Ontario Turfgrass Research Foundation Final Report, June 2011

PROJECT TITLE: Stimulating host defences for control of turfgrass diseases

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Summary:
A two-year study on stimulating turfgrass defences to diseases was funded by the Ontario Turfgrass Research Foundation, and the work was done at the University of Guelph. The purpose of the work was to investigate alternatives to pesticides for controlling turfgrass diseases by using plant defense activators to stimulate the inherent ability of plants to resist pathogen attack. Past research has found that resistance can be induced in susceptible plants by micro-organisms and their metabolites or by synthetic analogs of natural products. We found that certain chemicals such as Civitas from Petro Canada did not have direct inhibitory effects on over a dozen fungal pathogens, but could increase the ability of the plants to withstand attack. However, the compound, benzothiadiazole which is the active ingredient in the fungicide Actigard was found to directly inhibit fungal growth at concentrations recommended for use in the field. In lab tests, Civitas was found to reduce dollar spot by 50 to 100%, while BTH only showed a 10% reduction in dollar spot. In field tests, Civitas and BTH were both found to reduce dollar spot disease levels by over 50%. We also began studies on gene expression on a genome scale (transcriptomics using RNA-seq), and found thousands of genes in bentgrass that were affected by the exposure to resistance-activating chemicals, some of them going up many fold, while others went down many fold. Our continuing work to is characterize some of these genes to get a better handle on what resistance-activating chemicals are doing to the turfgrass plants and what other effects these chemicals may have.

Direct effects of Activators on turfgrass pathogens
The turfgrass pathogens, *Colletotrichum graminicola, Microdochium nivale, Rhizoctonia solani, Sclerotinia homoeocarpa,* and *Magnaporthe grisea*, were tested for their direct sensitivity to Civitas, BTH and BD by growing these fungi on agar plates amended with different concentrations of the activators. None of the fungi were found to be directly inhibited by Civitas or BD (butanediol, an induced systemic resistance activator), but all were affected by BTH (benzothiadiazole, a systemic acquired resistance activator), at concentrations recommended for use in the field.

Indirect effects of BTH and BD on turfgrass diseases in the lab
Both Civitas and BD provided enhanced resistance to creeping bentgrass against the dollar spot pathogen (*Sclerotinia homoeocarpa*) in lab tests, with disease reduction in the range of 50% to almost 100% elimination of disease, but BTH only gave a 10% reduction in dollar spot on bentgrass in these first laboratory tests. BTH did not give any significant reduction in the level of *Microdochium nivale*, the cause of Fusarium patch. Only one cultivar was used in those studies, and therefore it was repeated with a collection of creeping bentgrass breeding lines. They were screened for their ability to respond to applications of BTH or BD for inducing defenses to Fusarium patch. A continuous range of the response to these defense activators was observed with some lines showing no effect of BTH or BD on inducing defences, while others show a high degree of induced defenses and good disease control. The responses to BD and BTH were independent, meaning that some lines responded well to BD but not BTH and vice versa, and some lines responded well to both activators. Gene expression studies were begun on selected lines for BTH and BD effects, and these are described below.

Indirect effects of BTH and BD on turfgrass diseases in the field
Field plots were set up at the Guelph Turfgrass Institute to test Civitas and BTH on diseases of creeping bentgrass, including dollar spot, Fusarium patch and snow molds. Induced resistance against Fusarium

patch and snow mold could not be properly evaluated because conditions were not favourable for disease development in both seasons of the trials. Dollar spot was reduced by over 50% with Civitas and with BTH.

Other defense activating agents

In the second year of the project, tests were been done with 12 different chemicals thought to activate resistance, but only one showed some disease inhibition: a green copper-complex pigment dispersion produced by PetroCanada now registered as Harmonizer. Experiments with the green copper-complex pigment have shown consistent levels of control in both the lab and field, but other products, such as Regalia, an extract from Giant Knotweed, have shown some control in the lab and field, but the results have been too variable to be considered promising. The other products tested, such as an extract of fungal cell walls, called oligo-chitin, BABA, an unusual amino acid, and azelaic acid, a plant compound, never showed disease control in repeated experiments, even though they have previously been reported to be effective in other plants, like tomato.

Gene expression studies

Two creeping bentgrass lines that showed a high induction of defenses by BD and two lines that showed no induction of defenses by BD were analyzed for changes in expression of specific resistance-related genes. Lines that respond well to BD showed changes in gene expression, but no significant changes in gene expression were observed in lines that showed no induced defenses by BD. Two creeping bentgrass lines that showed a high induction of defenses by BTH and two lines that showed no induction of defenses by BTH were also analyzed. Mass scale gene expression studies were initiated with RNA-seq of bentgrass samples treated with pigment or with BTH to identify genes that were affected. The results showed that thousands of genes in bentgrass were affected by the exposure to resistance-activating chemicals, some of them going up many fold, while others went down many fold. Our continuing work to is characterize some of these genes to get a better handle on what resistance-activating chemicals are doing to the turfgrass plants and the implications for disease and general turfgrass management.

PRESENTATIONS related to this study

Hsiang T, Goodwin P, Cortes-Barco A, Nash B. 2011. Enhancing the resistance of turfgrasses against diseases. Canadian International Turfgrass Conference. Vancouver, B.C., March 6-8, 2011.

Nash B, Goodwin P, Hsiang T. 2011. Harmonizer for control of turfgrass diseases. Ontario Turfgrass Symposium. Guelph, Ontario, February 23-24, 2011.

Hsiang T. 2010. Induced Systemic Resistance Against Three Foliar Diseases of Agrostis stolonifera. China Agricultural University Seminar, October 25, 2010, Beijing, China.

Hsiang T. 2010. Pathology Green Projects: disease control. Guelph Turfgrass Institute Field Day, August 19, 2010, Guelph, Ontario.

Cortes A, Hsiang T, Goodwin PH. 2010. Induced Systemic resistance against three foliar diseases of *Agrostis stolonifera* by an isoparaffin mixture. Can. Phytopath. Soc. Ann. Mtg., Vancouver, BC, June 23, 2010.

Nash B, Hsiang T and Goodwin P. 2010. Wax and pigment for turfgrass disease control. Can. Phytopath. Soc. Ann. Mtg., Vancouver, BC, June 21, 2010.

Cortes A, Hsiang T, Goodwin PH. 2010. Induced Systemic resistance against three foliar diseases of *Agrostis stolonifera* by an isoparaffin mixture. Reinhardsbrunn Symposium, Friedrichroda, Germany. April 29, 2010.

Cortes A, Hsiang T, Goodwin PH. 2010. Induced Systemic resistance against three foliar diseases of *Agrostis stolonifera* by an isoparaffin mixture. European Turfgrass Society, Annual Meeting, Angers, France. April 13, 2010.

Nash B, Hsiang T, Goodwin PH. 2010. Civitas™, a novel product for turfgrass disease control. Ontario Turfgrass Symposium. Guelph, Ontario, February 15-16, 2010.

Hsiang T. 2009. Biological control of plant diseases in cold environments. Conference on Plant and Microbe Adaptation to Cold, Oslo, Norway, Dec 4-8, 2009. (Invited Speaker, Research)

Hsiang T. 2009. Turf in tough times. University of Guelph, First Year Diploma Students Symposium. Nov. 27, 2009, University of Guelph. (Invited Speaker, Research)

Hsiang T, Cortes A and Goodwin PH. 2009. PC1, a novel reduced-risk fungicide: field testing. International Turfgrass Research Conference, Santiago, Chile. July 26-31, 2009. (Research).

Cortes A, Hsiang T and Goodwin PH. 2009. Induced systemic resistance in *Agrostis stolonifera* and *Nicotiana benthamiana* by the volatile organic compound, (2R,3R)-butanediol, is associated with priming of gene expression. XIV International Congress on Molecular Plant-Microbe Interactions, Quebec, Quebec. July 19-23, 2009. (Research).

Nash B, Hsiang T and Goodwin P. 2009. A novel product for turfgrass disease control. Can. Phytopath. Soc. Ann. Mtg., Winnipeg, MN, June 22-25, 2009. (Research).

PUBLICATIONS related to this study

Hsiang T, Goodwin PH, Cortes-Barco AM. 2011. Plant defense activators and control of turfgrass diseases. Outlooks on Pest Management 22:xx-xx. Accepted 2011/6/2.

Cortes-Barco AM, Hsiang T, Goodwin PH. 2010. Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (2R,3R)-Butanediol or an isoparaffin mixture. Annals of Applied Biology 157:179-189.

Cortes-Barco AM, Goodwin PH and Hsiang T. 2010. A comparison of induced resistance activated by benzothiadiazole, (2R,3R)-butanediol and an isoparaffin mixture against anthracnose of *Nicotiana benthamiana.* Plant Pathology 59:643-653.