Efficacy of *Trichoderma* fungal biocontrol agents for the control of snow mould disease in turf grass

Final Report:
March 2021 revised September 2021

For

From
Institute for Sustainable Horticulture
Kwantlen Polytechnic University
12666 72nd Ave., Surrey, BC, V3W 2M8

Director:
Deborah Henderson PhD
LEEF Regional Innovation Chair
604-599-3460
deborah.henderson@kpu.ca

Research Team:
Matilda Tabert, BSc
Michelle Franklin, PhD
Lisa Wegener, MSc

Funding for this project has been provided by the Western Canada Turfgrass Association

ABSTRACT

Pink snow mould, caused by *Microdochium nivale*, is a major issue in Western Canada, causing reductions in revenue due to reduced turf quality on golf greens. Interest in alternative control measures to chemical pesticides has increased over the last few decades due to environmental concerns and pesticide bans and restrictions. One agent of keen interest is the beneficial fungal genus *Trichoderma*. Two local isolates of *T. atroviride* and *T. harzianum* from the Fraser Valley were investigated for potential control of *M. nivale* in laboratory assays and in a field trial. *In vitro* laboratory bioassays showed that the strain of *T. atroviride*, TA-222, exhibited suppression in the growth of *M. nivale* of 31%-37% and was able to overgrow the pathogen and sporulate abundantly. The *T. harzianum* strain, TH-238, reduced the growth of the pathogen by 25-28% and was also able to overgrow the pathogen and sporulate. When comparing the two isolates, *T. atroviride* (TA-222) always caused greater suppression. In the field, turf grass plots were treated with liquid cultures of both isolates, pure spore powder of *T. atroviride*, and three commercial products, namely Rootshield Plus™ and Rhizovital™ (*Bacillus amyloliquifaciens*), a positive control (Banner Maxx™) and a negative control (water). Treatment applications began in May 2020 and continued monthly until October 2020. Percent area damage was analysed prior to treatment application and after one month of application. A final assessment in February 2021 was conducted to determine the effect of 6 months of treatment application. Mean percent damage decreased one month after treatment application for all plots with no effect by treatment. The final analysis, in February 2021, demonstrated no treatment effect on the percent damage caused by pink snow mould. The reasons for the lack of performance of all control measures were likely due to heavy rains in October and very high disease pressure. Strategies for using biologicals for snow mould control were discussed.

INTRODUCTION

Snow moulds cause serious problems in turf grass in regions where the winters are cold and turf is snow covered (Dicklow, 2011; Nelson, 2004). There are several causal agents of snow mould; the three main fungi are *Typhula ishikariensis, T. incarnate* and *Microdochium nivale* (Jung et al, 2007). These pathogens cause patches of discoloured and water-soaked grass and delay turf

recovery after winter, which, in turn, reduces the quality of the turf and playability on golf courses (Jung et al, 2007; Nelson, 2004). For this reason, golf courses in the Pacific Northwest spend approximately $20,000 annually on fungicides for the control of snow mould diseases (Mattox et al, 2015).

Pink snow mould, *Microdochium nivale*, is one of the most common snow moulds affecting turf grass in Western Canada. This pathogen does not require snow cover in the winter to facilitate disease progression and can be active all year long in areas with cool, wet climates (Latin, 2019; Jung et al 2007; McBeath, 2002). At temperatures less than 5°C, *M. nivale* is not as aggressive as the other snow moulds, however, it can grow rapidly in temperatures up to 20°C on turf with abundant surface moisture, and has an optimal growth temperature of 25°C *in vitro* (McBeath, 2002; Dicklow, 2011). *M. nivale* survives suboptimal conditions in decaying turf debris and infected leaf material (Latin, 2019; McBeath, 2002).

The search for alternative methods for the control of snow mould has been ongoing for several decades (Nelson, 2004). The rise in pesticide bans and restrictions have made it difficult for turf management that relies heavily on the use of fungicides to control this disease (Mattox et al, 2015; Nelson, 2004). Several biological agents have been investigated for the control of snow moulds, including *Typhula phacorrhiza, Pseudomonas aureofaciens* and *Trichoderma atroviride* (Nelson, 2004; Hsiang, 2000; McBeath, 2002).

Many members of the *Trichoderma* genus are hyperparasites that have the potential to suppress plant pathogens (Harman, 2006). An Alaskan isolate of *T. atroviride* has been found to suppress *M. nivale*. *T. atroviride* penetrates the cell walls of the hyphae and feeds directly on pathogenic fungi (McBeath, 2002). It is possible that other isolates of this species, and of other species in the genus *Trichoderma*, could be potential biological control agents for *M. nivale*.

The objectives of this study are:

1. To survey and identify the fungi responsible for snow mould disease on the KPU golf course and other golf courses throughout British Columbia,

2. To compare the interactions of two native BC *Trichoderma* isolates (TA-222 and TH-238) and collected snow mould strains in laboratory in-vitro assays.

3. To determine the efficacy of two native *Trichoderma* species applied as soil drenches (*T. atroviride,* TA-222 and *T. harzianum*, TH-238) for the control of pink snow mould on a golf course (KPU golf course)

METHODS

Survey

The golf industry was made aware of the project through sharing the proposal in spring 2020. A letter was sent out across British Columbia for the submission of snow mould samples in September 2020 (a copy of the letter can be found in Appendix 1).

Identification of snow mold pathogens

Plugs were collected from a golf green on the Kwantlen Polytechnic University (KPU) School of Horticulture teaching golf course (KPU Langley campus, 20901 Langley Bypass, Langley British Columbia) and incubated in a plastic bag lined with wet paper towel at room temperature in the dark for one week. Samples were observed visually for fungal growth. Blades of grass and roots exhibiting fungal growth were placed on Potato Dextrose Agar (PDA) amended with Streptomycin (0.005%). Plates were incubated at room temperature in the dark for two days and observed for fungal growth. Snow moulds caused by *Typhula* spp. are not common in the Fraser Valley (J. Elmhirst, pers. comm.) and therefore samples with fungal growth characteristic of snow mould, were treated with a protocol used for *M. nivale;* culturing on fresh PDA plates and incubated at room temperature in the dark for one week. To extract DNA, a sterile scalpel was used to scrape each culture (~ 1cm area) and the fungal material was placed into a Fast Prep Lysing Matrix tube containing 1 ml of DNA lysis buffer, and DNA was extracted following the Macherey-Nagel Nucleospin Kit ™ instructions. Subsequently, PCR was performed using ITS 1 and ITS 4 primers (White et al. 1990). Amplified DNA was visualized on a 1.5% agarose gel to confirm the presence of DNA. Samples were sent to an external lab (Psomagen, Maryland USA) for bidirectional DNA sequencing. Forward and reverse sequences were aligned using DNASTAR ™ software, and the

consensus sequence was analyzed using the National Centre for Biotechnology Information online search tool, BLAST (Altschul, et al, 1990).

The same method was used for the identification of snow mould pathogens from samples submitted by BC golf courses.

Laboratory antagonism assays

The antagonism of *Microdochium nivale* by *Trichoderma atroviride* TA-222 and *T. harzianum* TH-238 was investigated using dual culture assays. A 5mm plug of a 4-day old culture of either *Trichoderma* isolate was placed 9mm away from the edge of the petri dish containing PDA. On the opposite edge of the plate a 5mm plug of a 4-day old culture of *M. nivale* was placed 9 mm from the edge of the plate. Control plates contained a single 5mm plug of either *M. nivale* TA-222 or TH-238 placed 9mm away front the edge of the plate in a method similar to that used by Rahman et al. (2009). Each treatment consisted of 10 replicates. Plates were incubated at 20°C in the dark. Growth of *M. nivale* and the *Trichoderma* isolates was recorded daily for four days by measuring the radius of growth of the fungi growing toward each other. Radial growth of the controls was also measured. After four days of incubation, the percent inhibition of radial growth (PIRG) was calculated using the following formula (Rahmen et al, 2009):

$$PIRG = \frac{R_1 - R_2}{R_1} \, x \, 100$$

Where,

R_1 = the radial growth of the control and

R_2 = the radial growth of the pathogen growing toward the antagonist.

Dual cultures were observed after the 4 days of incubation and the behaviour of the *Trichoderma* isolates was rated on day 10 after inoculation using a scale from 1-3 where: 1 = *Trichoderma* overgrows the pathogen with no sporulation, 2= *Trichoderma* overgrows the pathogen with sparse sporulation and 3 = *Trichoderma* overgrows the pathogen with abundant sporulation. Refer to Appendix 2 for a visual representation of the rating scale.

Dual culture assays were conducted three times.

Growth and percent inhibition of growth (PIRG) data were square root transformed and analysed using CoStat statistical software (CoHort) with a two-way ANOVA to determine if there was an interaction between treatment and assay. If there was a significant interaction, each assay was analysed separately using a one way-ANOVA followed by Tukey's Honestly Significant Difference (HSD) multiple comparisons test ($p<0.05$). Ratings were analysed using the Fisher Exact Test using RStudio (RStudio, 2016).

Field trial

This trial was conducted at the KPU teaching golf course. One golf green with a high level of snow mould symptoms was divided into 100 plots measuring 1 m^2 each. Photographs of each plot were taken before treatment commenced to assess the initial damage due to *M. nivale*. Eight treatments were included (Table 1).

Table 1. Treatments applied to turf for control of snow mould (*M. nivale*).

	Treatment	Rate (per Litre)	Frequency
1	Negative Control (water)	--	Monthly
2	Positive Control (Banner Maxx™)	Label rate	Once in the fall
3	Rootshield Plus™	2.25 g	Every 14 days
4	*T. atroviride* TA-222 (liquid)	2.0 x 10^9 CFU	Monthly
5	*T. atroviride* TA-222 (spores)	3.0 x 10^9 CFU	Monthly
6	*T. atroviride* TA-222 (spores)	3.0 x 10^9 CFU	Every 14 days
7	*T. harzianum* TH-238 (liquid)	2.0 x 10^9 CFU	Monthly
8	Rhizovital™	0.5 mL	Monthly for 4 months

Each treatment was assigned to 12 replicate plots of 1 m^2 in a completely randomized design. Each plot received 200 mL of treatment solution. Treatments were mixed with tap water and applied using a 5.7 L Green Gorilla™ hand-held battery powered sprayer (Lee Valley Tools, Vancouver, British Columbia).

Rootshield Plus™, a blend of *T. harzianum* and *T. virens*, was purchased from Terralink Horticulture (Abbotsford, British Columbia) and applied following the recommended label rate. Rhizovital™ (*Bacillus amyloliquifaciens*) was supplied by Sylvar Technologies Ltd. (Fredericton, New Brunswick) and applied following the manufacturer's instructions (Sylvar Technologies, pers. comm). The positive control used in this study was Banner Maxx™ (Syngenta Canada Inc., Ontario).

Figure 1. Field trial set up. The golf green was divided into $1m^2$ plots for treatment application.

Liquid *Trichoderma* inoculum was produced one week before application. Cultures were grown in a liquid media known to support the growth of *Trichoderma* (20 g/L molasses, 15 g/L yeast extract, 1 mL/L gentamycin mixed in tap water). Liquid media was sterilized at 121°C for 30 minutes and allowed to cool to room temperature prior to inoculation. For the first application, spores from a culture of *Trichoderma atroviride* TA-222 and *T. harzianum* TH-238 were scraped into separate tubes containing 12 mL of sterile reverse osmosis water (RO water). The suspensions were mixed with a vortex mixer for 30 seconds at 3000 rpm and then placed in a sonicator for 15 minutes to break up spore clumps. After sonication, the suspensions were mixed again for 30 seconds at 3000 rpm. Three millilitres of spore suspension were inoculated into one litre of liquid media in 2 L Erlenmeyer flasks.

For the second round of liquid inoculum production, and all subsequent rounds of production, each 2 L Erlenmeyer flask containing 1 L of liquid media was inoculated with eight 1 cm plugs of culture of either TA-222 or TH-238.

Flasks were placed on a shaker table at 150 rpm at 27 °C in the dark and incubated for 6-8 days. After incubation, mycelia and liquid media were blended in a Waring Laboratory Commercial blender to obtained a homogenous slurry. Ten-fold serial dilutions were made for each flask and 100µL were spread on petri dishes with PDA media for colony-forming unit (CFU) counts. For ease of counting, this was changed for the second round of application to *Trichoderma* selective media (PDA amended with 0.3 g/L chloramphenicol, 0.02 g/L streptomycin sulfate, 0.02 g/L rose bengal) since the colonies grow more densely on this media in comparison to PDA. Plates were incubated in the dark at room temperature for 1-3 days. CFU were counted from the 10^{-4} dilution. The number of CFU/mL were calculated using the following formula,

$$No. CFU\ per\ mL = \frac{No. CFU}{plate}\, x\, \frac{plate}{0.1mL}\, x\ 10^{4}$$

Solutions were kept at 4 °C for 3-4 days, until application.

For the treatment using spores of *T. atroviride* TA-222, spores from recent mass production runs were used. The spore count and viability were calculated following the method of Grace and Jaronski (2005). Spore powder (0.1 g) was suspended in 10 mL of sterile RO water. Tubes were mixed with a vortex mixer at 3000 rpm for 30 seconds and then placed in a sonicator for 15 minutes to break up spore clumps. After sonication, the tubes were mixed again for 30 seconds with a vortex mixer at 3000 rpm. Two 10-fold serial dilutions were made from the initial suspension and the initial number of spores in the solution (10^{-2} dilution) was counted using a Neubauer haemocytometer. A sterile cotton swab was then dipped into the spore suspensions (10^{-2} dilution) and streaked on petri dishes containing PDA. Petri dishes were incubated in the dark at room temperature. After 17 hours, the proportion of viable spores was calculated using the formula,

$$Viability\ (\%) = \frac{a-b}{a}\, x\ 100$$

Where,

a = the total number of spores counted (at least 300 spores) and,

b = the number of un-germinated spores.

Viabilities were counted prior to each application. The number of viable spores per gram of TA-222 was kept constant for each application by multiplying the original number of spores per gram of product by the proportion of viable spores counted.

The positive control, Banner Maxx™, was applied on October 23, 2020.

Photographs of the plots were taken four days prior to each monthly treatment application. Percent area damage was evaluated using NIS-Elements software (Nikon). Analysis was conducted on the photographs taken before the study commenced, one month after the first treatment and in February 2021 after the treatment period.

Data were analysed using SAS statistical software with a generalized linear model followed by Tukey-Kramer's post hoc multiple comparisons test ($p<0.05$). Data from the pre-treatment plots and one month after first treatment were square root transformed before analysis.

RESULTS & DISCUSSION

Survey

One turf sample was submitted from the BC golf course industry for identification. This sample came from Capilano Golf Club (West Vancouver).

Identification of snow mold pathogens

The identity of the pathogen causing snow mould in turf grass on the KPU golf course was *Microdochium nivale*, the causal agent of pink snow mould. *M. nivale* is the only snow mould found in the Fraser Valley and Lower Mainland. *Typhula* snow mould occurs in the Okanagan and has not been reported in this area since the winter does not reach low enough temperatures to support its growth (J. Elmhirst, pers. comm).

The sample from Capilano Golf Club was also identified as *Microdochium nivale*.

Laboratory antagonism assays

There was a statistical interaction between treatment and assay for both growth of the pathogen and percent inhibition of growth ($p=0.0011$ and $p<0.001$, respectively) which indicates that the trend in the effect of TA-222 and TH-238 on the growth and percent inhibition of growth of *M. nivale* was not statistically the same for all three bioassays. Therefore, assays were analysed separately.

In all three assays, after three days of growth, *T. atroviride* TA-222 inhibited the growth of *M. nivale* significantly more than *T. harzianum* 238 (26.85mm-28.40mm and 29.9mm-31.1mm total pathogen growth, respectively; Fig. 2). Even before reaching the pathogen, *T. atroviride* TA-222 suppressed the growth of *M. nivale* significantly. *T. harzianum* 238 suppressed the growth on day 3 significantly from the *M. nivale* control in the first assay but in the second and third assays, the mean growth was less than the control but was not statistically significant. By day four, the percent inhibition of growth of *M. nivale* by both *T. atroviride* TA-222 and *T. harzianum* 238 was significant in all three assays (31.05-37.21% and 25.62-27.91% inhibition, respectively; Fig. 3). Consistently, *T. atroviride* TA-222 inhibited the growth significantly more than *T. harzianum* 238. *T. atroviride* TA-222 also had a higher antagonism rating than *T. harzianum* 238 on the basis of sporulation over the pathogen (Fig. 4). In the second assay there was no significant difference in the antagonism rating between the two isolates, however, the difference was significant in the first and third assays.

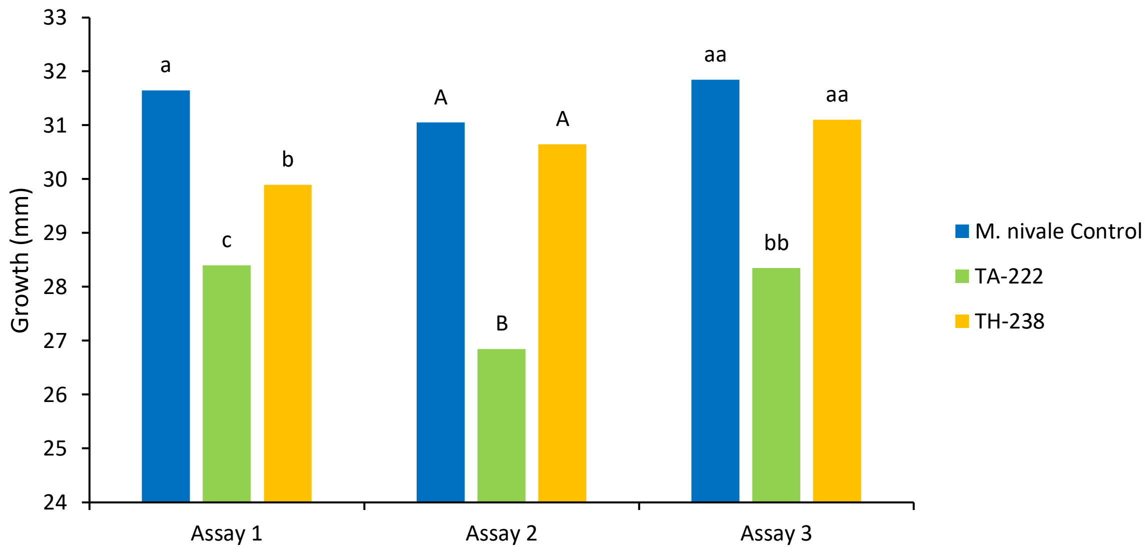


Figure 2. Mean growth of *M. nivale* in the presence of *T. atroviride* (TA-222) and *T. harzianum* (TH-238) in three dual culture assays measured on day 3 before the *Trichoderma* isolate started overgrowing the pathogen. Values with the same letter are not significantly different (Tukey's HSD, $p<0.05$). Each assay was analysed separately.

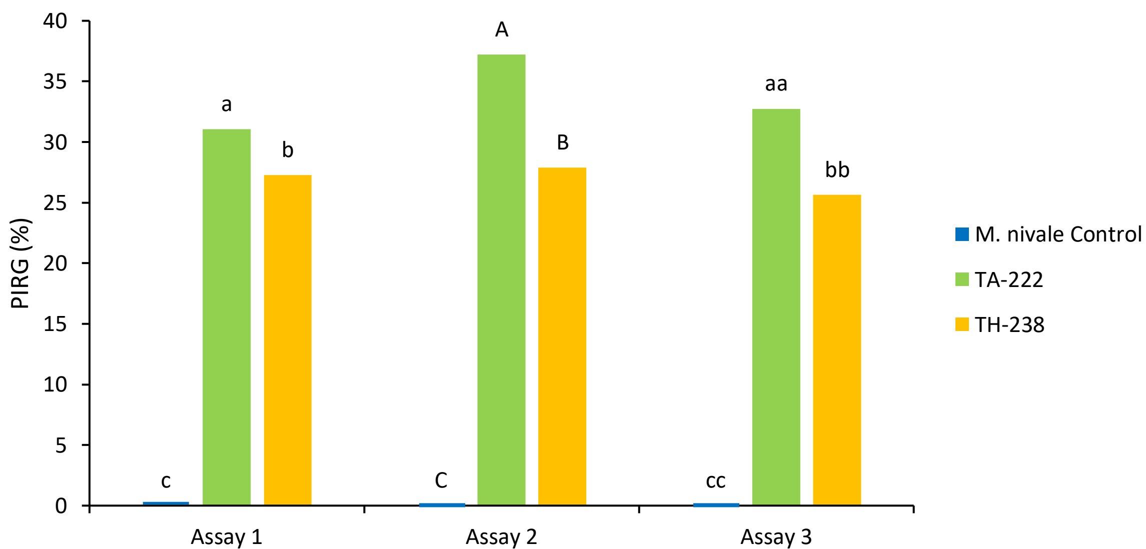


Figure 3. Mean percent inhibition of radial growth (PIRG) of *M. nivale* in the presence of *T. atroviride* (TA-222) and *T. harzianum* (TH-238) in three dual culture assays measured on day 4 after the *Trichoderma* isolate touched the pathogen. Values with the same letter are not significantly different (Tukey's HSD, $p<0.05$). Each assay was analysed separately.

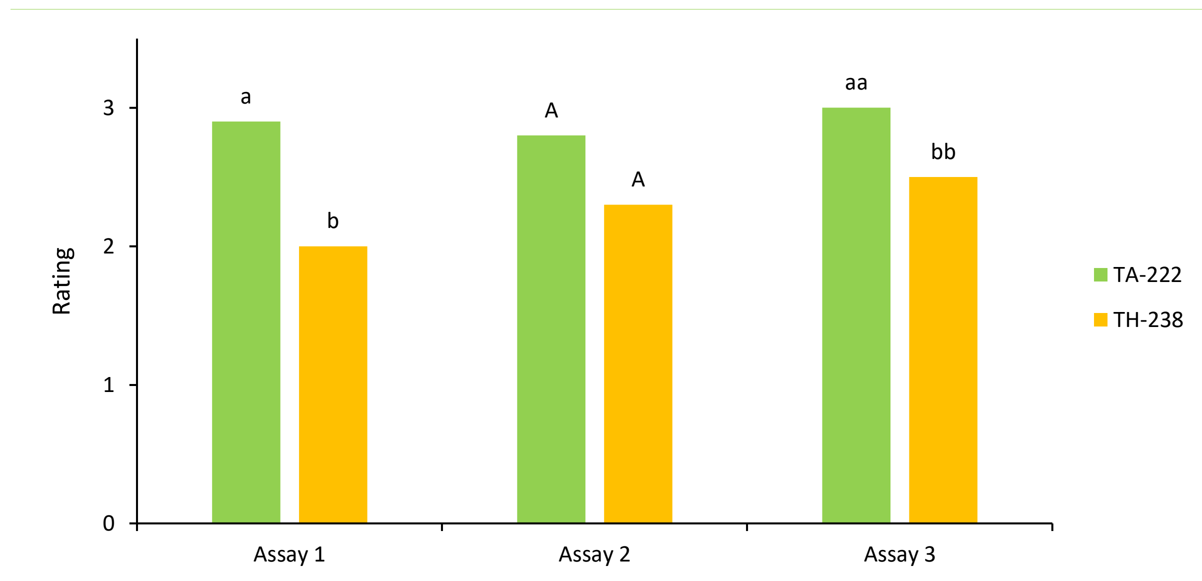


Figure 4. Mean antagonism rating of *T. atroviride* (TA-222) and *T. harzianum* (TH-238) in contact with *M. nivale* in three dual culture assays **r**ated on day 10. Rating based on the behaviour of Trichoderma in contact with the pathogen. Values with the same letter are not significantly different (Fisher's Exact Test, $p<0.05$). Each assay was analysed separately.

From these results, it is clear that after three days of growth *in vitro, T. atroviride* (TA-222) supresses the growth of *M. nivale,* whereas *T. harzianum* (TH-238) did not show consistent suppression. Both isolates inhibited the growth of the pathogen upon contact, with *T. atroviride* (TA-222) causing significantly greater inhibition than *T. harzianum* (TH-238) and were antagonistic to *M. nivale*. Of the two isolates, *T. atroviride* (TA-222) shows the most promise as a biocontrol agent against *M. nivale.*

Field trial

Damage on the turf plots ranged between 20-30% prior to treatment application and decreased to less than 5% one month after the initial application for all treatments (Fig. 5). The overall decrease in damage over time was significant ($p<0.0001$). However, there was no significant effect of treatment on the disease severity and no interaction between time and treatment after one month ($p=0.9729$ and $p=0.7935$, respectively). It is not surprising that all plots, including the plots treated with just water and those designated for treatment with the positive control, which have not yet

received treatment, had less damage one month into application. Snow mould damaged turf does recover over the late spring and summer when the temperature increases and the pathogen lays dormant in plant debris (Jung et al, 2007; Dicklow, 2011).

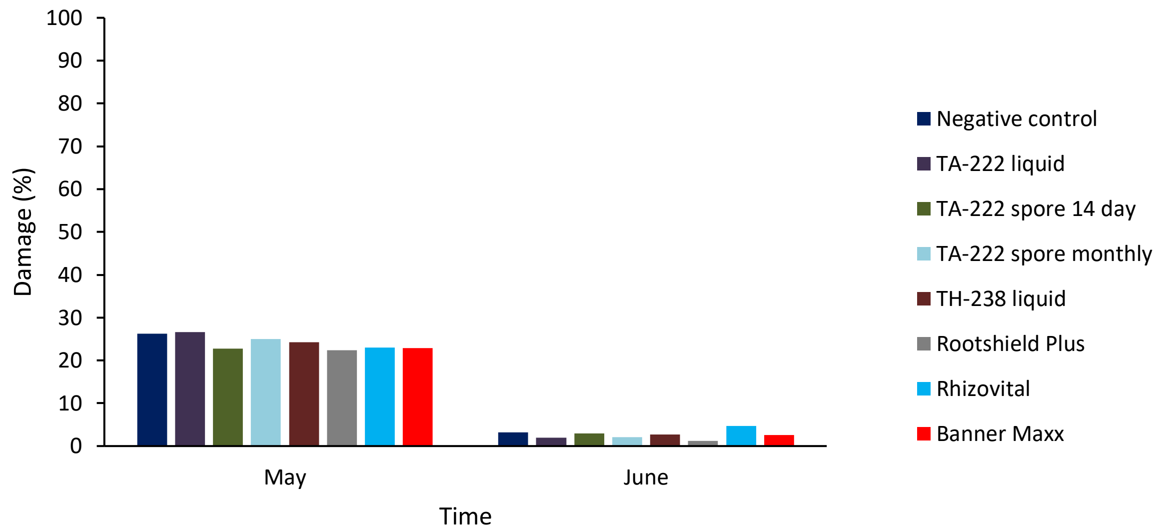


Figure 5. Mean percent area damage to turf plots by *M. nivale* prior to treatment application (May) and one month after first treatment application (June).

In February 2021, after 6 months of treatment application (May-October 2020), damage to the turf plots had increased from the summer recovery and ranged from 20%-30% (Fig. 6). Refer to Appendix 3 for photographs of mean disease damage for each treatment. Plots treated with local isolates TA-222 and TH-238 and Rhizovital™ had higher mean percent damage than the water treated negative control, Rootshield Plus™ and Banner Maxx™, a chemical registered and commonly used for the control of pink snow mould. However, this difference was not statistically significant ($p = 0.0804$). One concern is that the positive control did not have any effect on the damage caused by *M. nivale*. In the Fraser Valley, the fall and winter months usually experience a large amount of rainfall. It is possible that after application, subsequent rainfall washed the chemical out of the root zone. The label states to "avoid application when heavy rainfall is forecast;" however, this product is to be applied in the late fall and, in this region, that is a period of rain. The total amount of precipitation on October 23, 2020 was between 12.8mm and 13.6mm (totals for Vancouver and Abbotsford, respectively; climate.weather.gc.ca). Banner Maxx was applied in the morning and, at the time of application, there was no precipitation.

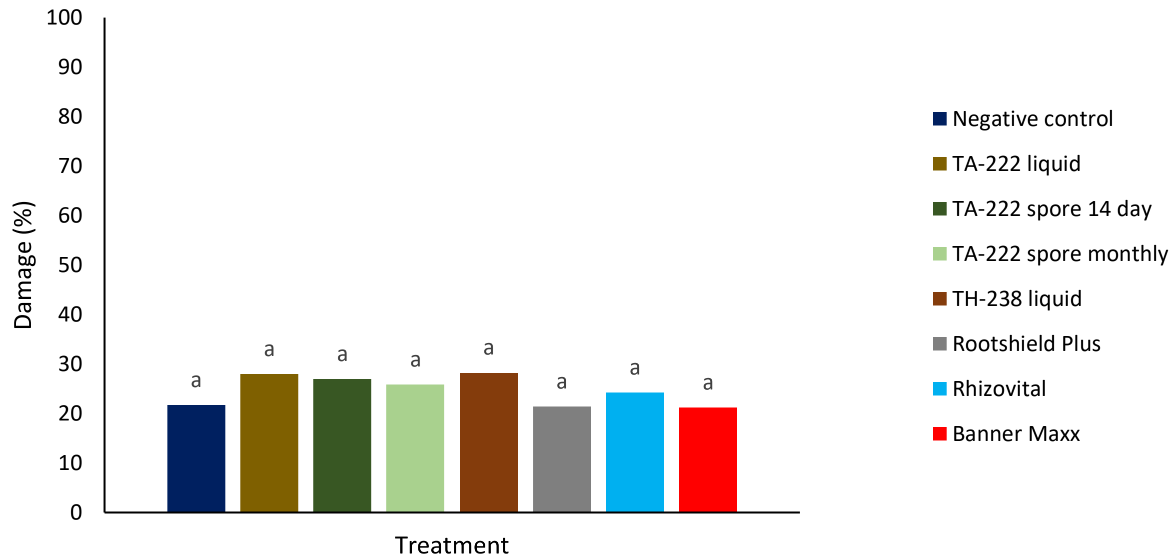


Figure 6. Mean percent area damage to turf plots by *M. nivale* in February 2021, after 6 months of treatment applications during the spring, summer and fall. Bars connected with the same letter are not significantly different (Tukey-Kramer, $p<0.05$).

Although *Trichoderma atroviride* TA-222 showed promise for suppression of *Microdochium nivale*, pink snow mould, in laboratory assays, this result was not observed in the field trial. In laboratory assays, fungi are grown in optimal conditions, whereas in the field, environmental conditions are often not optimal and heavily influence the performance of fungi applied as treatments. Many factors, including temperature, UV and soil moisture can play an important role in determining the success of biocontrol applications.

REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410. PubMed.

Dicklow, M.B. 2011. The snow molds. *Center for Agriculture, Food and the Environment*. Retrieved June 16, 2020 from https://ag.umass.edu.

Harman, G.E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* 96:190-194. doi: 10.1094/PHYTO-96-0190.

Hsiang, T. 2002. Research leads to new snow mold inhibitor. Mountain West Turf. 2: 37-39.

Jung, G., Chang, S.W., and Jo, Y. 2007. A fresh look at fungicides for snow mold control. Golf Course Superintendents Association of America.

Latin, R. 2019. Pink Snow Mold and Microdochium Patch. In *Turfgrass Disease Profiles*. Retrieved October 30, 2019, from https://www.extension.purdue.edu/extmedia/bp/bp-102-w.pdf

Mattox, C., Kowalewski, A., and McDonald, B. 2015. Final report: fungicide alternative management practices for Microdochium patch. In *Western Canada Turfgrass Association.* Retrieved October 30, 2019, from http://wcta-online.com/turfgrass-research/1374-final-report-fungicide-alternative-management-practices-for-microdochium-patch

McBeath, J.H. 2002. Snow mold-plant-antagonist interactions: survival of the fittest under the snow. Plant Health Inst., https://www.apsnet.org/edcenter/apsnetfeatures/Pages/SnowMold.aspx

Nelson, M. 2004. Carving an edge in snow mold. Green Section Record.

Rahman, M.A., Begum, M.F., and M.F. Alam. 2009. Screening of *Trichoderma* isolates as a biological control agent against *Ceratocystis paradoxa* causing pineapple disease in sugarcane. Mycobiology. 37: 277-285.

RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M.A Innis, D.H Gelfand, J.J Sninsky and T.J White (eds) *PCR protocols: a guide to methods and applications,* pp. 315-322. Academic Press, New York.

APPENDIX 1: LETTER TO GOLF COURSES REQUESTING SNOW MOULD SAMPLES

September 2020

Re: Turf samples

Dear golf course superintendent,

The Institute for Sustainable Horticulture, Kwantlen Polytechnic University (KPU) with support from the Western Canada Turfgrass Association (WTCA) is conducting a study to examine the 'Efficacy of fungal biocontrol agents for the control of snow mold disease in turfgrass.' Several fungal pathogens are responsible for snow mold disease. As such, one of our project objectives is to survey and identify fungi that are the causal agent of snow mold disease on golf courses throughout British Columbia.

This year, we will compare the efficacy of three beneficial fungal strains of Trichoderma to control snow mold on the KPU teaching golf greens. Information on the prevalence and distribution of fungal species causing snow mold symptoms across BC golf courses will help us determine if Trichoderma strains that prove promising at KPU could be useful for widespread snow mold control on BC golf courses.

Collection of a snow mold sample from your golf course is easy! We require a small core (around 2" wide) of turf grass along with the roots and soil from an area with snow mold disease symptoms. This can be placed in a bag and sent to the Institute for Sustainable Horticulture, KPU. We will extract fungus from the root/soil sample and perform a genetic analysis to identify the fungal pathogen causing snow mold on your golf course. We will follow up to communicate our findings to you. If you would like to participate please email Lisa Wegener at lisa.wegener@kpu.ca. The cost of shipping samples will be covered by the WCTA research grant.

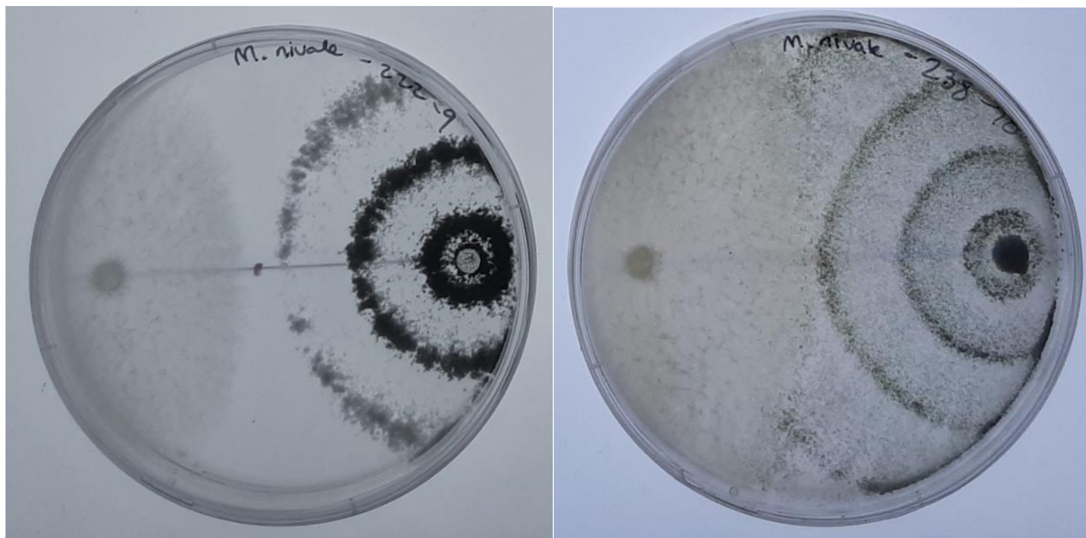
Sincerely,

Lisa Wegener,
Research and Laboratory Coordinator
School of Horticulture and Institute for Sustainable Horticulture
Kwantlen Polytechnic University
12666 72nd Ave. Surrey, BC, V3W 2M8

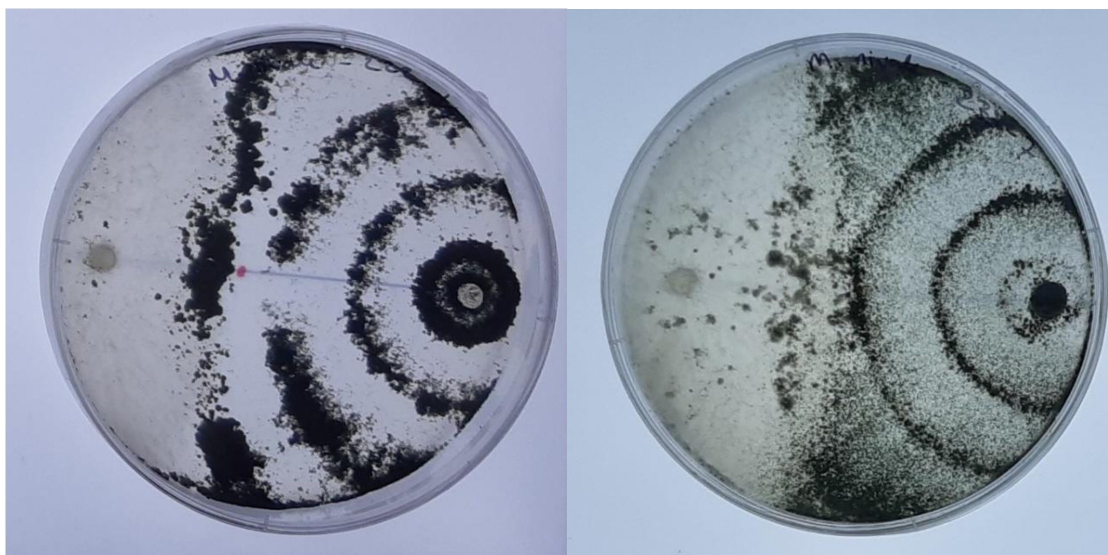
APPENDIX 2: ANTAGONISM RATING SCALE

M. nivale is on the left side of the petri dishes and Trichoderma on the right (dark areas are heavily sporulated). Bands show the response of Trichoderma night and day, with dark bands (sporulation) occurring in the daytime. When dark areas are seen on top of the pathogen, this confirms that Trichoderma has been aggressive in growing over the pathogen and is sporulating.

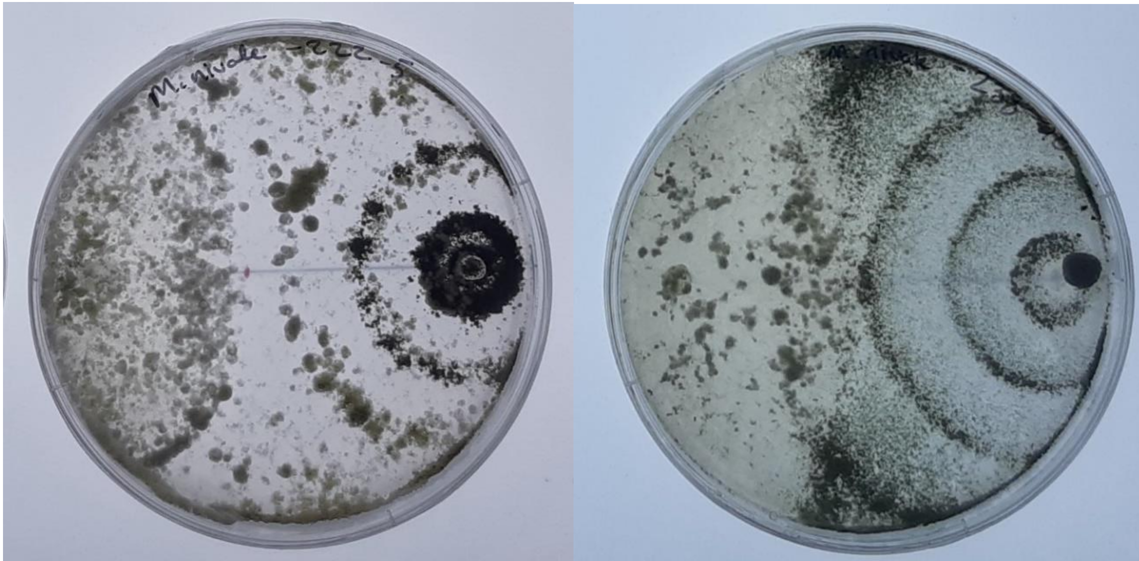
Rating: 1 –*Trichoderma* overgrows the pathogen with no sporulation.



Rating: 2 – *Trichoderma* overgrows the pathogen with sparse sporulation



Rating: 3 – *Trichoderma* overgrows the pathogen with abundant sporulation



APPENDIX 3: AVERAGE DAMAGE ON TURF PLOTS FOR EACH TREATMENT

Figure 7. Negative Control plot (Plot 1) showing mean damage on February 11, 2021.

Figure 8. Plot treated with TA-222 liquid (Plot 41) showing mean damage on February 11, 2021. (the plots were in the process of being aerated the day of this photo)

Figure 9. Plot treated with TA-222 spores at 14 day intervals (Plot 15) showing mean damage on February 11, 2021.

Figure 10. Plot treated with TA-222 spores at monthly intervals (Plot 23) showing mean damage on February 11, 2021

Figure 11. Plot treated with TH-238 liquid (Plot 4) showing mean damage on February 11, 2021

Figure 12. Plot treated with Rootshield Plus™ at 14 day intervals (Plot 15) showing mean damage on February 11, 2021

Figure 13. Plot treated with Rhizovital™ (Plot 75) showing mean damage on February 11, 2021

Figure 14. Plot treated with one application of Banner Maxx™ (Plot 40) showing mean damage on February 11, 2021