceptible varieties. It is important to note that analysis of AUDPC showed a low correlation to yield (<0.55 in 2001, <0.52 in 2002, <0.11 in 2003) in all years. This is most likely because disease levels, as determined by CLS ratings, did not surpass the economic threshold in the majority of plots every year. Therefore, yield differences occurred under moderate (2001 and 2002) and light (2003) CLS pressure. With only a few exceptions, the results indicate clearly that moderately resistant varieties (KWS<5) can yield as well as their susceptible counterparts can. This stands in contrast to the published results of Smith and Campbell. This difference can be explained by the progress that sugar beet breeders have made in maintaining high yields and incorporating better CLS resistance into varieties since the 1996 Smith and Campbell publication.

In 2001, yields from treatments on the susceptible variety Beta 2185 (KWS=6.3) were not significantly better than the same treatments on the more resistant varieties HH 111 (KWS=5.3) and HM 7054 (KWS=4.1). Extractable sucrose from plots of the four-spray treatment (tetraconazole, benomyl, TPTH, and TPTH) on Beta 2185 was not significantly better than any other treatment on all varieties. The yields of extractable sucrose are reported in Table 2.6.

Yields for Beta 2185, HH 111 and HM 7054 in 2002 are shown in Table 2.6 as well. As in 2001, yields from treatments on Beta 2185 were not statistically greater than those on the more resistant variety HM 7054. However, differences did occur in the untreated control. Beta 2185 (KWS=6.3) had greater yield than the more resistant variety

HH 115 (KWS=4.9) (Table 2.10). Also, Monarch (KWS=5) did not yield as well as Beta 2185 when both were treated with tetraconazole and trifloxystrobin in the two-spray program.

Table 2.8: Variety trial yield as kilograms of extractable sucrose per hectare for common varieties in 2001 and 2002.

	Variety (KWS)					
Treatment[1]	2001			2002		
(beginning at disease onset and followed at 14 day intervals)	Beta 2185 (6.3)	HH 111 (5.3)	HM 7054 (4.1)	Beta 2185 (6.3)	HH 111 (5.3)	HM 7054 (4.1)
1) Untreated	8377	7718	8462	9761	8962*	9462
2) 1 spray	9029	8987	8468	9724	9761	9456
3) 2 sprays	8041	8822	7199	9804	9231	9609
4) 3 sprays	8743	8084	7089	9261	9389	9505
5) 4 sprays	8596	7529	7974	9987	9652	10,512
6) 4 sprays BmJ + tetraconazole	8590	9042	7309	9261	9084*	8743*
7) 4 sprays BmJ	7773	8828	7687	9554	9188	9310
$LSD_{(0.05)}$		*1883*			*877.9*	

1- In 2001, first spray = tetraconazole (0.15 L/ha); second spray = benomyl (92 g/ha); third and fourth sprays = triphenyltin hydroxide (57 g/ha). The 4 spray treatment results are an average of two identical 4 spray treatments. In 2002, first spray = tetraconazole (0.15 L/ha); second spray = trifloxystrobin (84 ml/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (110 ml/ha).

- In 2001 and 2002, the BmJ + tetraconazole treatment was a first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}).
- In 2001 and 2002, BmJ was four sprays of BmJ (10^7 cfu ml^{-1}) beginning at disease onset.

* - statistically different than the four-spray fungicide treatment of the susceptible variety, Beta 2185 at P=0.05 (expected to give highest yields).

Yields of common varieties from 2002 and 2003 are shown in Table 2.9. The most susceptible variety in this group is Monarch (KWS=5). Yields from treatments on Monarch were not statistically different from the same treatments on the more resistant variety Beta 3820 in 2002 (Table 2.10). This was also true when comparing all other varieties to Monarch in 2003 (Table 2.11).

Treatments of BmJ alone or in various combinations with tetraconazole at half the label rate gave varying yields. Treatments including BmJ consistently gave yields not significantly different from the majority of fungicide treatments. BmJ-only treatments generally gave moderate yields with all varieties in all years. The integrated treatment of BmJ with tetraconazole generally gave yields equal to BmJ alone in all years. The integrated BmJ treatment also yielded as well as the majority of fungicide treatments on all varieties.

These results show that growing moderately resistant varieties will allow for one to two fewer fungicide applications without sacrificing overall yield when compared to susceptible varieties. BmJ also gives better disease control when applied to more resistant varieties, especially when mixed with a reduced rate of tetraconazole. Yields of BmJ treated plots also were competitive overall. This reduction in fungicide application is important in reducing the cost of disease control, and should result in less selection pressure for fungicide resistance in the treated populations of *C. beticola.*

Table 2.9: Variety Trial results of yield as kilograms of extractable sucrose per hectare for common varieties in 2002 and 2003.

Treatment[1] (beginning at disease onset and followed at 14 day intervals)	Variety (KWS) 2002 Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)	2003 Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)
1) Untreated	9292	8834*	9029*	11,616	12,214	11,455
2) 1 spray	9707	9432	9200	11,891	11,927	11,171
3) 2 sprays	8840*	9505	9603	11,250	11,915	11,708
4) 3 sprays	9371	9408	9804	11,610	13,446*	11,628
5) 4 sprays	10,256	9267	10,036	11,805	12,019	11,207
6) 4 sprays BmJ + tetraconazole	9249	9243	9603	11,110	11,695	12,019
7) 4 sprays BmJ	9517	9395	9383	11,429	11,671	11,634
$LSD_{(0.05)}$		*877.9*			*1065.8*	

1- In 2002, first spray = tetraconazole (0.15 L/ha); second spray = trifloxystrobin (84 ml/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (110 ml/ha). In 2003, first spray = trifloxystrobin (108 ml/ha); second spray = tetraconazole (0.15 L/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (84 ml/ha).

- In 2002, BmJ + tetraconazole was a first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays of BmJ (10^7 cfu ml^{-1}). In 2003, BmJ + tetraconazole was applied in two different ways. Results are an average of these two treatments. One BmJ + tetraconazole treatment began with a spray of BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset; then the first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}). The other BmJ + tetraconazole treatment was a first spray of BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}).

- In 2002, the BmJ treatment was four sprays of BmJ (10^7 cfu ml^{-1}) beginning at disease onset. In 2003, the BmJ treatment began with a spray of BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset followed by four sprays of BmJ (10^7 cfu ml^{-1}) beginning @ disease onset.

* - statistically different than the four-spray fungicide treatments of the most susceptible varieties, Beta 2185 in 2002 and Monarch in 2003 at P=0.05.

Table 2.10: 2002 Variety trial yield as kilograms of extractable sucrose per hectare.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Beta 2185 (6.3)	HH 111 (5.3)	Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)	HM 7054 (4.1)
1) Untreated Control	9761	8962*	9292	8834*	9029*	9462
2) 1[1]: tetraconazole (0.15 L/ha)	9724	9761	9707	9432	9200	9456
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	9804	9231	8840*	9505	9603	9609
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	9261	9389	9371	9408	9804	9505
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (110 ml/ha)	9987	9652	10,256	9267	10,036	10,512
6) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	9261	9084*	9249	9243	9603	8743*
7) 1-4: BmJ (10^7 cfu ml^{-1})	9554	9188	9517	9395	9383	9310
$LSD_{(0.05)}$			*877.9*			

1 – numbers preceding fungicides/BmJ indicate order of sprays
2 – TPTH = triphenyltin hydroxide
* - statistically lower than the four-spray fungicide treatment on Beta 2185 at P=0.05 (expected to give the highest yield).

Table 2.11: 2003 Variety trial yield as kilograms of extractable sucrose per hectare.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Monarch (5)	HH 115 (4.9)	VDH 66556 (4.9)	Beta 3820 (4.4)	Trophy (4.2)	AC 927 (4)
1) Untreated Control	11,616	12,214	12,104	11,455	10,908	11,756
2) 1[1]: trifloxystrobin (108 ml/ha)	11,891	11,927	12,428	11,171	11,714	12,459
3) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha)	11,250	11,915	11,769	11,708	11,170	11,457
4) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH[2] (57 g/ha)	11,610	13,446*	12,556	11,628	11,403	12,245
5) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (84 ml/ha)	11,805	12,019	12,623	11,207	11,500	12,550
6) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	11,232	11,964	12,098	11,805	11,720	11,970
7) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1-4: BmJ (10^7 cfu ml^{-1})	11,427	11,671	12,806	11,634	10,652	12,391
8) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	10,988	11,433	12,714	12,226	10,805	12,781
$LSD_{(0.05)}$			*1065.8*			

1 – numbers preceding fungicides/BmJ indicate order of sprays

2 – TPTH = triphenyltin hydroxide

* - statistically higher than the 4-spray treatment on the more susceptible variety, Monarch at P=0.05 (expected to give highest yields).

Treatment X Variety Effects for Each Year:

A more detailed look at each year's variety trial is presented below. Disease control is addressed separately from yield.

Disease Control: Results from the 2001 Variety trials are summarized in Table 2.4. Disease levels were cut in half for all varieties with a single application of tetraconazole, indicating the importance of a fungicide application during the early stages of the epidemic. For the most resistant variety, HM 7054, a single spray performed just as well as two, three, or four sprays and controlled CLS to levels comparable with those reached with four sprays on the susceptible Beta 2185.

Results of disease control in 2002 (Table 2.6) showed treatment effects similar to those seen in 2001. Once again, a single application of tetraconazole greatly reduced disease, although by only approximately 33% as compared to approximately 50% in 2001. Subsequent chemical applications reduced disease levels consecutively with the largest reductions occurring when using one spray and three sprays. Disease control equal to that of the four-spray treatment was achieved with one to two less sprays on the more resistant HH 115 and Beta 3820 varieties. Beta 2185 performed surprisingly well in 2002 when compared to the other varieties, especially the more resistant varieties HH 111, HH 115, and Monarch that had published KWS scores lower than Beta 2185. The one-spray treatment on the most resistant varieties, HM 7054 and Beta 3820, controlled the disease at comparable levels to that of two sprays on Beta 2185.

Individual treatment and variety effects on CLS levels were significantly different from other treatment and variety effects in 2003 even though the overall disease levels were quite low (Table 2.7). As seen in the previous two years, the first fungicide application greatly reduced AUDPC values for all varieties. Additional sprays continued to reduce disease levels with some significance. Equal disease levels were once again achieved with one less spray on more resistant varieties (KWS<4.9) when compared to the more susceptible varieties in the trial (KWS≥4.9).

The results for the use of BmJ were mixed but promising in all years. The disease control performance of BmJ alone was not as good as the fungicide applications in 2001, but did increasingly improve when applied to HH 111 and HM 7054, respectively (Table 2.4). This treatment performed better in 2002 and gave results statistically equivalent to the one-spray treatment on all varieties (Table 2.6). When BmJ was applied on Beta 3820 and HM 7054, results were comparable to the 2-spray treatment on Beta 2185, Beta 3820 and HM 7054. In 2003, the BmJ-only treatment performed better than the untreated control in 4 of 6 varieties (Table 2.7). Over all three years of testing, BmJ performed better when applied to more resistant varieties (KWS<5). This shows the potential for use of BmJ on resistant varieties as an effective CLS control method.

Treatments that integrated BmJ with tetraconazole, performed well when applied to all varieties in 2001 and 2002. Disease control was comparable to that from all fungicide treatments in 2001 (Table 2.4). CLS control in 2002 was not significantly different from at least one fungicide spray and sometimes two sprays depending on the variety (Table 2.6). Results from 2003 indicated integrated BmJ treatment (Table 2.7)

performance varied with the variety. Potential differences in efficacy between the two integrated BmJ spray programs are important to this study. One program consisted of BmJ sprayed prior to disease onset, followed by BmJ with tetraconazole (half the label rate) at disease onset, then three additional sprays of BmJ at two-week intervals. The other integrated program omitted the application of BmJ before disease onset. Treatments began at disease onset with an application of the full rate of tetraconazole followed by three sprays of BmJ at two-week intervals. AUDPC values were lower for the integrated program that included BmJ applications before or at disease onset on all varieties, suggesting the importance of early treatments of BmJ.

Yield: The effects of variety and CLS control treatments on yield are shown in Tables 2.8, 2.10-2.17. Measurements of the percent sucrose in the beet (Table 2.12), gross yield in metric tons per hectare (Table 2.13), and kilograms of extractable sucrose per hectare (Table 2.8) were not significantly different from the untreated plot of the susceptible variety Beta 2185 in the 2001 trial. The one exception was the four-spray treatment (tetraconazole, benomyl, TPTH, TPTH) on HH 111 that had a significantly lower percent sucrose than the untreated control of Beta 2185. The four-spray treatment (tetraconazole, benomyl, TPTH, TPTH) on Beta 2185 was not significantly different from other treatments on all varieties in percent sucrose and both gross and net yield. Yields attained for HH 111 and HM 7054 when using a single spray were comparable to the best yields of Beta 2185. BmJ treatments gave statistically equal yields to fungicide treatments. Yield response to BmJ alone remained at high levels in Beta 2185 and HH 111 even though CLS levels were higher. This may indicate that BmJ allows the plant to

tolerate higher levels of disease without effecting yield or may be controlling other diseases through the induction of systemic resistance. Yields were generally highest in plots receiving one application of tetraconazole.

Table 2.12: Variety trial results of percent sucrose for common varieties in 2001 and 2002.

	Variety (KWS)					
Treatment[1]	2001			2002		
(beginning at disease onset and followed at 14 day intervals)	Beta 2185 (6.3)	HH 111 (5.3)	HM 7054 (4.2)	Beta 2185 (6.3)	HH 111 (5.3)	HM 7054 (4.2)
1) untreated control	16.8	16.4	16.5	17.9	17.9	17.7
2) 1 spray	17.0	17.2	16.9	17.7	18.0	17.2
3) 2 sprays	16.9	16.6	16.2	17.8	17.5	17.8
4) 3 sprays	16.8	16.6	16.5	17.2	17.9	17.6
5) 4 sprays	16.6	16.1*	17.1	17.3	17.6	17.6
6) 4 sprays BmJ + tetraconazole	16.7	16.3	16.4	17.6	17.7	17.3
7) 4 sprays BmJ	16.8	16.2	16.4	17.4	17.3	17.6
$LSD_{(0.05)}$		*0.65*			*0.76*	

1- In 2001, first spray = tetraconazole (0.15 L/ha); second spray = benomyl (92 g/ha); third and fourth sprays = triphenyltin hydroxide (57 g/ha). The 4 spray treatment results are an average of two identical 4 spray treatments. In 2002, first spray = tetraconazole (0.15 L/ha); second spray = trifloxystrobin (84 ml/ha); third spray = triphenyltin hydroxide (57 g/ha); fourth spray = trifloxystrobin (110 ml/ha).

- In 2001 and 2002, the BmJ + tetraconazole treatment was a first spray = BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha); second, third and fourth sprays = BmJ (10^7 cfu ml^{-1}).
- In 2001 and 2002, BmJ was four sprays of BmJ (10^7 cfu ml^{-1}) beginning at disease onset.

*- significantly lower than untreated control of Beta 2185 at P=0.05.

Table 2.13: Variety trial yield results as metric tons of sugar beets per hectare for common varieties in 2001 and 2002.

Treatment[1] (beginning at disease onset and followed at 14 day intervals)	Variety (KWS) 2001 Beta 2185 (6.3)	HH 111 (5.3)	HM 7054 (4.2)	2002 Beta 2185 (6.3)	HH 111 (5.3)	HM 7054 (4.2)
1) Untreated	50.6	53.5	54.8	55.3	52.2	57.1
2) 1 spray	58.2	56.9	53.6	58.3	59.2	60.5
3) 2 sprays	57.1	50.4	47.6	57.6	58.0	56.7
4) 3 sprays	54.3	56.7	45.8	57.6	57.4	58.0
5) 4 sprays	48.3	54.4	51.1	58.0	59.8	62.1
6) 4 sprays BmJ + tetraconazole	58.7	50.1	49.9	56.0	56.7	54.9
7) 4 sprays BmJ	58.7	54.7	47.5	60.5	57.6	56.2
$LSD_{(0.05)}$		*11.9*			*4.7*	

1- In 2001, first spray = tetraconazole (0.15 L/ha); second spray = benomyl (92 g/ha); third and fourth sprays = TPTH (57 g/ha). The 4-spray treatment results are an average of two identical 4-spray treatments. In 2002, first spray = tetraconazole (0.15 L/ha); second spray = trifloxystrobin (84 ml/ha); third spray = TPTH (57 g/ha); fourth spray = trifloxystrobin (110 ml/ha).
- In 2001 and 2002, the BmJ + tetraconazole treatment = 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1}).
- In 2001 and 2002, 4 sprays BmJ = 1-4: BmJ (10^7 cfu ml^{-1}) beginning at disease onset.

Only the untreated plots of HH 115 had a significantly lower sucrose percentage than the untreated control with the susceptible variety Beta 2185 in 2002 (Table 2.14). Percent sucrose after the four-spray treatment (tetraconazole, trifloxystrobin, TPTH, trifloxystrobin) on Beta 2185 was not significantly different from all other treatments on all varieties. Gross yield in metric tons per hectare of sugar beet was not significantly lower in all other varieties than Beta 2185 with the same CLS control treatment (Table 2.15). In addition, gross sugar beet yield of all varieties with all treatments was statistically equal to the four-

spray fungicide treatment on Beta 2185. Yields of extractable sucrose in 2002 showed that a one-spray or two-spray fungicide program on all varieties gave yields statistically equal to the four-spray fungicide treatment on Beta 2185 (Table 2.10). The highest yields in most varieties were in plots treated with the four-spray fungicide treatment. These results from 2001 and 2002 show varieties with higher levels of CLS resistance give competitive yields under moderate disease pressure.

Table 2.14: 2002 Variety trial results of percent sucrose for treatments on each variety.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Beta 2185 (6.3)	HH 111 (5.3)	Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)	HM 7054 (4.1)
1) Untreated Control	17.9	17.9	17.7	17.1*	17.4	17.7
2) 1[1]: tetraconazole (0.15 L/ha)	17.7	18.0	17.7	17.5	17.3	17.2
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	17.8	17.5	17.6	17.8	17.5	17.8
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	17.2	17.9	17.6	17.7	17.9	17.6
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (110 ml/ha)	17.3	17.6	17.6	17.6	17.7	17.6
6) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	17.6	17.7	17.6	17.7	17.6	17.3
7) 1-4: BmJ (10^7 cfu ml^{-1})	17.4	17.3	17.5	17.5	17.5	17.6
$LSD_{(0.05)}$			*0.76*			

1 – numbers preceding fungicides/BmJ indicate order of sprays.
2 – TPTH = triphenyltin hydroxide
***** - significantly lower than untreated control of Beta 2185 at P=0.05.

Table 2.15: 2002 Variety trial yield results in metric tons per hectare for treatments on each variety.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Beta 2185 (6.3)	HH 111 (5.3)	Monarch (5)	HH 115 (4.9)	Beta 3820 (4.4)	HM 7054 (4.1)
1) Untreated Control	55.3	52.2*	58.5	57.1	56.0	57.1
2) 1[1]: tetraconazole (0.15 L/ha)	58.3	59.2	57.1	56.9	58.3	60.5
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	57.6	58.0	55.3	57.8	57.8	56.7
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	57.6	57.4	56.2	56.5	58.3	58.0
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (110 ml/ha)	58.0	59.8	59.6	58.9	64.3	62.1
6) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	56.0	56.7	57.8	56.5	54.5	54.9
7) 1-4: BmJ (10^7 cfu ml^{-1})	60.5	57.6	58.3	56.0	58.0	56.2
$LSD_{(0.05)}$			*4.7*			

1 – numbers preceding fungicides/BmJ indicate order of sprays.
2 – TPTH = triphenyltin hydroxide
***** - significantly lower than the 4-spray fungicide treatment on Beta 2185 at P=0.05.

Similarly, yield data from 2003 showed that varieties that are more resistant are competitive under very light disease pressure as well. The percent sucrose for AC 927 receiving the BmJ only treatment and the four-spray fungicide treatment (trifloxystrobin, tetraconazole, TPTH and trifloxystrobin) was significantly better than the four-spray fungicide treatment on the most susceptible variety, Monarch (Table 2.16). Also having significantly greater percent sucrose than the four-spray fungicide treatment on

Monarch, was the treatment of BmJ with tetraconazole (half the label rate) followed by three sprays of BmJ on Beta 3820. No treatments on all varieties were statistically lower in percent sucrose from the four-spray fungicide treatment on Monarch.

Table 2.16: 2003 Variety trial results of percent sucrose for treatments on each variety.

	Variety (KWS)					
Treatment (starting at disease onset and followed at 14 day intervals)	Monarch (5)	HH 115 (4.9)	VDH 66556 (4.9)	Beta 3820 (4.4)	Trophy (4.2)	AC 927 (4)
1) Untreated Control	18.1	18.6	18.4	18.6	18.6	18.5
2) 1[1]: trifloxystrobin (108 ml/ha)	17.9	18.2	18.4	18.5	18.6	18.7
3) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha)	17.5	18.3	18.1	18.5	18.6	18.4
4) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH[2] (57 g/ha)	17.9	18.2	17.6	18.3	18.3	18.7
5) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (84 ml/ha)	18.1	17.7	18.2	18.2	18.3	18.7
6) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	17.9	18.1	17.9	18.5	18.6	18.6
7) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1-4: BmJ (10^7 cfu ml^{-1})	17.5	18.2	18.2	18.8*	18.1	19.0*
8) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	17.6	17.9	18.0	18.8*	18.1	18.6
$LSD_{(0.05)}$	*0.64*					

1 – numbers preceding fungicides/BmJ indicate order of sprays

2 – TPTH = triphenyltin hydroxide

***** - significantly higher than untreated control and 4-spray fungicide treatments on Monarch at P=0.05.

Table 2.17: 2003 Variety trial yield results in metric tons per hectare for treatments on each variety.

Treatment (starting at disease onset and followed at 14 day intervals)	Variety (KWS) Monarch (5)	HH 115 (4.9)	VDH 66556 (4.9)	Beta 3820 (4.4)	Trophy (4.2)	AC 927 (4)
1) Untreated Control	68.3	69.0	69.2	64.7	61.7*	66.8
2) 1[1]: trifloxystrobin (108 ml/ha)	70.1	69.0	71.0	63.4	66.6	70.2
3) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha)	68.4	68.8	68.5	66.8	63.4	65.5
4) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH[2] (57 g/ha)	68.9	77.6	75.0	66.8	65.6	69.9
5) 1: trifloxystrobin (108 ml/ha), 2: tetraconazole (0.15 L/ha), 3: TPTH (57 g/ha), 4: trifloxystrobin (84 ml/ha)	69.3	71.7	73.1	65.0	66.3	70.4
6) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	66.8	69.5	71.1	67.3	66.3	67.5
7) BmJ (10^7 cfu ml^{-1}) 7 days prior to disease onset, 1-4: BmJ (10^7 cfu ml^{-1})	69.7	67.4	74.1	67.2	62.6*	68.5
8) 1: BmJ (10^7 cfu ml^{-1}) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu ml^{-1})	66.7	67.8	74.2	68.5	63.3	72.2
$LSD_{(0.05)}$			*6.1*			

1 – numbers preceding fungicides/BmJ indicate order of sprays

2 – TPTH = triphenyltin hydroxide

***** - significantly lower than the 4-spray fungicide treatment on Monarch at P=0.05.

Only the untreated control and BmJ-only treatments on Trophy had statistically lower gross yield than the four-spray fungicide treatment on Monarch (Table 2.17). Almost all treatments on all varieties resulted in extractable sucrose yields that were statistically equal to or greater than those of Monarch (KWS 5.0) with the four-spray fungicide treatment (Table 2.11). The two exceptions were the untreated control and the BmJ-only treatments on Trophy. Yield results once again did not reflect a significant loss in yield capacity due to higher disease resistance in the moderately resistant varieties (KWS<5).

This series of variety studies reveals that growing more resistant varieties can allow reduced fungicide applications without sacrificing yield potential. In addition, BmJ was shown to be a viable CLS control tool, especially when applied with reduced levels of fungicides (reduced rate or single application) on resistant varieties.

Discussion

The use of sugar beet varieties with moderate Cercospora leaf spot (CLS) resistance gave improved disease control without sacrificing yield. Varieties with a KWS score <4.9 consistently allowed for one to two fewer fungicide applications as compared to more susceptible varieties (Tables 2.4, 2.6, and 2.7). Yields of moderately resistant varieties were comparable to those of the susceptible varieties (Tables 2.8, 2.10, and 2.11). Analysis showed no correlation between lower KWS values (increased CLS resistance) in moderately resistant hybrids and changes in yield of extractable sucrose. This situation contrasts with the low yields experienced with earlier generations of

moderately resistant hybrids (Smith and Campbell, 1996) and indicates the improvements made in yield potential in sugar beet cultivars with high levels of CLS resistance. Agronomic quality of moderately resistant cultivars will continue to improve as breeding programs integrate CLS resistance into higher yielding hybrids.

The disease progress curves of the untreated control plots of each variety in 2002 and 2003 show differences in the shape of the curves of different varieties (Figures 2.2 and 2.3). The KWS value given to each variety by the Betaseed nurseries in Shakopee and Rosemount MN did not consistently describe the performance of the variety in Sidney MT. The KWS value is assigned after averaging five to seven KWS ratings from late July through late August (Windels *et al*., 1998). A measurement of AUDPC in addition to the KWS score may be a valuable tool in accurately describing varietal resistance to CLS because it takes into account the disease progression over time (shape of disease progress curve). Under higher disease levels than experienced in this study, a measurement of the AUDPC value above the economic threshold of 3% leaf damage may be advantageous for determining CLS resistance levels.

Analysis of the timing and intensity of CLS infection periods as indicated by the Cercospora prediction model show that conditions were more favorable for CLS progression in 2001 and 2002 than in 2003 (Figures 2.7, 2.8, and 2.9). Infection periods generally coincided with flood irrigation events in 2001. The final irrigation on August 20 may have resulted in higher relative humidity and a higher likelihood of infection in the following 3-4 days than the model predicted. The increased probability of high relative humidity in the canopy during this time may have been underestimated by

weather sensors because they were not placed in the canopy. The increase in CLS symptoms after August 20 presented in Figures 2.1 and 2.4 would suggest that the infection period after August 20 was stronger than indicated by the model. It is important to note that the first two fungicide applications occurred during the strongest infection periods and maintained CLS symptoms below the economic threshold of 3% leaf damage. Additional applications of fungicide were not needed, indicating the utility of properly timed fungicide sprays early in disease development.

The Cercospora prediction model indicated intermittent infection periods from early July to early September in 2002. The first two spray applications were once again timed to give good control of CLS early in the disease development (Figure 2.8). Additional sprays, although not required to maintain CLS levels below the economic threshold (Figure 2.5), continued to improve CLS control because they were applied near the occurrence of the strongest infection period indicated by the model on August 8 and 9 (Figure 2.8).

The low levels of CLS in 2003 reflect the reduced intensity and number of infection periods as well as the later onset of these periods compared to previous years. CLS should have increased at a higher rate in September in accordance with an infection period in early September (Figures 2.3 and 2.9), based upon a comparison of the disease progress curve with the timing of infection periods in 2003. The absence of a faster increase suggests that a more detailed review is needed of the Cercospora prediction model as currently employed in the Sidney area. It is possible that daytime high temperatures greater than 35˚ C (95˚ F) are detrimental to *C. beticola*. From August 11 to

16, daytime highs were greater than 35˚ C. Cercospora leaf spot did increase during August as shown in the September 5 CLS rating and should have resulted in an increase of inoculum available for the next infection period (Figures 2.3 and 2.9). Yet, when the next infection period was predicted on September 9, CLS infection did not occur as determined by the final CLS rating on September 25. If infections had occurred, the September 25 CLS rating allowed enough time (two weeks from the September 9 infection period) for infections to mature into visible symptoms. Increased daytime high temperatures from August 11 to 16 may have affected conidiation during this time or the ability of conidia to infect the leaf. Further analysis and study is needed to elucidate the unexpected low incidence of CLS in September of 2003.

The level of CLS resistance in the varieties grown in 2003 may have played an important role in the overall disease levels. The highest KWS rating of varieties grown in 2003 (KWS=5) was much lower than in previous years (KWS=6.3) and may have resulted in a lower amount of *C. beticola* in the field, especially at the end of the season. Higher levels of infection may have taken place had varieties that are more susceptible been grown in the experiment, such as in 2001 and 2002. In addition, commercial sugar beet fields surrounding the EARC grew varieties with lower published KWS ratings in 2003, potentially reducing the overall amount of *C. beticola* conidia in the area as the season progressed relative to previous years. Since the CLS resistance available affects infection efficiency, latent period and sporulation, it is likely that KWS scores and AUDPC ranking of varieties should be incorporated into the model. Researchers in Germany (Wolf and Verreet, 2002) have incorporated variety resistance into their model.

The use of the Cercospora prediction model for proper management of fungicide use continues to be an important tool along with moderately resistant varieties. With the cost of a single fungicide application approaching $50 per hectare including the cost of aerial application, any reduction in the number of needed applications is favorable to the producer. More importantly, fewer applications of fungicide reduce the selection pressure placed on the *C. beticola* population. This has been suggested to be an important factor in managing the development of fungicide resistance (Köller 1996). Induced systemic resistance (ISR) has also been suggested as a potential tool for use in fungicide resistance management (Ortega *et al*. 1998). *Bacillus mycoides* isolate BmJ has been determined to be an inducer of systemic acquired resistance in sugar beet (Bargabus et al. 2002).

The CLS control efficacy of BmJ applied alone or in combination with tetraconazole at half the label rate was tested in all field trials reported. Previous studies have indicated that BmJ can give disease control comparable to fungicide programs, especially when applied in combination with reduced rates of fungicide (Bargabus et al. 2002; Jacobsen et al. 2001). Results presented here confirm the utility of BmJ as a viable CLS control tool.

When BmJ was used with moderately resistant cultivars in 2001 and 2002, disease control equaled that of the susceptible variety, Beta 2185, with four-spray and two-spray fungicide treatments, respectively. The combination of BmJ and moderately resistant varieties gave promising disease control results. AUDPC values indicate that this combination controls CLS to levels less than half of what is experienced in the

untreated control of the susceptible variety (Tables 2.4, 2.8 and 2.9). BmJ gave significant disease control in all trials conducted in 2001 and 2002 (Tables 2.4 and 2.8). BmJ treatments slow the progression of disease as shown by reduced progress curve slopes (Figures 2.4 and 2.5), indicating that the effects of systemic acquired resistance as induced by BmJ (Bargabus et al. 2002) may function in controlling CLS in a manner similar to the rate-reducing functions of host-plant resistance (Rossi et al, 1998). Light disease pressure made it difficult to determine the effectiveness of BmJ in 2003.

Yields of BmJ treated plots were consistently competitive with those of fungicide treated plots. No detrimental effects on yield were observed. The induction of systemic acquired resistance in sugar beet by BmJ may also give control of other diseases as indicated by the high yields of the BmJ treated plots of HH 111 and Beta 2185 in 2001 (Table 2.6). Overall, BmJ has been demonstrated to be an efficacious CLS management tool. The approved sugar beet varieties will continue to increase in CLS resistance, so using BmJ on varieties that are more resistant could be an attractive disease control option in the future.

In summary, the reported field trial results indicate that effective CLS management can be attained by integrating moderately resistant sugar beet hybrids with properly timed applications of fungicide and/or BmJ. The use of moderately resistant hybrids allows one to two fewer fungicide applications without affecting yields. Reductions in fungicide use can also be accomplished by using BmJ. This integrated disease control approach should lead to greater longevity of fungicide efficacy and overall sustainability of CLS control measures if implemented. Reductions in the

selection pressure placed on the *C. beticola* population by fungicides should slow the development of fungicide resistance (Ortega et al, 1998). Further studies will monitor the success of these strategies to determine pertinent changes in the *C. beticola* population that may affect disease control success.

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CHAPTER 3

FUNGICIDE RESISTANCE IN *CERCOSPORA BETICOLA* IN MONTANA

Introduction

Current strategies for the management of Cercospora leaf spot (CLS) rely on crop rotation, a few registered fungicides, a CLS prediction model, scouting (Whitney and Duffus 1986; Windels et al. 1998) and partially resistant varieties (Miller et al. 1994; Smith and Campbell 1996). Future availability of fungicides may be limited due to implementation of the Food Quality Protection Act of 1996 or development of fungicide resistance in *Cercospora beticola* Sacc. (L), the causative agent of CLS (Weiland and Halloin 2001; Karaoglanidis et al. 2003). Studies from 1998 through 2002 designed to measure the levels of sensitivity to fungicides have indicated increased fungicide resistance in *C. beticola* populations in eastern Montana. Tests have shown the presence of complete resistance to benomyl, a benzimidazole class of systemic fungicide (Jacobsen et al. 2003). Tolerance to 10 ppm of the triazole and strobilurin classes of systemic fungicides has been recorded, although in a much smaller proportion of the isolates tested. Tolerance to 5 ppm of the protectant fungicide triphenyltin hydroxide (TPTH) has also been identified in these tests (Jacobsen et al. 2003). Other surveys of fungicide resistance in the northern plains of the United States have recently identified the presence of *C. beticola* resistance to another benzimidazole, thiophanate methyl; an ethylene bis dithiocarbamate, mancozeb; and a strobilurin, azoxystrobin (Weiland and Halloin, 2001;

Briere et al. 2003). Tolerance of Minnesota-derived *C. beticola* isolates to TPTH has been documented by Bugbee (1995). The results presented here indicate the baseline levels of fungicide resistance to benomyl, azoxystrobin, and tetraconazole in the *C. beticola* population of eastern Montana.

Strategies for management of fungicide resistance examined in this study include rotation of fungicides with different modes of action, the use of a *Bacillus mycoides* biological control agent (BmJ), and the use of host plant resistance to control CLS. BmJ acts as an inducer of systemic acquired resistance in sugar beet (Bargabus *et al*. 2002). Induced systemic resistance has been suggested to be an effective tool in fungicide resistance management (Ortega et al. 1998).

Field isolations of *C. beticola* made from experimental plots at the Eastern Agricultural Research Center in Sidney MT in 1999 to 2002 were tested for fungicide resistance to determine the effects of sugar beet variety, and various treatments of fungicide and BmJ on fungicide resistance development. Two tests were employed. First, a spore germination test determined the ability of conidia to germinate in the presence of 0, 1 and 10 ppm concentrations of fungicide amended into water agar plates and second, a mycelial growth test measured the ability of mycelium to grow in the presence of these same fungicide concentrations in potato dextrose agar over a two-week period. These two assay techniques were used to determine the prevalence of fungicide resistance or reduced sensitivity in *C. beticola* isolates from eastern Montana.

Materials & Methods

Spore Germination Screen:

Relative tolerance of field collected *Cercospora beticola* to 1 and 10 ppm concentrations of benomyl, azoxystrobin, and tetraconazole was assessed by observations of conidial germination on media amended with fungicide. Just prior to harvest, generally mid to late September of each year, 10 heavily infected leaves were removed from the treated rows of every plot from selected field trials. Leaves were placed in paper bags and allowed to dry at room temperature (24° C). Portions of the leaves with lesions were then placed in small humidity chambers for at least 48 hours to allow for *C. beticola* sporulation. Humidity chambers consisted of a damp piece of paper towel in a sterile petri plate. Conidia were removed from individual lesions on several leaves by placing a 5-10 μL droplet of sterile distilled water on the lesion and agitating the surface with the pipet tip to dislodge the conidia before removing the suspension. Conidia from as many as 30 individual lesions were pooled together before being spread on a series of 1.5% water agar (WA) plates amended with benomyl, azoxystrobin, or tetraconazole at 0, 1, and 10 ppm. All WA plates contained streptomycin sulfate and tetracycline at a concentration of 10 ppm to inhibit bacterial growth. After inoculation, the plates were incubated 16-20 hours at 28° C at which time 100 spores per WA plate were examined for germination using a Ziess compound microscope at 100X magnification. The incubation time did not exceed 20 hours because hyphal growth became extensive enough beyond this point to complicate proper identification of individual spores and germination. All plates inoculated with the same conidial suspension were counted at the

same time. Spores with germ tubes equal to the spore length were considered germinated. Spore germination counts on WA plates without fungicide were used as the baseline values. Relative germination levels from each fungicide-amended plate were calculated as percent of untreated control.

Mycelial Growth Screen:

Ten single germinated spore isolates per plot sample were transferred from WA plates without fungicide onto potato dextrose agar (PDA) amended with 10 ppm streptomycin sulfate and tetracycline. Conidia were transferred from WA plates without fungicide to avoid biased selection of conidia expressing fungicide tolerance. To transfer the conidia, a scope mounted spore cutter (Microscore Inc.) was used to cut a cylindrical portion of agar containing a germinated conidium that was then transferred to PDA using a small probe. After transfer, each isolate was incubated a minimum of 6 days at 28° C in the dark. Mycelial plugs were then taken from the edge of each colony with a sterile #2 cork borer and placed on PDA plates amended with benomyl, azoxystrobin, or tetraconazole at 0, 1 and 10 ppm, and streptomycin sulfate and tetracycline at 10 ppm each. Colony diameters were measured in millimeters after 7 and 14 days at 28° C. with growth on fungicide-amended plates compared to that of the control plate. Measurements were corrected to compensate for the original size (5 mm diameter) of the transferred mycelial plug. Growth equal to or greater than 70% of control was considered to represent resistance to the respective level of the fungicide. Growth below this level was considered to represent sensitivity.

Statistical Analysis:

Analysis was performed using the SAS® (SAS, 1988) see previous chapter for citation and Statistix7® (Analytical Software, 2002) Analytical Software. 2002. Statistix 7, v. 7.1 Tallahassee FL statistical programs. Data were analyzed to elucidate possible effects of general categories of field treatments and variety type on the development of fungicide resistance. Data from the spore germination screens were analyzed using the general linear model for analysis of variance. A Students *t* Test (Fisher's) was used to determine the level of least significance difference (LSD) and organize significance groupings among field treatments at the P=0.05 level for the spore germination screen data.

Raw data from the mycelial growth screens, defined as percent growth as compared to the control, represented fungicide resistance if the value was equal to or greater than 70%. Data with a value less than 70% was determined to represent fungicide sensitivity. A Chi-square test was conducted to determine the possible significance of field treatments and varieties on fungicide resistance development.

Results & Discussion

Tests of fungicide effects on germination of *Cercospora beticola* conidia showed an increase in fungicide tolerance from 1999 to 2002 in combined tested populations taken from field samples of select treatments of field trials. A single representative fungicide from the benzimidazole, strobilurin and triazole classes of systemic fungicides was sufficient in all tests due to the documentation of cross-resistance within each class (Ruppel, 1975; Percich et al. 1984; Cunha and Rizzo 2003; Hildebrand et al. 1988;

Kendall et al. 1993; Karaoglanidis and Thanassoulopoulos, 2003; Chin *et al*. 2001; Briere et al, 2003). These test fungicides were benomyl and axozystrobin (all years), propiconazole in 1999, and tetraconazole in 2001-2002. The ability of conidia to germinate on fungicide-amended water agar was considered reduced sensitivity and not resistance, due to the inability of the test used to quantify the level of response for individual isolates. The overall results of spore germination screens for 1999, 2001, and 2002 are summarized in Table 3.1.

Table 3.1: Percentage of fungicide-tolerant isolates in the entire tested *Cercospora beticola* population of each year determined by spore germination tests.

Year	benomyl		azoxystrobin		tetraconazole	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
1999	-	6.3	2.8	2.1	1.8	-
2001	55.9	53.3	3.5	3.1	46.9	25.9
2002	72.9	67.2	0.04	0.00	43.6	6.2

Limited samples of *C. beticola* from field trials in 1999 were tested against benomyl, azoxystrobin, and the triazole fungicide, propiconazole. Results showed that the number of isolates able to germinate on 1 ppm and/or 10 ppm of these fungicides was 6.3% or less of all isolates tested in 1999 (66 field samples; 100 isolates per fungicide concentration for each field sample). Samples were not taken in 2000. In 2001, a larger number of field samples were tested (139 field samples; 100 isolates per fungicide concentration for each field sample). The percentages of all tested isolates from all field samples that were tolerant to benomyl and tetraconazole (triazole) at 1 ppm and 10 ppm were much greater in 2001. In 2002, the percentage of all tested isolates (361 field samples; 100 isolates per fungicide concentration for each field sample) that were tolerant

to benomyl increased again. However, this trend did not continue for tetraconazole, a sterol biosynthesis inhibitor (SBI). While the proportion of the population tolerant to 1 ppm remained similar to that of 2001, at approximately 45%, the proportion tolerant to 10 ppm dropped from 26% in 2001 to 6% in 2002.

This reduction in tolerance to tetraconazole may be due to the cessation of consecutive sprays of tetraconazole applied in the field in 2002. In 2001, tetraconazole was applied in four consecutive sprays in three spray programs resulting in significant selection pressure. Although tetraconazole was applied in approximately the same number of spray programs in 2002 as in 2001, it was always applied as a single spray or in rotation with other fungicides having a different mode of action or with BmJ. Selection pressure for resistance to tetraconazole would have been slightly lower in 2002.

Another potential reason for the drop in tetraconazole tolerance may be due to the use of more resistant varieties in 2002 than in 2001. The majority of field samples in 2001 were taken from the susceptible variety, Beta 2185 with a KWS of 6.3. In 2002, approximately one half of the field samples were taken from moderately resistant varieties with a KWS score less than 5. Although Karaoglanidis et al. (2003) determined that host plant resistance did not have a direct effect on the development of resistance to another sterol biosynthesis inhibiting fungicide, flutriafol, they did conclude that the use of more resistant hybrids would ultimately aide in the management of fungicide resistance by reducing the required number of fungicide applications and resulting selection pressure. The use of varieties with greater CLS resistance also reduces the population of *C. beticola* conidia that are exposed to fungicide application.

A third possible reason for the reduction in tolerance to tetraconazole is the documentation by Karaoglanidis et al. (2002) that *C. beticola* isolates exhibiting resistance to flutriafol had increased sensitivity to flutriafol after cold exposure (3° C for 10 months). The increase in sensitivity was most pronounced in isolates exhibiting moderate to high initial levels of resistance to flutriafol. With winter temperatures in eastern Montana often dropping well below the freezing mark, an increase in sensitivity to the SBI, tetraconazole, in some resistant *C. beticola* isolates after cold exposure during winter may explain the reduction in resistance levels from 2001 to 2002 at the higher concentration of 10 ppm.

Mycelial growth screens were also used to test for fungicide tolerance/resistance. These screens evaluated the ability of individual field isolates of *Cercospora beticola* to grow in the presence of 0, 1 and 10 ppm of benomyl, azoxystrobin and tetraconazole (propiconazole in 1999). Measurements of radial growth were used to quantify fungicide sensitivity. Growth of isolates at ≥70% of control represented resistance to the corresponding concentration of fungicide with growth <70% of control representing sensitivity. Isolates were taken from the same field samples of infected sugar beet leaves that were used for spore germination testing. The overall results obtained from the mycelial growth screens correspond well to results from spore germination screens with benomyl (Table 3.2). A higher percentage of isolates tested in mycelial growth screens (19.7%) were resistant to benomyl in 1999 than was seen in the spore germination screens (6.3%). The overall trend of increasing benomyl resistance was consistent in both types of screens.

Persistence and even increase of benomyl resistance in years when a benomyl fungicide was used very little (only two of thirty spray programs in 2002), is consistent with earlier findings that indicate little to no fitness cost from this resistance (Ruppel 1975, Dovas et al. 1976, Ruppel *et al*. 1980, Weiland *et al*. 2001, Karaoglanidis *et al*. 2003). This allows resistant isolates to maintain prevalence in the population, even in the absence of selection pressure.

Table 3.2: Mycelial growth screen results shown as the percentage of fungicide-resistant isolates in the entire tested population of each year.

Year	benomyl		azoxystrobin		tetraconazole	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
1999	19.7	19.7	33.6	21.9	0.0	0.0
2001	69.6	68.1	0.4	0.0	2.1	1.0
2002	87.1	86.5	0.4	0.1	1.6	1.0

It is also important to note that benomyl resistance levels were not affected by dose. Ruppel et al. (1980) indicated resistance to the highest dose tested of benomyl (10 ppm) was to be sufficient to cause severe leaf spot in sugar beet sprayed with field rates of benomyl. This is due to the qualitative nature of this resistance. The cause of resistance to the benzimidazole class of fungicides (including benomyl) was identified in *Cercospora kikuchii* (Matsumoto & Tomoyasu) M.W. Gardner to be a single mutation in the gene for the cytoplasmic protein β-tubulin, conferring complete resistance (Upchurch et al. 1991). The most common mutation is the substitution of glutamic acid by alanine, glycine, lysine, or glutamine at the 198th codon. This has been determined for other pathogens showing benzimidazole resistance (Albertini et al. 1999, Koenraadt et al. 1992, Cunha and Rizzo 2003).

Testing with azoxystrobin and tetraconazole gave conflicting results from the spore germination and mycelial growth screens. Isolates resistant to azoxystrobin were detected by mycelial growth screens at much higher levels than with spore germination in 1999. This anomaly may be due to the specific activity of azoxystrobin, a strobilurin class of fungicide known to inhibit the Q_o binding site of complex III of cytochrome b (Q_oI fungicide). Recent studies have determined resistance to the strobilurin class of fungicides to be dependent on the ability of fungi to use an alternative oxidase (AOX) or one of two mutations in cytochrome b, G143A and F129L (Wood and Hollomon 2003; Avila-Adame and Köller 2003). Strobilurin efficacy in *Magnaporthe grisea* is strongest during spore germination and is reduced as the fungus enters vegetative growth, both *in planta* and *in vitro*. Alternative oxidase is less efficient in oxidation than the standard oxidation by the Q_o and Q_i sites of the mitochondrial complex III. During periods of high metabolic activity, such as spore germination, AOX is insufficient for fungal survival when the Q_o site has been blocked by a strobilurin.

Studies of AOX importance in strobilurin resistance have used salicylhydroxamic acid (SHAM) to inhibit the activity of AOX during strobilurin resistance testing (Avila-Adame and Köller 2002, 2003). By inhibiting AOX, resistance to the strobilurins becomes dependent on mutations in the cytochrome b gene. In our studies, SHAM was not included and therefore limited our ability to determine the nature of resistance to strobilurin in the *C. beticola* isolates tested. A small number of isolates exhibiting resistance to azoxystrobin in the mycelial growth screens were subsequently tested in the same manner on PDA amended with azoxystrobin at 0, 1 and 10 ppm and SHAM at 0

and 1 ppm. In these tests, all isolates were inhibited at varying degrees by SHAM alone. For all isolates, growth did not reach 70% of control on 1 or 10 ppm azoxystrobin whether in combination with SHAM or not. The combination of SHAM and azoxystrobin gave enhanced inhibition of mycelial growth in all isolates. One isolate was completely inhibited by the presence of SHAM independent on the presence of azoxystrobin, which may indicate complete reliance on AOX in this isolate. Further studies of resistance to azoxystrobin or other Q_oI inhibiting fungicides will include tests using SHAM alone, SHAM with azoxystrobin, and azoxystrobin alone to better delineate the source of the resistant phenotype.

The cytochrome b mutations G143A and F129L give different levels of strobilurin resistance. Studies of *M. grisea* and *M. fijienses* indicate that G143A, a substitution of alanine for glycine at the 143 position, confers very strong resistance approaching complete immunity, while F129L, a substitution of phenylalanine by leucine, is less effective (Wood and Hollomon 2003; Avrila-Adame and Köller, 2003). In 2001 and 2002, resistance levels differed from those of 1999 and were very low in mycelial growth screens, corresponding to the levels seen in the spore germination screens of these years. Because different localized populations were studied from year to year, it is possible that differing results in azoxystrobin resistance are due to differences in *C. beticola* genotypes carrying different mutations or relying on AOX to differing degrees.

Tetraconazole resistance was much lower in the mycelial growth screens of all field samples. This may reflect the influence of sample size on fungicide resistance

screens. Spore germination screens tested one hundred isolates per fungicide concentration for each field sample, while mycelial growth screens only tested up to ten isolates. The smaller sample size used for the mycelial growth screens may not have been adequate to detect tetraconazole resistance. Sterol biosynthesis inhibiting fungicides such as tetraconazole seem to be most effective during the mycelial growth stage of *C. beticola* (Karaoglanidis et al. 2002). This would explain the increased sensitivity to tetraconazole recorded in the mycelial growth screens.

Resistance to the sterol biosynthesis inhibitors (SBIs) has been attributed to mutations in the gene for 14α-demethylase (*CYP51A1*), the target enzyme for SBI activity. A single nucleotide substitution in the substrate recognition site resulting in an amino acid change from phenylalanine to tyrosine has been correlated with resistance to SBIs in *Uncinula necator*, *Pennicillium italicum*, and *Erysiphe graminis* (Deleye et al. 1997; de Waard 1996; and Deleye et al. 1998). Recent studies by Schnabel and Jones (2000) have determined overexpression of the *CYP51A1* in *Venturia inequalis* to also confer resistance to myclobutanil, a SBI fungicide. These studies have shown that resistance to the SBI fungicides is stepwise in nature and increases with the presence of a mutation in *CYP51A1* and/or its overexpression, resulting in the need for higher doses of an SBI to obtain control of resistant isolates equivalent to that achieved with a lower dose in controlling sensitive isolates.

Treatment Effects on Fungicide Resistance:

Potential effects of field treatments on fungicide resistance in 2001 and 2002 were analyzed by combining treatments into four general categories: untreated control,

fungicides, BmJ integrated with fungicides, and BmJ used alone. Percentage of populations resistant to benomyl, azoxystrobin and tetraconazole at concentrations of 1 and 10 ppm are shown: spore germination results for 1 and 10 ppm concentrations in Table 3.3 and mycelial growth screen for 1 and 10 ppm concentrations in Table 3.4.

Spore Germination Screens: General treatment categories showed effects on tolerance levels to all three fungicides in spore germination screens in 2001. Benomyl, azoxystrobin, and tetraconazole tolerance was reduced compared to the untreated control by treatments of fungicides with or without BmJ. The largest reductions in benomyl and tetraconazole tolerance were attained by treatments of BmJ with fungicide. Azoxystrobin tolerance was reduced the most with fungicide treatments. BmJ applied alone was not effective in reducing tolerance levels determined by spore germination screens. The use of BmJ in the field results in higher disease incidence of CLS than most fungicide spray programs. The likelihood of the presence of isolates with a mutation conferring fungicide resistance is increased as the population increases. However, with a lack of selection pressure by fungicides in plots treated only with BmJ, results should not have identified resistant isolates at levels comparable to plots receiving selection pressure from fungicide applications. This is because the portion of the *C. beticola* population exhibiting fungicide resistance within each BmJ treated plot should not have increased relative to sensitive isolates. A more plausible explanation for the high levels of fungicide resistant isolates in BmJ treated plots is the movement of resistant isolates from plots sprayed with fungicide to BmJ treated plots. This is possible due to the ability of conidia to travel up

to 100 m (Windels et al. 1998) and the small plot sizes (3.66 m by 9.14 m) used in the field experiments.

Table 3.3: Percentage of conidia germinating on 1 ppm (A) and 10 ppm (B) of each fungicide from all field samples tested in each year according to general treatments.

A	benomyl		azoxystrobin		tetraconazole	
Field Treatment	1 ppm		1 ppm		1 ppm	
	2001	2002	2001	2002	2001	2002
Untreated Control	72.4 ab[1]	67.9 a	13.8 a	0.05 a	62.9 ab	45.6 ab
Fungicide	54.1 b	73.5 a	0.2 c	0.06 a	45.5 bc	40.3 b
BmJ + Fungicide	37.0 c	75.7 a	5.5 b	0.0 a	34.7 c	46.3 ab
BmJ	86.2 a	71.2 a	18.6 a	0.0 a	70.0 a	51.9 a

B	benomyl		azoxystrobin		tetraconazole	
Field Treatment	10 ppm		10 ppm		10 ppm	
	2001	2002	2001	2002	2001	2002
Untreated Control	72.2 a[1]	63.4 a	11.8 a	0.05 a	44.6 a	8.5 ab
Fungicide	49.8 b	68.5 a	0.1 b	0.0 b	20.1 b	5.0 b
BmJ with Fungicide	45.6 b	66.8 a	1.3 b	0.0 b	16.4 b	4.9 b
BmJ	82.7 a	65.2 a	21.6 a	0.0 b	60.3 a	9.6 a

1- values with the same letter are not significantly different at the P=0.05 level.

Treatment effects were not present in 2002. This was probably caused by the overall reduction in field applications of benomyl, azoxystrobin, and tetraconazole. Benomyl was not applied in 2002 and the benzimidazole, thiophanate methyl, was only applied twice. Azoxystrobin was applied in five sprays in 2001 and two sprays in 2002; however, trifloxystrobin (strobilurin class) was applied numerous times in both years. Tetraconazole was applied in four consecutive sprays in three spray programs in 2001. In 2002, tetraconazole was applied only in rotation with other fungicides or only once if alone. These changes in fungicide application may have a large impact on the way the general treatment categories affected tolerance levels in each year. A reduction in multiple applications of a single fungicide and the resulting reduction in selection

pressure from 2001 to 2002 may have reduced the differences in the response of fungicide resistance to the general treatment categories.

Overall levels of tolerance to 10 ppm concentrations of azoxystrobin and tetraconazole in spore germination screens were much lower in 2002 (Tables 3.1 and 3.3). The difference in tetraconazole tolerance may be due to the above-mentioned use of repeated applications (4 sprays) of tetraconazole in spray programs in 2001. This practice was not continued in the 2002 season. Repeated applications of tetraconazole would increase selection pressure resulting in a larger portion of tolerant *C. beticola* in the field population.

Mycelial Growth Screens: Results for the mycelial growth screens show a reduction in benomyl resistance by treatments of BmJ alone in 2001 (Table 3.4). This reduction contradicts the spore germination screen, and may be the result of insufficient sample sizes in the mycelial growth screens. Other treatment effects were not detected for benomyl resistance in both years, including BmJ alone in 2002. These variable results may be largely due to the high level of benomyl resistance already established in the population. In the two-year period from 1999 to 2001, practical resistance to benomyl in the Sidney growing area developed to the point that benzimidazole fungicides including benomyl were no longer effective (B.J. Jacobsen, personal communication, 2002). The high levels of stable resistance may have greatly confounded the results of treatment effects on resistance screens.

Differences in azoxystrobin resistance levels were small and seemed to contradict spore germination results. As mentioned above, azoxystrobin efficacy is not as strong

during vegetative growth. Resistance levels in the mycelial growth screens should therefore have been at least as high as the spore germination screens for each general treatment category in 2001. This did not occur. Sample size may once again have been insufficient. The type of resistance response seen in *M. grisea* (Avrila-Adame and Köller 2003) may not adequately describe strobilurin resistance in *C. beticola*.

Table 3.4: Mycelial Growth Screen Results. Percentage of *Cercospora beticola* isolates resistant to 1 ppm (A) and 10 ppm (B) concentrations of each fungicide for each year according to general treatment categories. The total number of isolates tested is shown on the far right of the table.

A	benomyl		azoxystrobin		tetraconazole		# of isolates	
Field Treatment	1 ppm		1 ppm		1 ppm		tested	
	2001	2002	2001	2002	2001	2002	2001	2002
Untreated Control	74.0	88.3	0.0	0.0	1.0	0.0	100	222
Fungicide	74.4	89.0	0.0	0.7	1.5	3.0	797	1103
BmJ with Fungicide	81.4	81.9	2.1	0.0	7.9	0.3	140	288
BmJ	43.7	85.1	0.8	0.0	2.5	0.0	119	471

B	benomyl		azoxystrobin		tetraconazole		# of isolates	
Field Treatment	10 ppm		10 ppm		10 ppm		tested	
	2001	2002	2001	2002	2001	2002	2001	2002
Untreated Control	62.0	87.4	0.0	0.0	0.0	0.0	100	222
Fungicide	74.2	88.2	0.0	0.3	0.1	1.8	797	1103
BmJ with Fungicide	80.0	81.9	0.0	0.0	7.1	0.0	140	288
BmJ	42.0	84.7	0.0	0.0	1.7	0.0	119	471

In 2002, azoxystrobin resistance loosely followed the *M. grisea* model with very low spore germination. Mycelial growth was less sensitive to azoxystrobin but was often insufficient to be classified as full resistance (growth ≥70% of control). Further investigation is needed to determine the type of azoxystrobin resistance in *C. beticola*. As mentioned earlier, these tests should include SHAM to aid in determining the

influence of AOX in resistance of *C. beticola* to the strobilurins. However, since toxicity from SHAM alone was detected, rates between 0 and 1 ppm should be examined.

General treatment effects on tetraconazole resistance as measured by mycelial growth screens showed treatments of BmJ with fungicides to give the highest resistance in 2001. However, this was not the case in 2002. Overall levels were lower in 2002 with only a few isolates showing resistance. The majority of resistant isolates were found in the fungicide treatment category.

A dose response may have been present with spore germination and mycelial growth of *C. beticola* in the presence of tetraconazole. Spore germination and mycelial growth were slowed by tetraconazole increasingly as dose was increased. Existence of a dose response explains the differences in results from the two types of screens used in this study. The rate of spore germination was often not hindered enough to show susceptibility with the spore germination screen, especially at the 1 ppm concentration. However, mycelial growth was inhibited enough to keep growth at less than 70% control in most instances, resulting in a susceptible determination. Therefore, differential levels of tolerance/resistance in the two types of screens occur. Also of importance to the differing results from each screen are the previously reported results that sterol biosynthesis inhibitors like tetraconazole are most effective against the mycelial growth stage of *C. beticola* (Karaoglanidis et al. 2002).

Treatment X Variety Effects on Fungicide Resistance:

Effects of specific spray programs applied to two sugar beet varieties on fungicide tolerance/resistance were determined for the Variety trial of 2002. The results for both

the spore germination and mycelial growth screens are shown in Tables 3.5 and 3.6. Important to this study is the increased level of fungicide resistance in *C. beticola* isolates from the spray program consisting of four sprays on the susceptible variety, Beta 2185 namely tetraconazole #1, trifloxystrobin #2, TPTH #3 and trifloxystrobin #4. In spore germination screens, *C. beticola* isolates from this spray program, along with the three-spray program, of tetraconazole followed by trifloxystrobin and TPTH on Beta 2185, had high levels of tolerance to benomyl. *C. beticola* isolates from these spray programs also had the highest levels of tolerance to azoxystrobin and tetraconazole. This same spray program did not result in higher tolerance levels to azoxystrobin and tetraconazole with the moderately resistant variety, HM 7054.

Mycelial growth screen results roughly matched the results obtained with spore germination screens. Benomyl resistant isolates accounted for very high proportions of the population from each treatment. Isolates resistant to 1 and 10 ppm azoxystrobin numbered less then ten and were mostly identified in fungicide-only spray programs on Beta 2185. Two resistant isolates were also found in the spray program of tetraconazole followed by trifloxystrobin on HM 7054. Tetraconazole resistance at 1 and 10 ppm was identified in only three field treatments. The majority of resistant isolates were from the three-spray and four-spray programs on Beta 2185. Several resistant isolates were also identified in the two-spray treatment on HM 7054.

Table 3.5: Spore germination screen results by field treatment for the susceptible and resistant varieties Beta 2185 (KWS=6.3) and HM 7054 (KWS=4.1), respectively. The percentage of spores germinating on 1 ppm (A) and 10 ppm (B) concentrations of fungicide.

A Field Treatment	benomyl 1 ppm		azoxystrobin 1 ppm		tetraconazole 1 ppm	
	Beta 2185	HM 7054	Beta 2185	HM 7054	Beta 2185	HM 7054
1) Untreated Control	60.82	63.00	0.00	0.00	42.44	43.25
2) 1[1]: tetraconazole (0.15 L/ha)	65.57	60.66	0.00	0.00	42.84	36.91
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	86.53	55.65	0.00	0.00	38.11	39.89
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	91.54	85.90	0.00	0.00	43.45	41.10
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha), 4: trifloxystrobin (110 ml/ha)	86.99	78.48	1.39	0.00	61.38	40.36
6) 1: BmJ (10^7 cfu/ha) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu/ha)	76.89	68.37	0.00	0.00	56.96	39.12
7) 1-4: BmJ (10^7 cfu/ha)	70.03	85.96	0.00	0.00	47.35	37.60
$LSD_{(0.05)}$	*30.73*		*1.05*		*30.22*	

B Field Treatment	benomyl 10 ppm		azoxystrobin 10 ppm		tetraconazole 10 ppm	
	Beta 2185	HM 7054	Beta 2185	HM 7054	Beta 2185	HM 7054
1) Untreated Control	60.84	60.31	0.00	0.00	3.58	3.05
2) 1[1]: tetraconazole (0.15 L/ha)	67.23	60.39	0.00	0.00	3.40	2.31
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	78.68	54.89	0.00	0.00	3.31	9.08
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	74.02	82.12	0.00	0.00	6.18	3.61
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha), 4: trifloxystrobin (110 ml/ha)	82.00	59.99	0.00	0.00	24.53	1.05
6) 1: BmJ (10^7 cfu/ha) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu/ha)	70.30	61.25	0.00	0.00	5.24	1.15
7) 1-4: BmJ (10^7 cfu/ha)	62.13	69.79	0.00	0.00	3.42	4.11
$LSD_{(0.05)}$	*30.44*		*0.00*		*11.06*	

1 – numbers following fungicides/BmJ indicate order of application.
2 – TPTH = triphenyltin hydroxide

Table 3.6: Mycelial growth screen results by field treatment for the susceptible and resistant varieties Beta 2185 (KWS=6.3) and HM 7054 (KWS=4.2), respectively. The percentage of isolates resistant to 1 ppm (A) and 10 ppm (B) concentrations of fungicide.

A Field Treatment	benomyl 1 ppm		azoxystrobin 1 ppm		tetraconazole 1 ppm		# of isolates tested	
	Beta 2185	HM 7054	Beta 2185	HM 7054	Beta 2185	HM 7054		
1) Untreated Control	85.7	98.1	0.0	0.0	0.0	0.0	56	53
2) 1[1]: tetraconazole (0.15 L/ha)	75.5	78.2	1.9	0.0	0.0	0.0	53	55
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	85.0	83.6	0.0	3.6	0.0	14.5	60	55
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	100.0	100.0	1.7	0.0	1.7	0.0	60	60
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha), 4: trifloxystrobin (110 ml/ha)	96.6	89.7	6.9	0.0	36.2	0.0	58	39
6) 1: BmJ (10^7 cfu/ha) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu/ha)	85.0	68.3	0.0	0.0	0.0	0.0	60	60
7) 1-4: BmJ (10^7 cfu/ha)	77.2	96.6	0.0	0.0	0.0	0.0	57	59

B Field Treatment	benomyl 1 ppm		azoxystrobin 1 ppm		tetraconazole 1 ppm		# of isolates tested	
	Beta 2185	HM 7054	Beta 2185	HM 7054	Beta 2185	HM 7054		
1) Untreated Control	85.7	96.2	0.0	0.0	0.0	0.0	56	53
2) 1[1]: tetraconazole (0.15 L/ha)	71.7	78.2	1.9	0.0	0.0	0.0	53	55
3) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha)	85.0	83.6	0.0	3.6	0.0	7.3	60	55
4) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha)	100.0	96.7	0.0	0.0	1.7	0.0	60	60
5) 1: tetraconazole (0.15 L/ha), 2: trifloxystrobin (84 ml/ha), 3: TPTH[2] (57 g/ha), 4: trifloxystrobin (110 ml/ha)	93.1	89.7	0.0	0.0	22.4	0.0	58	39
6) 1: BmJ (10^7 cfu/ha) + tetraconazole (75 ml/ha), 2-4: BmJ (10^7 cfu/ha)	85.0	68.3	0.0	0.0	0.0	0.0	60	60
7) 1-4: BmJ (10^7 cfu/ha)	77.2	94.9	0.0	0.0	0.0	0.0	57	59

1 – numbers following fungicides/BmJ indicate order of application.
2 – TPTH = triphenyltin hydroxide

These results indicate that a moderately resistant sugar beet hybrid may be a valuable tool in a fungicide resistance management program. This value is most likely due to the overall reduction in disease levels and *C. beticola* spore numbers occurring on a resistant hybrid compared to a susceptible hybrid as suggested by Karaoglanidis et al. (2003). Lower numbers of fungal spores in a field reduce the chance that a resistant genotype will be present in numbers large enough to alter the overall tolerance of the population. The increased levels of resistance to all three fungicides in the four-spray program on Beta 2185 suggest that the use of multiple classes of fungicide in rotation may not be sufficient to reduce resistance development when applied to a susceptible variety.

Multiple-Fungicide Resistance:

While the mycelial growth screen did not provide as robust an assay as the spore germination screen for detecting fungicide resistance due to smaller sample sizes, the ability of the mycelial growth screen to test a single isolate for resistance to more than one fungicide allowed the detection of resistance to multiple fungicides. Resistance to benomyl was widely distributed within the population of all isolates tested in 2001 and 2002 (Tables 3.3-6). Resistance to tetraconazole and azoxystrobin often occurred in isolates also exhibiting resistance to benomyl. In 1999, three isolates showed resistance to benomyl and azoxystrobin. In 2001, all 21 isolates showing resistance to tetraconazole were also resistant to benomyl. All three isolates resistant to azoxystrobin were also resistant to benomyl and tetraconazole. In 2002, 38 of 39 isolates resistant to tetraconazole were also resistant to benomyl. All ten isolates resistant to azoxystrobin

were also resistant to benomyl with seven of these resistant to tetraconazole as well. These results indicate that benomyl resistance in the *C. beticola* isolates tested does not interfere with the development of resistance to other modes of action. Similarly, resistance to tetraconazole seems not to interfere with resistance to azoxystrobin, whether being caused by dependence on AOX or mutations in the cytochrome b gene. Studies of the apple scab fungus, *Venturia inaequalis,* by Köller and Wilcox (2001) suggested that resistance to one fungicide might influence the genetic plasticity of a fungus resulting in increasing the speed of development for resistance to another, unrelated fungicide. This may be the case in this study since resistance to benomyl is common and may act as an accelerator of resistance development to tetraconazole and azoxystrobin. Forced efflux (detoxification) of fungicides by a molecular pump produced by the fungus is another possible explanation proposed for the development of resistance to multiple fungicides with differing modes of action (Del Sorbo et al. 1997; Sanglard et al. 1997).

The multinucleate nature of *C. beticola* cells and the multi-celled nature of the conidia (Pons et al. 1985) may allow for a high probability of a nucleus with a fungicide resistance mutation being present in a single conidium. The potential for genetic recombination through the parasexual cycle may also aid in the ability of *C. beticola* to quickly develop fungicide resistance if enough selection pressure is applied. Resistance to multiple fungicides in a single conidium may be possible due to the presence of multiple nuclei, each conferring resistance to an individual fungicide. The possibility of high variation in genotype demands that precautions are taken to properly manage the development of fungicide resistance in *C. beticola*.

The techniques used in this study have attempted to gauge the levels of fungicide sensitivity/resistance in *C. beticola* isolates from a single experimental farm, the Eastern Agricultural Research Center in Sidney, MT. Intensive field sampling and subsequent fungicide resistance testing using two screening methods have shown that fungicide resistance in *C. beticola* is variable and complex. The methodology of this study differs from previous fungicide resistance studies of *C. beticola* by Ruppel (1975), Ruppel et al. (1980), Percich et al. 1984; Bugbee (1995), Campbell et al. (1998), Weiland and Smith (1999), Weiland and Halloin (2001), and Briere et al. (2003). These cited examples monitored fewer samples taken from several commercial fields in a general growing area. The majority of resistance testing of *C. beticola* has used vegetative growth assays similar to the mycelial growth screens described here (Georgopoulus and Dovas 1973; Ruppel 1975; Ruppel et al. 1980; Percich et al. 1984; and Briere et al. 2003). In these studies, growth at a predetermined discriminatory dose or the determination of EC50 values identified whether an isolate was resistant or not.

Techniques similar to the spore germination screen employed here were used by Smith et al. (1997), Bugbee (1996), and Weiland and Halloin (2001). While a collective spore suspension from several leaf spots on several leaves from a single field sample was used to inoculate plates with differing concentrations of fungicide in our study, these other studies assayed the germination and subsequent vegetative growth of conidia from an individual lesion. Macroscopic identification of spore germination and vegetative growth over a five-day period was used in these studies rather than microscopic identification of germination as was done here. Our spore germination screening

technique determined the germination of 100 individual spores per fungicide concentration for each field sample (a total of 700 spores per sample) and relied on the ability to gather hundreds of conidia for each screen. Combining spores from several lesions was therefore necessary. This combination may have contributed to the variability of the results in the spore germination screens because the number of lesions sampled for each test varied from approximately 5 to 30, with each lesion potentially being genotypically and phenotypically different. Future studies will analyze the variability of lesions on the scale of an individual leaf and experimental plot to determine the potential variability in the spore germination screen as employed in this study.

Table 3.7: Coefficient of variation values for spore germination screens in both years.

	benomyl		azoxystrobin		tetraconazole	
Year	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
2001	56.2	57.7	100	100	58.8	79.6
2002	36.7	40.5	100	100	60.4	100

In summary, the use of fungicide rotation, the biological control agent BmJ applied with reduced rates of fungicide, and moderately resistant varieties have indicated their potential in managing fungicide resistance in these studies. These strategies seem most effective in controlling the emergence of fungicide resistance as seen in the cases of azoxystrobin and tetraconazole resistance. The effectiveness of these strategies may be correlated to the ability to reduce the overall level of disease in the field, thus lowering the odds that a competitive resistant genotype will be present. It is important to note that the overall variability was high in all spore germination screen results (Table 3.7). This may be due to the small size of the experimental plots (3.66 m by 9.14 m) allowing cross-

plot movement of *C. beticola* during the growing season or variation inherent in the screening method. Tests of samples taken from commercial fields or large experimental plots are needed to elucidate the cause of variability in results of tetraconazole resistance. Further studies on the nature and respective levels of resistance to azoxystrobin and tetraconazole in *C. beticola* isolates from eastern Montana are needed in the future. Establishment of stable fungicide resistance, e.g., benomyl resistance, makes recovery of fungicide efficacy difficult (Ruppel et al. 1980, Karaoglanidis et al. 2003) and may allow accelerated development of resistance to other fungicides (Köller and Wilcox, 2001). Therefore, proper management is most important when resistant populations are still at low levels. Prevention through proper cultivation, crop rotation, the rotation of fungicides with different modes of action, growing moderately resistant varieties and applying BmJ when available is the most effective and sustainable way of managing fungicide resistance in *C. beticola* in eastern Montana.

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APPENDIX

CERCOSPORA LEAF SPOT DAMAGE CATEGORIES

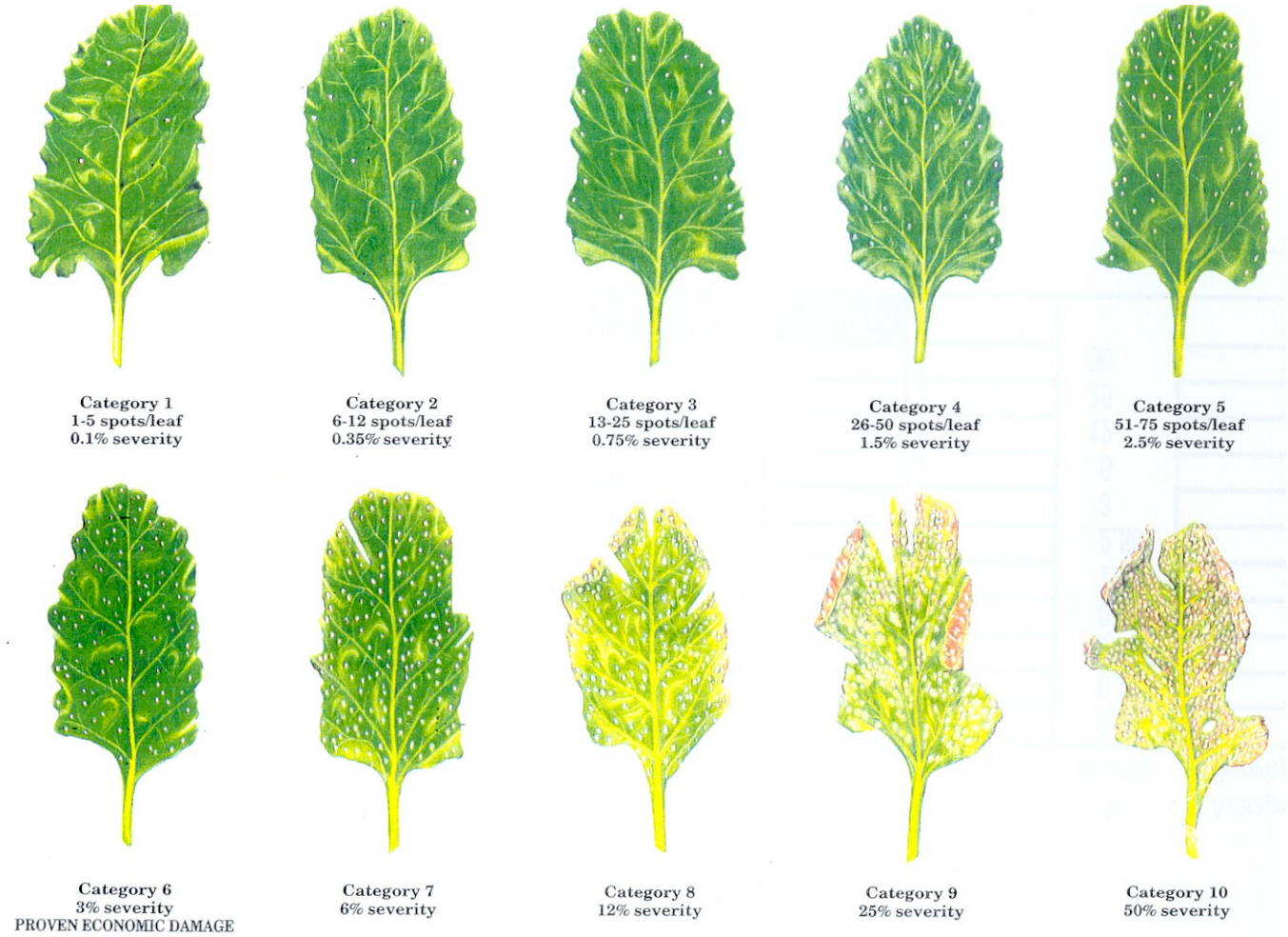

Figure A: Cercospora Leaf Spot Damage Categories.