DISSERTATION

RELATIONSHIP OF GRAY SNOW MOLD DEVELOPMENT IN *POA ANNUA/POA PRATENSIS* TO PERSISTENCE OF CHLOROTHALONIL AND FLUDIOXONIL UNDER SNOW COVER AND EFFECT OF SNOW REMOVAL ON GRAY SNOW MOLD DEVELOPMENT AT HIGH ALTITUDE GOLF COURSES.

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ABSTRACT

RELATIONSHIP OF GRAY SNOW MOLD DEVELOPMENT IN *POA ANNUA/POA PRATENSIS* TO PERSISTENCE OF CHLOROTHALONIL AND FLUDIOXONIL UNDER SNOW COVER AND EFFECT OF SNOW REMOVAL ON GRAY SNOW MOLD DEVELOPMENT AT HIGH ALTITUDE GOLF COURSES.

Gray snow mold caused by Typhula incarnata and T. ishikariensis (speckled snow mold) is a major problem on golf courses where snow cover persists for periods exceeding 60 days. The disease is primarily managed by preventive fungicide applications in fall prior to winter snow cover. However, fungicide rates necessary to control snow mold are often much higher than those needed to suppress other turf diseases during the summer. Studies were undertaken to determine the sensitivity of T. incarnata and T. ishikariensis isolates to chlorothalonil and fludioxonil, two fungicides commonly used for Typhula blight control. Growth of most isolates (70%) on potato dextrose agar (PDA) amended with 1 µg/ml chlorothalonil was inhibited by more than 50% relative to growth on non-amended PDA. However, almost all isolates exhibited at least some growth at concentrations as high as 500 µg/ml. Growth of most isolates was inhibited by more than 50% on PDA amended with 0.1 μ g/ml fludioxonil and > 90% were inhibited by more than 80% at 1 µg/ml. The persistence of chlorothalonil and fludioxonil residues in the turf during winter was measured by gas chromatography/mass spectrometry. In 2005-2006, chlorothalonil concentrations decreased by approximately 50% the first week after application and then decreased at a rate of 0.7-1.0 µg/g tissue/day during snow cover. The rapid decrease in

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chlorothalonil concentration after the first week was not observed in other years. Instead, concentrations of chlorothalonil and fludioxonil in the verdure decreased at less than 1.0 µg/g tissue/day or remained nearly the same at most sampling dates indicating these fungicides did not dissipate rapidly under snow. Despite this, only marginal control of Typhula blight was observed in the fungicide-treated plots. In non-fungicide treated plots, Typhula blight patches developed rapidly between the December and January sampling dates, sometimes to a level of plot damage equal to that observed in late April or May.

Golf course superintendents at high altitudes in Colorado apply fungicides in late October before permanent snow cover to prevent gray or speckled snow mold development. Snow is also removed from putting greens in March and kept snow-free through spring to help suppress snow mold. However, the benefits of spring snow removal in snow mold suppression compared to potential turfgrass damage caused by exposure to low temperatures following removal have not been documented. We compared snow mold severity and turfgrass health on a Kentucky bluegrass fairway at Breckenridge and annual bluegrass plots at Vail, CO with permanent snow cover through winter to plots where snow was removed from late October through mid-November and to plots where snow was removed in mid-March and maintained snowfree through the spring. Both gray and speckled snow molds were observed at both locations and in each year, although the majority of the damage was caused by speckled snow mold. Snow removal influenced temperatures at the turfgrass surface during the winter and a similar trend was observed for each treatment in each year and at both locations.

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CHAPTER 1

RELATIONSHIP OF GRAY SNOW MOLD DEVELOPMENT IN *POA ANNUA/POA PRATENSIS* TO PERSISTENCE OF CHLOROTHALONIL AND FLUDIOXONIL UNDER SNOW COVER

SUMMARY

Typhula blight is a major problem on golf courses where snow cover persists for periods exceeding 60 days. The disease is primarily managed by preventive fungicide applications in fall prior to winter snow cover. However, fungicide rates necessary to control snow mold are often much higher than those needed to suppress other turf diseases during the summer. Studies were undertaken to determine the sensitivity of Typhula incarnata and T. ishikariensis isolates to chlorothalonil and fludioxonil, two fungicides commonly used for Typhula blight control. Growth of most isolates (70%) on potato dextrose agar (PDA) amended with 1 µg/ml chlorothalonil was inhibited by more than 50% relative to growth on non-amended PDA. However, almost all isolates exhibited at least some growth at concentrations as high as 500 µg/ml. Growth of most isolates was inhibited by more than 50% on PDA amended with 0.1 µg/ml fludioxonil and > 90% were inhibited by more than 80% at 1 µg/ml. The persistence of chlorothalonil and fludioxonil residues in the turf during winter was measured by gas chromatography/mass spectrometry. In 2005-2006, chlorothalonil concentrations decreased by approximately 50% the first week after application and then decreased at a rate of 0.7-1.0 µg/g tissue/day during snow cover. The rapid decrease in chlorothalonil concentration after the first week was not observed in other years. Instead, concentrations of chlorothalonil and fludioxonil in the verdure decreased at less than 1.0 µg/g tissue/day or remained nearly the same at most sampling dates indicating

these fungicides did not dissipate rapidly under snow. Despite this, only marginal control of Typhula blight was observed in the fungicide-treated plots. In non-fungicide treated plots, Typhula blight patches developed rapidly between the December and January sampling dates, sometimes to a level of plot damage equal to that observed in late April or May.

Introduction:

Gray snow mold caused by Typhula incarnata and speckled snow mold caused by Typhula ishikariensis, collectively called Typhula blight when they occur together, are major problems on golf courses in areas where snow cover persists for long durations (Hsiang et al., 1999). However, gray snow mold may also develop in a mild form during winter in the absence of snow cover (Smith, 1989). Symptoms of snow molds are evident at snowmelt and are usually exhibited as light yellow, straw-colored or grayishbrown turf from a few centimeters to a meter or more in diameter (Smith, 1989). Leaves in the affected areas are matted together and often are covered with gravish-white mycelium. The mycelium desiccates as the turf grass dries out leaving areas of what appears to be dead turfgrass. Most often, crowns of the turf plants are not affected and will produce new leaves within two-three weeks after snowmelt (Smiley, 2005). Gray snow mold will produce faintly pink sclerotia up to 5 mm in diameter and may be found on or within the infected tissues (Smith, 1989). They will darken with age to a reddishbrown or dark-brown, wrinkling as they dry. Sclerotia of gray snow mold can be variable in shape, ranging from subglobose to elongated, flattened or irregular (Smith, 1989).

Speckled snow mold develops where winters are longer and more severe, usually requiring a snow cover duration of at least 90 days and generally upwards of 120 days (Chang et al., 2006). Sclerotia of speckled snow mold are readily detached from the leaf and are much smaller than gray snow mold. They can be subglobose or slightly flattened, light brown to almost black as they dry (Smith, 1989). A study done in Wisconsin on the distribution of *Typhula* spp. found that in areas of longer snow cover days (northern Wisconsin), *Typhula ishikariensis* occurred more frequently and in areas of fewer days of snow cover (southern Wisconsin), *T. incarnata* was the dominant species of snow mold (Chang et al., 2006).

Typhula is a basidiomycetous genus which has been assigned to different taxonomic families over time (Hsiang et al., 1999). In the GenBank taxonomy web page (http://www.ncbi.nlm.nih.gov/taxonomy), the genus *Typhula* is placed in the order Thelephorales, family Typhulaceae (Hsiang et al., 1999). The classification of *Typhula ishikariensis* and its varieties have been through several iterations. Pathogenicity, distribution, basidiocarp size and mating experiments (Bruehl and Cunfer, 1975), (Bruehl et al., 1975) split *T. idahoensis* from *T. ishikariensis*. Later studies regarded these two species as conspecific and designated *T. idahoensis* as a variety of *T. ishikariensis* and designated a variant of *T. ishikariensis* found in Canada as *T. ishikariensis* var. *canadensis* (Arsvoll & Smith, 1978). In 1980, Bruehl and Machtmes maintained that *T. ishikariensis* and *T. idahoensis* were separate species based on mating experiments between *Typhula* isolates. *T. ishikariensis* var. *canadensis* was rejected as a distinct variety (Bruehl and Machtmes, 1980). The *T. ishikariensis* complex

was divided into two biological species by Matsumoto; var. ishikariensis from turf in North America belongs to biological species I (Matsumoto, 1982) and Biotype A (Hsiang et al., 1999), however, the Japanese biotype A (Biological Species I, Biotype A and Group I) does not cause disease on turf under natural conditions; Biological species II (Biotype C, Biotype Bss and Group II) includes the North American var. *canadensis*; and the Japanese biotype B, classified as Typhula idahoensis by Bruehl (Bruehl and Cunfer, 1975), is also Group III and Biological species I (Hsiang et al., 1999). A publication by Hsiang using genetic markers found that, even though T. phacorrhiza, T. incarnata, and T. ishikariensis are distinct species, the varieties of T. ishikariensis do not show genetic differences at the species level (Hsiang & Wu, 2000). In 2006, the development of species specific PCR primers designed to identify T. incarnata, T. phacorrhiza, T. ishikariensis, T. ishikariensis var. ishikariensis and T. ishikariensis var. canadensis for samples collected from golf courses throughout Wisconsin were able to identify 1,835 isolates to one of the Typhula species and/or its variety (Chang et al., 2006).

Cultural practices play an important role in the development of snow mold diseases. Grasses need nitrogen fertility in order to overwinter, but excessive nitrogen fertilization near the end of the growing season promotes lush growth and prevents the turfgrass from becoming winter hardy (Hsiang et al., 1999). However, fertilization with nitrogen after the growth of leaf blades has stopped in the fall may promote turfgrass recovery and rapid growth in the spring (Couch, 1995). Fertilization with inorganic fertilizers may reduce snow mold severity in *Agrostis* spp. as compared with using

organic fertilizers (Hsiang et al., 1999). Since gray and speckled snow mold requires prolonged snow cover, attempts have been made to reduce snow mold severity by removing snow from the turf surface (Hsiang et al., 1999) (see Chapter 2). Recommendations for dealing with damage from snow molds in spring include improving drainage, replanting or re-sodding, promoting rapid drying and warming of the turf or lightly fertilizing the affected areas (Smiley, 2005).

The type of turfgrass also influences the severity of Typhula blight. Bentgrasses, especially creeping bentgrasses (*Agrostis palustris*), are often severely damaged by Typhula blight (Smith, 1989, Vargas, 1994, Wang et al., 2005) found genotypic variation in creeping bentgrass clones to damage caused by *T. ishikariensis*. Clones from fairways tended to be more resistant than those from putting greens. Kentucky bluegrass (*Poa pratensis*) and annual bluegrass (*Poa annua*), often found on golf course fairways or putting greens in Colorado, are considered intermediate in susceptibility to Typhula blight (Vargas et al., 1972, Hsiang et al., 1999) although this conclusion appears to be based on observational, rather than experimental evidence.

Recent research into biocontrol and organisms antagonistic to *Typhula* spp. shows promise. *Typhula phacorrhiza* shows the most promise as a potential biocontrol. Isolates have been identified that could inhibit disease caused by *T. ishikariensis* on creeping bentgrass. A culture filtrate of *Pseudomonas flourescens* was inhibitory to mycelial growth of *T. ishikariensis* and in field trials, a bacterial suspension was able to reduce snow mold damage (Hsiang et al., 1999). *Trichoderma atroviride* is a cold tolerant, versatile hyperparasite that was isolated from the sub-arctic region of Alaska

and has been found to suppress a wide range of economically important plant pathogens, including *Typhula incarnata, T. idahoensis* and *T. ishikariensis* (McBeath, 2002.). Introducing these antagonists, as well as establishment, has been the largest hurdle to practicing biocontrol. These antagonists have to be able to survive the harsh winter environment under the snow cover as well as be low-temperature tolerant. Economics also factor into biocontrol applications. These treatments may need to be applied annually or bi-annually in order to build populations high enough to be antagonistic to snow mold pathogens. This may result in costs even greater than fungicide use, however environmental risks and societal concerns may justify these costs if efficacy of the biocontrol is acceptable (Hsiang et al., 1999).

Typhula blight on bentgrass or annual bluegrass putting greens and fairways in Colorado is primarily controlled with fungicide applications applied just before snow cover in the fall. Nevertheless, Typhula blight can still produce varying degrees of damage to the golf course. In spring, after snow melt, Typhula blight damage can pose economic and aesthetic problems for the golf course superintendent to get the course in shape in time for spring play. During the 1970's and early 1980's, inorganic fungicides containing mercury and cadmium were widely and effectively used to control snow molds. However, due to their effect on people and their persistence in the environment, they fell into disfavor and their use was discontinued (Skoglund, 1998). The contact fungicide quintozene replaced the mercurial compounds as the product of choice for snow mold control because of its efficacy and price. However, quintozene is phytotoxic to creeping bentgrass (Vargas, 1994). In 2010 the use of quintozene was briefly halted

by the Environmental Protection Agency due to potential human health effects, but it was re-authorized for sale in August of 2011. Nevertheless, ongoing concerns about quintozene resulted in continued research into alternative fungicides for Typhula blight control.

Many other fungicide chemistries have been used in controlling Typhula blight (Appendix 2). These include chlorothalonil, chloroneb, iprodione, fludioxonil, thiophanate methyl, vinclozolin, and some of the triazole and strobiluron fungicides (Hsiang et al., 1999, Jung, 2007, Vargas, 1994,). In most cases a combination of active ingredients are used since several different fungal pathogens including *Microdochium nivale*, *T. ishikariensis* and *T. incarnata* may be active under snow cover (Hsiang et al., 1999). The combination of chlorothalonil, fludioxonil and propiconazole has effectively controlled Typhula blight in Colorado (Appendix 3) and is widely used by superintendents. Chlorothalonil and fludioxonil are non-polar, broad-spectrum fungicides (Putnam et al., 2003), whereas propiconazole is a triazole. These fungicides are also sold in a commercially available formulation called Instrata (Syngenta Professional Products, Greensboro, NC).

Fungicide rates for Typhula blight control usually are several times that required for controlling other turf diseases (Hsiang et al., 1999). The reason for this isn't entirely understood, although factors such as insensitivity of fungal isolates to selected fungicides, chemical inactivity or effectiveness at low temperatures, fungicide dissipation from melting snow, or photo- or biodegradation during winter may play a role (Monadjemi et al., 2011, Sigler et al., 2003, Stromqvist and Jarvis, 2005, Wu et al., 2002). Fungicide dissipation or degradation is of particular interest, since a fungicide

applied in the fall must persist and remain at high enough concentrations, sometimes for periods of 4-5 months, to inhibit snow mold fungi. There have been many studies looking at the fate of fungicides after application to turf (Carroll, 1993, Carroll, 2001, Sigler et al., 2003, Stromqvist and Jarvis, 2005), although these studies have been conducted during the summer when turfgrass is actively growing.

The objectives of this study were to identify the *Typhula* isolates collected from different golf courses in Colorado, determine the sensitivity of a subset of these isolates to chlorothalonil and fludioxonil, to monitor the persistence of these fungicides on the turf during the winter, and evaluate the efficacy of these products in controlling Typhula blight.

Methods and Materials:

Collection of Typhula isolates

Turf samples containing *Typhula* sclerotia were arbitrarily collected from snow mold patches that had developed in the fungicide-treated plots at Vail and Breckenridge during 2007 (Appendix 1). Individual sclerotia were disinfested in a 0.05% sodium hypochlorite solution for 30 seconds (Jo et al., 2006) then rinsed with sterile distilled water before plating on half-strength potato dextrose agar. Pure cultures were maintained on ½ PDA at 10°C. *Typhula* isolates were also collected from various locations in Colorado or were obtained from other sources (Appendix 1), and maintained on ½ PDA as described. Some isolates were collected from fairways that in past years had been treated with fungicides, including chlorothalonil, propiconazole and quintozene, whereas other isolates were collected from fairway roughs and other sites

where fungicides were less likely to have been applied. *Typhula* isolates were speciated by morphological characteristics of the sclerotia (Smith, 1989). The species of a smaller set of isolates (Table 3) was verified by amplifying the rDNA ITS region with the universal primers ITS4 and ITS5, sequencing the amplicon, then comparing it with known *Typhula* isolates using BLASTN (<u>http://blast.ncbi.nlm.nih.gov/Blast.cgi</u>). In all cases, the sequencing verified species identification based on morphology in culture.

Fungicide residue field plots

2005-06

To test the persistence of chlorothalonil in the turf through the winter, fungicide plots were established on a Kentucky bluegrass (*Poa pratensis*) fairway at the Breckenridge Golf Course, Breckenridge, CO, on 24 Oct 2005. The grass was mowed at a height of approximately 1.5 cm during the growing season but was not mowed after fungicide application. Plots measuring 3 m by 3 m were left untreated or were sprayed with chlorothalonil (Daconil Ultrex 82.5WDG, Syngenta Professional Products, Greensboro, NC) at 3.0 or 3.7 kg/ha or quintozene (Turfcide 400, American Vanguard Corporation, Newport Beach, CA) at 15.12 kg/ha. Quintozene, a commonly used snow mold fungicide, was included for comparison of efficacy to chlorothalonil, but residues were not determined for this fungicide during the study. Applications were made using a CO₂ sprayer pressurized to 138kPa and equipped with a spray boom with four 8004 flat fan nozzles to deliver water at 815 L per ha. Plots were arranged in a randomized complete block design with four replicates. The fairway was snow-free on the date of

application and fungicides were allowed to dry on the foliage before collecting the first samples.

Plot corners were marked with 2.4-m-long orange fiberglass poles such that the boundaries of each treatment could be identified during snow cover. A 6.35-cm-diameter X 5-cm-deep turf core sample was arbitrarily removed from each treatment using a 1.2-meter-long soil core remover. Samples were placed in plastic bags, labeled and stored at -80° C until they were processed for fungicide residue analysis. Samples were collected approximately weekly for the first month after application, biweekly for the following month, and monthly thereafter until the final collection on 2 May 2006.

2006-07

Fungicide trials were repeated in 2006 on a Kentucky bluegrass fairway at the Breckenridge Golf Course and on an annual bluegrass fairway at the Vail Golf Club, CO. The experimental design was identical to that in 2005-06, with an additional treatment of the commercial fungicide mixture Instrata 3.61SE (Syngenta Professional Products, Greensboro, NC) containing 3.7 kg chlorothalonil, 0.006 kg fludiozonil and 0.093 kg propiconazole per ha. Fungicide applications were made 24 Oct 2006 at both locations. The turf had been mowed to a height of approximately 1.5 cm during the growing season but was not mowed following fungicide application. Sample removal and processing were the same as described above. Final sample removal and visual

evaluations at Vail Golf Club occurred on 26 March 2007, whereas final sample extraction and visual evaluations at Breckenridge was 1 May 2007.

2007-08

On 24 Oct 2007, plots on an annual bluegrass fairway at Vail were left untreated or treated with applications of fludioxonil (Medallion 50WP, Syngenta Professional Products, Greensboro, NC) at 0.008 kg/ha, propiconazole (BannerMAXX 1.3ME, Syngenta Professional products, Greensboro, NC) at 1.08 kg/ha or two rates of the Instrata as used in 2006-2007. Sample collection and processing for fungicide residue analysis were the same as described above. Final samples were collected 12 May 2008.

Fungicide residues

Several extraction solvents and protocols were initially used to try to maximize extraction of chlorothalonil and fludioxonil from turf samples (Appendix 3). The final extraction procedure was based on research conducted by Mastovska (Mastovska and Lehotay, 2004) using toluene as the solvent. Living, green turfgrass leaves and shoots, hereafter referred to as the verdure (Beard, 1973) were clipped at a height just above the thatch layer from the chlorothalonil or fludioxonil-treated turf cores collected at each sampling date. The thatch layer is defined as the tightly intermingled layer of dead and living stems and roots that develops between the verdure and soil surface (Beard,

1973). Three, 0.2 g (fresh weight) sub-samples of verdure from the core removed from each fungicide-treated plot were placed in 50 ml glass centrifuge tubes with Teflon-lined caps. Ten ml of water-saturated toluene and 10 ml of distilled water were added to each 50 ml tube. The tubes were agitated on a wrist-action shaker for 2 hours and then centrifuged for 20 minutes at 6000 x g and 4°C to separate the toluene from water. A 100 µl aliquot of the toluene phase containing the chlorothalonil extract was placed in a 2 ml volumetric tube to which 10 µl of a 0.1 mg/ml butylate internal standard solution and 1890 µl water-saturated toluene had been added. In the case of fludioxonil, 10 µl of 0.1 mg/ml metalochlor internal standard solution was added to 1990 µl of the toluene phase containing the fludioxonil extract (no dilution was needed). Tubes were then hand-shaken to mix the butylate and toluene or metalochlor and toluene; then approximately 1 ml of the mixture was transferred to vials for analysis by gas chromatography/mass spectrometry (GC/MS, Hewlett-Packard 5890 Series II Plus Gas Chromatograph, Hewlett-Packard 5972 Series Mass Selection detector, Agilent Technologies, Palo Alto, CA, USA).

Fungicide concentrations in the toluene phase were analyzed with the GC/MS by monitoring the masses for butylate (m/e 146,156,174) and chlorothalonil (m/e 264, 266, 268 270), or metolachlor (m/e 238, 240) and fludioxonil (m/e 248, 249). An HP 5MS 30 mm x 0.25 mm column was used with a flow of helium at 1.5 ml/min. The injection temperature for the chlorothalonil runs was 225°C and the detector temperature was 280°C. The program for detecting chlorothalonil was as follows: initial oven temperature 80°C (hold for 1 minute), ramped at 20°C min to 230°C, then held at 230°C

for 1.5 minutes with a run time of 16 min. For each sampling date, analytical samples of chlorothalonil at 50, 100, 250, 500 and 1000 ng/-ml⁻¹ or fludioxonil at 0.25, 0.5, 1.0, 2.0 and 5.0 ng/-ml⁻¹ in toluene were processed. The R^2 values of the peak area ratio of chlorothalonil/butylate or fludioxonil/metalochlor to concentrations of chlorothalonil or fludioxonil, respectively, were always >97%.

Fludioxonil and chlorothalonil residues extracted from the 2007-08 Instrata treated plots were evaluated in a slightly different manner. Samples of verdure were prepared as above using the toluene/water in 50 ml glass tubes. A 100 µl aliguot of the toluene phase and 20 µl of a 0.5 mg/ml atrazine as the internal standard was placed in a 2 ml volumetric tube for the chlorothalonil extraction after which 1880 µl of toluene was added to bring to volume. A 1980 µl aliquot of the toluene phase was added to 20 µl of 0.5 mg/ml atrazine as the internal standard for the fludioxonil extraction from Instrata. Fungicide concentrations in the toluene phase were analyzed with the GC/MS by monitoring the masses for atrazine (m/e 200, 215) and chlorothalonil (m/e 266) or fludioxonil (m/e 248, 249). An HP 5MS 30 mm x 0.25 mm column was used with a flow of helium at 1.5 ml/min. The injection temperature for the fungicide runs was 250°C and the detector temperature was 280°C. The program for detecting chlorothalonil was as follows: initial oven temperature 150°C (hold for 1 minute), ramped to 250°C at a rate of 5°/min with a total run time of 20 min. For each sampling date, analytical samples of chlorothalonil or fludioxonil at 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 µg/ml in toluene were processed. The R^2 values of the peak area ratio of chlorothalonil/atrazine or

fludioxonil/atrazine to concentrations of chlorothalonil or fludioxonil, respectively, were always >99%.

Quality control (QC) samples consisted of verdure without fungicide or spiked with 50 or 100 μ l of a 767 μ g/ml chlorothalonil (made from the formulated Daconil Ultrex product) in 2005-06 and 2006-07. Formulated product was initially used because of the concern that inert ingredients might influence recovery. In 2006-07 and 2007-08 verdure spiked with a 0.5, 1 and 2 μ g/ml of analytical fludioxonil or chlorothalonil were also included in each run. Mean recovery rates for chlorothalonil were 90.1% (n = 33, SD ± 9.0) from Daconil Ultrex in 2005-06, 90.4 % (n=51, SD ± 9.6) from Daconil Ultrex in 2006-07, 90.2% (n=27, SD±7.9) from Instrata in 2006-2007 and 81.7% (n=24, SD ± 9.5) from Instrata in 2007-2008. Mean recovery rates for fludioxonil were 94.2% (n=9, SD±3.4%) from Instrata in 2006-07 and 94.1% (n=32, SD ± 5.9) from Instrata and Medallion in 2007-08.

Data from all GC/MS were converted to fungicide concentrations (µg/g) using the ratio of butylate to chlorothalonil, metolachlor to fludioxonil or atrazine to chlorothalonil/fludioxonil and then to µg/g fresh plant tissue. Linear regression analysis (SAS Inc., NC) was performed between fungicide concentrations and sampling dates. To more accurately assess fungicide dissipation under snow cover, the initial sampling date as well as those that occurred more than two weeks after snow melt in the spring, were omitted from the final regression analysis.

Efficacy of chlorothalonil and fludioxonil at high field application rates

Field studies from 2005-06, 2006-07 and 2007-08 indicated that labeled rates of chlorothalonil and fludioxonil applied to the fungicide plots did not control Typhula blight (Figure 1.12 and 1.13, Table 1.1). Therefore, the possibility of controlling Typhula blight using higher fungicide rates (i.e. rates 2, 5 and 10 times the concentration of the highest labeled rate) was evaluated in an annual bluegrass fairway at Vail in 2008-09. The fairway had been maintained at a height of 1.5 cm prior to the experiment, but was not mowed following the fungicide applications. Chlorothalonil (Daconil Ultrex) at 12.5, 22.5, 63.0 and 126.0 and fludioxonil (Medallion) at 0.38, 0.77, 1.91 and 3.86 kg/ha were applied on 29 Oct 2008 in a randomized complete block design with four replications. Applications were made in the manner previously described. Plots were rated 27 Apr 2009 for Typhula blight severity. Residue samples were not collected during the winter and the experiment was not repeated.

In vitro fungicide inhibition assays

A 7 mm plug of agar containing mycelium of one of 180 *Typhula ishikariensis* or 15 *T. incarnata* isolates was placed individually in the center of a petri plate containing $\frac{1}{2}$ PDA amended with chlorothalonil (Daconil Ultrex) at 0 and 1 µg/ml. An arbitrarily selected subset of *T. ishikariensis* (57 isolates at 10 and 100 µg/ml and 20 isolates at 500 µg/ml) and *T. incarnata* (11 isolates at 10, 100 and 500 µg/ml) was tested at other concentrations. These concentrations were selected because they were in the range of chlorothalonil residues detected in the verdure of fungicide-treated plots. Growth *T.*

ishikariensis was determined in ½ PDA plates amended with fludioxonil (Medallion) at 0, 0.1 and 1.0 µg/ml (199 isolates) or 0.01 and 0.001 µg/ml (39 isolates). Fifteen T. incarnata isolates were tested at the same concentrations. Growth was recorded of 179 T. ishikariensis and 15 T. incarnata isolates in agar amended with propiconazole (BannerMAXX) at 0 and 1 µg/ml. A subset of 57 T. ishikariensis and 11 T. incarnata isolates were also grown on agar amended with propiconazole at 10 and 100 µg/ml. All plates were incubated in a growth chamber with no internal light at 10°C for 22 or 30 days, and radial growth of the fungus on the plates was measured. The radius of the initial agar plug was subtracted from each measurement. The percent relative mycelial growth (RMG) at each fungicide concentration was calculated as the radial growth (on fungicide-amended $\frac{1}{2}$ PDA/radial growth or non-amended $\frac{1}{2}$ PDA) x 100 (Jo et al., 2006). The relationships between RMG and fungicide concentrations were analyzed by linear regression analysis (Minitab 14). However, R^2 values were often low (< 0.80) and there was no linear relationship at the fungicide concentrations we tested for many isolates. Therefore EC₅₀ values were not determined by regression analysis. Instead the percentage of growth inhibition of each isolate at each fungicide concentration was determined.

Results:

Typhula isolates

A systematic survey of the distribution and frequency of *Typhula* species in Colorado golf courses was not conducted, but isolates were collected from several

locations in the state (Appendix 1). *T. ishikariensis* was present at most locations whereas *T. incarnata* and *T. phacorrhiza* were less frequently recovered. A more intensive collection was made from within plots at Vail and Breckenridge and 467 of the isolates collected were identified as *T. ishikariensis* based on morphological characteristics of their sclerotia. Although *T. ishikariensis* was dominant at both sites, eleven isolates arbitrarily collected from an annual bluegrass rough adjacent to the fungicide plots at Vail in 2007 were identified as *T. incarnata*.

A subset of 110 *Typhula* isolates were further characterized based on rDNA ITS sequencing results (Table 1.3). Of these, 52% of the isolates were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishikariensis* biological species 1 and 19% were identified as *T. ishik*

In addition to *Typhula* species, other fungi associated with snow mold damage were collected. *Myriosclerotinia borealis* was identified at a single site in Wolcott, CO, *Waitea circinata* was recovered from damaged turf at Granby, CO and *Fibularhizoctonia carotae* was associated with mold on leaf debris on fairways at Loveland and Fort Collins, CO. Another fungus that was apparently associated with snow mold damage on Kentucky bluegrass fairways and that developed sclerotia resembling *Typhula* was collected from Loveland and Colorado Springs. However, the rDNA ITS sequence was less than 80% similar to any of the *Typhula* species and it remained unidentified.

Residue trials

2005-06

Chlorothalonil residues in the verdure immediately following application of the low and high rates of Daconil Ultrex on 24 Oct 2005 were 132 and 75 µg/g tissue, respectively (Figure 1.1). Residues decreased by >50% after the first week and during a period of no snow cover. Snow covered the plots after the first week in November and remained for 134 consecutive days. Chlorothalonil residues decreased at a much slower rate of -0.7 ($R^2 = .90$, P < 0.001) and -1.0 ($R^2 = .88$, P < 0.001) µg/g tissue/day in the low and high Daconil Ultrex-treated plots, respectively, during this period. On 14 Mar 2006, the sampling date just prior to spring snow melt, chlorothalonil concentrations of 7 and 24 µg/g tissue at the low and high application rates were detected in the verdure. Chlorothalonil residues also were detected at levels <20µg/g tissue in samples collected after snowmelt. Despite continued presence of chlorothalonil residues in the verdure throughout the winter, chlorothalonil-treated plots sustained >50% damage from *Typhula* spp. (Table 1.1).

2006-07

Chlorothalonil residues in the verdure immediately following applications of Daconil Ultrex at the low and high rates on 24 Oct 2006 were 54 and 97, and 27 and 66 µg/g tissue at Vail and Breckenridge, respectively (Figure 1.2). Residues at Breckenridge were similar to those detected during the initial sampling date at the same site in 2005 (Figure 1.1). Unlike 2005, there was no rapid decrease in chlorothalonil

residues the first week after application at either site. Instead, slightly higher chlorothalonil concentrations were measured after the first week at Vail. There was no permanent snow cover at either location until mid-November 2006 and approximately the top 1.5 cm of the soil froze during this period. The soil subsequently thawed after permanent snow cover as determined by verdure temperatures recorded by thermistors during the winter (data not shown). Snow melt occurred during the first week in Mar 2007 after which light rain and snow continued to fall through May, but any snow that fell was not permanent on the plots. There was consistently higher chlorothalonil residues on the annual bluegrass plots at Vail compared to the Kentucky bluegrass plots at Breckenridge throughout the permanent snow cover period. Chlorothalonil concentrations from 1 Nov 2006 through 26 March 2007 decreased at a rate of -0.2 (R^2 = .86, P=0.002) and -0.3 (R^2 =.62, P=0.02) µg/g tissue/day at Vail and -0.1 (R^2 = .76, P=0.01) and -0.2 ($R^2 = .90$, P = 0.001) at the low and high application rates, respectively, (Figure 1. 2), and chlorothalonil concentrations at the end of this period were 11 and 24, and 25 and 59 µg/g tissue for the low and high chlorothalonil application rates at Breckenridge and Vail, respectively. Similar chlorothalonil residues at both sites were measured on 12 May 07. As in 2005-06, all plots at both locations treated with the two rates of chlorothalonil sustained injury from Typhula spp. (Table 1.1), although damage was much more severe on the annual bluegrass fairway at Vail (50-80%) compared to Breckenridge (24-40%).

In 2006, chlorothalonil was also applied to plots as part of the formulation of the commercial fungicide mixture Instrata. The chlorothalonil application rate (3.7 kg/ha) in the mixture was approximately half that of the low application rate of Daconil Ultrex (8

kg/ha) in the same trial. Nevertheless, chlorothalonil concentrations in the verdure at most sampling dates were similar (Figure. 1.3). Chlorothalonil concentrations in the Instrata-treated plots were consistently lower at Breckenridge than Vail. Chlorothalonil concentrations in the verdure of Instrata-treated plots were consistently lower at Breckenridge than Vail. There was no clear trend in chlorothalonil residue changes in the verdure during snow cover at either site (Figure 1.3). For example, chlorothalonil concentrations from the Instrata application were 31 and 44 µg/g tissue on 24 Oct 2006 compared to 28 and 61 µg/g tissue on 26 Mar 2007 at Breckenridge and Vail, respectively. Residues of fludioxonil, another component of the Instrata fungicide, were <4µg/g tissue immediately following applications at both sites. There was a slight trend toward decreasing concentrations through the winter, although fludioxonil could be detected at all sampling dates. Plots treated with Instrata had approximately 8% Typhula blight damage at Breckenridge compared to 48% at Vail (Table 1.1).

2007-08

On 24 Oct 2007, chlorothalonil was applied to plots at Vail as part of the commercial fungicide mixture Instrata. Chlorothalonil residues in the verdure immediately after application were 57 and 75 µg/g tissue for the low and high Instrata rates, respectively, and did not change appreciably after the first week (Figure 1.4). There was no permanent snow cover until 20 Nov 2007, and the verdure and top 1.5 cm of soil froze by 15 Nov. The soil and verdure thawed following permanent snow cover. Snow melt occurred during the first week in May 2008. Chlorothalonil residues

fluctuated during the winter with no clear trend until 1 Apr 2008 when concentrations at the two application rates dropped to approximately 25 μ g/g tissue. Initial fludioxonil residues in verdure resulting from low and high Instrata application rates were 0.5 and 0.8 μ g/g tissue, respectively, and residues below 1.1 μ g/g tissue were detected Until 1 Apr (Figure 1.4). No fludioxonil residue was detected in May at either application rate. Application of fludioxonil alone (Medallion) to plots at 1.0 kg/ha resulted in much higher initial residues (13 μ g/g tissue) than Instrata applications. Residues remained above 10 μ g/g tissue until 6 Mar and then decreased rapidly at the last two sampling dates. Plots treated with fludioxonil only (Medallion 50WP) sustained more (76%) Typhula blight damage than plots treated with a combination of fludioxonil, chlorothalonil and propiconazole (Instrata) at the low (36%) and high (22%) application rate (Table 1.1).

Efficacy of chlorothalonil and fludioxonil at high application rates

Because labeled rates of chlorothalonil and fludioxonil failed to provide adequate Typhula blight control in the residue experiments, applications of up to 10X the highest labeled rates of these fungicides were applied. Typhula blight was not effectively controlled at any rate of fludioxonil (Table 1.2). Only the highest rate of chlorothalonil provided some measure of control (14% damage), but this rate also caused phytotoxicity to the annual bluegrass (Figure 1.11).

In-vitro fungicide inhibition assays

Chlorothalonil was inhibitory to the growth of *T. ishikariensis* to some extent at all concentrations tested (Figure 1.5). Although growth in 21% of the isolates was inhibited by >90% at 1 µg/ml chlorothalonil, 53% (96/180) had a growth reduction <80%. Increasing the chlorothalonil concentrations to 10 and 100 µg/ml increased growth inhibition in some of the isolates, but not in others. For example, the proportion of isolates that were inhibited by >90% remained similar at 10 (22%), 100 (19%) and 500 (18%) µg/ml. Thus increasing the chlorothalonil concentration to 500 µg/ml did not result in complete inhibition of all isolates. *Typhula incarnata* isolates were even less sensitive to chlorothalonil. Twelve of 15 isolates were only slightly or not inhibited (<10%) at 1 µg/ml, and only 3 of 11 isolates had a growth inhibition >90% at a concentration of 500 µg/ml (Figure 1.6).

Growth in approximately half of the *T. ishikariensis* isolates was slightly or not inhibited (<10%) at 0.001 and 0.01 µg/ml fludioxonil, and none were completely inhibited at these concentrations (Figure 1.7). Increasing the fludioxonil concentration to 1 µg/ml resulted in growth reductions >80% in a larger proportion (85%) of isolates. Nevertheless, only 12% of the isolates were completely inhibited or nearly so (>90%) at 1 µg/ml. Growth reduction in *T. incarnata* followed a similar pattern with very little inhibition (<10%) in all isolates at 0.001 µg/ml, increasing inhibition at 0.01 and 0.1 µg/ml, but still not complete inhibition at 1 µg/ml (Figure 1.8). Growth of all *T. ishikariensis* and *T. incarnata* isolates were completely inhibited at 10 µg/ml fludioxonil (data not shown). Propiconazole at 1µg/ml reduced growth by varying amounts in *T*.

ishikariensis isolates, but only 3 isolates were inhibited >90% (Figure 1.9). Although growth was further reduced in many isolates at 10 μ g/ml, only 3 additional isolates were inhibited by >90%. Growth inhibition in *T. incarnata* followed a similar pattern with increasing but not complete inhibition at 10 μ g/ml propiconazole (Figure 1.10). Growth of all *T. ishikariensis* and *T. incarnata* isolates were completely inhibited at 100 μ g/ml propiconazole (data not shown).

Discussion

The collection of *Typhula ishikariensis* and *T. incarnata* from many sites in Colorado is in agreement with previous studies (Chang et al., 2006, Smith, 1989) that showed these species are widely distributed in North America. There was a general trend for *T. ishikariensis* to be more widely distributed at high elevations in Colorado (Vail, Breckenridge), although this may have been an artifact of the sampling procedure. Also, both species were recovered from golf courses at lower elevations (Ft. Collins and Loveland).

Only *T. ishikariensis* was recovered from the fungicide plots at Vail and Breckenridge in a more intensive sampling in 2007, even though *T. incarnata* was consistently collected from blighted turfgrass in the fairway rough less than 100 m away. The dominance of *T. ishikariensis* on bentgrass greens and fairways has also been observed in Wisconsin and Utah (Chang et al., 2006). The reason for this isn't known, but it is unlikely the apparent exclusion of *T. incarnata* from the fungicide plots was a result of environmental differences such as snow depth or length of snow cover. It is also unlikely that a difference in fungicide sensitivity between the species is the cause,

even though the roughs are rarely treated with fungicides and fairways are treated annually. *Typhula incarnata* was similar in sensitivity to propiconazole, and less sensitive to fludioxonil and chlorothalonil than *T. ishikariensis*. *T. incarnata* is considered a weak pathogen, but is better able to survive in a saprophytic phase than *T. ishikariensis* (Matsumoto, 1994). It also adapts to less favorable locations for snow mold development (Smith, 1992) with a major saprotrophic phase. In contrast, the more aggressive *T. ishikariensis* may be better able to colonize highly maintained turfgrass on the fairway and thus displace *T. incarnata* from these sites.

During the general survey, a *Typhula*-like fungus was recovered from blighted Kentucky bluegrass patches in Colorado Springs, Fort Collins and Loveland. The fungus produced sclerotia with somewhat similar characteristics to *T. incarnata*. The rDNA ITS sequences of the isolates from the different locations were the same, but were distinctly different from published sequences of *T. incarnata* and *T. ishikariensis*. However, the sequences matched (>98% similarity) those of an unidentified snow mold fungus collected in Oklahoma (Conway and Williams, 1986) and Wisconsin (Millett, 1999). The fungus was slightly pathogenic to creeping bentgrass in a Wisconsin study (Millett, 1999), whereas no evidence of pathogenicity was detected in laboratory studies conducted in Oklahoma (Conway and Williams, 1986) and Colorado (Jorge Ibarra and Ned Tisserat, Colorado State University, personal communication). The fungus probably has a mostly saprophytic phase colonizing dead turfgrass damaged by Typhula blight

This general survey also correlated with the survey that Chang et al. (Chang et al., 2006) conducted in Wisconsin, Minnesota and Utah to identify *Typhula* species from

golf courses in these states. They found that when there were an increased number of snow cover days, the frequency of *Typhula ishikariensis* increased. This is very similar to what was found in the survey of golf courses in Colorado. Golf courses in the mountains of Colorado generally have upwards of 120 days of snow cover each winter, and the primary species that was recovered from these areas was *Typhula ishikariensis* (Biological species I). The frequency of *Typhula ishikariensis* may also be due to its' greater inoculum potential (Chang et al., 2006), as well as environmental conditions.

Fungicides have not always provided the desired level of control of Typhula blight even though they may be applied at very high rates (Appendix 2), (Hsiang et al., 1999). One possible explanation is that fungicides may dissipate in the verdure prior to snow cover or during the long period of snow cover. Wu et al. (2002) found that chlorothalonil persisted in the turf for only 12 days, with a dissipation half-life of 4.9 days, following application to a bentgrass putting green in summer. Mondadjemi et al. (2011) reported a similar simulated half-life of 5.3 days for chlorothalonil and concluded photodegradation played an important role. Thus, applying a fungicide too far in advance of snow cover might result in reduced fungicide residues in the verdure. However, I did not observe significant (>50%) decreases in chlorothalonil residues, except at Vail in 2005, following application; even though, in some cases, fungicides were applied up to three weeks before snow cover. Furthermore, chlorothalonil and fludioxonil persisted throughout the winter under snow cover with minimal loss in concentration.

The lack or slow rate of chlorothalonil and fludioxonil dissipation during late fall and early winter may be a result of several factors. Fairways were not mowed, and as a

result, clippings containing fungicide residue were not dislodged to the thatch or soil following fungicide application. Both bio- and photodegradation can be a factor in fungicide dissipation (Monadjemi et al., 2011, Sigler et al., 2003, Wang et al., 2011), but cold temperatures inhibiting microbial growth, shorter day lengths, and a diminished light intensity under snow likely reduced losses by these factors. Fungicides can also be washed from the foliage. Carroll et al. (Carroll, 1993 and 2001) found that chlorothalonil washoff was relatively slow and constant once the fungicide dried; however, significant loss could occur during the first hour if a simulated rainfall occurred. Any precipitation at high elevations in Colorado following fungicide application would likely occur as snow. Melting snow should not have the same washoff potential as rainfall. It was observed that the ground beneath the snow remained frozen during winter and a thin film of water was present on leaves. It was also noted on the samples that were collected that the turfgrass continued to grow, as evidenced by the new shoots of annual bluegrass or Kentucky bluegrass. This apparently was not sufficient to dislodge or reduce a significant amount of fungicide during the winter. A practical implication of these results is that while it is still desirable to apply fungicides as close to snow cover as possible, applications within 1-2 weeks before permanent snow should not cause significant fungicide dissipation.

Chlorothalonil and fludioxonil concentrations in the verdure immediately following application in each year were approximately 8-12 times lower than predicted (880 and 90 μ g/g tissue for the highest chlorothalonil and fludioxonil rates, respectively) based on calculations using the average weight of the verdure collected from the turf cores. Wu et al. (Wu et al., 2002) also observed an eight fold difference between observed and

predicted concentrations of chlorothalonil on a bentgrass green. In their study, approximately 200 µg chlorothalonil/g tissue were recovered immediately after application of the fungicide at a rate of 24.5 kg/ha compared to the 68-115 µg/g tissue I recovered following application of 13.9 kg/ha (44% less) of the fungicide. In contrast, Carroll et al. (2001) reported recovery of about 5,000 µg/g chlorothalonil in foliage following application of the fungicide at 9.2 kg/ha.

The reason for the apparent discrepancies between theoretical and observed fungicide residues isn't known. Chlorothalonil is a lipophilic compound and presumably penetrates waxy leaf surfaces making extraction more difficult. Nevertheless, extraction efficiency from bluegrass leaves spiked with known concentrations of chlorothalonil was always greater than 81% in my studies. The turfgrass is not a flat surface, and it is possible some fungicide did not adhere to leaves during application. For example, Carroll et al. (Carroll, 2001) estimated that only 51-55% of chlorothalonil was intercepted in the turfgrass canopy. However Wu et al. (Wu et al., 2002) found only a very small concentration of chlorothalonil was present in thatch one day after application, and no detectable amounts were found in soil. In the preliminary GC optimization runs, there was no detection of chlorothalonil in thatch samples, although the sampling was not extensive.

All of the fungicides tested were inhibitory to *T. ishikariensis* and *T. incarnata in vitro*. However, while concentrations of chlorothalonil as low as 1 μ g/ml inhibited most of the isolates to some degree, a majority of isolates exhibited at least some growth at concentrations as high as 500 μ g/ml. Similarly, most *T ishikariensis* and *T. incarnata* isolates exhibited at least some *in vitro* growth at fludioxonil and propiconazole

concentrations detected in the field. There was no evidence of a 'qualitative' resistance whereby an isolate exhibited complete insensitivity to the fungicide (Deising et al., 2008) . Instead, isolates exhibited more 'quantitative' effects in which they may tolerate increasing concentrations of fungicide until some critical threshold is reached. Efflux transporters in fungal membranes are responsible for removing toxic chemicals (e.g. phytoalexins) from their cells (Deising et al., 2008) and it is possible that this mechanism is reducing fungicide levels in the fungal cells to sub-lethal levels.

I conducted the *in vitro* assays at 10°C and it is possible that results would have been different had they been done at 1°C. *Typhula* species are psychrophilic and are more metabolically active at low temperatures than most fungi. The possible combination of slower absorption of the fungicide and an active detoxification or efflux system might have allowed the fungus to tolerate relatively high fungicide concentrations.

The fungicide concentrations used in the *in vitro* studies were similar to those observed in the turfgrass verdure in field plots. Given that snow cover may persist for 4 months or longer, these data suggest that even limited fungal growth could result in significant turf colonization. For example, *T. ishikariensis* isolates had an approximate diameter growth of 3 mm/day on non-amended agar at 10°C. Even with a 50% growth reduction resulting from fungicide inhibition, a *Typhula* colony would attain a diameter of 18 cm after 4 months. Obviously many other factors, including near freezing temperatures under the snow could influence the growth rate, but it is plausible that a growth-inhibited fungus could cause damage to the turfgrass provided the fungicide did not affect pathogenicity. A high sclerotial population in the fungicide-treated turfgrass,

and subsequently the development of many small infection centers, could still result in major damage unless fungicide concentrations in the verdure exceeded that needed for complete inhibition. This may explain why increasing fludioxonail and chlorothalonil application rates in the field did not result in complete control of Typhula blight.

But even at the higher concentrations of fludioxonil and chlorothalonil, Typhula blight was not totally inhibited. And in the *in-vitro* studies, with higher concentrations of fludioxonil, chlorothalonil and propiconazole, it was noted that what appeared to be sporocarps were growing on the original mycelial plug, although this was never confirmed. It appeared that the fungus was trying to grow away from the fungicide-amended agar. So it may be that the fungus is able to 'grow out' of the fungicide layer in the turfgrass in order to cause disease. These structures were not observed in the field or on any of the collected samples, but they may not have survived for very long in the verdure. Since both fludioxonil and chlorothalonil are contact fungicides, new turf growth under snow cover may also reduce fungicide efficacy. Even though recovery of sufficient amounts of fungicide residue from the verdure should theoretically inhibit fungal growth, this was not inhibitory to the fungus. This may be why, in the fungicide trials (Appendix 2), two or more fungicides combined in one application seem to work better than individual compounds.

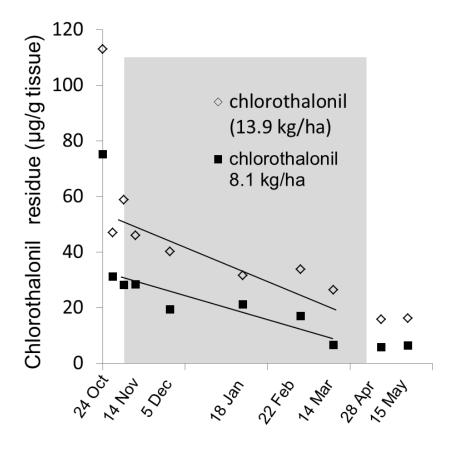


Figure 1.1. Relationship between chlorothalonil residues in the verdure and sampling dates during the winter of 2005-06 following application of two rates of chlorothalonil to a Kentucky bluegrass fairway at Breckenridge, CO on 24 Oct 2006. The rates of chlorothalonil loss from the verdure from 14 Nov 2005 to 14 Mar 2006 were not different (*P*>0.10) between the high (-1.0 µg/g tissue/day, $R^2 = 0.88$, *P*<0.001) and low (-0.7 µg/g tissue/day, $R^2 = 0.90$, *P*<0.001) application rates of chlorothalonil. Shaded area represents period of snow cover during the winter.

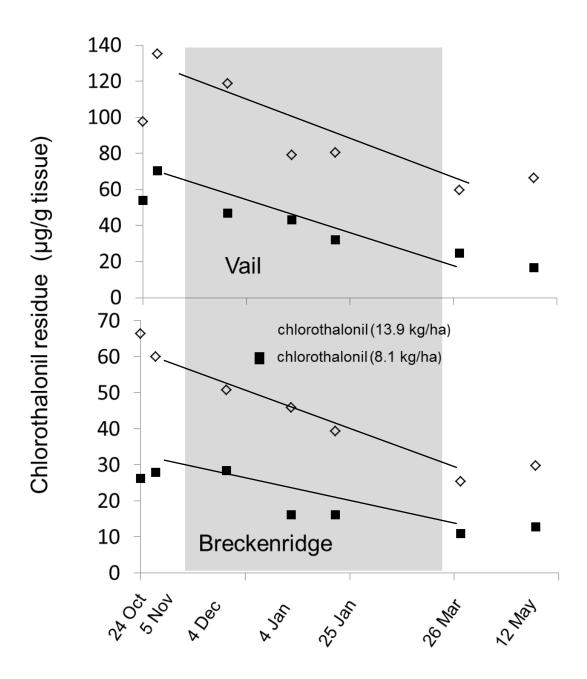


Figure 1.2. Relationship between chlorothalonil residues in the verdure and sampling dates during the winter of 2006-07 following application of two rates of chlorothalonil on 24 Oct 2006 to an annual bluegrass fairway at Vail, and a Kentucky bluegrass fairway at Breckenridge, CO. Rates of chlorothalonil loss from the verdure at Vail from 5 Nov 2006 to 26 Mar 2007 were not different (P > 0.10) between the high (-0.3 µg/g tissue/day, $R^2 = 0.62$, P = 0.02) and low (-0.24 µg/g tissue/day, $R^2 = 0.86$, P = 0.002) rates. Similarly chlorothalonil loss rates were not different (P > 0.10) between high (-0.2 µg/g tissue/day, $R^2 = 0.90$, P = 0.001) and low rates (-0.09 µg/g tissue/day, $R^2 = 0.76$, P = .001) at Breckenridge. Shaded area represents period of snow cover during the winter.

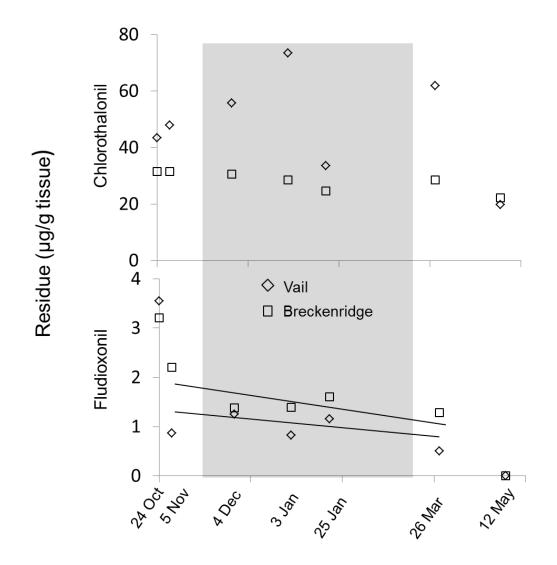


Figure 1.3. Relationship between chlorothalonil and fludioxonil residues in the verdure and sampling dates during the winter of 2006-2007 following application of the commercial fungicide mixture Instrata containing chlorothalonil (3.7 kg/ha), fludioxonil (0.006 kg/ha) and propiconazole (0.093 kg/ha), to an annual bluegrass fairway at Vail and a Kentucky bluegrass fairway at Breckenridge, Colorado. The rate of fludioxonil loss from the verdure 5 Nov 2006 to 26 Mar 2007 were not different (P > 0.10) between Vail (-0.004 µg/g tissue/day, $R^2 = 0.44$, P = 0.09) and Breckenridge (-0.006 µg/g tissue/day, $R^2 = 0.65$, P = 0.03). Shaded area represents period of snow cover.

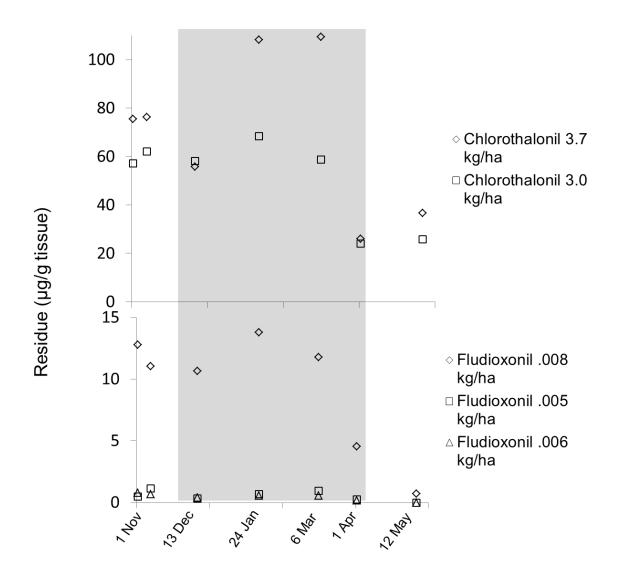


Figure 1.4. Relationship between chlorothalonil and fludioxonil residues in the verdure and sampling dates during the winter 2007-08 following application of two rates of the commercial fungicide mixture Instrata containing chlorothalonil (3.0 and 3.7 kg/ha), fludioxonil (0.005 and 0.006 kg/ha) and propiconazole (0.076 and 0.093 kg/ha), and fludioxonil (0.008 kg/ha) applied alone (as the commercial fungicide Medallion 50WP) to an annual bluegrass fairway at Vail, Colorado. Shaded area represents period of permanent snow cover.

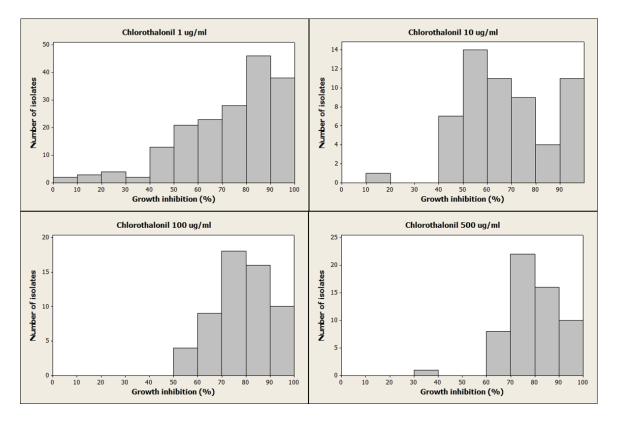


Figure 1.5. Inhibition in diameter growth of *Typhula ishikariensis* isolates on potato dextrose agar amended with 1,10, 100, and 500 μ g/ml chlorothalonil relative to growth in non-amended agar. A subset of isolates was screened at the higher concentrations.

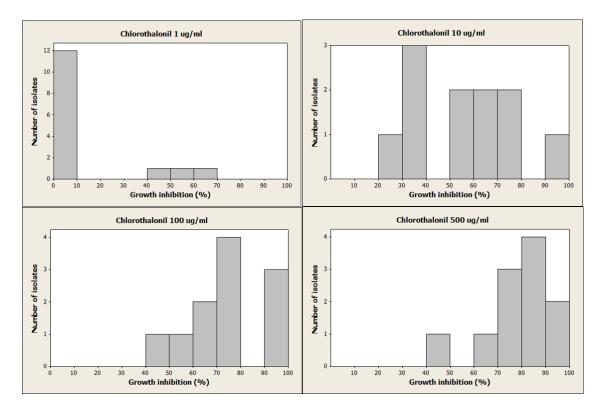


Figure 1.6. Inhibition in diameter growth of *Typhula incarnata* isolates on potato dextrose agar amended with 1, 10, 100, and 500 μ g/ml chlorothalonil relative to growth in non-amended agar.

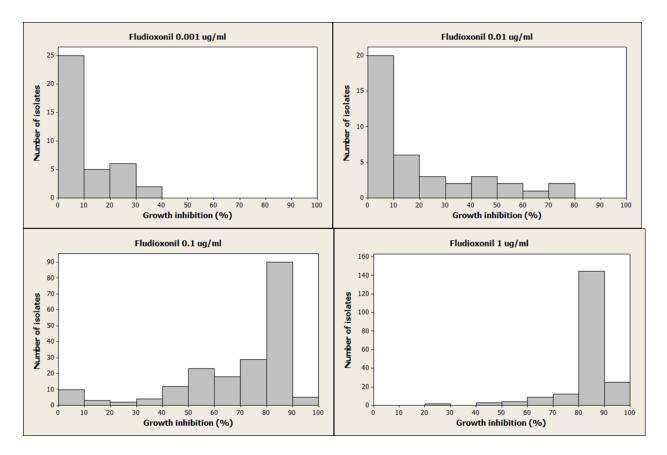


Figure 1.7. Growth inhibition of *Typhula ishikariensis* on half-strength potato dextrose agar amended with 0.001, 0.01, 0.1 and 1 μ g/ml fludioxonil relative to growth of isolates in non-fungicide amended agar.

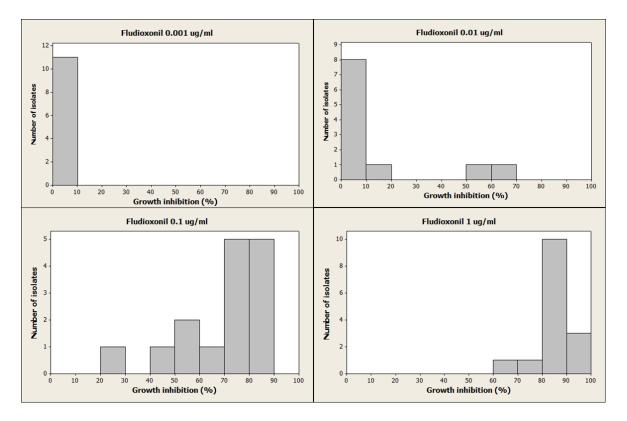


Figure 1.8. Growth inhibition of *Typhula incarnata* isolates on half-strength potato dextrose agar amended with 0.001, 0.01, 0.1 and 1 μ g/ml fludioxonil relative to growth of isolates in non-fungicide amended agar.

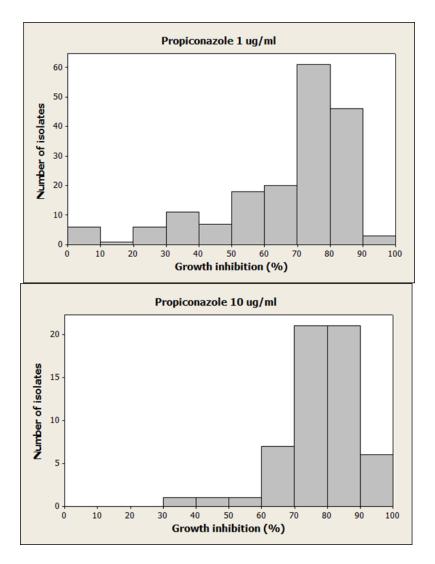


Figure 1.9. Growth inhibition of *Typhula ishikariensis* isolates on half strength potato dextrose agar amended with 1 and 10 μ g/ml propiconazole relative to growth in non-amended agar. A subset (57) of all isolates (179) was screened at the higher concentration.

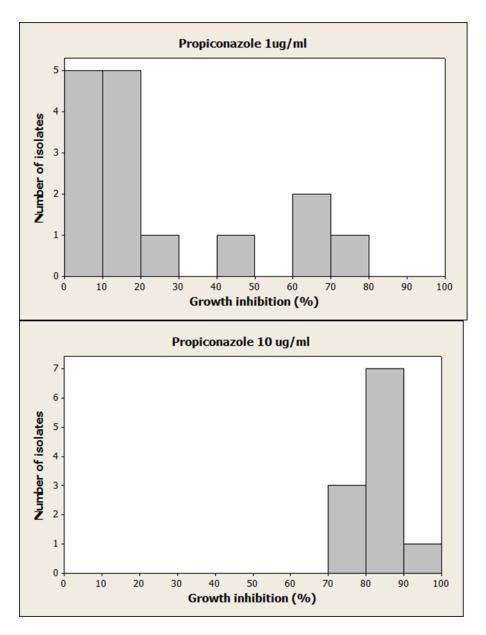


Figure 1.10. Growth inhibition of *Typhula incarnata* isolates on half strength potato dextrose agar amended with 1 and 10 μ g/ml propiconazole relative to growth in non-amended agar.



Figure 1.11. Concentration study (2008-09) using chlorothalonil applied at 10X the recommended rate showing that development of snow mold was inhibited; however, phytotoxicity damage to turfgrass was exhibited.

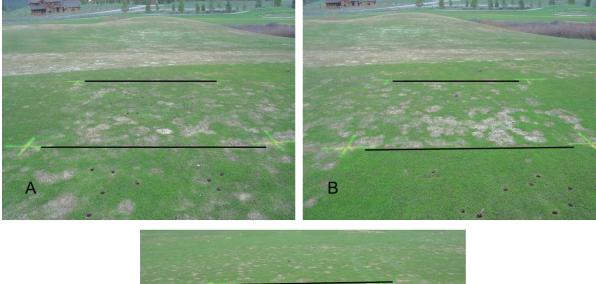




Figure 1.12. Typhula blight damage to A) chlorothalonil treated (8.1 kg/ha), B) chlorothalonil treated (13.9 kg/ha), and C) an untreated plot on a Kentucky bluegrass fairway at Breckenridge, CO in May 2006. Area between the lines is an individual treatment plot.

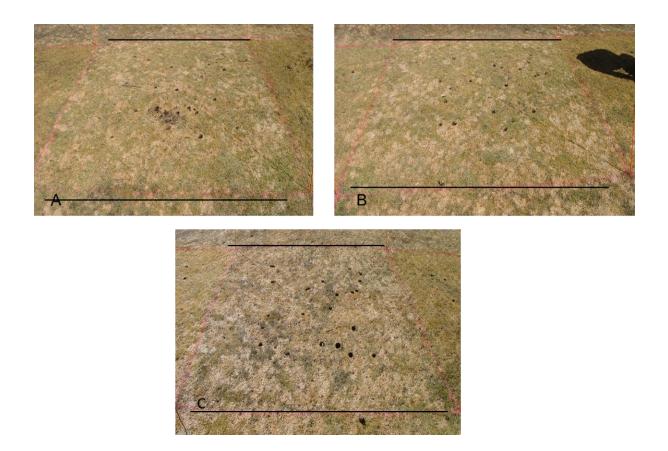


Figure 1.13. Typhula blight damage to A) chlorothalonil-treated plot (8.1 kg/ha), B) chlorothalonil treated plot (13.9 kg/ha), and C) an untreated plot on an annual bluegrass fairway at Vail, CO in March 2007. Area between the black lines is an individual treatment plot.

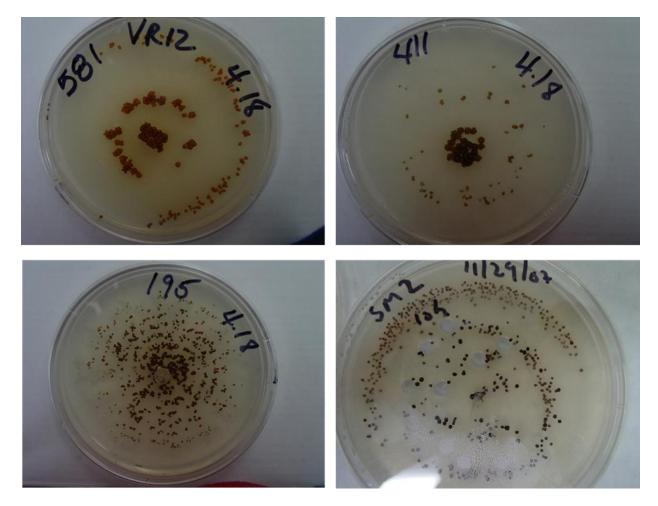


Figure 1.14 Representative isolates of *Typhula* spp. Plate labeled 581 is *Typhula incarnata*; Plate labeled 411 is *Typhula ishikariensis* var. *canadensis*/BSII; Plate labeled 195 is *Typhula idahoensis*/BSI; and Plate labeled SM2 is *Typhula ishikariensis* var. *ishikariensis*/BSI. Note differences in color and size of the sclerotia.

Treatment and Rate	% of Plot Damaged [×]					
	Breckenridge 2005-06	Breckenridge 2006-07	Vail 2006-07	Vail 2007-08		
Quintozene						
15.12 kg/ha						
(Turfcide 400)	17.5b	24.0bc	50.0c			
Chlorothalonil						
8.06 kg/ha						
(Daconil Ultrex						
82.5WDG)	58.8a	20.0bc	73.75b			
Chlorothalonil						
13.86 kg/ha	50.0a	40.0b	82.0ab			
Chlorothalonil						
3.7 kg/ha,						
Propiconazole						
0.093 kg/ha,						
Fludioxonil						
0.006 kg/ha		7.5c	48.75c	22.5d		
(Instrata 3.61SE) Chlorothalonil		7.00	40.750	22.5U		
2.98 kg/ha, Propiconazole						
0.076 kg/ha,						
Fludioxonil						
0.005 kg/ha				36.3cd		
Fludioxonil				00.000		
0.765 kg/ha						
(Medallion 50WP)				75.8b		
Propiconazole				10.00		
1.075 kg/ha						
(BannerMAXX 1.3ME)				52.5c		
Untreated Control	42.5a	71.25a	87.0a	98.8a		

Table 1.1. Typhula blight damage to a Kentucky Bluegrass fairway at Breckenridge, and an annual bluegrass fairway at Vail, CO, following fungicide applications.

^xPercent damage is the mean of 4 replications. Means not followed by the same letter in the same column are different (P<0.05) according to a F-least significant difference test.

Table 1.2. Typhula blight damage to an annual bluegrass fairway at Vail, CO, in 2009 following applications of chlorothalonil and fludioxonil at labeled and 2X, 5X and 10X labeled rates.

		% of Plot Damaged (means of 4
	Treatment and Rate	treatments)
Labeled Rate		
Daconil Ultrex	Chlorothalonil	
82.5WDG	12.51 kg/ha	83.0ab
	Chlorothalonil	
2X	25.22 kg/ha	52.5c
	Chlorothalonil	
5X	63.08 kg/ha	58.8bc
	Chlorothalonil	
10X	126.04 kg/ha	14.3d
	Untreated Control	94.0a
Labeled Rate		
Medallion		
50WP	Fludioxonil 0.38 kg/ha	74.3a-c
2X	Fludioxonil 0.77 kg/ha	78.0a-c
5X	Fludioxonil 1.91 kg/ha	82.5ab
10X	Fludioxonil 3.86 kg/ha	62.5bc

^xValues are means of four replications. Means not followed by the same letter are different (P<0.05) according to an F-least significant difference test.

^yTurfgrass in plots was extensively discolored as a result of phytotoxicity from the high rate of chlorothalonil.

Table 1.3. Snow mold isolates identified by ITS sequencing. Biological species I is *Typhula ishikariensis* var. *ishikariensis* or *T. ishikariensis* var. *idahoensis*; Biological species II is *T. ishikariensis* var. *canadensis*.

Snow Mold Isolates Sequenced						
Vail	Breckenridge	Other Locations				
40	17	16				
14	7	9				
8	0	13				
0	0	4				
0	0	1				
1 0	0 0	0 1				
	Vail 40 14 8 0	Vail Breckenridge 40 17 14 7 8 0 0 0				

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CHAPTER 2

EFFECT OF SNOW REMOVAL ON GRAY SNOW MOLD DEVELOPMENT AT HIGH ALTITUDE GOLF COURSES.

SUMMARY

Golf course superintendents at high altitudes in Colorado apply fungicides in late October before permanent snow cover to prevent gray (Typhula incarnata) or speckled snow mold (Typhula ishikariensis) development. Snow is also removed from putting greens in March and kept snow-free through spring to help suppress snow mold. However, the benefits of spring snow removal in snow mold suppression compared to potential turfgrass damage caused by exposure to low temperatures following removal have not been documented. We compared snow mold severity and turfgrass health on a Kentucky bluegrass fairway at Breckenridge and annual bluegrass plots at Vail, CO with permanent snow cover through winter to plots where snow was removed from late October through mid-November and to plots where snow was removed in mid-March and maintained snow-free through the spring. Both gray and speckled snow molds were observed at both locations and in each year, although the majority of the damage was caused by speckled snow mold. Snow removal in the fall resulted in high fluctuation of temperatures at the turfgrass surface until permanent snow cover stabilized them for the remainder of the winter. Snow removal in the spring also resulted in extreme temperature fluctuations, but damage to the turfgrass by freezing

temperatures was not apparent. A similar trend in temperature fluctuations was observed for each treatment in each year and at both locations.

Introduction:

Gray snow mold caused by *Typhula incarnata* and speckled snow mold caused by T. ishikariensis, collectively called Typhula blight, are major diseases of turfgrasses at high elevations in Colorado. These fungi oversummer in the thatch as hardened sclerotia. Scleortia germinate in fall and the mycelium colonizes the above-ground portions of plants. Sclerotia of *T. incarnata* may also germinate to form sporocarps that produce basidiospores. However, sporocarp formation is apparently restricted to regions with rainy or foggy fall weather (Smith, 1989), and has not been observed in Colorado. Although turf can be damaged by *T. incarnata* from late autumn to early spring in wet, cold climates with little to no snow, both T. incarnata and T. ishikariensis are more often active under deep, prolonged snow cover (Smith, 1989). In spring after snowmelt, patches of matted turfgrass with gray mycelium are apparent. In severe cases, coalescing patches may cover the entire sward. Numerous sclerotia are formed on diseased leaf surfaces or are embedded in leaf tissue. Damage from Typhula blight is variable; in some years only the leaves are killed, whereas in others large areas of the turfgrass may be killed.

Typhula blight is primarily controlled with preventive fungicide applications in fall just prior to snow cover. Efficacy can be influenced by the active ingredient used, application timing, and disease severity. Numerous fungicides and fungicide

combinations have been tested for Typhula blight control (Koch, 2010a, b, and c, Koch, 2011, Popko, 2011a, b, and c) (Appendix 2). In general, fungicide combinations having different modes of action are recommended with the assumption that these mixtures provide a broader spectrum of control against a variety of snow mold pathogens (Chang et al., 2006, Jo et al., 2006, Jung, 2007, Vargas, 1994). Fungicides are often applied as a single application as close to snow cover as possible to prevent chemical dissipation or photodegradation. For example, the dissipation half-life of chlorothalonil on a bentgrass putting green in the summer was 5 days (Wu et al., 2002). In another study, the field-extrapolated half-life of chlorothalonil photolysis on vegetation was also estimated to be 5 days (Monadjemi et al., 2011). In contrast, chlorothalonil persisted for up to five months under snow without appreciable dissipation (Chapter 1). It has been recommended that systemic fungicides should be applied before leaf growth completely stops in the fall to allow for uptake and movement, although details on timing weren't provided (Smiley, 2005). This premise has been adopted and modified by some Colorado superintendents into the practice of making two fall applications for Typhula blight control; one fungicide application containing a systemic triazole or strobiluron fungicide in late September or early October, followed by a second application in late October or early November just prior to snow cover.

Late winter or early spring snow removal is often cited as a control strategy for Typhula blight (Hsiang et al., 1999, Smiley, 2005). This is based on observations that Typhula blight is more severe in areas with a longer duration of snow cover (Chang et al., 2006, Hsiang et al., 1999, Smith, 1989). Late winter snow removal from putting

greens has been widely implemented by Colorado superintendents as a way to reduce Typhula blight, yet there is no experimental data to support this practice.

Superintendents also remove snow in late winter to prevent ice or freeze damage to the turfgrass. Winter injury, resulting from low temperatures or ice cover is important in cold climate areas (Beard and Olien, 1963), including Colorado. The injury is often exacerbated if the predominant grass species is annual bluegrass (*Poa annua*) (Tompkins et al., 2004), which is the most common species of turfgrass on high elevation golf courses in Colorado. Contrary to the practice of early snow removal, Tompkins et al. (Tompkins et al., 2000) concluded that it was important to maintain a snow cover on putting greens in order to maintain turfgrass dormancy for as long as possible.

The objectives of my study were to examine the efficacy of various fungicides against Typhula blight, and to determine whether two fall fungicide applications enhanced control relative to a single application just before snow cover. I also determined whether certain cultural practices, including fall and spring snow removal, and snow compaction influenced Typhula blight development.

Methods and Materials:

Effect of fungicide and snow removal on snow mold development 2005-06.

Plots were established on an annual bluegrass golf course fairway at Vail and a Kentucky bluegrass fairway at Breckenridge, CO on 24 Oct 2005 to determine the effects of fungicide applications and snow removal timing on Typhula blight damage. Plots were arranged in a split-plot randomized complete-block design with 4 replications. Whole plots measured 1.5 X 4.3 m and were left untreated or treated with guintozene (Turfcide 400F, American Vanguard Corporation, Newport Beach, CA) at 15.12 kg a.i./ha. Applications were made using a CO₂ sprayer pressurized to 138 kPa and equipped with a spray boom with four 8004 flat fan nozzles to deliver water at 815 L per ha. Subplots measured 1.4 X 2.1 m and consisted of; no snow removal during the experiment; snow removal at approximately weekly intervals from the application date until the end of November (Fall) and thereafter allowing snow to accumulate on plots until spring melt, or; snow removal at approximately weekly intervals from 1 Mar 2006 until the final rating date on 27 Apr 2006 (Spring). Snow was removed with shovels (Figure 2.3). Snow covered the plots on 21 Nov 2005 and remained until 20 Apr 2006. Temperatures at the turfgrass surface in each snow-removal treatment were recorded at two hour intervals for the duration of the experiment using thermistors (Spectrum Technologies, Plainfield, IL).

Typhula blight severity was determined at the last sampling date on 7 May 2006 by visually estimating the percent damage in each plot. Data were subjected to analysis of variance and means separated using Fisher's protected least significant difference (LSD) (P=0.05). Typhula blight severity in the non-fungicide, non-snow removal subplots was also collected at approximately monthly intervals through the winter. On the first and last sampling dates with no snow cover, sub-plots were rated visually. However, Typhula blight severity in plots on other dates with snow cover were estimated by arbitrarily removing two, 6.35-cm-diameter X 5-cm-deep turf cores from

each plot using a 1.2-meter-long soil core remover (Figure 2.4) and determining percentage of dead grass in each core. The percent damage in the two cores from each subplot were averaged; those percentages were then used to determine the mean snow mold damage among the four replicates in each treatment.

The experiment was repeated at Vail and Breckenridge in 2006-2007 using the same plot design as previously described. The commercial fungicide mixture Instrata 3.6SE (Syngenta Professional Products, Greensboro, NC) containing 3.7 kg chlorothalonil, 0.006 kg fludioxonil and 0.093 kg propiconazole a.i. per ha was applied at Vail, while quintozene (Turfcide 400F, American Vanguard Corporation, Newport Beach, CA) at 15.12 kg a.i./ha was applied at Breckenridge. Fungicide applications were made on 24 Oct 2006 at both locations and final snow mold damage ratings were made on 1 May 2007. Temperature measurements and turfgrass core collections also were the same as in 2005-06, except that cores were placed in a layer of moistened sand in 30-cm-diameter plastic pots and incubated in the greenhouse for two weeks after which they were rated for percentage turf damage (Figure 2.5).

A third experiment was conducted at Vail in 2007-08. Plot sizes were the same as in 2005-06. On 24 Oct 2007 the commercial fungicide mixture Instrata 3.6SE (Syngenta Professional Products, Greensboro, NC) at the same application rates of 2006-2007 was applied to whole plots. Subplots consisted of no snow removal or a spring removal as previously described. A fall snow removal treatment was not included and turf cores were not collected during the winter. The final plot rating was on 12 May 2008.

Effect of snow compaction on temperatures at the turfgrass surface

Nordic track ski trails were created for cross-country skiing and snowshoeing at the Breckenridge and Vail golf courses by compacting snow covering the fairways and tee boxes using a track-driven vehicle (snow cat). Trails were groomed and compacted following each snow event during the winter. The level of snow compaction was not measured. Temperatures at the snow/turfgrass interface beneath the Nordic track and an adjacent non-compacted area on a golf course fairway at each course were measured at 2-hour intervals with thermistors (Spectrum Technologies, Plainfield, IL) throughout the winter in 2006-07 and again in 2007-08.

Fungicide efficacy trials

Fungicide efficacy trials have been conducted each year since 1984 to determine which chemical/biological treatments provide the best control of Typhula blight. Fungicides were applied to a Kentucky bluegrass fairway at Breckenridge or to an annual bluegrass fairway or putting green at Vail, CO. Treatments were arranged in a randomized complete block with three to four replications. Individual plots measured 1.5 x 1.5 m on the fairways and 0.9 x 0.9 m on the putting green. Fungicides were applied in late October to early November with a CO₂ powered sprayer as previously described: granular formulations were spread using a hand-held shaker. In some cases sand was added to the granular formulations to increase volume and allow for more uniform coverage of the plot during application. Plots were rated in April or May

after snow melt for percentage turfgrass damage by Typhula blight. Methods and results of these studies from 2005-2011 are presented in Appendix 2.

In addition to comparing the efficacy of different fungicide products, studies were conducted to determine whether two fall fungicide applications were superior to a single application just before snow cover in controlling Typhula blight. In these studies, a combination of fungicides were applied in late September or early October approximately one month before snow cover. A second application of these same fungicide combinations, or in some cases a slightly different fungicide combination, was applied in late October (Table 2.1). These treatments were compared by paired T-tests to plots treated with the same fungicide combinations applied in late October only (Table 2.1).

Results

Effect of fungicide and snow removal on snow mold development

Both gray and speckled snow molds were observed at both locations and in each year, although the majority of the damage was caused by speckled snow mold. Pink snow mold was not common (<1% of the patches) in any year.

Typhula blight severity was consistently higher on an annual bluegrass fairway at Vail compared to a Kentucky bluegrass fairway at Breckenridge (Figure 2.6). Applications of quintozene at Vail and Breckenridge in 2005-2006 and 2006-2007, and the formulated product Instrata at Vail in 2007-08 (Figure 2.10) reduced (P<0.05), but

did not completely suppress Typhula blight (Figure 2.6 and 2.7). There were no interactions in Typhula blight damage between fungicide and snow removal treatments, except at Vail (P<0.5) in 2005-06 where severe damage (86%) was observed in the fungicide-treated plots that had snow removed in the fall. Most of the damage was a result of freeze injury and not Typhula blight as determined by the lack of mycelium and sclerotia in the dead grass. Fall snow removal did not increase winter damage or Typhula blight in Kentucky bluegrass at Breckenridge in either year (Figure 2.6 and 2.7). Spring snow removal also did not reduce (P>0.10) snow mold severity in fungicidetreated or non-treated subplots compared to subplots with no snow removal.

Snow removal treatments influenced temperatures at the turfgrass surface during the winter; a similar trend was observed for each treatment in each year and at both locations (Figure 2.11, 2.12 and 2.13). In the no-snow removal plots, surface temperatures fluctuated prior to snow cover, and then stabilized between -1°C and 0°C for the duration of winter until snow melted in spring. In the fall snow removal plots, temperatures fluctuated widely, with temperatures reaching lows of -18°C on some days in late November 2005 at Vail (Figure 2.11). Similar trends were seen in other years (Figure 2.12 and 2.13). After snow cover, temperatures slowly increased and then stabilized by mid-December to near freezing for the remainder of the winter. The freezing and thawing cycles in late November resulted in increased damage to the annual bluegrass at Vail, but not Kentucky bluegrass at Breckenridge. Temperatures in the spring-snow removal plots mimicked those in the no-snow removal plots from November until March. Then temperatures at the turfgrass surface fluctuated widely, and multiple freezing and thawing cycles occurred after snow removal. Yet no

increased turf damage was observed as a result of fluctuating temperatures in the spring-snow removal plots (Figure 2.6, 2.7 and 2.10). Maximum temperatures recorded at the surface during periods of no snow cover were likely higher than what actually occurred because the thermistors may have been heated by direct exposure to sunlight.

Temporal development of Typhula blight was monitored in the no-snow removal plots in 2006-07 and 2007-08 at Vail and Breckenridge (Figure 2.8 and 2.9). No mycelium or sclerotia of *Typhula* spp. were observed on cores removed during November in either year. On 23 Dec 2006, a very sparse mycelium of *Typhula* (determined by microscopic confirmation of clamp connections) was observed on cores collected at Vail and Breckenridge, however, turf necrosis was not observed. Similarly, no turf damage was observed on 6 Dec 2007 at either location. However, Typhula blight severity had increased dramatically at both locations and in both years by the January sampling period (Figure 2.8 and 2.9). In fact, the disease severity recorded in January, with the exception of Vail in 2007-08, was similar to the final severity ratings taken in April or May.

Effect of snow compaction on temperatures at the turfgrass surface

In 2005-06, Typhula blight was observed in a non-snow compacted area adjacent to, but not under, a snow-compacted Nordic ski track path that had been established on the Kentucky bluegrass fairways and tees at Breckenridge. This phenomenon was observed again in 2006-07 (Figure 2.14 and 2.15). Temperatures at the turfgrass surface in the non-compacted areas remained near 0°C, whereas

temperatures under the compacted areas were continuously below freezing and consistently 3-7°C colder during the majority of the winter (Figure 2.16 and 2.17). Although the turfgrass under the Nordic track exhibited few Typhula blight patches following snow melt, it was discolored and took several weeks longer than the non-compacted areas to resume growth in the spring (Figure 2.14 and 2.15).

Fungicide Efficacy Trials

Results of the fungicide efficacy trials from 2005-06 through 2010-11 are included in Appendix 2. Although a detailed analysis of each experiment is not provided here, some general observations can be made. Typhula blight damage (>90% each year) on an untreated annual bluegrass fairway at Vail was always more severe than a Kentucky bluegrass fairway at Breckenridge. Many of the treatments provided only fair to poor control at Vail, although some resulted in adequate (<10% damage) suppression of Typhula blight (Appendix 2). Several biological control products were tested, but none provided satisfactory control of Typhula blight.

One of the key comparisons in each year of the study was the efficacy of a late-September application of a fungicide combination followed by a second application in late October, compared to a single fungicide application prior to snow cover (Table 2.1). Twenty paired comparisons from two golf courses over a 7-year period were analyzed and in only one case did the two application regime provide better (P<0.05) control of Typhula blight than the single fungicide application.

Discussion

Kentucky bluegrass and annual bluegrass have been reported as intermediate in susceptibility to *T. incarnata* and *T. ishikariensis* (Wu and Hsiang, 1998). In my studies, annual bluegrass was consistently more severely damaged by Typhula blight than Kentucky bluegrass. Not only were the patches less numerous in Kentucky bluegrass, they were more superficial and the turfgrass appeared to recover quickly once the turfgrass resumed growth. Golf course superintendents in Colorado that manage Kentucky bluegrass fairways and that have limited fungicide budgets may be able to forego fall fungicide applications without suffering significant turfgrass damage from Typhula blight.

Golf course superintendents in the Colorado Mountains routinely remove snow from putting greens beginning in late February and keep the greens snow-free for the duration of the winter. Many do this in the belief that it helps suppress snow mold development. Snow molds are reported to be more severe during winters with extended periods of snow cover (Hsiang et al., 1999; McBeath, 2002; Nissinen, 1996) and snow removal is often recommended as a control (Hsiang et al., 1999; Smiley, 2005; Smith, 1989). Repeat fungicide applications after a mid-winter thaw or snow removal also have been suggested (Burpee et al., 1990; Fushtey, 1980; Smiley, 2005). However, in these experiments, Typhula blight severity at the time of snow removal in early March in the non-fungicide treated subplots was very similar to that observed in the no-snow removal plots rated in late April or May. For example, the non-fungicide treated annual bluegrass subplots had already been damaged >90% by Typhula blight

at the time of snow removal in March. Residue studies (Chapter 1) also indicated that fungicide concentrations in the verdure did not appreciably decline from November through March, thus obviating the need to re-treat the turfgrass after snow removal. Therefore, this research did not support the practice of late winter snow removal in the early spring to help suppress Typhula blight development on fungicide-treated turfgrass.

Another reason superintendent's remove snow from putting greens in late winter is to prevent ice formation resulting from melting snow under snowpack. The combination of crown hydration followed by ice formation is a major cause of winter damage to annual bluegrass in some regions (Dionne et al., 2001; Fry, 2004; Tompkins et al., 2000, 2004). There was no ice formation or freeze injury to annual bluegrass or Kentucky bluegrass in the no-snow removal subplots in any year of these studies. Turf quality was similar to that in the spring snow removal plots. Thus, my results did not support the need for early snow removal to prevent freeze injury. However, plots were established on a level fairway rather than a putting green and it is possible that the higher mowing height may have protected crowns from freeze injury. Furthermore, ice damage may be an issue in low areas of fairways or putting greens where water is more likely to puddle and freeze.

Late winter snow removal can expose turfgrass to extreme temperature fluctuations and multiple subfreezing events as evidenced by temperature data in the snow removal subplots. Annual bluegrass quality immediately following snow removal in early March was excellent in all subplots; surprisingly there was no evidence of ice formation or freeze injury to the turfgrass in any year. The turfgrass in the spring snow removal subplots continued to remain healthy throughout the spring even though it was

exposed to multiple freezing/thawing events. Cold-acclimated plants are more tolerant of freezing temperatures (Fry, 2004), and it is possible that the extended exposure of turf under snow cover led to a high level of cold-acclimation.

The hypothesis was that snow removal in late fall would freeze the upper soil profile and result in surface temperatures during the winter too cold for snow mold development. Although *Typhula* spp. are psychrophilic, their growth is inhibited when soil temperatures drop below 0°C (Cunfer and Bruehl, 1973; Dejardin and Ward, 1971; Hoshino et al., 1997; McBeath, 2002.). In these plots, snow removal in November resulted in temperatures fluctuating between a low of 15-18°C and a high of 25°C at the turfgrass surface until permanent snow cover provided temperature stabilization. However, temperatures eventually increased and remained near 0°C, and similar to the no-snow removal plots, throughout the winter. Therefore there was no advantage, in terms of reducing surface temperatures below freezing for long periods, with this treatment. However, fall snow removal did result in significant freeze damage to the annual but not Kentucky bluegrass in 2005-06. Golf course superintendents in Colorado often apply nitrogen fertilizers and continue to irrigate late into fall to encourage turfgrass recovery and to promote golf play for as long as possible. These practices may inhibit cold-acclimation and limit winter hardiness (Fry, 2004; Smith, 1989).

Typhula sclerotia germinate in the soil or thatch during cool, wet weather just prior to or immediately following snow cover to produce mycelium (Hsiang et al., 1999; Vargas, 1994). The mycelium then colonizes plant tissue directly or through stomata (Oshiman et al., 1995), although the rate at which this occurs is variable depending on

environmental conditions. Although mycelial growth apparently begins soon after snow cover in late fall, long snow durations are required for plant colonization and significant plant damage (Hsiang et al., 1999; Smith, 1989; Vargas, 1994). The rate at which these fungi colonize tissue has not been thoroughly studied. McBeath (2002.) reported that maximum snow mold activity in Alaska occurred in late spring as the snow began to melt and when soil temperatures were near 0°C and the soil/snow interface was saturated. These same conditions were observed soon after snow cover in late fall and in the absence of snow melt. Yet there was no evidence of mycelial growth or plant colonization in November in cores removed under snow cover, and only sparse fungal growth and no plant damage in December. By early to mid-January, extensive mycelial growth on plants was observed, suggesting that plant colonization occurred over a relatively short time period. Whether the turfgrass had been colonized by *Typhula* at a level not visually observable is not known, but these results suggest plant colonization is not necessarily a gradual process.

Preventing snow compaction has been offered as a management tool for suppressing Typhula blight (Smiley, 2005). Snow compaction may prevent rapid snow melting in spring and could result in longer periods of snow cover. However, it was noted that snow compaction lowered the surface temperature to a level that is inhibitory to *Typhula* growth (Hsiang et al., 1999; McBeath, 2002;, Matsumoto, 2009; Smith, 1989,). These data were corroborated by the lack of Typhula blight development under the Nordic track. Although snow compaction could be an effective strategy for reducing Typhula blight, its application may have other detrimental effects. For example, annual and Kentucky bluegrass in compacted areas exhibited more discoloration and a slower

spring greenup relative to non-compacted areas. Compaction also resulted in irrigation line damage at Breckenridge in 2006 as a result of freezing pipes. Thus compaction may be of limited use on golf courses for control of snow molds caused by *Typhula*.

Another control strategy for Typhula blight that is of questionable value in Colorado, but isstill used, is the practice of making a fungicide application in late September or early October followed by a second application just before snow cover. The origin of this concept is unknown, although it may be based on the belief that sclerotia of *Typhula* germinate during wet, cool periods in the fall to form basidiospores that are subsequently dispersed by air currents. Fungicide applications presumably prevent this early inoculum buildup prior to snow cover. However, sporocarps of *T. ishikariensis* are rare in nature (Cunfer and Bruehl, 1973) and have never been observed in Colorado. In addition, it is assumed that uptake and movement of systemic fungicides is better when the turfgrass is actively growing. In my studies, early fall fungicide applications, with one exception, did not enhance Typhula blight control. The significant expense of this early fungicide application cannot be justified based on these results.



Figure 2.1. Golf Course superintendents at high elevation courses in Colorado routinely remove snow from putting greens in late February or early March.



Figure 2.2. Annual bluegrass putting green after snow removal in late February 2006. Note that there is no discoloration or evidence of freeze injury to the turfgrass.



Figure 2.3. Snow removal from plot in March 2008.

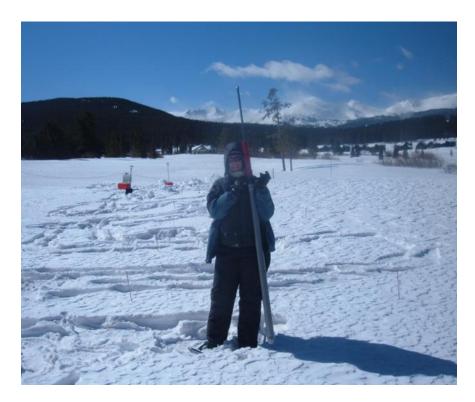


Figure 2.4. Removal of soil cores with a 1.2-meter-long soil core remover during 2005-06.



Figure 2.5. Example of gray and speckled snow mold damage to turfgrass cores removed from snow-covered plots and incubated in greenhouse for 2 weeks (2006-07).

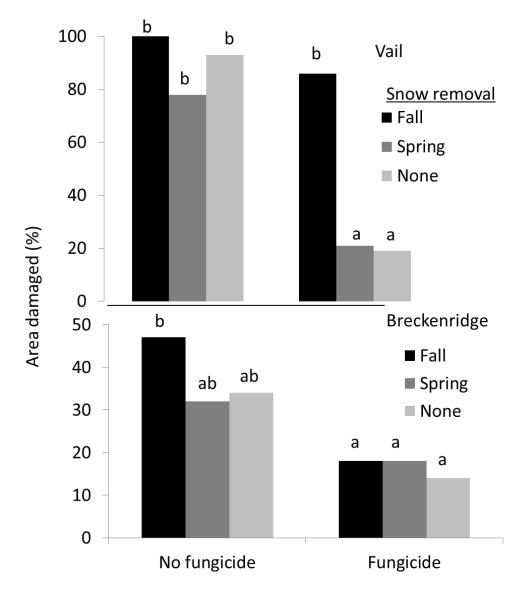
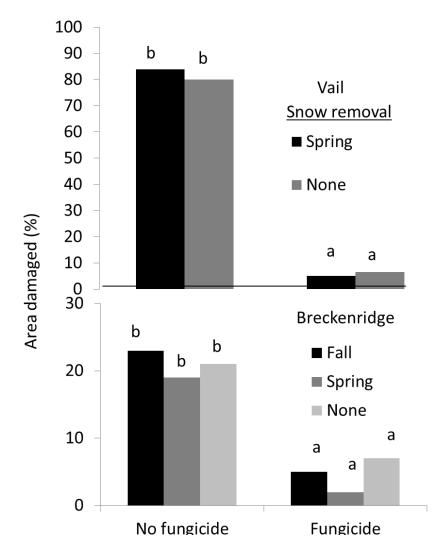
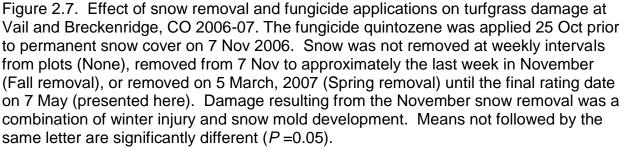


Figure 2.6. Effect of snow removal and fungicide applications on turfgrass damage at Vail and Breckenridge, CO 2005-06. The fungicide quintozene was applied 25 Oct prior to permanent snow cover on 21 Nov 2005. Snow was not removed (none), removed at weekly intervals during November (Fall), or removed at weekly intervals from 5 Mar, 2007 (Spring) until the final rating date on 7 May. Damage resulting from the November snow removal was a combination of winter injury and snow mold development. Means not followed by the same letter are significantly different (P =0.05) by Fisher's LSD test.





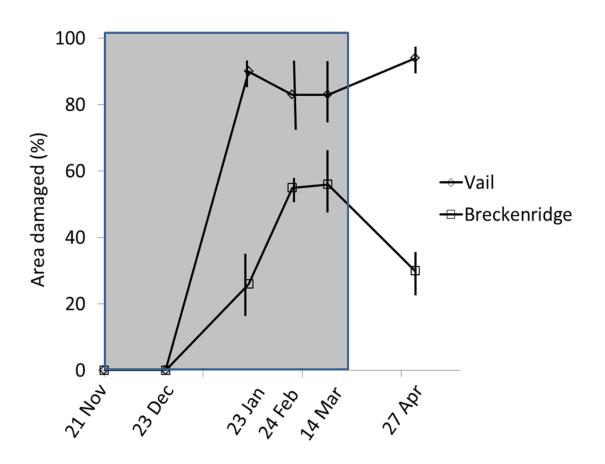


Figure 2.8. Seasonal development of gray and speckled snow mold in non-fungicide treated plots on an annual bluegrass and Kentucky bluegrass fairway at Vail and Breckenridge, CO respectively in 2005-06. Shaded area represents the period of snow coverage on plots. Snow mold damage on all dates except 27 Apr 2006 were determined by removing turf cores from underneath the snow cover and estimating plot damage based on injury to the cores. Bars for each mean represent standard error.

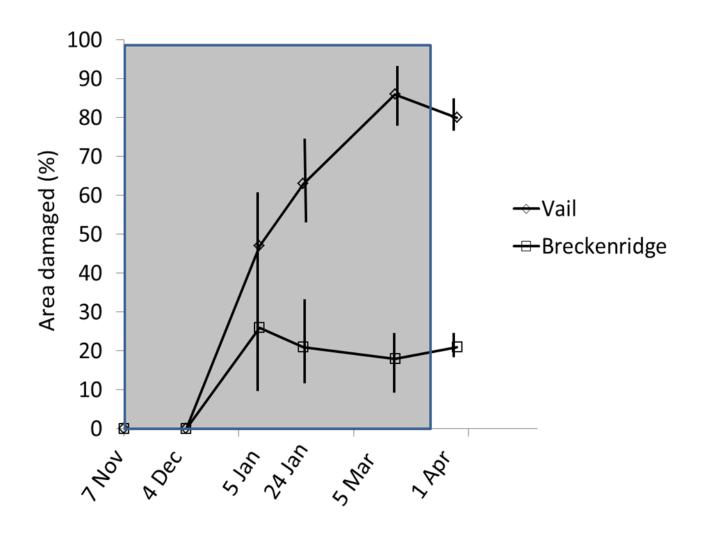


Figure 2.9. Seasonal development of gray and speckled snow mold in non-fungicide treated plots on an annual bluegrass and Kentucky bluegrass fairway at Vail and Breckenridge, CO, respectively, in 2006-07. Shaded area represents the period of snow coverage on plots. Snow mold damage on all dates except 1 Apr were determined by removing turf cores from underneath the snow cover and estimating plot damage based on injury to the cores. Bars for each mean represent standard error.

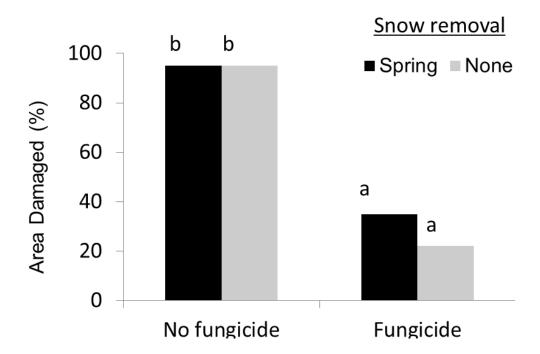


Figure 2.10. Effect of snow removal and fungicide applications on turfgrass damage at Vail, CO 2007-08. The commercial fungicide Instrata (containing the active ingredients chlorothalonil, propiconazole and fludioxonil) was applied 25 Oct 2006 prior to permanent snow cover on 7 Nov. Snow was not removed from plots (None), or removed on 26 Mar 2008 (Spring removal) and weekly thereafter until the final rating date 24 Apr 2008. Means not followed by the same letter are significantly different (*P* =0.05) by Fisher's LSD test.

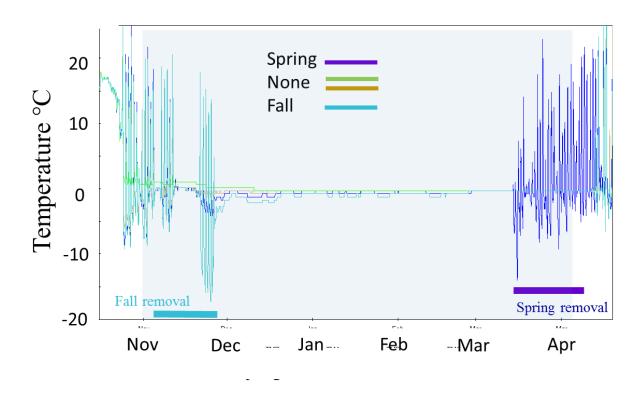


Figure 2.11. Temperatures at the turf surface on Kentucky bluegrass fairway at Breckenridge, CO during the winter 2005-06 for plots with no, fall (turquois), spring (purple), and no (brown and green) snow removal. Shaded area represents the period of snow cover.

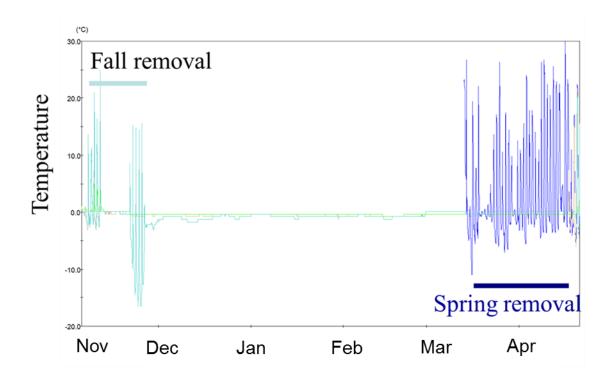


Figure 2.12. Temperatures at the turf surface on annual bluegrass fairway at Vail, CO during the winter 2005-2006 for plots with no, fall (turquois), and spring (purple) snow removal.

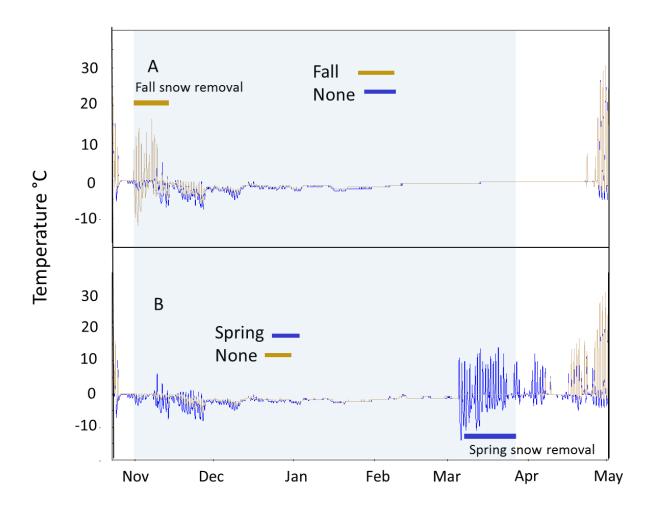


Figure 2.13. Temperatures at the turf surface on annual bluegrass fairway at Breckenridge, CO during the winter 2006-07 for plots with A) fall (brown line) and no snow removal (blue line) and B) spring (blue line) and no snow removal (brown line). The spring and fall snow removal bars indicate the period in which snow was physically removed from the plots at approximately weekly intervals. Shaded area represents the period of snow cover.



Figure 2.14. Snow mold damage was less severe on a Kentucky bluegrass tee at Breckenridge, CO where snow was compacted for Nordic track.



Figure 2.15. Snow mold damage was less severe on a Kentucky bluegrass fairway at Breckenridge, CO where snow was compacted for Nordic track. Note turfgrass discoloration associated with snow compaction.

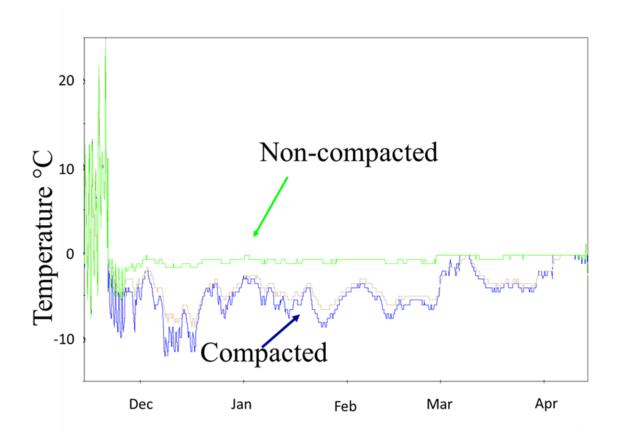


Figure 2. 16 Temperatures at the surface of Kentucky bluegrass in adjacent areas of a fairway where snow had not been compacted or had been compacted for the construction of a Nordic ski track at Breckenridge CO, 2006-07.

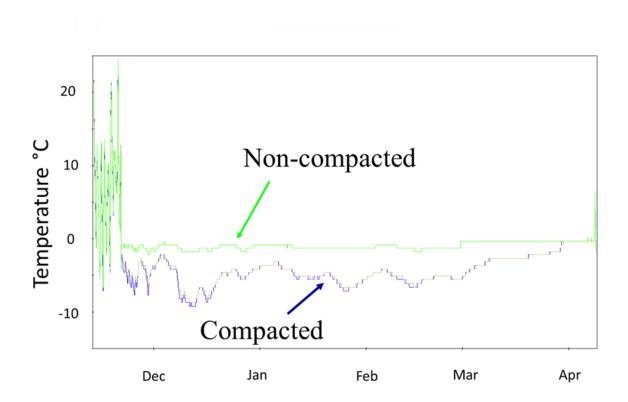


Figure 2.17. Temperatures at the surface of annual bluegrass in adjacent areas of a fairway where snow had not been compacted or had been compacted for the construction of a Nordic ski track at Vail CO, 2006-07.

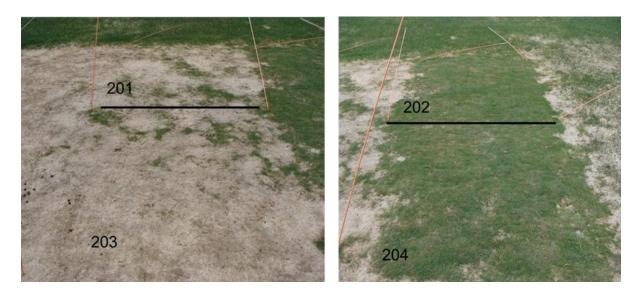


Figure 2.18. Damage to snow removal plots by snow mold at Vail, CO, 2007-2008. Plot 201 was no spring snow removal and no fungicide application, plot 203 was spring snow removal and no fungicide application. Plot 202 was no spring snow removal with a fungicide application and plot 204 was spring snow removal with a fungicide application. Table 2. 1 Comparison of one versus two fall fungicide applications for the control of Typhula blight at Vail and Breckenridge, CO 2005-2011.

Location ^w , year, treatment ^{X,Y} and rate	Percent Damage (± SE) ^Z
Breckenridge 2004-05	. ,
BannerMAXX 1.3MEC 4.0 fl oz followed by BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	11.7 ±9.4 7.5 ±2.5 NS
Spectro 90WDG 4.0 oz <i>followed by</i> Spectro 90WDG 4.0 oz + CL-EXP-4 1.0 oz Spectro 90WDG 5.76 oz + CL-EXP-4 1.0 oz	3.0 ±1.1 2.0 ±1.0 NS
Spectro 90WDG 4.0 oz <i>followed by</i> Spectro 90WDG 4.0 oz + Endorse 2.5WP 4.0 oz Spectro 90WDG 5.76 oz + Endorse 2.5WP 4.0 oz	11.8 ±6.2 9.0 ±4.1 NS
Untreated control	61.3 ±10.9
Vail 2004-05 BannerMAXX 1.3MEC 4.0 fl oz followed by BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	25.0 ±2.9 23.7 ±4.2 NS
Spectro 90WDG 4.0 oz <i>followed by</i> Spectro 90WDG 4.0 oz + CL-EXP-4 1.0 oz Spectro 90WDG 5.76 oz + CL-EXP-4 1.0 oz	3.8 ±2.4 4.3 ±2.1 NS
Spectro 90WDG 4.0 oz <i>followed by</i> Spectro 90WDG 4.0 oz + Endorse 2.5WP 4.0 oz Spectro 90WDG 5.76 oz + Endorse 2.5WP 4.0 oz	6.3 ±1.3 10.0 ±2.0 NS
Untreated control	93.8 ±4.7
Breckenridge 2005-06 BannerMAXX 1.3MEC 4.0 fl oz followed by BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	11.3 ±5.5 27.8 ±17.7 NS

Instrata 3.6SE 5.5 fl oz <i>followed by</i> Instrata 3.6 Instrata 3.6SE 11.0 fl oz	SE 5.5 fl oz	1.0 ±0.7 0.0	NS
Untreated control		38.8 ±12.1	
Vail 2005-06 BannerMAXX 1.3MEC 4.0 fl oz followed by Ba 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50 oz		25.0 ±6.4 33.7 ±5.9	NS
Instrata 3.6SE 5.5 fl oz <i>followed by</i> Instrata 3.6 Instrata 3.6SE 11.0 fl oz	SE 5.5 fl oz	20.5 ±6.1 30.0 ±13.3	NS
Untreated control		65.8 ±14.3	
Vail 2006-07 Spectro 90WDG 4.0 oz followed by Cleary 26 + Daconil Ultrex 82.5WDG 5.5 oz Spectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl o Ultrex 82.5WDG 5.5 oz		20.8 ±13.1 33.8 ±16.2	*
Untreated control		88.8 ±3.8	
<u>Vail 2007-08</u> Instrata 3.6SE 5.5 fl oz <i>followed by</i> Instrata 3. oz Instrata 3.6SE 11.0 fl oz	6SE 5.5 fl	27.5 ±7.5 14.3 ±6.6	NS
Untreated control		98.3 ±1.2	
Vail 2008-09 Instrata 3.6SE 5.5 fl oz followed by Instrata 3. oz Fairway Practice green Instrata 3.6SE 11.0 fl oz Fairway Practice green	6SE 5.5 fl	23.3 4.8 15.0 ±3.5 16.7 ±5.9 18.3 ±6.6	NS NS NS
Untreated control Fairway Practice green		92.3 ±5.9 98.3	

Vail 2009-10 Instrata 3.6SE 5.5 fl oz followed by Instrata 3.6SE 5.5 fl oz

Instrata 3.6SE 11.0 fl oz	10.0 ±2.9 11.7 ±1.7	NS
Curalan 50EG 1.0 oz + Daconil Ultrex 82.5WDG 3.2 oz followed by Insignia 20WG 0.7 oz + Trinity 19.2SC 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz Insignia 20SC 0.54 oz + Trinity 19.2SC 1.0 fl oz + Daconil	23.3 ±1.7	
Ultrex 82.5WDG 3.2 oz	16.7 ±3.3	NS
Untreated control	81.7 ±1.7	
Vail 2010-11		
Instrata 3.6SE 5.5 fl oz followed by Instrata 3.6 SE 5.5 fl	0.8 ±0.3	
oz Instrata 3.6 SE 11.0 fl oz	2.5 ±1.2 N	5
Pegasus HPX 2.75 fl oz + Kestrel MEX 1.3ME 2.0 fl oz + Dovetail 39.3EC 4.0 fl oz + Disarm SC 0.18 fl oz <i>followed</i> by Pegasus HPX 2.75 fl oz + Kestrel MEX 1.3ME 2.0 fl oz		
+ Dovetail 39.3EC 4.0 fl oz + Disarm SC 0.18 fl oz	0.0	
Pegasus HPX 5.5 fl oz + Kestrel MEX 1.3ME 4.0 fl oz + Dovetail 39.3EC 8.0 fl oz + Disarm SC 0.36 fl oz	4.0 ±3.7 N	6
Interface 24.5SC 3.0 fl oz + Triton Flo 367SC 0.5 fl oz followed by Interface 24.5SC 3.0 fl oz + Triton Flo 367SC	6.3 ±3.8	
0.5 fl oz	0.0 N/	-
Interface 24.5SC 6.0 fl oz + Triton Flo 367SC 0.85 fl oz	0.0 NS	5
Untreated Control	90.0 ±0.0	

^WPlots were established on a Kentucky bluegrass fairway at Breckenridge and at an annual bluegrass fairway at Vail except in 2008-09, when plots were placed on both an annual bluegrass fairway and practice putting green at Vail.

^xTreatments with a fungicide application made in late September and *followed by* a second application in late October or early November just before snow cover were compared to the same or similar fungicide combination applied only at the second date.

^YFungicide treatments were propiconazole 14.% a.i. (Banner MAXX and Kestrel MEX), fludioxonil 50.0% a.i (Medallion 50WP), polyoxin D Zinc Salt 2.5% a.i. (Endorse 2.5WP), chlorothalonil 82.5% a.i. (Daconil Ultrex 82.5WDG), chlorothalonil 54.0% a.i. (Pegasus HPX), vinclozolin 50.0% a.i. (Curalan 50EG), pyraclostrobin 20.0% a.i. (Insignia 20WG), triticonazole 19.2% a.i. (Trinity 19.2SC), fluoxastrobin 40.3% a.i. (Disarm 480SC), triticonazole 30.1% a.i. (Triton Flo), chlorothalonil 72% a.i. and thiophanate-methyl 18% a.i. (Spectro 90WDG), iprodione 19.65% a.i. and thiphanate-methyl 19.65% a.i. (Cleary 26/36 and Dovetail EC), iprodione 23.1% a.i. and trifloxystrobin 1.44% a.i. (Interface), chlorothalonil 29.9%, propiconazole 4.7% and fludioxonil 1.2% (Instrata 3.61SE).

^z Damage ratings are the means of 4 replications. A paired T test was used to compare the single fall fungicide application to two fall applications. A * indicates a significant (P<0.05) and NS a non-significant (P>0.10) difference between treatments. Damage from the untreated control plots was included as a reference of overall snow mold severity (pressure) in the experiment.

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APPENDIX 1

LIST OF SNOW MOLD ISOLATES

Isolate No.	Collected From	Date Collected	ld ^x	Isolate No.	Collected From	Date Collected	ld [×]
SM101	Vail, CO	5-Mar-07	TISH	SM147	Loveland, CO	No Date	TISH
SM102	Vail, CO	5-Mar-07	TISH	SM148	Vail, CO	26-Mar-07	TISH
SM103	Vail, CO	5-Mar-07	TISH	SM149	Vail, CO	26-Mar-07	TISH
SM104	Vail, CO	5-Mar-07	TISH	SM150	Vail, CO	26-Mar-07	TISH
SM105	Vail, CO	5-Mar-07	TISH	SM151	Vail, CO	26-Mar-07	TISH
SM106	Vail, CO	5-Mar-07	TISH	SM152	Vail, CO	26-Mar-07	TISH
SM107	Vail, CO	5-Mar-07	TISH	SM153	Vail, CO	26-Mar-07	TISH
SM108	Vail, CO	5-Mar-07	TISH	SM154	Vail, CO	26-Mar-07	TISH
SM109	Vail, CO	5-Mar-07	TISH	SM155	Vail, CO	26-Mar-07	TISH
SM110	Fort Collins, CO	No Date	TINC	SM156	Vail, CO	26-Mar-07	TISH
SM111	Fort Collins, CO	No Date	TINC	SM157	Vail, CO	26-Mar-07	TISH
SM112	Fort Collins, CO	No Date	TINC	SM158	Vail, CO	26-Mar-07	TISH
SM113	Fort Collins, CO	No Date	TINC	SM159	Vail, CO	26-Mar-07	TISH
SM114	Fort Collins, CO	No Date	TINC	SM160	Vail, CO	26-Mar-07	TISH
SM115	Fort Collins, CO	No Date	TINC	SM161	Vail, CO	26-Mar-07	TISH
SM116	Fort Collins, CO	No Date	TPHA	SM162	Vail, CO	26-Mar-07	TISH
SM117	Fort Collins, CO	No Date	TINC	SM163	Vail, CO	26-Mar-07	TISH
SM118	Fort Collins, CO	No Date	TINC	SM164	Vail, CO	26-Mar-07	TISH
SM119	Fort Collins, CO	No Date	TINC	SM165	Vail, CO	26-Mar-07	TISH
SM120	Fort Collins, CO	No Date	TINC	SM166	Vail, CO	26-Mar-07	TISH
SM121	Fort Collins, CO	No Date	TINC	SM167	Vail, CO	26-Mar-07	TISH
SM122	Loveland, CO	No Date	TISH	SM168	Vail, CO	26-Mar-07	TISH
SM123	Fort Collins, CO	No Date	TISH	SM169	Vail, CO	26-Mar-07	TISH
SM124	Fort Collins, CO	No Date	TISH	SM170	Vail, CO	26-Mar-07	TISH
SM125	Loveland, CO	No Date	TISH	SM171	Vail, CO	26-Mar-07	TISH
SM126	Fort Collins, CO	No Date	TISH	SM172	Vail, CO	26-Mar-07	TISH
SM127	Fort Collins, CO	No Date	TISH	SM173	Vail, CO	26-Mar-07	TISH
SM128	Fort Collins, CO	No Date	TISH	SM174	Vail, CO	26-Mar-07	TISH
SM129	Loveland, CO	No Date	TINC	SM175	Vail, CO	26-Mar-07	TISH
SM130	Loveland, CO	No Date	TINC	SM176	Vail, CO	26-Mar-07	TISH
SM131	Loveland, CO	No Date	TINC	SM177	Loveland, CO	No Date	TISH
SM132	Loveland, CO	No Date	TINC	SM178	Loveland, CO	No Date	TISH
SM133	Loveland, CO	No Date	TINC	SM179	Loveland, CO	No Date	TISH
SM134	Loveland, CO	No Date	TINC	SM180	Fort Collins, CO	No Date	TISH
SM135	Loveland, CO	No Date	TINC	SM181	Vail, CO	26-Mar-07	TISH
SM136	Fort Collins, CO	No Date	TINC	SM182	Vail, CO	26-Mar-07	TISH
SM137	Loveland, CO	No Date	TINC	SM183	Vail, CO	26-Mar-07	TISH
SM138	Fort Collins, CO	No Date	TISH	SM184	Vail, CO	26-Mar-07	TISH
SM139	Fort Collins, CO	No Date	TINC	SM185	Vail, CO	26-Mar-07	TISH
SM140	Loveland, CO	No Date	TISH	SM186	Vail, CO	26-Mar-07	TISH
SM141	Fort Collins, CO	No Date	TISH	SM187	Vail, CO	26-Mar-07	TISH
SM142	Fort Collins, CO	No Date	TISH	SM188	Vail, CO	26-Mar-07	TISH
SM143	Loveland, CO	No Date	TISH	SM189	Vail, CO	26-Mar-07	TISH
SM144	Loveland, CO	No Date	TISH	SM190	Vail, CO	26-Mar-07	TISH
SM145	Loveland, CO	No Date	TISH	SM191	Vail, CO	26-Mar-07	TISH
SM146	Loveland, CO	No Date	TISH	SM192	Vail, CO	26-Mar-07	TISH

Isolate No.	Collected From	Date Collected	ld ^x	Isolate No.	Collected From	Date Collected	ld ^x
SM193	Vail, CO	26-Mar-07	TISH	SM241	Vail, CO	26-Mar-07	TISH
SM194	Vail, CO	26-Mar-07	TISH	SM242	Vail, CO	26-Mar-07	TISH
SM195	Vail, CO	26-Mar-07	TISH	SM243	Vail, CO	26-Mar-07	TISH
SM196	Vail, CO	26-Mar-07	TISH	SM244	Vail, CO	26-Mar-07	TISH
SM197	Vail, CO	26-Mar-07	TISH	SM245	Vail, CO	26-Mar-07	TISH
SM198	Vail, CO	26-Mar-07	TISH	SM246	Vail, CO	26-Mar-07	TISH
SM199	Vail, CO	26-Mar-07	TISH	SM247	Vail, CO	26-Mar-07	TISH
SM200	Vail, CO	26-Mar-07	TISH	SM248	Vail, CO	26-Mar-07	TISH
SM201	Vail, CO	26-Mar-07	TISH	SM249	Vail, CO	26-Mar-07	TISH
SM202	Vail, CO	26-Mar-07	TISH	SM250	Vail, CO	26-Mar-07	TISH
SM203	Vail, CO	26-Mar-07	TISH	SM251	Vail, CO	26-Mar-07	TISH
SM204	Vail, CO	26-Mar-07	TISH	SM252	Vail, CO	26-Mar-07	TISH
SM205	Vail, CO	26-Mar-07	TISH	SM253	Vail, CO	26-Mar-07	TISH
SM206	Vail, CO	26-Mar-07	TISH	SM254	Vail, CO	26-Mar-07	TISH
SM207	Vail, CO	26-Mar-07	TISH	SM255	Vail, CO	26-Mar-07	TISH
SM208	Vail, CO	26-Mar-07	TISH	SM256	Vail, CO	26-Mar-07	TISH
SM209	Vail, CO	26-Mar-07	TISH	SM257	Vail, CO	26-Mar-07	TISH
SM210	Vail, CO	26-Mar-07	TISH	SM258	Vail, CO	26-Mar-07	TISH
SM211	Vail, CO	26-Mar-07	TISH	SM259	Vail, CO	26-Mar-07	TISH
SM212	Vail, CO	26-Mar-07	TISH	SM260	Vail, CO	26-Mar-07	TISH
SM213	Vail, CO	26-Mar-07	TISH	SM261	Vail, CO	26-Mar-07	TISH
SM214	Vail, CO	26-Mar-07	TISH	SM262	Vail, CO	26-Mar-07	TISH
SM215	Vail, CO	26-Mar-07	TISH	SM263	Vail, CO	26-Mar-07	TISH
SM216	Vail, CO	26-Mar-07	TISH	SM264	Vail, CO	26-Mar-07	TISH
SM217	Vail, CO	26-Mar-07	TISH	SM265	Vail, CO	26-Mar-07	TISH
SM218	Vail, CO	26-Mar-07	TISH	SM266	Vail, CO	26-Mar-07	TISH
SM219	Vail, CO	26-Mar-07	TISH	SM267	Vail, CO	26-Mar-07	TISH
SM220	Vail, CO	26-Mar-07	TISH	SM268	Vail, CO	26-Mar-07	TISH
SM221	Vail, CO	26-Mar-07	TISH	SM269	Vail, CO	26-Mar-07	TISH
SM222	Vail, CO	26-Mar-07	TISH	SM270	Vail, CO	26-Mar-07	TISH
SM223	Vail, CO	26-Mar-07	TISH	SM271	Vail, CO	26-Mar-07	TISH
SM224	Vail, CO	26-Mar-07	TISH	SM272	Vail, CO	26-Mar-07	TISH
SM225	Vail, CO	26-Mar-07	TISH	SM273	Vail, CO	26-Mar-07	TISH
SM226	Vail, CO	26-Mar-07	TISH	SM274	Vail, CO	26-Mar-07	TISH
SM227	Vail, CO	26-Mar-07	TISH	SM275	Vail, CO	26-Mar-07	TISH
SM228	Vail, CO	26-Mar-07	TISH	SM276	Vail, CO	26-Mar-07	TISH
SM229	Vail, CO	26-Mar-07	TISH	SM277	Vail, CO	26-Mar-07	TISH
SM230	Vail, CO	26-Mar-07	TISH	SM278	Vail, CO	26-Mar-07	TISH
SM231	Vail, CO	26-Mar-07	TISH	SM279	Vail, CO	26-Mar-07	TISH
SM232	Vail, CO	26-Mar-07	TISH	SM280	Vail, CO	26-Mar-07	TISH
SM233	Vail, CO	26-Mar-07	TISH	SM281	Vail, CO	26-Mar-07	TISH
SM234	Vail, CO	26-Mar-07	TISH	SM282	Vail, CO	26-Mar-07	TISH
SM235	Vail, CO	26-Mar-07	TISH	SM283	Vail, CO	26-Mar-07	TISH
SM236	Vail, CO	26-Mar-07	TISH	SM284	Vail, CO	26-Mar-07	TISH
SM237	Vail, CO	26-Mar-07	TISH	SM285	Vail, CO	26-Mar-07	TISH
SM238	Vail, CO	26-Mar-07	TISH	SM286	Vail, CO	26-Mar-07	TISH
SM239	Vail, CO	26-Mar-07	TISH	SM287	Vail, CO	26-Mar-07	TISH
SM240	Vail, CO	26-Mar-07	TISH	SM288	Vail, CO	26-Mar-07	TISH

Isolate No.	Collected From	Date Collected	ld ^x	Isolate No.	Collected From	Date Collected	ld ^x
SM289	Vail, CO	26-Mar-07	TISH	SM336	Vail, CO	26-Mar-07	TISH
SM290	Vail, CO	26-Mar-07	TISH	SM337	Vail, CO	26-Mar-07	TISH
SM291	Vail, CO	26-Mar-07	TISH	SM338	Vail, CO	26-Mar-07	TISH
SM292	Vail, CO	26-Mar-07	TISH	SM339	Vail, CO	26-Mar-07	TISH
SM293	Vail, CO	26-Mar-07	TISH	SM340	Vail, CO	26-Mar-07	TISH
SM294	Vail, CO	26-Mar-07	TISH	SM341	Vail, CO	26-Mar-07	TISH
SM295	Vail, CO	26-Mar-07	TISH	SM342	Vail, CO	26-Mar-07	TISH
SM296	Vail, CO	26-Mar-07	TISH	SM343	Vail, CO	26-Mar-07	TISH
SM297	Vail, CO	26-Mar-07	TISH	SM344	Vail, CO	26-Mar-07	TISH
SM298	Vail, CO	26-Mar-07	TISH	SM345	Vail, CO	26-Mar-07	TISH
SM299	Vail, CO	26-Mar-07	TISH	SM346	Vail, CO	26-Mar-07	TISH
SM300	Vail, CO	26-Mar-07	TISH	SM347	Vail, CO	26-Mar-07	TISH
SM301	Vail, CO	26-Mar-07	TISH	SM348	Vail, CO	26-Mar-07	TISH
SM302	Vail, CO	26-Mar-07	TISH	SM349	Vail, CO	26-Mar-07	TISH
SM303	Vail, CO	26-Mar-07	TISH	SM350	Vail, CO	26-Mar-07	TISH
SM304	Vail, CO	26-Mar-07	TISH	SM351	Vail, CO	26-Mar-07	MNIV
SM305	Vail, CO	26-Mar-07	TISH	SM352	Vail, CO	26-Mar-07	TISH
SM306	Vail, CO	26-Mar-07	TISH	SM353	Vail, CO	26-Mar-07	TISH
SM307	Vail, CO	26-Mar-07	TISH	SM354	Vail, CO	26-Mar-07	TISH
SM308	Vail, CO	26-Mar-07	TISH	SM355	Vail, CO	26-Mar-07	TISH
SM309	Vail, CO	26-Mar-07	TISH	SM356	Vail, CO	26-Mar-07	TISH
SM310	Vail, CO	26-Mar-07	TISH	SM357	Vail, CO	26-Mar-07	TISH
SM311	Vail, CO	26-Mar-07	TISH	SM358	Vail, CO	26-Mar-07	TISH
SM312	Vail, CO	26-Mar-07	TISH	SM359	Vail, CO	26-Mar-07	TISH
SM313	Vail, CO	26-Mar-07	TISH	SM360	Vail, CO	26-Mar-07	TISH
SM314	Vail, CO	26-Mar-07	TISH	SM361	Vail, CO	26-Mar-07	TISH
SM315	Vail, CO	26-Mar-07	TISH	SM362	Vail, CO	26-Mar-07	TISH
SM316	Vail, CO	26-Mar-07	TISH	SM363	Vail, CO	26-Mar-07	TISH
SM317	Vail, CO	26-Mar-07	TISH	SM364	Vail, CO	26-Mar-07	TISH
SM318	Vail, CO	26-Mar-07	TISH	SM365	Vail, CO	26-Mar-07	TISH
SM319	Vail, CO	26-Mar-07	TISH	SM366	Vail, CO	26-Mar-07	TISH
SM320	Vail, CO	26-Mar-07	TISH	SM367	Vail, CO	26-Mar-07	TISH
SM321	Vail, CO	26-Mar-07	TISH	SM368	Vail, CO	26-Mar-07	TISH
SM322	Vail, CO	26-Mar-07	TISH	SM369	Breckenridge, CO	1-May-07	TISH
SM323	Vail, CO	26-Mar-07	TISH	SM370	Breckenridge, CO	1-May-07	TISH
SM324	Vail, CO	26-Mar-07	TISH	SM371	Breckenridge, CO	1-May-07	TISH
SM325	Vail, CO	26-Mar-07	TISH	SM372	Breckenridge, CO	1-May-07	TISH
SM326	Vail, CO	26-Mar-07	TISH	SM373	Breckenridge, CO	1-May-07	TISH
SM327	Vail, CO	26-Mar-07	TISH	SM374	Breckenridge, CO	1-May-07	TISH
SM328	Vail, CO	26-Mar-07	TISH	SM375	Breckenridge, CO	1-May-07	TISH
SM329	Vail, CO	26-Mar-07	TISH	SM376	Breckenridge, CO	1-May-07	TISH
SM330	Vail, CO	26-Mar-07	TISH	SM377	Breckenridge, CO	1-May-07	TISH
SM331	Vail, CO	26-Mar-07	TISH	SM378	Breckenridge, CO	1-May-07	TISH
SM332	Vail, CO	26-Mar-07	TISH	SM379	Breckenridge, CO	1-May-07	TISH
SM333	Vail, CO	26-Mar-07	TISH	SM379	Breckenridge, CO	1-May-07	TISH
SM334	Vail, CO	26-Mar-07	TISH	SM380	Breckenridge, CO	1-May-07	TISH
SM335	Vail, CO Vail, CO	26-Mar-07	TISH	SM381	Breckenridge, CO	1-May-07	TISH

Isolate No.	Collected From	Date Collected	ld ^x	Isolate No.	Collected From	Date Collected	ld ^x
SM383	Breckenridge, CO	1-May-07	TISH	SM430	Breckenridge, CO	1-May-07	TISH
SM384	Breckenridge, CO	1-May-07	TISH	SM430	Breckenridge, CO	1-May-07	TISH
SM385	Breckenridge, CO	1-May-07	TISH	SM431	Breckenridge, CO	1-May-07	TISH
SM386	Breckenridge, CO	1-May-07	TISH	SM432	Breckenridge, CO	1-May-07	TISH
SM387	Breckenridge, CO	1-May-07	TISH	SM433	Breckenridge, CO	1-May-07	TISH
SM388	Breckenridge, CO	1-May-07	TISH	SM434	Breckenridge, CO	1-May-07	TISH
SM389	Breckenridge, CO	1-May-07	TISH	SM435	Breckenridge, CO	1-May-07	TISH
SM390	Breckenridge, CO	1-May-07	TISH	SM436	Breckenridge, CO	1-May-07	TISH
SM391	Breckenridge, CO	1-May-07	TISH	SM437	Breckenridge, CO	1-May-07	TISH
SM392	Breckenridge, CO	1-May-07	TISH	SM438	Breckenridge, CO	1-May-07	TISH
SM393	Breckenridge, CO	1-May-07	TISH	SM439	Breckenridge, CO	1-May-07	TISH
SM394	Breckenridge, CO	1-May-07	TISH	SM440	Breckenridge, CO	1-May-07	TISH
SM395	Breckenridge, CO	1-May-07	TISH	SM441	Breckenridge, CO	1-May-07	TISH
SM396	Breckenridge, CO	1-May-07	TISH	SM442	Breckenridge, CO	1-May-07	TISH
SM397	Breckenridge, CO	1-May-07	TISH	SM443	Breckenridge, CO	1-May-07	TISH
SM398	Breckenridge, CO	1-May-07	TISH	SM444	Breckenridge, CO	1-May-07	TISH
SM399	Breckenridge, CO	1-May-07	TISH	SM445	Breckenridge, CO	1-May-07	TISH
SM400	Breckenridge, CO	1-May-07	TISH	SM446	Breckenridge, CO	1-May-07	TISH
SM401	Breckenridge, CO	1-May-07	TISH	SM447	Breckenridge, CO	1-May-07	TISH
SM402	Breckenridge, CO	1-May-07	TISH	SM448	Breckenridge, CO	1-May-07	TISH
SM403	Breckenridge, CO	1-May-07	TISH	SM449	Breckenridge, CO	1-May-07	TISH
SM404	Breckenridge, CO	1-May-07	TISH	SM450	Breckenridge, CO	1-May-07	TISH
SM405	Breckenridge, CO	1-May-07	TISH	SM451	Breckenridge, CO	1-May-07	TISH
SM406	Breckenridge, CO	1-May-07	TISH	SM452	Breckenridge, CO	1-May-07	TISH
SM407	Breckenridge, CO	1-May-07	TISH	SM453	Breckenridge, CO	1-May-07	TISH
SM408	Breckenridge, CO	1-May-07	TISH	SM454	Breckenridge, CO	1-May-07	TISH
SM409	Breckenridge, CO	1-May-07	TISH	SM455	Breckenridge, CO	1-May-07	TISH
SM410	Breckenridge, CO	1-May-07	TISH	SM456	Breckenridge, CO	1-May-07	TISH
SM410	Breckenridge, CO	1-May-07	TISH	SM450 SM457	Breckenridge, CO	1-May-07	TISH
SM412	Breckenridge, CO	1-May-07	TISH	SM458	Breckenridge, CO	1-May-07	TISH
SM413	Breckenridge, CO	1-May-07	TISH	SM459	Breckenridge, CO	1-May-07	TISH
SM414	Breckenridge, CO	1-May-07	TISH	SM460	Breckenridge, CO	1-May-07	TISH
SM415	Breckenridge, CO	1-May-07	TISH	SM460	Breckenridge, CO	1-May-07	TISH
SM416	Breckenridge, CO	1-May-07	TISH	SM462	Breckenridge, CO	1-May-07	TISH
SM417	Breckenridge, CO	1-May-07	TISH	SM463	Breckenridge, CO	1-May-07	TISH
SM418	Breckenridge, CO	1-May-07	TISH	SM463	Breckenridge, CO	1-May-07	TISH
SM419	Breckenridge, CO	1-May-07	TISH	SM465	Breckenridge, CO	1-May-07	TISH
SM419 SM420	Breckenridge, CO	1-May-07	TISH	SM466	Breckenridge, CO	1-May-07	TISH
SM420 SM421	Breckenridge, CO	1-May-07	TISH	SM467	Breckenridge, CO	1-May-07	TISH
SM422	Breckenridge, CO	1-May-07	TISH	SM468	Breckenridge, CO	1-May-07	TISH
SM422 SM423	Breckenridge, CO	1-May-07	TISH	SM469	Breckenridge, CO	1-May-07	TISH
SM423	Breckenridge, CO	1-May-07	TISH	SM409	Breckenridge, CO	1-May-07	TISH
SM424 SM425	Breckenridge, CO	1-May-07	TISH	SM470 SM471	Breckenridge, CO	1-May-07	TISH
SM425 SM426	U ,		TISH	SM471 SM472	U ,	-	TISH
SM426 SM427	Breckenridge, CO	1-May-07	TISH		Breckenridge, CO	1-May-07	TISH
	Breckenridge, CO	1-May-07		SM473	Breckenridge, CO	1-May-07	TISH
SM428	Breckenridge, CO	1-May-07	TISH	SM474	Breckenridge, CO	1-May-07	TISH
SM429	Breckenridge, CO	1-May-07	TISH	SM475	Breckenridge, CO	1-May-07	IISH

Isolate No.	Collected From	Date Collected	ld [×]	Isolate No.	Collected From	Date Collected	ld ^x
SM476	Breckenridge, CO	1-May-07	TISH	SM523	Breckenridge, CO	1-May-07	TISH
SM477	Breckenridge, CO	1-May-07	TISH	SM524	Breckenridge, CO	1-May-07	TISH
SM478	Breckenridge, CO	1-May-07	TISH	SM525	Breckenridge, CO	1-May-07	TISH
SM479	Breckenridge, CO	1-May-07	TISH	SM526	Breckenridge, CO	1-May-07	TISH
SM480	Breckenridge, CO	1-May-07	TISH	SM527	Breckenridge, CO	1-May-07	TISH
SM481	Breckenridge, CO	1-May-07	TISH	SM528	Breckenridge, CO	1-May-07	TISH
SM482	Breckenridge, CO	1-May-07	TISH	SM529	Breckenridge, CO	1-May-07	TISH
SM483	Breckenridge, CO	1-May-07	TISH	SM530	Breckenridge, CO	1-May-07	TISH
SM484	Breckenridge, CO	1-May-07	TISH	SM531	Breckenridge, CO	1-May-07	TISH
SM485	Breckenridge, CO	1-May-07	TISH	SM532	Breckenridge, CO	1-May-07	TISH
SM486	Breckenridge, CO	1-May-07	TISH	SM533	Vail, CO	26-Mar-07	TISH
SM487	Breckenridge, CO	1-May-07	TISH	SM534	Breckenridge, CO	1-May-07	TISH
SM488	Breckenridge, CO	1-May-07	TISH	SM535	Breckenridge, CO	1-May-07	TISH
SM489	Breckenridge, CO	1-May-07	TISH	SM536	Vail, CO	26-Mar-07	TISH
SM490	Breckenridge, CO	1-May-07	TISH	SM537	Breckenridge, CO	1-May-07	TISH
SM491	Breckenridge, CO	1-May-07	TISH	SM538	Breckenridge, CO	1-May-07	TISH
SM492	Breckenridge, CO	1-May-07	TISH	SM539	Breckenridge, CO	1-May-07	TISH
SM493	Breckenridge, CO	1-May-07	TISH	SM540	Breckenridge, CO	1-May-07	TISH
SM494	Breckenridge, CO	1-May-07	TISH	SM541	Breckenridge, CO	1-May-07	TISH
SM495	Breckenridge, CO	1-May-07	TISH	SM542	Breckenridge, CO	1-May-07	TISH
SM496	Breckenridge, CO	1-May-07	TISH	SM543	Breckenridge, CO	1-May-07	TISH
SM497	Breckenridge, CO	1-May-07	TISH	SM544	Breckenridge, CO	1-May-07	TISH
SM498	Breckenridge, CO	1-May-07	TISH	SM545	Breckenridge, CO	1-May-07	TISH
SM499	Breckenridge, CO	1-May-07	TISH	SM546	Breckenridge, CO	1-May-07	TISH
SM500	Breckenridge, CO	1-May-07	TISH	SM547	Breckenridge, CO	1-May-07	TISH
SM501	Breckenridge, CO	1-May-07	TISH	SM548	Breckenridge, CO	1-May-07	TISH
SM502	Breckenridge, CO	1-May-07	TISH	SM549	Breckenridge, CO	1-May-07	TISH
SM503	Breckenridge, CO	1-May-07	TISH	SM550	Breckenridge, CO	1-May-07	TISH
SM504	Breckenridge, CO	1-May-07	TISH	SM551	Breckenridge, CO	1-May-07	TISH
SM505	Breckenridge, CO	1-May-07	TISH	SM552	Breckenridge, CO	1-May-07	TISH
SM506	Breckenridge, CO	1-May-07	TISH	SM553	Breckenridge, CO	1-May-07	TISH
SM507	Vail, CO	26-Mar-07	TISH	SM554	Breckenridge, CO	1-May-07	TISH
SM508	Vail, CO	26-Mar-07	TISH	SM555	Breckenridge, CO	1-May-07	TISH
SM509	Vail, CO	26-Mar-07	TISH	SM556	Breckenridge, CO	1-May-07	TISH
SM510	Vail, CO	26-Mar-07	TISH	SM557	Breckenridge, CO	1-May-07	TISH
SM511	Vail, CO	26-Mar-07	TISH	SM558	Breckenridge, CO	1-May-07	TISH
SM512	Vail, CO	26-Mar-07	TISH	SM559	Breckenridge, CO	1-May-07	TISH
SM513	Breckenridge, CO	1-May-07	TISH	SM560	Breckenridge, CO	26-Mar-07	TISH
SM514	Breckenridge, CO	1-May-07	TISH	SM561	Breckenridge, CO	1-May-07	TISH
SM515	Breckenridge, CO	1-May-07	TISH	SM562	Vail, CO	26-Mar-07	TISH
SM516	Breckenridge, CO	1-May-07	TISH	SM563	Breckenridge, CO	1-May-07	TISH
SM517	Breckenridge, CO	1-May-07	TISH	SM564	Breckenridge, CO	1-May-07	TISH
SM518	Breckenridge, CO	1-May-07	TISH	SM565	Breckenridge, CO	1-May-07	TISH
SM519	Breckenridge, CO	1-May-07	TISH	SM566	Breckenridge, CO	1-May-07	TISH
SM520	Breckenridge, CO	1-May-07	TISH	SM567	Breckenridge, CO	1-May-07	TISH
SM521	Breckenridge, CO	1-May-07	TISH	SM568	Breckenridge, CO	1-May-07	TISH
SM522	Breckenridge, CO	1-May-07	TISH	SM569	Breckenridge, CO	1-May-07	TISH

Isolate No.	Collected From	Date Collected	ld ^x	Isolate No.	Collected From	Date Collected	ld ^x
SM570	Breckenridge, CO	1-May-07	TISH	SM617	Canada	Acquired 2007	TISH
SM571	Breckenridge, CO	1-May-07	TISH	SM618	Canada	Acquired 2007	TINC
SM572	Breckenridge, CO	1-May-07	TISH	SM619	Canada	Acquired 2007	TINC
SM573	Breckenridge, CO	1-May-07	TISH	SM620	Canada	Acquired 2007	TISH
SM574	Breckenridge, CO	1-May-07	TISH	SM621	Canada	Acquired 2007	TISH
SM575	Breckenridge, CO	1-May-07	TISH	SM622	Canada	Acquired 2007	TISH
SM576	Breckenridge, CO	1-May-07	TISH	SM623	Canada	Acquired 2007	TISH
SM577	Breckenridge, CO	1-May-07	TISH	SM624	Breckenridge, CO	1-May-07	TISH
SM578	Breckenridge, CO	1-May-07	TISH	SM625	Breckenridge, CO	1-May-07	TISH
SM579	Breckenridge, CO	1-May-07	TISH	SM626	Breckenridge, CO	1-May-07	TISH
SM580	Breckenridge, CO	1-May-07	TISH	SM627	Vail, CO	26-Mar-07	TISH
SM581	Vail, CO	26-Mar-07	TINC	SM628	Edwards, CO	12-14 May 2007	TINC
SM582	Vail, CO	26-Mar-07	TISH	SM629	Wolcott, CO	12-14 May 2007	MBOR
SM583	Vail, CO	26-Mar-07	TINC	SM630	Edwards, CO	12-14 May 2007	TPHA
SM584	Vail, CO	26-Mar-07	TINC	SM631	Telluride, CO	12-14 May 2007	TISH
SM585	Vail, CO	26-Mar-07	TINC	SM632	Basalt, CO	12-14 May 2007	TISH
SM587	Vail, CO	26-Mar-07	TINC	SM633	Telluride, CO	12-14 May 2007	TINC
SM588	Vail, CO	26-Mar-07	TINC	SM634	Wolcott, CO	12-14 May 2007	TINC
SM589	Vail, CO	26-Mar-07	TINC	SM635	Wolcott, CO	12-14 May 2007	TINC
SM590	Vail, CO	26-Mar-07	TINC	SM636	Wolcott, CO	12-14 May 2007	TINC
SM591	Vail, CO	26-Mar-07	TINC	SM637	Telluride, CO	12-14 May 2007	TINC
SM592	Vail, CO	26-Mar-07	TINC	SM638	Telluride, CO	12-14 May 2007	TINC
SM593	Michigan	Acquired 2007	TISH	SM639	Edwards, CO	12-14 May 2007	TINC
SM594	Wisconsin	Acquired 2007	TISH	SM640	Granby, CO	12-14 May 2007	TINC
SM595	Michigan	Acquired 2007	TISH	SM641	Vail, CO	12-14 May 2007	TISH
SM596	Michigan	Acquired 2007	TISH	SM642	Vail, CO	12-14 May 2007	TISH
SM597	Wisconsin	Acquired 2007	TISH	SM643	Vail, CO	12-14 May 2007	TISH
SM598	Wisconsin	Acquired 2007	TISH	SM644	Vail, CO	12-14 May 2007	TISH
SM599	Wisconsin	Acquired 2007	TISH	SM645	Granby, CO	12-14 May 2007	WCIR
SM600	Michigan	Acquired 2007	TINC	SM646	Telluride, CO	12-14 May 2007	TISH
SM601	Minnesota	Acquired 2007	TINC	SM647	Basalt, CO	12-14 May 2007	TISH
SM602	Wisconsin	Acquired 2007	TINC	SM648	Edwards, CO	12-14 May 2007	TISH
SM603	Michigan	Acquired 2007	TINC	SM649	Vail, CO	12-14 May 2007	TISH
SM604	Michigan	Acquired 2007	TISH	SM650	Vail, CO	12-14 May 2007	TISH
SM605	Michigan	Acquired 2007	TISH	SM651	Vail, CO	12-14 May 2007	TISH
SM606	Michigan	Acquired 2007	TISH	SM652	Vail, CO	12-14 May 2007	TISH
SM607	Idaho	Acquired 2007	TINC	SM653	Vail, CO	12-14 May 2007	TISH
SM608	Idaho	Acquired 2007	TINC	SM654	Vail, CO	12-14 May 2007	TISH
SM609	Idaho	Acquired 2007	TINC	SM655	Vail, CO	12-14 May 2007	TISH
SM610	Idaho	Acquired 2007	TINC	SM656	Vail, CO	12-14 May 2007	TISH
SM611	Idaho	Acquired 2007	TISH	SM657	Vail, CO	12-14 May 2007	TISH
SM612	Idaho	Acquired 2007	TISH	SM658	Edwards, CO	12-14 May 2007	TPHA
SM613	Idaho	Acquired 2007	TISH	SM659	Edwards, CO	12-14 May 2007	TISH
SM614	Idaho	Acquired 2007	TISH	SM660	Edwards, CO	12-14 May 2007	TISH
SM615	Canada	Acquired 2007	TISH	SM661	Basalt, CO	12-14 May 2007	TISH
SM616	Canada	Acquired 2007	TISH	SM662	Telluride, CO	12-14 May 2007	TISH

Isolate No.	Collected From	Date Collected	ld ^x	Isolate No.	Collected From	Date Collected	ld ^x
SM663	Edwards, CO	12-14 May 2007	TISH				
SM664	Keystone, CO	12-14 May 2007	TISH				
SM665	Wolcott, CO	12-14 May 2007	TISH				
SM667	Granby, CO	12-14 May 2007	TISH				
SM668	Granby, CO	12-14 May 2007	TISH				
SM669	Edwards, CO	12-14 May 2007	TPHA				
SM670	Edwards, CO	12-14 May 2007	TISH				
SM671	Granby, CO	12-14 May 2007	TISH				
SM672	Edwards, CO	12-14 May 2007	TISH				
SM673	Vail, CO	12-14 May 2007	TISH				
SM674	Granby, CO	12-14 May 2007	TISH				
SM675	Granby, CO	12-14 May 2007	TISH				
SM676	Keystone, CO	12-14 May 2007	TISH				
SM677	Vail, CO	12-14 May 2007	TISH				
SM678	Vail, CO	12-14 May 2007	TISH				
SM679	Vail, CO	12-14 May 2007	TISH				
SM680	Vail, CO	12-14 May 2007	TISH				
SM681	Telluride, CO	12-14 May 2007	TISH				
SM682	Keystone, CO	12-14 May 2007	TISH				
SM683	Keystone, CO	12-14 May 2007	TISH				

[×] Identification of *Typhula* isolates; TISH = *Typhula* ishikariensis, TINC = *Typhula* incarnata, TPHAC = *Typhula* phacorrhiza, MNIV = Microdochium nivale, MBOR = Myriosclerotinia borealis, and WCIR = Waitea circinata.

APPENDIX 2

FUNGICIDE TRIAL RESULTS FOR BRECKENRIDGE, CO 2004-05 AND 2005-06

FUNGICIDE TRIAL RESULTS FOR VAIL, CO 2004-05, 2005-06, 2006-07, 2007-08, 2008-09, 2009-10, 2010-11

KENTUCKY BLUEGRASS (*Poa pratensis* cv. unknown) ANNUAL BLUEGRASS (*Poa annua* cv. unknown) Pink Snow Mold (*Michrodium nivale*) Gray Snow Mold (*Typhula* spp.) T.D. Blunt, N.A. Tisserat and S. Fichtner Bioagricultural Sciences and Pest Management Colorado State University Fort Collins, CO 80523-1177

Fungicide evaluation for the preventive control of snow molds on Kentucky Bluegrass and Annual Bluegrass, 2004-2005.

Experiments for control of pink and gray snow mold were performed on a Kentucky Bluegrass/Annual Bluegrass fairway at Breckenridge Golf Club, Fairway 4 Beaver, Breckenridge, CO (average elevation 9600 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of three treatments of fungicides were applied 1 Oct 04. A second application of the three treatments and the remainder of the treatments were applied 18 Oct 04. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 04 May 05.

Evaluations of the plots were later than the Vail plots due to the fact the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated differently this year than in previous years. Our rating system was change to a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Breckenridge, CO showed results consistent with disease pressure but not as severe of disease pressure as Vail. The Breckenridge course is mainly Kentucky Bluegrass which differs from Vail (mainly *Poa annua*) Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Table 1. Snow mold fungicide trials at Breckenridge Golf Club, Breckenridge, CO

Treatment and Rate/1000 sq. ft. Disea	se Rating
CL-EXP-2 4.0 oz + Daconil WS 6FL 5.5 fl oz	1.38k
CL-EXP-4 1.0 oz + Spectro 90WDG 5.76 oz	1.88jk
Syngenta Eperimental A14036 43.3SE 9.3 fl oz	
Spectro 90WDG 4.0 oz FB Spectro 90WDG 4.0 oz + CL-EXP-4 1.0 oz	2.50i-k
Medallion 50WDG 0.142 oz + Daconil WS 6FL 4.80 fl oz + BannerMAXX 1.3MEC 1.71 fl oz	3.38 i-k
Syngenta Experimental A14036 43.3SE 18.6 fl oz	4.50h-k
CL-EXP-2 4.0 oz + Daconil WS 6FL 5.5 fl oz + Magnum 3.5 fl oz	4.50h-k
Chipco 26GT 4.0 fl oz + Signature 80WDG 8.0 oz + Daconil Ultrex 82.5WDG 3.2 oz	5.25g-k
Endorse 2.5WP 4.0 oz + Spectro 90WDG 5.76 oz	
Syngenta Experimental A14212C 1.67EC 5.24 fl oz	6.63f-k
BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	
Syngenta Experimental A14036 43.3SE 4.7 fl oz	7.38f-k
Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz + Turfcide 400 8.0 fl oz	7.50e-k
Daconil WS 6FL 5.5 fl oz + Medallion 50WDG 0.5 oz	7.50e-k
BannerMAXX 1.3MEC 4.0 fl oz FB BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	8.63e-k
Chipco 26GT 4.0 fl oz + Compass 50WDG 0.25 oz + Signature 80WDG 8.0 oz +	
Turfcide 400 8.0 fl oz	
Syngenta Experimental A14212C 1.67EC 3.0 fl oz	
AND 4322G 10.0 lb	11.25e-k
Chipco 26GT 4.0 fl oz + Signature 80WDG 8.0 oz + Turfcide 400 8.0 fl oz	12.63e-k

AND 3118G 6.0 lb + AND 3237G 12.0 lb + AND 3238G 7.2 lb	12.75e-k
Spectro 90WDG 4.0 oz FB Spectro 90WDG 4.0 oz + Endorse 2.5WP 4.0 oz	15.25d-j
AND 4311G 6.6 lb	16.25d-i
AND 4309G 6.0 lb + AND 3237G 12.0 lb + AND 3238G 7.2 lb	17.50d-h
AND 4323G 6.6 lb	18.63c-g
Heritage 50WDG 0.4 oz + Medallion 50WDG 0.5 oz	19.38c-f
AND 4333G 9.0 lb	19.38c-f
Chipco 26GT 4 fl oz + Compass 50WDG 0.25 oz + Turfcide 400 8.0 fl oz	19.38c-f
Eagle 1.67EW 2.4 oz	21.25b-e
AND 4310G 8.0 lb	27.50b-d
AND 4334G 9.0 lb	28.75b-d
AND3223G 6.6 lb	31.88bc
	33.88b
Untreated Control	61.88a
LSD (α=0.05)	

ANNUAL BLUEGRASS (*Poa annua* cv. unknown) Pink Snow Mold (*Michrodium nivale*) Gray Snow Mold (*Typhula* spp.) T.D. Blunt, N. A. Tisserat and S. Fichtner Bioagricultural Sciences and Pest Management Colorado State University Fort Collins, CO 80523-1177

Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2004-2005.

Experiments for control of pink and gray snow mold were performed on an Annual Bluegrass fairway at Vail Golf Club, Fairway 14, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of three treatments of fungicides were applied 1 Oct 04. A second application of the three treatments and the remainder of the treatments were applied 15 Oct 04. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 22 Apr 05.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated differently this year than in previous years. Our rating system was change to a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

	ease Rating
Chipco 26GT 4 fl oz + Daconil Ultrex 82.5WDG 3.2 oz + Turfcide 400 8 fl oz	
Spectro 90WDG 4.0 oz FB Spectro 90WDG 4.0 oz + CL-EXP-4 1.0 oz	
Spectro 90WDG 4.0 oz FB Spectro 90WDG 4.0 oz + Endorse 2.5WP 4.0 oz	
Spectro 90WDG 5.76 oz + CL-EXP-4 1.0 oz	
Daconil WS 6FL 5.5 fl oz + CL-EXP-2 4.0 oz + Magnum 3.5 fl oz	
Daconil WS 6FL 5.5 fl oz + CL-EXP-2 4.0 oz	9.00l-o
Chipco 26GT 4.0 fl oz + Compass 50WDG 0.25 oz + Signature 80WDG 8.0 oz +	
Turfcide 400 8.0 fl oz	
Spectro 90WDG 5.76 oz + Endorse 2.5WP 4.0 oz	
Daconil WS 6FL 5.5 fl oz + Medallion 50WDG 0.5 oz	
Chipco 26GT 4.0 fl oz + Compass 50WDG 0.25 oz + Turfcide 400 8.0 fl oz	
Syngenta Experimental A14036 43.3SE 18.7 fl oz	
Syngenta Experimental A14036 43.3SE 9.3 fl oz	19.38j-o
Chipco 26GT 4.0 fl oz + Signature 80WDG 8.0 oz + Daconil Ultrex 82.5WDG 3.2 oz	20.63h-o
Medallion 50WDG 0.142 oz + Daconil WS 6FL 4.8 fl oz + BannerMAXX 1.3MEC 1.71 fl oz	22.50h-m
BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	•
Chipco 26GT 4.0 fl oz + Signature 80WDG 8.0 oz + Turfcide 400 8.0 fl oz	25.63f-l
Syngenta Experimental A14036 43.3SE 4.7 fl oz	25.63f-l
AND 3223G 6.6 lb	25.63f-l
BannerMAXX 1.3MEC 4.0 fl oz FB BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WDG 0.5 oz	28.375f-k
AND 4311G 6.6 lb	29.38f-k
AND 4322G 10 lb	32.50g-j
Heritage 50WDG 0.4 oz + Medallion 50WDG 0.5 oz	32.50g-j

AND 4323G 6.6 lb	36.88e-i
Syngenta Experimental A14212C 167EC 5.24 fl oz	38.13e-h
AND 4309F 6.0 lb + AND 3237G 12.0 lb + AND 3238G 7.2 lb	43.13e-g
AND 3118G 6.0 lb + AND 3237G 12.0 lb + AND 3238G 7.2 lb	43.75ef
Syngenta Experimental A14212C 167EC 3.0 fl oz	51.88de
AND 4334G 9.0 lb	62.50cd
AND 4310G 8.0 lb	65.63cd
AND 4333G 9.0 lb	70.63bc
Eagle 1.67EW 2.4 fl oz	86.25ab
Eagle 1.67EW 1.4 fl oz	89.38a
Untreated Control	
LSD (α=0.05)	18.37_

KENTUCKY BLUEGRASS (*Poa pratensis* cv. unknown) ANNUAL BLUEGRASS (*Poa annua* cv. unknown) Pink Snow Mold (*Michrodium nivale*) Gray Snow Mold (*Typhula* spp.)

T.D. Blunt, N.A. Tisserat Bioagricultural Sciences and Pest Management Colorado State University Fort Collins, CO 80523-1177

Fungicide evaluation for the preventive control of snow molds on Kentucky Bluegrass, 2005-2006.

Experiments for control of pink and gray snow mold were performed on a Kentucky Bluegrass fairway at Breckenridge Golf Club, Fairway 4 Beaver, Breckenridge, CO (average elevation 9300 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of three treatments of fungicides were applied 9 Sept 05. A second application of the three treatments and the remainder of the treatments were applied 24 Oct 05. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 03 May 06.

Evaluations of the plots were about average for Breckenridge even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated this year based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Breckenridge, CO showed results consistent with increased disease pressure as evidenced by the untreated control results, although Breckenridge had less overall disease incidence than Vail. There was also significant vole damage on the trial plots. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Table 1. Snow mold fungicide trials at Breckenridge Golf Club, Breckenridge, CO

Tuble 1. Show mold fulgicide thats at breekenninge oon elds, breekenninge, eo	
Treatment and Rate/1000 sq. ft. (unless otherwise noted) Dis	ease Rating
Instrata 3.61SE 11.0 fl oz	
Headway 167EC 5.25 fl oz + Daconil WeatherStik 6F 5.5 fl oz	
Insignia 20WP 0.7 oz + Rever 4F 8.0 fl oz + Manicure Ultrex 82.5WDG 3.2 oz	0.00k
Medallion 50WP 0.14 oz + Daconil WeatherStik 6F 2.36 fl oz + Banner MAXX 1.3ME 1.7 fl oz	0.25k
Insignia 20WP 0.7 oz + Iprodione Pro 2SE 4.0 fl oz + Manicure Ultrex 82.5WDG 3.2 oz	0.25k
Cleary 26/36 4.0 fl oz + Endorse 2.5WP 4.0 oz + Daconil Ultrex 82.5WDG 5.5 oz	0.25k
Endorse 2.5WP 4.0 oz + Spectro 90WDG 5.75 oz	0.50jk
Cleary 26/36 4.0 fl oz + CL-EXP-4 1.0 oz + Daconil Ultrex 82.5WDG 5.5 oz	
BannerMAXX 1.3ME 2.0 fl oz + Daconil WeatherStik 6F 5.5 fl oz	0.75jk
Insignia 20WDG 0.9 oz + Iprodione Pro 2SE 4.0 fl oz + Manicure Ultrex 82.5WDG 3.2 oz	0.75jk
18 Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz + Revere 4F 12.0 fl oz	0.75jk
Instrata 3.61SE 5.5 fl oz FB Instrata 3.61SE 5.5 fl oz	1.00jk
Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz + Turfcide 400 8.0 fl oz	1.25jk
Insignia 20WDG 0.7 oz FB 18 Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz	1.25jk
Spectator Ultra 1.3ME 4.0 fl oz + Insignia 20WDG 0.7 oz FB Manicure Ultra 82.5WDG 5.0 oz	1.25jk
CL-EXP-4 1.0 oz + Spectro 90WDG 5.75 oz	1.25jk
Insignia 20WDG 0.9 oz + Manicure Ultrex 82.5WDG 3.2 oz	
Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz	1.50jk
Rubigan AS 8.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	
Cleary 26/36 4.0 fl oz + Endorse 2.5WP 4.0 oz	3.00h-k
Instrata 3.61SE 9.3 fl oz	

Insignia 20WDG 0.9 oz + Iprodione Pro 2SE 4.0 fl oz + Revere 4F 8.0 fl oz	3.75h-k
Instrata 3.61SE 4.7 fl oz	5.00g-k
Heritage TL 0.8ME 2.0 fl oz + Medallion 50WP 0.5 oz + Daconil WeatherStik 6F 5.5 fl oz	5.00g-k
Daconil WeatherStik 6F 5.5 fl oz + Medallion 50WP 0.5 oz	8.25f-k
Turfcide 400 8.0 fl oz + Daconil Ultrex 82.5 WDG 3.2 oz	8.75f-k
18 Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz FB Revere 4F 12.0 fl oz	9.25f-k
Manicure Ultra 82.5WDG 5.0 oz FB PCNB 12.5 Plus 10-3-23E 6 lb	9.25f-k
Instrata 3.61SE 6.0 fl oz	9.50f-k
Chipco 26GT 4.0 fl oz + Lynx 22FL 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	9.50f-k
Headway 167EC 5.25 fl oz	
Heritage TL 0.8ME 2.0 fl oz + Medallion 50WP 0.5 oz	
Insignia 20WDG 0.7 oz FB Manicure Ultra 82.5WDG 5.0 oz + Revere 4F 12.0 fl oz	10.50f-k
Daconil Ultrex 82.5WDG 5.0 oz	
Banner MAXX 1.3ME 4.0 fl oz FB Banner MAXX 1.3ME 4.0 fl oz + Medallion 50WP 0.5 oz	11.25f-k
Rubigan AS 8.0 fl oz + Heritage 50WDG 0.4 oz	12.50f-k
Turfcide 400 12.0 fl oz + Lysone 20 ml/100 ft2	12.50f-k
Rubigan AS 8.0 fl oz + Turfcide 400 8.0 fl oz	12.75f-k
Turfcide 400 8.0 fl oz + Chipco 26GT 4.0 fl oz	13.25f-k
Revere 4F 12.0 fl oz	14.00f-k
Tartan 25SC 2.0 fl oz + Turfcide 400 8.0 fl oz	
Daconil Ultrex 82.5WDG 5.0 oz + Lysone 20 ml/100 ft2	L4.50e-k
Headway 167EC 5.25 fl oz + Medallion 50WP 0.5 oz	
Rubigan AS 8.0 fl oz	
Turfcide 400 16.0 fl oz	
Spectator Ultra 1.3ME 4.0 fl oz FB Revere 4F 12.0 fl oz	17.75d-k
Plant Helper 423 oz wt/a FB Plant Helper 423 oz wt/a	
Banner MAXX 1.3ME 2.0 fl oz + Turfcide 400 6.0 fl oz	21.75b-k
Turfcide 400 8.0 fl oz	
Turfcide 400 12.0 fl oz	
Turfcide 400 8.0 fl oz + Lysone 20 ml/100 ft2	
Chipco 26GT 4.0 fl oz + Lynx 22FL 1.0 fl oz + Signature 80WDG 4.0 oz	
Banner MAXX 1.3ME 4.0 fl oz + Medallion 50WP 0.5 oz	•
Chipco 26GT 4.0 fl oz + Lynx 22FL 1.0 fl oz	20 75h_f
Lysone 20 ml/100 ft2	31.25b-f
Lysone 20 ml/100 ft2 Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz	31.25b-f 37.50b-e
Lysone 20 ml/100 ft2 Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz Untreated Control	31.25b-f 37.50b-e 38.75b-d
Lysone 20 ml/100 ft2 Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz Untreated Control Plant Helper 1 lb a.i./100 gal FB Plant Helper 1 lb a.i./100 gal	31.25b-f 37.50b-e 38.75b-d 43.75a-c
Lysone 20 ml/100 ft2 Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz Untreated Control Plant Helper 1 lb a.i./100 gal FB Plant Helper 1 lb a.i./100 gal Untreated Control	31.25b-f 37.50b-e 38.75b-d 43.75a-c 45.00ab
Lysone 20 ml/100 ft2 Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz Untreated Control Plant Helper 1 lb a.i./100 gal FB Plant Helper 1 lb a.i./100 gal	31.25b-f 37.50b-e 38.75b-d 43.75a-c 45.00ab <u>64.00a</u>

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Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2005-2006.

Experiments for control of speckled and gray snow mold were performed on a Kentucky Bluegrass/Annual Bluegrass fairway at Vail Golf Club, Fairway 14, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of three treatments of fungicides were applied 9 Sept 05. A second application of the three treatments and the remainder of the treatments were applied 10 Oct 05. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 28 Apr 06.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated this year based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Table 1. Snow mold fungicide triais at Vali Golf Club, Vali, CO	
Treatment and Rate/1000 sq. ft. (unless otherwise noted)	Disease Rating
18Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz + Revere 4F	
Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz + Turfcide 400 8.0 fl oz	15.88uv
Insignia 20WDG 0.7 oz FB Manicure Ultra 82.5WDG 5.0 oz + Revere 4F 12.0 fl oz	17.50uv
BannerMAXX 1.3MEC 4.0 fl oz FB BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WP 0.5 o	oz 19.63uv
Insignia 20WDG 0.7 oz + Revere 4F 8.0 fl oz + Manicure Ultra 82.5WDG 3.2 oz	21.38uv
Instrata 3.61SE 5.5 fl oz FB Instrata 5.5 3.61SE 5.5 fl oz	24.63t-v
Cleary 26/36 4.0 fl oz + CL-EXP-4 1.0 oz + Daconil Ultrex 82.5WDG 5.5 oz	25.88s-v
CL-EXP-4 1.0 oz + Spectro 90WDG 5.75 oz	
Headway 167EC 5.25 fl oz + Medallion 50WP 0.5 oz	27.00r-v
Cleary 26/36 4.0 fl oz + Endorse 2.5WP 4.0 oz + Daconil Ultrex 82.5WDG 5.5 oz	29.25r-v
Tartan 25SC 2.0 fl oz + Turfcide 400 8.0 fl oz	31.75q-v
BannerMAXX 1.3MEC 4.0 fl oz + Medallion 50WP 0.5 oz	
Insignia 20WDG 0.9 oz + Iprodione Pro 2SE 4.0 fl oz + Revere 4F 8.0 fl oz	32.00p-v
Insignia 20WDG 0.9 oz + Iprodione Pro 2SE 4.0 fl oz + Manicure Ultra 82.5WDG 3.2 oz	32.50p-v
Instrata 3.61SE 11.0 fl oz	36.25o-v
Headway 167EC 5.25 fl oz	37.38n-v
Insignia 20WP 0.7 oz + Iprodione Pro 2SE 4.0 fl oz + Manicure Ultra 82.5WDG 3.2 oz	39.75m-v
Chipco 26GT 4.0 fl oz + Lynx 22F 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	
Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz	50.00k-t
Endorse 2.5WP 4.0 oz + Spectro 90WDG 5.75 oz	50.38k-t
Rubigan AS 8.0 fl oz + Heritage 50WDG 0.4 oz	
Revere 4000f 12.0 fl oz	53.00k-r

Headway 167EC 5.25 fl oz + Daconil WS 6F 5.5 fl oz	
Instrata 3.61SE 9.3 fl oz	
Heritage TL 0.8ME 2.0 fl oz + Medallion 50WP 0.5 oz+ Daconil WeatherStik 6F 5.5 fl oz	
Turfcide 400 12.0 fl oz + Lysone 20ml/100ft ²	•
Hertiage TL 0.8ME 2.0 fl oz + Medallion 50WP 0.5 oz	
0	0.1
Spectator Ultra 1.3MEC 4.0 fl oz + Insignia 20WDG 0.7 oz FB Manicure Ultra 82.5WDG 5.0 fl o	
Turfcide 400 8.0 fl oz + Chipco 26GT 4.0 fl oz	• •
Instrata 3.61SE 6.0 fl oz	•
Turfcide 400 12.0 fl oz	-
Turfcide 400 16.0 fl oz	0
Insignia 20WDG 0.7 oz FB 18Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz	
Daconil WeatherStik 6F 5.5 fl oz + Medallion 50WP 0.5 oz	
Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz	
Spectator Ultra 1.3MEC 4.0 fl oz FB Revere 4F 12.0 fl oz	
Rubigan AS 8.0 fl oz + Turfcide 400 8.0 fl oz	
Chipco 26GT 4.0 fl oz + Lynx 22FL 1.0 fl oz + Signature 80WDG 4.0 oz	
Turfcide 400 8.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	
Cleary 26/36 4.0 fl oz + Endorse2.5WP 4.0 oz	
Untreated Control	
Insignia 20WDG 0.9 oz + Manicure Ultrex 82.5WDG 3.2 oz	
Headway 167EC 3.0 fl oz FB Headway 167EC 3.0 fl oz	
Turfcide 400 8.0 fl oz + Lysone 20 ml/100 ft ²	. 77.13a-j
Instrata 3.61SE 4.7 fl oz	79.75a-i
Untreated Control	80.75a-i
Turfcide 400 8.0 fl oz	81.38a-i
Medallion 50WP 0.14 oz + Daconil WeatherStik 6f 2.36 fl oz + Banner MAXX 1.3ME 1.7 fl oz	82.13a-i
Banner MAXX 1.3ME 2.0 fl oz + Turfcide 400 6.0 fl oz	82.13a-i
Chipco 26GT 4.0 fl oz + Lynx 22FL 1.0 fl oz	82.50a-h
Banner MAXX 1.3ME 2.0 fl oz + Daconil WeatherStik 6F 5.5 fl oz	
18Plus 23.3F 4.0 fl oz + Manicure Ultra 1.3ME 5.0 fl oz FB Revere 4F 12.0 fl oz	83.75a-g
Manicure Ultra 1.3ME 5.0 fl oz FB PCNB 12.5 Plus 10-3-23E 6 lb	. 87.50a-f
Rubigan AS 8.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	89.38a-f
Plant Helper 1 lb a.i./100 gal FB Plant Helper 1 lb a.i./100 gal	
Rubigan AS 8.0 fl oz	
Daconil Ultrex 82.5WDG 5.0 oz	
Lysone 20ml/100 ft ²	
Plant Helper 423 oz wt/z FB Plant Helper 423 oz wt/a	
Daconil Ultrex 82.5WDG 5.0 oz + Lysone 20 ml/100 ft^2	
LSD (α=0.05)	

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Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2006-2007.

Experiments for control of speckled and gray snow mold were performed on an Annual Bluegrass fairway at Vail Golf Club, Fairway 14, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of three treatments of fungicides were applied 26 Sept 06. A second application of the three treatments and the remainder of the treatments were applied 24 Oct 06. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 26 Mar 07.

Snow had to be removed from plots prior to fungicide application in October. After application plots remained snow-free for two weeks. Snowmelt in late March occurred approximately two weeks earlier than normal. Nevertheless, disease pressure was hit with untreated plots averaging 89% plot area damaged. All fungicides except DPX-H6573 at 0.44 fl oz, DPX-LEM17 at 0.5 and 1.0 oz and DPX-YT669 at 0.425 fl oz reduced severity. However, most fungicide combinations did not provide acceptable levels of control (<10% damage). Plots were re-evaluated on 1 May 07 (results not shown) with most plots showing an increase in turf recovery from snow mold damage.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Table 1. Show mold fungicide trials at vali Gon Club, vali, CO	
Treatment and Rate/1000 sq. ft. (unless otherwise noted)	Disease Rating
Insignia 20WDG 0.7 oz FB Manicure Ultra 82.5WDG 5.0 oz + Revere 4F 12 fl oz	6.63n
Spectro 90WDG 4.0 oz FB Cleary 26/36 4 fl oz + Endorse 2.5WP 4.0 oz	8.00nm
Lynx 22FL 1.0 fl oz + Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5 WDG 5.0 oz	8.63l-n
Cleary 26/36 4.0 fl oz + CL-EXP-9 1.2 oz	8.75l-n
Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + CL-EXP-9 1.2 oz	9.00l-n
Insignia 20WDG 0.7 oz + Revere 4F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz	9.00l-n
Cleary 26/36 4.0 fl oz + Endorse 2.5 WP 4.0 WP	9.50k-n
Tartan 288SC 2.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	10.00k-n
TBZ + TFS Green 2.0 fl oz + Chipco 26GT 4.0 fl oz	14.38j-n
Insignia 20WDG 0.7 oz + BAS595 1.0 oz + Manicure Ultrex 82.5WDG 5.0 oz	15.50j-n
Spectro 90WDG 5.75 oz + CL-EXP-9 1.2 oz	16.50j-n
Tartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz	16.75j-n
Instrata 3.61SE 11.0 fl oz	18.00i-n
TBZ + TFS Green 2.0 fl oz	18.75i-n

balmer MAXA 1.5ME 2.0 H 02 + Datolili WeatherStik 6F 3.5 H 02 + STNC	Banner MAXX 1.3ME 2.0 fl oz + Daconil WeatherStik 6F 5.5 fl oz + SYNC	10 C2h n
Insignia 20WDG 0.7 oz FB Manicure Ultra 82.5WDG 5.0 oz + 18Plus 23.3F 4.0fl oz21.25h-nTartan 2885C 2.0 fl oz + Prostar 70DF 2.2 fl oz22.75g-nInsignia 20WDG 0.7 oz + Revere 4F 4.0 fl oz + Manicure Ultra 82.5WDG 4.0 oz23.00g-nInstrata 3.615E 9.0 fl oz23.50g-nMedallion 50WP 0.15 oz + Daconil WeatherStik 6f 2.5 fl oz + Banner Maxx 1.3ME 1.8fl oz25.00g-nSpectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz26.88f-n18 Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz + Revere 4F 12.0 fl oz27.25f-nBanner MAXX 1.3ME 2.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz + Medallion 50WP 0.5 oz28.63f-nBanner MAXX 1.3ME 2.0 fl oz + Daconil Ultrex 82.5 WDG 5.0 oz + Medallion 50WP 0.5 oz30.25f-nInsignia 20WDG 60.7 oz + Iprodione Pro2SE 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz38.36f-nTartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz33.75f-nTartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz33.75f-nTartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz34.63f-nDPX-LEM17 20SC 1.0 fl oz + DPX YT669 22.5SC 0.425 fl oz35.88f-lSpectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz44.63e-iSpectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz45.38e-iTurtricide 400 6.0 fl oz46.32e-iSpectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz45.38e-iSpectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz + SYNC45.38e-iSpectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz + SYNC45.32e-i		
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Insignia 20WDG 0.7 oz + Revere 4F 4.0 fl oz + Manicure Ultra 82.5WDG 4.0 oz 23.00g-n Instrata 3.615E 9.0 fl oz. 23.50g-n Medallion 50WP 0.15 oz + Daconil WeatherStik 6f 2.5 fl oz + Banner Maxx 1.3ME 1.8fl oz 25.00g-n Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz 26.88f-n 18 Plus 23.3F 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz + Revere 4F 12.0 fl oz 27.25f-n Banner MAXX 1.3ME 2.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz + Medallion 50WP 0.5 oz 30.25f-n Insignia 20WDG 0.7 oz + prodione Pro2SE 4.0 fl oz + Manicure Ultra 82.5WDG 5.0 oz 31.50f-n Armada 50WP 1.2 oz FB Revere 4F 12.0 fl oz. 33.75f-n Tartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz. 33.75f-n Tartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz. 35.88f-l Spectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz 41.25e-j Instrana 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz. 33.75f-n Tartan 288SC 2.0 fl oz + Chipco 26GT 6.0 fl oz. 35.88f-l Spectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz 41.25e-j Instrana 3.615E 5.0 fl oz. 44.63e-i Spectro 90WDG 4.0 oz + Cleary 26/36 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz + SYNC 45.38e-i Turfcide 400 1.0 fl oz. 46.35e-n		
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$\begin{aligned} & Medallion 50WP 0.15 \ oz + Daconil WeatherStik 6f 2.5 \ fl oz + Banner Maxx 1.3ME 1.8fl oz \\ & Spectro 90WDG 4.0 \ oz FE Cleary 26/36 4.0 \ fl oz + Daconil Ultrex 82.SVDG 5.5 \ oz \\ & Z6.88f - n \\ & B Plus 23.3F 4.0 \ fl oz + Manicure Ultra 82.SWDG 5.0 \ oz + Revere 4F 12.0 \ fl oz \\ & Z7.25f - n \\ & Cleary 26/36 4.0 \ fl oz + Daconil Ultrex 82.SWDG 5.5 \ oz \\ & Z7.50f - n \\ & Cleary 26/36 4.0 \ fl oz + Daconil Ultrex 82.SWDG 5.0 \ oz + Medallion SOWP 0.5 \ oz \\ & Z8.63f - n \\ & Banner MAXX 1.3ME 2.0 \ fl oz + Daconil Ultrex 82.SWDG 5.0 \ oz + Medallion SOWP 0.5 \ oz \\ & Z8.63f - n \\ & Banner MAXX 1.3ME 2.0 \ fl oz + Daconil Ultrex 82.SWDG 5.0 \ oz + Medallion SOWP 0.5 \ oz \\ & Z8.63f - n \\ & Insignia 20WDGG 0.7 \ oz + Iprodione Pro2SE 4.0 \ fl oz + Manicure Ultra 82.SWDG 5.0 \ oz \\ & Z8.63f - n \\ & Intra Insignia 20WDGG 0.7 \ oz + Iprodione Pro2SE 4.0 \ fl oz + Manicure Ultra 82.SWDG 5.0 \ oz \\ & Z3.563f - N \\ & Arrada 25SSC 2.0 \ fl oz + Clapco 26GT 6.0 \ fl oz \\ & Z9.5C 0.425 \ fl oz \\ & Z9.5 C0.22 \\ & Z3.588f - Z9.55 C0.4255 I Oz \\ & Z9.5 C0.4255 Oz \\ & Z9.5 Oz \\$		•
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Oilseed radish seed 1 day germination 100g/100 ft ²	Oilseed radish seed 6 day germination 500 g/100 ft ²	95.63a
Mustard seed 1 day germination 500 g/100 ft ²		
LSD (α=0.05)	Mustard seed 1 day germination 500 g/100 ft ²	<u>98.75a</u>
	<u>LSD (α=0.05)</u>	27.42

T.D. Blunt and N. A. Tisserat Bioagricultural Sciences and Pest Management Colorado State University Fort Collins, CO 80523-1177

Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2007-2008.

Experiments for control of speckled and gray snow mold were performed on an Annual Bluegrass fairway at Vail Golf Club, Fairway 14, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of several treatments of fungicides were applied 27 Sept 07. A second application of the initial treatments and the remainder of the treatments were applied 24 Oct 07. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 12 May 08.

Snow cover this field season was delayed. The first permanent snow was on or about 19 Nov 07. This season had greater snow cover than had been seen in the previous 3 years. The depth of the snow, at one time, was about 4 feet deep. Final snow melt from the trial plots was about 10 May 08, which was about two weeks later than normal. At evaluation time, there was still snow cover on shaded parts of the golf course. Due to the length and amount of snow cover, disease pressure was high. The untreated plots averaged 98% area damaged. Even though there were several fungicide combinations that provided adequate control (<10% damage) and there were a few combinations that were close to the 10% control, the majority of the fungicide combinations did not provide acceptable levels of control.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Table 1. Snow mold fungicide trials at Vail Golf Club, Vail, CO	
Treatment and Rate/1000 sq. ft. (unless otherwise noted) Disea	ase Rating
Spectro 90WDG 4.0 oz FB Clearys 26/36 8.0 fl oz + CL-EXP-9 1.2 oz	1.50i
Spectro 90WDG 4.0 oz FB CL-EXP-9 1.2 oz + Endorse 2.5WP 4.0 oz	2.25i
Reserve 575SC 3.8 fl oz + Compass 50WDG 0.25 oz	2.25i
Spectro 90WDG 5.75 oz + CL-EXP-9 1.2 oz	2.75i
Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + CL-EXP-9 1.2 oz	4.50hi
Lynx 240SC 1.5 fl oz + Compass 50WG 0.25 oz + Daconil Ultrex 82.5WDG 5.0 oz	7.00g-i
Instrata 3.61SE 9.3 fl oz + Medallion 50WP 0.25 oz	7.00g-i
Spectro 90WDG 4.0 oz FB Clearys 26/36 8.0 fl oz + Endorse 2.5WP 4.0 oz	7.50g-i
Reserve 575SC 7.6 fl oz + Chipco 26GT 4.0 fl oz	10.50f-i
Spectro 90WDG 4.0 FB Cleary 26/36 4.0 fl oz + Endorse 4.0 oz	10.50f-i
Instrata 3.61SE 11.0 fl oz + Medallion 50WP 0.25oz	12.50f-i
Insignia 20WDG 0.7 oz + Trinity 19.2SC 1.0 fl oz + Turfcide 400 6.0 fl oz	13.75e-i
Instrata 3.61SE 11.0 fl oz	14.25e-i

Lynx 240SC 1.5 fl oz + Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	18.75d-i
Insignia 20WDG 0.7 oz + Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	
Trinity 19.2SC 1.0 fl oz + Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	20.00d-i
Insignia 20WDG 0.7 oz + Chipco 26GT 4.0 fl oz + Turfcide 400 6.0 fl oz	22.50d-i
Banner MAXX 1.3ME 4.0 fl oz + Daconil Ultrex 82.5WDG 4.5 oz + Medallion 50WP 0.33 oz	25.00c-h
Instrata 3.61SE 5.5 fl oz FB Instrata 3.61 5.5 fl oz	27.50c-g
Instrata 3.61SE 7.0 fl oz FB Instrata 3.61SE 7.0 fl oz	30.00c-f
Instrata 3.61SE 9.3 fl oz	31.25c-f
Banner MAXX 1.3ME 3.25 fl oz + Daconil Ultrex 82.5WDG 4.5 oz + Medallion 50WP 0.27 oz	31.25c-f
Tartan 288SC 2.0 fl oz + Turfcide 400 6.0 fl oz	34.25c-e
Insignia 20WDG 0.7 oz + Trinity 19.2SC 1.0 fl oz + Chipco 26GT 4.0 fl oz	36.25cd
Insignia 20WDG 0.7 oz + Trinity 19.2SC 1.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	37.25cd
Tartan 288SC 2.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	45.75bc
Reserve 575SC 7.6 fl oz	59.50
Instrata 3.61SE 9.3 fl oz	65.00b
Untreated Control	98.25a
LSD (α=0.05)	

T.D. Blunt and N. A. Tisserat **Bioagricultural Sciences and Pest Management** Colorado State University Fort Collins, CO 80523-1177

Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2008-2009.

Experiments for control of speckled and gray snow mold were performed on an Annual Bluegrass fairway at Vail Golf Club, Fairway 14, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of several treatments of fungicides were applied 26 Sept 08. A second application of the initial treatments and the remainder of the treatments were applied 29 Oct 08. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO^2 -powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 27 April 09.

The first permanent snow was on or about 15 Nov 08. This season had as much snow cover as the previous year. At evaluation time, there was still snow cover on parts of the golf course. At the time of evaluation, there was still a small amount of snow on the trial plots that needed to be shoveled off in order to rate the effectiveness of the treatments. Disease pressure was high again this year. The untreated plots averaged 92% area damaged. Even though there were several fungicide combinations that provided adequate control (<10% damage) and there were a few combinations that were close to the 10% control, the majority of the fungicides or combinations did not provide acceptable levels of control.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α=0.05).

Table 1. Snow mold fungicide trials at Vail Golf Club, Vail, CO	
Treatment and Rate/1000 sq. ft. (unless otherwise noted) Disea	ase Rating_
Spectro 90WDG 4.0 oz FB Cleary 26/36 8.0 fl oz + CL-EXP-9 1.2 oz	4.50s
Tartan 299SC 2.0 fl oz + Daconil Ultrex 82.5WDG 5.0 oz	5.25rs
Spectro 90WDG 4.0 FB Instrata 3.61SE 11.0 fl oz + Medallion 50WP 0.25oz	6.25rs
Pegasus HPX 3.6 fl oz + Dovetail 39.3EC 4.0 fl oz + KestralMEX 1.3ME 3.0 fl oz +	
Disarm SC 0.36 fl oz	6.50rs
Triton Flo 367SC 0.85 fl oz + Compass 50WG 0.25 oz + Daconil Ultrex 82.5WDG 5.0 oz	6.50rs
Reserve 4.8SC 5.4 fl oz + Compass 50WG 0.25 oz	6.75q-s
Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + CL-EXP-9 1.2 oz	7.50q-s
Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + Endorse 2.5WP 4.0 oz + CL-EXP-11 6.0 fl oz .	8.50p-s
Instrata 3.61SE 8.0 fl oz + Headway 167EC 1.5 fl oz	10.00o-s
Headway 167EC 3.0 fl oz + Concert 4.3SC 8.25 fl oz	12.00n-s
USF26019T 6.0 fl oz + Triton Flo 367SC 0.85 fl oz	12.00n-s
Spectro 90WDG 4.0 oz FB CL-EXP-9 1.2 oz + Endorse 2.5WP 4.0 oz	12.00n-s

Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + Endorse 4.0 2.5WP 4.0 oz Triton Flo 367SC 1.1 fl oz + Compass 50WG 0.2 oz + Daconil Ultrex 82.5WDG 5.0 oz Spectro 90WDG 5.75 oz + CL-EXP-9 1.2 oz USF26019T 5.0 fl oz + Triton Flo 367SC 0.85 fl oz Instrata 3.61SE 5.5 fl oz FB Instrata 3.61SE 5.5 fl oz Spectro 90WDG 4.0 oz FB Cleary 26/36 4.0 fl oz + CL-EXP-9 1.2 oz Curalan EG 50WP 1.0 oz + Daconil Ultrex 82.5WDG FB	12.75n-s 13.00n-s 13.25n-s 15.00m-s 15.50m-s
Insignia 20WDG 0.5 oz + Trinity 1.67SC 1.0 fl oz	
Banner MAXX 1.3ME 2.0 fl oz FB Instrata 3.61SE 11.0 fl oz + Medallion 50WP 0.25 oz	
Instrata 3.61SE 11.0 fl oz.	18.25m-s
Banner MAXX 1.3ME 4.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz + Medallion 50WP +	10.75
Chipco 26GT 4.0 fl oz	
Pegasus HPX 5.5 fl oz + Dovetail 39.3EC 4.0 fl oz + Disarm 0.25G 0.36 oz	
Banner MAXX 1.3 ME 4.0 fl oz + Medallion 50WP 0.4 oz + Chipco 26GT 4.0 fl oz	
USF26019T 4.0 fl oz + Triton Flo 367SC 0.85 fl oz Disarm 480SC 3.0 fl oz + Chipco 26GT 4.0 fl oz	
Instrata 3.61SE 9.3 fl oz	
Tourney 50WDG 0.44 oz + Cleary's 3336 4.0 fl oz	
Curalan EG 50WG 1.0 oz + Daconil Ultrex 82.5WDG 3.2 oz FB Trinity 1.67SC 1.5 fl oz +	51.251-0
Daconil Ultrex 82.5WDG 3.2 oz.	33 75h-n
Turfcide 400 6.0 fl oz + Spectro 90WDG 4.0 oz	
Tourney 50WDG 0.44 oz + Daconil Ultrex 82.5WDG 3.2 oz FB Tourney 50WDG 0.44 oz +	57100 <u>5</u> III
Daconil Ultrex 82.5WDG 3.2 oz.	42.00f-l
Trinity 1.67SC 1.0 fl oz + Insignia 20WDG 0.5 oz	
AND3150 5 lb FB AND8076 5.95 lb + AND5017 6.66 lb	
Trinity 1.67SC 1.5 fl oz + Insignia 20WDG 0.5 oz	48.00f-j
Tourney 50WDG 0.44 oz FB Tourney 50WDG 0.44 oz	51.25e-i
AND5017 6.66 lb	55.00e-h
Turfcide 400 9.0 fl oz	57.50d-g
AND3150 5.0 lb FB AND5017 6.66 lb	62.00c-f
Turfcide 400 12.0 fl oz	
AND3150 3.75 lb FB AND5017 3.33 lb	70.50b-e
Disarm-G 4.5 lb	
AND5017 3.33 lb	77.75a-d
Disarm 480SC 0.36 fl oz	
Insignia 20WDG 0.7 oz	
Rhapsody 60EC 5.0 fl oz + Insignia 20WDG 0.35 oz	
Turfcide 400 6.0 fl oz	
Untreated Control	
Untreated Control	
LSD (α=0.05)	22.25

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Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2009-2010.

Experiments for control of speckled and gray snow mold were performed on an Annual Bluegrass fairway at Vail Golf Club, Fairway 14, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of several treatments of fungicides were applied 2 Oct 09. A second application of the initial treatments and the remainder of the treatments were applied 05 Nov 09. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 10 May 10.

Snow cover this field season was delayed. The first permanent snow was on or about 19 Nov 10. First snow melt was in Apr 10 with additional snow cover after the first melt. Final snow melt from the trial plots was about 06 May 10, which was about two weeks later than normal. At evaluation time, there was still snow cover on shaded parts of the golf course. Disease pressure on the fairway was consistent with previous years. Even though there were several fungicide combinations that provided adequate control (<20% damage) and there were a couple combinations that were close to the 10% control threshold, about half of the treatment combinations did not provide acceptable levels of control.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Treatment and Rate/1000 sq. ft. (unless otherwise noted)	Disease Rating
Reserve 4.8SC 5.4fl oz + Compass 50WG 0.25 oz	9.67h
Instrata 3.61SE 5.5 fl oz FB Instrata 3.61SE 5.5 fl oz	10.00h
Instrata 3.61SE 5.5 fl oz + Fore 80WP 8.0 oz FB Instrata 3.61SE 5.5 fl oz + Fore 80WP 8.0	oz 10.33gh
Insignia 20WDG 0.54 oz + Trinity 1.67SC 1.0 oz + Iprodione Pro 2SE 4.0 fl oz	11.00gh
Tourney 50WDG 0.37 oz + Cleary 3336 4.0 fl oz FB	
Tourney 50WDG 0.37 oz + Cleary 3336 4.0 fl oz	11.67gh
Instrata 3.61SE 11.0 fl oz	11.67gh
Interface 24.5SC 4.0 fl oz + Turfcide 400 8.0 fl oz	
Interface 24.5SC 5.0 fl oz + Triton Flo 367SC 0.85 fl oz	12.67f-h
Daconil Ultrex 82.5WDG 5.5 oz + Medallion 50WP 0.50 oz + Banner MAXX 1.3ME 4.0 fl	oz 12.67f-h
Interface 24.5SC 6.0 fl oz + Triton Flo 367SC 0.85 fl oz	14.33f-h
V10277 45G 2.4 lb + Cleary 3336 4.0 fl oz FB V10277 45G 2.4 lb + Cleary 3336 4.0 fl oz	15.00f-h
Turfcide 400 16.0 fl oz	16.00e-h
Insignia 20SC 0.54 oz + Trinity 1.67SC 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	16.67e-h

Reserve 4.8SC 4.5 fl oz + Compass 50WG 0.20 oz	16.67e-h
Kestrel MEX 1.3ME 4.0 fl oz + Pegasus HPX 5.5 fl oz FB	
Kestrel MEX 1.3ME 4.0 oz + Raven 2SC 4.0 fl oz	16.67e-h
Daconil Ultrex 82.5WDG 3.2 oz + Medallion 50WP 0.50 oz + Banner MAXX 1.3ME 4.0 fl oz	16.67e-h
Turfcide 400 12 fl oz + Rhapsody 60EC 5.0 fl oz	18.33e-h
Peregrine 66WDG 4.0 oz + Pegasus HPX 3.0 fl oz + Kestrel MEX 1.3ME 4.0 fl oz +	
Raven 2SC 4.0 fl oz	18.33e-h
Instrata 3.61SE 11.0 fl oz + Medallion 50WP 0.17 oz	19.33e-h
Interface 24.5SC 4.0 fl oz + Triton Flo 367SC 0.85 fl oz	20.00e-h
Honor 28WG 0.83 oz + Trinity 1.67SC 1.0 oz + Daconil Ultrex 82.5WDG 3.2 oz	20.00e-h
Insignia 20WG 0.7 oz + Tourney 50WDG 0.37 oz + Daconil Ultrex 82.5WDG 5.5 oz	
Curalan EG 50WDG 1.0 oz + Daconil Ultrex 82.5WDG 3.2 oz FB	
Insignia 20WG 0.7 oz + Trinity 1.67SC 1.0 oz + Daconil Ultrex 82.5WDG 3.2 oz	23.33d-h
Reserve 4.8SC 4.5 fl oz + Compass 50WG 0.25 oz	23.33d-h
Disarm 480SC 5.8 fl oz + Raven2SC 4.0 fl oz	23.33d-h
V10277 45G 2.4 lb FB V10277 45G 2.4 lb	26.67d-h
Chipco 26GT 4.0 fl oz + Medallion 50WP 0.5 oz + Banner MAXX 1.3ME 4.0 fl oz	26.67d-h
Kestrel MEX 1.3ME 4.0 fl oz FB Disarm 480SC 5.8 fl oz + Raven 2SC 4.0 fl oz	28.33d-g
Chipco 26GT 4.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz + Medallion 50WP 0.5 oz +	
Banner MAXX 1.3ME 4.0 fl oz	28.33d-g
Disarm M 1.0 fl oz + Pegasus DF 3.6 fl oz	30.00d-f
Daconil Ultrex 82.5WDG 5.5 oz + Medallion 50WP 0.25oz + Banner MAXX 1.3ME 4.0 fl oz	
Pegasus L 3.6 fl oz + Kestrel MEX 1.3ME 3.0 fl oz + Dovetail 39.3EC 4.0 fl oz +	
Disarm 480SC 0.36 fl oz	40.00cd
Tourney 50WDG 0.37 oz FB Tourney 50WDG 0.37 oz	53.33bc
Rhapsody 60EC 5.0 fl oz	
Untreated Control	81.67a
LSD (α=0.05)	

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Fungicide evaluation for the preventive control of snow molds on Annual Bluegrass, 2010-2011.

Experiments for control of speckled and gray snow mold were performed on an Annual Bluegrass fairway at Vail Golf Club, Fairway 16, Vail, CO (average elevation 8150 ft.). Treatments were arranged in a randomized complete block design with four replications. Individual plots measured 5 ft. X 5 ft. First applications of several treatments of fungicides were applied 1 Oct 10. A second application of the initial treatments and the remainder of the treatments were applied 02 Nov 10. All fungicides were applied in the equivalent of 5 gal. water/1000 sq. ft. A CO²-powered sprayer equipped with 8003 Tee-jet nozzles delivered the treatments at 30 psi. Fungicide effectiveness was evaluated after snowmelt on 06 May 10.

Snow cover this field season was earlier than in previous years. The first snow was on or about 25 Oct 10. Final snow melt from the trial plots was about 06 May 11, which was about two weeks later than normal. At evaluation time, there was still snow cover on shaded parts of the golf course. This year we had a La Niña weather pattern which provided a total of 524 inches of snow over the entire season. Disease pressure on the fairway was consistent with previous years. The majority of the treatments provided control of <20% disease incidence.

Evaluations of the plots were about average for Vail even though the amount of snow cover received in the central Rocky Mountains was substantial. The plots were evaluated based on a percent of disease incidence per treatment that more accurately represents the actual disease present in each treatment/plot. The plots at Vail, CO showed results consistent with increased disease pressure as evidenced by the untreated control results. Results were calculated using SAS system proc glm calculating the means of the four replications and using Fisher's Protected Least Significant Difference (α =0.05).

Treatment and Rate/1000 sq. ft. (unless otherwise noted)	Disease Rating
Interface 24.5SC 6.0 fl oz + Triton Flo3667SC 0.85 fl oz	0.00g
Pegasus HPX 2.75 fl oz + Kestrel MEX 1.3ME 2.0 fl oz + Dovetail 39.3 4.0 fl oz +	
Disarm 480SC 0.18 fl oz FB Pegasus HPX 2.75 fl oz + Kestrel MEX 1.3ME 2.0 fl oz +	
Dovetail 39.3 4.0 fl oz + Disarm 480SC 0.18 fl oz	0.00g
Torque 3.6SE 0.9 fl oz + Clearys 26/36 4.0 fl oz	0.75g
Instrata 3.61SE 5.5 fl oz FB Instrata 3.61SE 5.5 fl oz	0.75g
Reserve 4.8SC 5.4 fl oz + Interface 24.5SC 6.0 fl oz	1.00g
Torque 3.6SE 0.6 fl oz + Clearys 26/36 4.0 fl oz	1.00g
Instrata 3.61SE 5.0 fl oz + Medallion 50WP 0.25 oz + Banner MAXX 1.3ME 2.0 fl oz +	
Daconil Ultrex 82.5WDG 2.0 oz	1.25g
Peregrine 66WDG 6.0 oz + Kestrel MEX 1.3ME 2.0 fl oz FB	
Peregrine 66WDG 6.0 oz + Kestrel MEX 1.3ME 2.0 fl oz	1.25g
Instrata 3.61SE 11.0 fl oz + Medallion 50WP 0.25 oz	1.75g
Instrata 3.61SE 11.0 fl oz	3.00e-g
Torque 3.6SE 0.6 fl oz + Affirm WDG 0.9 oz + Spectro 90WDG 3.67 oz	3.75e-g

Instrata 3.61SE 9.0 fl oz + Banner MAXX 1.3ME 2.0 fl oz + Daconil Ultrex 82.5WDG 2.0 oz Pegasus HPX 5.50 fl oz + Kestrel MEX 1.3ME 4.0 fl oz + Dovetail 39.3 8.0 fl oz +	3.75e-g
Disarm 480SC 0.36 fl oz	4.00e-g
Reserve 4.8SC 5.4 fl oz + Compass 50WG 0.25 oz	4.50e-g
Torque 3.6SE 0.6 fl oz + Affirm WDG 0.9 oz	4.50e-g
Instrata 3.61SE 9.0 fl oz + Medallion 50WP 0.25 oz + Banner MAXX 1.3ME 2.0 fl oz +	
Daconil Ultrex 82.5WDG 2.0 oz	5.00e-g
Curalan EG 50WG 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz FB	
Insignia 20WDG 0.54 oz + Trinity 1.67SC 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	5.25e-g
Torque 3.6SE 0.6 fl oz + Clearys 26/36 4.0 fl oz + Spectro 90WDG 3.67 oz	6.25e-g
Interface 24.5SC 3.0 fl oz + Triton Flo 367SC 0.5 fl oz FB	
Interface 24.5SC 3.0 fl oz + Triton Flo 367SC 0.5 fl oz	-
Torque 3.6SE 0.9 oz + Clearys 26/36 4.0 fl oz + Spectro 90WDG 3.67 oz	7.50e-g
Insignia 20WDG 0.7 oz + Trinity 1.67SC 1.5 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	8.50e-g
Banner MAXX 1.3ME 2.0 fl oz + Daconil Ultrex 82.5WDG 5.5 oz	8.75e-g
Interface 24.5SC 5.0 fl oz + Reserve 4.8SC 5.4 fl oz	
Spectro 90WDG 5.0 oz	19.25d-f
Insignia 20WDG 0.54 oz + Trinity 1.67SC 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	20.00с-е
Instrata 3.61SE 5.0 fl oz + Banner MAXX 1.3ME 2.0 fl oz + Daconil Ultrex 82.5WDG 2.0 oz	32.50cd
Honor 28WG 0.84 oz + Trinity 1.67SC 1.0 fl oz + Daconil Ultrex 82.5WDG 3.2 oz	34.50cd
Torque 3.6SE 0.9 fl oz + Daconil Ultrex 82.5WDG 5.5 oz	36.25c
Torque 3.6SE 0.6 fl oz + Daconil Ultrex 82.5WDG 5.5 oz	60.00b
Untreated Control	90.00a
LSD (α=0.05)	16.37

APPENDIX 3

OPTIMIZING THE GC/MS PROTOCOL

The process of optimizing the GC/MS protocol using chlorothalonil analytical standards was started in August of 2005. The objective was to determine the amount of chlorothalonil recovery we could expect. Initially, the concentrations used were 10, 25, 50, 75 and 100 ng/ml dilutions of a 0.1 mg/ml analytic standard of chlorothalonil in acetylnitrile combined with 10 µl of a 0.5 ng/ml butylate internal standard stock solution (50 µl of butylate in 100 ml toluene). This initial run on the GC/MS was satisfactory and the process continued. The analytical standards along with the internal standards were run three separate times and worked well, so the next step was to see which solvent(s) would work best to extract chlorothalonil from leaf tissue. Since chlorothalonil is a nonpolar solution, water is the extraction medium of choice to get it off the turf tissue, however, water cannot be used in the GC/MS runs. Toluene is generally the solvent of choice for the machine. A mixture of acetone or methanol and water at various percentages (25, 50 or 75%) as first solvent solutions were attempted. After the extraction process, the acetone or methanol was evaporated using a RapidVac at 50°C, 60 vortex speed, then adding toluene to the remaining water to extract the chlorothalonil into the toluene. These results were acceptable, but not good. The next step was to try acidifying the solution of acetone and water using 0.5% phosphoric acid. With this extraction method, we only recovered approximately 80% of the chlorothalonil standard. Using acetone as an extraction solvent also increased the amount of chlorophyll extracted when using inoculated turf. After experimenting with several iterations of acetone/water or methanol/water extraction methods, it was determined that neither acetone or methanol would be an acceptable solvent to extract chlorothalonil. Also, because of the increased amount of chlorophyll extracted along with the chlorothalonil,

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the results of acetone and methanol were not acceptable. Also, in the presence of acetone and acetonitrile (MeCN), chlorothalonil would degrade in the clear sampling vials but remain stable in amber colored vials (Mastovska and Lehotay, 2004). After experimenting with several iterations of acetone/water and methanol/water using inoculated turf and tomato plants, and still having to use toluene as the end solvent for the GC runs, it was decided to try extracting directly into toluene. Since the vials used for sampling were clear, toluene was the superior exchange solvent. It was also determined that the starting weight of 1g of turf tissue was too large a sample size. In optimizing the protocol, it was determined that 0.20g turf tissue in 10 ml diH2O and 10 ml toluene would extract an amount of chlorothalonil that would not overwhelm the GC/MS and would produce consistent results.