Implementation of a bioherbicide strategy for golf course environments

By

Geneviève Gagné

Department of Plant Science

McGill University

Montréal, Québec

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Abstract

The local pathogen *Sclerotinia minor* has been formulated to be used as a bioherbicide for broadleaf weed control in turfgrass. Prostrate knotweed (*Polygonum aviculare* L.), a major golf course weed, is difficult to control with chemical herbicides, but prostrate knotweed is susceptible to the bioherbicide. However, a golf course is a challenging environment for the bioherbicide due to the close and frequent mowing and concomitant reduced competitiveness of the grass. This research demonstrates that prostrate knotweed control in golf course is dose response sensitive. Studies have shown that when the bioherbicide is combined with a protective cover, the bioherbicide performance is enhanced. This research also demonstrates that the bioherbicide can be used with Daconil fungicide, but Banner fungicide adversely affects bioherbicide efficiency. This research involved collaboration with Beaconsfield Golf Club Inc., Ile Perrot Golf and Country Club Inc. and The Royal Montreal Golf Club.

Résumé

Sclerotinia minor est utilisé dans une stratégie de contrôle biologique par inondation pour les terrains gazonnés. La renouée des oiseaux (*Polygonum aviculare* L) est une des principales mauvaises herbes infestant les terrains de golf. Cette mauvaise herbe est difficile à contrôler avec des herbicides chimiques et elle est susceptible au bioherbicide. Les terrains de golf sont des environnements spécifiques qui sont susceptibles d'affecter le potentiel du bioherbicide. Cette recherche démontre que le contrôle de la renouée des oiseaux est positivement relié à l'augmentation de la dose de bioherbicide appliquée. Cette étude établit que l'utilisation d'un tissu protecteur augmente la performance du bioherbicide. Également, elle prouve que la performance du bioherbicide est affectée par le fongicide Banner. En revanche, le fongicide Daconil peut être utilisé avec le bioherbicide. Cette recherche a été effectuée en collaboration avec: Club de golf Beaconsfield Inc., Club de Golf Ile Perrot Inc. et le Royal Montréal.

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Chapter 1 - Introduction

A new bioherbicide, SARRITOR™, has been homologated to be used on commercial and residential turfgrass in Canada. The bioherbicide is a barley based formulation of the fungus *Sclerotinia minor* Jagger. Many studies show its efficiency at controlling dandelion in residential turfgrass (Abu-Dieyeh, 2006; Abu-Dieyeh et al., 2005; Abu-Dieyeh and Watson, 2005, 2006, 2007; Riddle et al., 1991; Schnick et al., 2002). When broadcast applied at 40 g/m^2, good weed control was obtained in several studies (Schnick et al., 2002; Abu-Dieyeh and Watson, 2007). No known research has addressed the potential of this new technology in golf course environments. New pesticide legislation and public concerns about pesticide use has opened an opportunity to integrate this new tool in the golf course management system.

Residential turfgrass and golf courses share common characteristics, but have enough differences to justify the need to test this bioherbicide in golf course environments. Both environments are Poaceae monocultures and they both receive basic components of turfgrass management including fertilization, irrigation and mowing (Turgeon, 1980). Despite these general similarities, golf course and residential turfgrass differ in many points. The intensity of the management in golf courses, the higher input requirements, the type of grass and the type of weeds change the environment where the bioherbicide is active.

In golf courses, grass is usually mowed daily on green and three times per week for the fairways. Residential turfgrass are mowed once a week like the

roughs area. A short grass environment, as in golf courses fairways and greens, increases the bioherbicide's exposure to many environmental factors including wind and ultra-violet (UV) light. Mycelium growth is affected by UV light (Nagy and Fischi, 2002) while wind increases evaporation and decreases water availability for the bioherbicide. Since the bioherbicide is a formulation of the local pathogen *S. minor*, moisture is one of the most critical factors for the bioherbicide germination and development (Watson, 2007). However, golf courses usually have good irrigation systems, but usage is limited to grass need and absence of players.

According to the three associated golf courses, prostrate knotweed, *Polygonium aviculare* L., is an important weed in golf course and it is difficult to control with chemical herbicides. Prostrate knotweed can also be found in poorly maintained and heavily trafficked residential and sports turfgrass, but there has been limited research to examine the ability of the bioherbicide to control this particular weed. Preliminary results demonstrated that prostrate knotweed is susceptible to the bioherbicide, but difficult to control, likely due to lack of direct contact between the weed foliage and the bioherbicide. Short grass might improve contact between the weed and the bioherbicide because weed growth habit often adapts to its environment (Costea and Tardif, 2004). We observed that prostrate knotweed has a tendency to grow upright in regular turf or in road side habitats. In golf courses, the same weed grows bigger leaves and spreads more laterally. In this position, the leaves present a larger surface and this surface is appressed to

the ground. This growth habit should facilitate contact between the weed and the bioherbicide.

A jute covering technique has been tested by Dr. M. Abu-Dieyeh during his research work on the bioherbicide at the Weed Laboratory of McGill University. This technique consists in the application of a protective jute cover on the area treated combined with the bioherbicide. The jute cover is applied for three to four days. This technique has been thought to lower temperature fluctuation, to increase water availability and to increase contact between the bioherbicide and the surface of leaves. Preliminary experiments demonstrated great potential for this technology combined with the bioherbicide for the control of prostrate knotweed in domestic turfgrass (Abu-Dieyeh, personal communication). However, other cover types have not been investigated. It is logical to compare the effect of jute cover combined with the bioherbicide on weed control, to other cover types used in agricultural and horticultural sectors.

Although golf courses are mandated to reduce their pesticide use; pesticides are still significant parts of the management system with herbicides and fungicides the major pesticide utilized. Previous research demonstrated that the bioherbicide can be combined with the main herbicides and fertilizer used in residential turf (Schinick et al. 2002; Abu-Dieyeh 2006). However, research has not addressed the potential impact of fungicides on bioherbicide performance.

Dollar spot disease, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is one of the most common disease in highly managed turf in eastern Canada

(Goodman and Burpee, 1991; Hsiang and Benedetto, 2007; Hsiang et al., 1997). Chlorothalonil [Daconil] and propiconazole [Banner] are two major active ingredients used in Québec to control this disease. Even if none of these fungicides are known to inhibit *Sclerotinia* disease, they might have negative impact on bioherbicide growth and development, thus fungicides might impede weed control.

This research aims at evaluating the potential of the bioherbicide, SARRITOR™, for control of prostrate knotweed in the golf course environment. This bioherbicide could be a valuable tool to incorporate in an integrated pest management system for golf courses. The integration of this new tool in golf course management system would touch many social, political and environmental issues. It is also scientifically important to test the bioherbicide in golf course environments because it has never been tested before. Also, no study addresses the potential effect of fungicides used in golf courses on the bioherbicide and the resulting level of weed control. Furthermore, more information has been collected on the use of jute covering and other cover types on the modification of the environment surrounding the bioherbicide, thus favouring its growth.

Research Hypotheses

A research program was established to implement knowledge regarding a bioherbicide strategy for golf courses.

Hypotheses tested:

1. The optimal bioherbicide rate for golf courses is 40g/m^2.
2. Jute material combined with the bioherbicide enhances weed control in golf course environments.
3. The jute material provides the best environment for the bioherbicide active ingredient to emerge and colonize the weeds.
4. Propiconazole and chlorotalonil will inhibit the growth of the bioherbicide.
5. The level of inhibition induced by fungicide application will depend on the length of time between the fungicide and the bioherbicide applications.

Research Objectives

Based on the preceding hypotheses, the following research objectives were defined.

1. Determine the optimum bioherbicide rate for golf course environments.
2. Investigate the effect of jute covering combined with the bioherbicide on prostrate knotweed control in golf courses.
3. Determine the best cover material to be used with the bioherbicide.
4. Examine the interactions between commonly used fungicides and the bioherbicide.
5. Determine the number of days between fungicides and bioherbicide application required to ensure no effect on bioherbicide performance.

Chapter 2 - General Literature Review

Biological control

Biological control, in general, can be divided into three approaches: the classical, the inundative and the conservation methods (Vincent et al., 2007). The classical strategy involves the introduction of a natural enemy or competitor in a new environment to provide long term control of a pest population. The inundative or augmentative strategy implies the release of a biocontrol organism in a sufficient amount to reduce the pest population at least temporarily. The conservation strategy encompasses efforts to favour natural enemy populations which are already present in the environment (Vincent et al., 2007). Biological control was first used to control insect pests in agricultural area (Huffaker, 1962). Logically, the first type of weed biocontrol involved the use of insects as biological agent. One of the first reported cases was the control of prickly pear cactus (*Opuntia stricta*.) by the moth *Cactoblastis cactorum* in Australia (Moran and Zimmermann, 1991). The moth larvae feed on the succulent pads of the cacti. Subsequently, many other insects have been evaluated and some have been released as weed biocontrol agents (Julien and Griffiths 1998).

Weed biological control with plant pathogens

The feasibility of using a plant pathogen as weed biological control agent has been widely promoted in the scientific literature (Charudattan, 1991; Inman, 1969; TeBeest et al., 1992; Templeton and TeBeest, 1979; Wilson, 1969). Weed control with plant pathogens can by divided into two approaches: the classical and

the bioherbicide approaches (Templeton and TeBeest, 1979). The two approaches are very different: they have different goals; they target different types of weed and the biocontrol agents are also different.

The classical approach with pathogens

Classical biological control of weeds involves the intentional introduction of natural enemies to regulate pest populations. Biological control in general holds its basis on the principle that natural enemies play a major role in the regulation of any given organism population (Lazarovits et al., 2007). Often introduced plants can become weeds because natural regulators of the population are not found in the new environment. Classical biological control with a pathogen aims at establishing a new equilibrium by introducing a pathogen to control one specific weed species. Thus, biocontrol agent used in the classical approach should be host specific.

The choice of the bioagent for classical control is very important to limit off target effects. Biocontrol agents are often specialist: they have only one host. In the classical approach, many introduced fungi were autoecious. The best known example is obviously the *Puccinia chondrillina* Bubak. & Syd. case. This rust was deliberately introduced in Australia to control *Chondrilla juncea* L. (Cullen et al., 1973; Hasan and Jenkins, 1972). The rust successfully controlled the weed. More examples of plant pathogens used as biological control agents in the classical approach can be found in Charudattan and Dinoor (2000).

The bioherbicide strategy

The bioherbicide strategy uses basic plant pathology knowledge to induce an infection in a weed population (TeBeest and Templeton, 1985; Templeton, 1982; Templeton et al., 1979; Templeton and Trujilio, 1981). The bioherbicide strategy uses massive inoculation to incite an epidemic in a restricted region. The presence of a local plant pathogen is artificially increased to a level able to compensate for constraints to the disease development in nature (Mortensen, 1986). The inoculation of a massive dose of a pathogen with the right timing may produce an epiphytotic in the weed population. Human intervention shortens the lag period during which the pathogen builds up its population (Charudattan, 1991). Native and alien weeds can be controlled with this strategy.

The application of a bioherbicide or microbial herbicide is effectuated just like a chemical herbicide (Templeton and TeBeest, 1979). The product is applied and the weed is controlled rapidly. A disease epidemic is induced in the weed population causing rapid collapse of undesirable plants. A bioherbicide usually has a narrower weed spectrum (Mortensen, 1986) and they are considered more environmentally-friendly than chemical herbicides (Tisdell et al., 1984). The success of a bioherbicide will depend on the plant-pathogen-environment interactions, cropping practices and suitable alternatives (TeBeest, 1985).

Bioherbicides first appeared in North America in the early 1980s with the commercialization of Collego, and DeVine and the registration of BIOMAL (Bowers, 1986; Kenney, 1986; Makowski and Mortensen 1992). Following the

relative success of these products, others bioherbicide were developed including Dr. BioSedge and Stumpout (Li et al., 2003). All the bioherbicides stated above are fungal spore preparations.

Many pathogens (including fungi, bacteria and actinomycetes) have been studied to determine their bioherbicide potential. An extensive list of other bioherbicides that have been on the market or are on their way can be found in previous reviews (Tebeest et al. 1992; Charudattan and Dinoor, 2000; Li et al., 2003). Most of the bioherbicides developed have a narrow host range. Recently, researchers have evaluated some broad host range pathogens as biocontrol agents including *Myrothecium verrucaria* to control kudzu (Hoagland et al., 2007) and *Sclerotinia minor* used in this research.

A bioherbicide requires formulation and mass production of the bioagent. These are important challenges for bioherbicide development. Formulation favours bioherbicide weed control efficacy by overcoming environmental limitations (mainly moisture requirement), increasing shelf life; enhancing stability, protecting bioherbicide against ultraviolet (UV), and enhancing virulence (Green et al., 1998).

***Sclerotinia minor* Jagger**

Description

Sclerotinia minor Jagger is a member of the Discomycetes class, Helotiales order and Sclerotiniaceae family. Three species of *Sclerotinia*, *S. minor* Jagger, *S. trifoliorum* Eriks and *S. sclerotiorum* (Lib.) de Bary, are considered valid (Bolton

et al., 2006). Both *S. minor* and S. *sclerotiorum* are found in Canada (Bardin and Huang, 2001). *Sclerotinia homoeocarpa* F.T. Bennett, agent causal of dollar spot disease, is not considered to be a valid species of the *Sclerotinia* genus (Bolton et al., 2006).

Both *S. minor* and *S. sclerotiorum* have a broad host range. Host plants of *S. minor* include 94 plants species and 66 genera in 21 families (Melzer et al., 1997). It is a local soil borne pathogen common in many countries around the world. No pathogenicity has been observed on different grass species (Kentucky bluegrass, creeping red fescue, chewings fescue, and perennial ryegrass). Although, it has been found that the fungus can infect many economic plants including lettuce, pepper and tomato (Melzer et al., 1997). Epidemics caused by this fungus has only been reported on lettuce crops in Canada (Melzer et al., 1997).

The first sign of a *Sclerotinia* disease is the appearance of fluffy mycelia and then the fungus spreads rapidly through the stem and the entire plant collapses, and soon after, black sclerotia bodies appear (Agrios, 2005). Sclerotia are the main infecting units. *Sclerotinia* fungi sexually reproduce by the formation of apothecia and release of ascospores (Agrios, 2005).

Sclerotia of *S. sclerotiorum,* can remain viable in the soil for up to eight years (Imolehin et al., 1980). The sclerotia of *S. sclerotiorum* can germinate to produce apothecia, whereas the sclerotia of *S. minor* have not been reported to produce ascospores in North America (Abawi and Grogan, 1979). Infection by *S.*

minor is mainly due to eruptive germination of sclerotia (Bolton et al., 2006; Melzer et al., 1997, Subbarao, 1997) The fungus overwinters as sclerotia and/or mycelium in infected plant tissues.

Sclerotinia spp. causes a wide range of diseases on several economic plants. For example on beans the disease is called white mold, on potatoes and tomatoes it is stem rot, on cabbage it is watery soft rot, on lettuce the disease is named drop and the disease is called nesting for post-harvest disease of bean. *Sclerotinia* spp. develops in cold and moist weather. Sclerotia germination of *S. minor* occurs between -0.03 and -0.3 MPa and between 5 and 25°C. The optimum conditions are -0.1 MPa at 15°C (Hao et al., 2003).

Mode of action

Members of the Sclerotiniaceae family share common features as far as pathogenicity is involved. *Sclerotinia* genus fungi are necrotrophic homothallic pathogens that consume dead and living plant material. Mycelium penetrates the cuticle of the host plant by three ways: by mechanical force via appressoria, by entering through the stomata and by secretion of enzymes that degrade plant leaf cuticle and other epidermal cells.

Oxalic acid and cell wall degrading enzymes are two important components of the infection process (Bolton et al., 2006; Brière et al., 2000). Oxalic acid production is common in several fungi. Oxalic acid affects plants by several ways. It deregulates stomata guard cell and it affects the pH at the leaf surface. Thus, stomata open, allowing water to evaporate and hyphae to enter the

host plant (Bolton 2006). As the oxalic acid reduces the pH at the leaf surface, the effect of pectolytic enzymes are enhanced (Brière et al., 2000). Low pH medium favours mycelium growth (Brière et al., 2000), so low pH induced by oxalic acid at the leaf surface should favour mycelium growth.

Control tools

Sclerotinia diseases can cause severe damage on numerous economic crops (Melzer et al., 1997). *S. minor* has been reported on lettuce crop in Québec (Reeleder and Charbonneau, 1987). Several techniques have been developed to control *S. minor*. Amongst them, pH adjustment (Wilson et al., 2005), crop rotation (Subbarao, 1998), drip irrigation (Hao et al., 2003), resistant cultivars (Abawi et al., 1985), soil sterilization (Lynch and Ebben, 1986), soil amendments with manure (Asirifi et al., 1994), and fungicides.

Recently, several soil organisms have been studied to test if they can reduce disease incidence of *S. minor*. *Serratia marcescens*, *Streptomyces viridodiasticus* and *Micromonospora carbonacea* can significantly reduce the growth of *S. minor in vitro* and the incidence of lettuce disease under greenhouse conditions (El-Tarabily, 2000). Other micro-organisms have been evaluated and *Coniothyrium minitans* (CONTANS *WG*) has been registered in Europe and the United-States to control *Sclerotinia sclerotiorum* and *S. minor* in agricultural soils (Watson, 2007).

The bioherbicide

Sclerotinia minor has been formulated to be used as a bioherbicide in turfgrass environments. The trade name is SARRITOR™. The bioherbicide is a living organism and it needs specific environmental conditions to germinate and to infect weeds. Environmental conditions needed are the same as the one recorded to predict disease infection in field. When used appropriately, the bioherbicide is very efficient at controlling dandelion (Abu-Dieyeh and Watson, 2006, 2007).

Sclerotinia minor isolate IMI 344141 used in this study was isolated from diseased lettuce plants from Sherrington, Quebec. Sclerotia are kept at 4^{o}C, germinated on potato dextrose agar (PDA) and mycelia are used to inoculate sterilized barley (*Hordeum vulgare* L.) grits. Production of the barley-based formulation is described in Abu-Dieyeh (2006).

The conditions required for satisfactory weed control in turfgrass are defined by the needs of the fungus. The optimal growth temperature is 17-21^{o}C and the humidity level needs to be higher to than 95% (Watson 2007). It is possible for *S. minor* to germinate from 6-30^{o}C (Imolehin et al., 1980). The temperature range limits the application timing. Thus, the bioherbicide treatments are normally associated with spring and fall seasons in Quebec. During these periods of the year, precipitation levels are generally favourable for positive functioning of the bioherbicide.

Efficiency of the bioherbicide in residential turfgrass

The bioherbicide is efficient at controlling dandelion (*Taraxacum officinale* Wiggers) and other broadleaf weeds in residential turfgrass. Dandelion accessions collected from the United States, Canada and Europe with significant morphological differences, were all susceptible to the bioherbicide treatment (Abu-Dieyeh and Watson, 2005). The bioherbicide negatively affects the reproductive potential of dandelion. Dandelion seeds from treated plants were significantly smaller, as compared to untreated plants, and the flowering period is shortened by the bioherbicide (Abu Dieyeh et al. 2005).

White clover *(Trifolium repens* L.), broadleaf plantain (*Plantago major* L.) and prostrate knotweed are also susceptible to the bioherbicide, but the control of prostrate knotweed is difficult in residential turfgrass (Abu-Dieyeh, personal communication). Prostrate knotweed control in golf course environments presents a much greater challenges for the bioherbicide.

The bioherbicide efficiently controls dandelion in turfgrass, even better than 2,4-D (2,4-dichlorophenoxy acetic acid) under field conditions (Schnick et al., 2002). At rates of 40 and 60 g/m^2, the bioherbicide alone achieves greater weed biomass reduction than 2,4 D at 0.3 and 0.6 kg ae ha^{-1}. Synergistic effects have also been observed; 40 and 60 g/m^2 of bioherbicide, combined with 0.6 kg ae ha^{-1} of 2,4-D, attained better weed control than the two applied separately. Interestingly, 40 and 60 g/m^2 of bioherbicide achieves more control than the combination of the same rates with 0.3 kg ae ha^{-1} of 2,4-D (Schnick et al., 2002).

Golf course environments

Golf courses include various environments that engender variation in weed species. For the purpose of this research, golf courses can be split in two parts according to the height of the grass. In the first part, the grass has a very short form, 0.3 cm to 5 cm, and includes the greens, the collars, the fairways, the tees and the semi-rough areas. The second part includes the rough area. The greens are usually mowed at 3 mm (it can be less during championship events). The collars are 76.2 cm (30 inches) wide between greens and fairways. The fairways grass is usually mowed at 1.6 cm. The tees are mowed to 0.6 to 0.8 cm. The semi-roughs have a grass height of 3.2 to 5 cm (Quast and Qtto, 2004). The semi-roughs are situated between fairways and roughs. In fairways and greens environments, creeping bentgrass (*Agrostis palustris Huds*.) is one of the best grass for North American climates (Dernoeden, 2000). The experiment described in this research will be done mainly in this first section (greens, tees, collars, fairways and semi-rough). Major broadleaf weeds infesting short grass environment are prostrate knotweed and white clover in the three associated golf courses.

The second part of a golf course is composed of the rough area. The roughs are mowed once or two times a week, depending on grass growth. The environment of the rough is similar to a residential turfgrass. In general the rough is composed of a turfgrass mix predominated by annual bluegrass (*Poa annua* L.), (this grass is considered as a weed), Kentucky blue grass (*Poa pratensis L.*) and perennial ryegrass (*Lolium perenne* L.). Major broadleaf weeds found in the longer grass are dandelion, white clover and plantain.

Golf course environments differ in many aspects from residential turfgrass. Golf turfgrass needs more input than domestic turfgrass. Golf courses usually utilize pesticides for playability. Intense mowing and clipping removal increase fertilizer need (Turgeon 1980). Very well fertilized grass can attract more pests and be more vulnerable. Pesticides are usually used to control or prevent pest problems, but golf courses have to reduce and limit their usage due to legislation and government pressure.

Pesticides legislation

In 2001, the Quebec Department of Environment held a public consultation on pesticide use in urban areas. The goal was to come up with a solution to reduce human pesticide exposure, specifically with regards to children. After this public consultation, Québec government voted a law to reduce pesticide used: *Loi sur les pesticides*. Golf courses are also directly targeted by this law, they have to reduce or minimize pesticides used.

Since April 3 2006, all golf courses in Québec must present a pesticide reduction plan to the Quebec Ministry of Sustainable Development, Environment and Parks. The document has to be renewed every three years and must be approved and signed by an agronomist (Anonymous 2003).

An integrated pest management strategy is suggested to golf superintendents by the ministry. Many tools are available to superintendents to increase grass competitiveness and discourage weeds, insects and diseases development. Management practices and techniques, including core aeration,

verticut, mowing, irrigation, fertilization, drainage and pesticides should be harmoniously used to achieve good grass quality, pesticide utilization being the last option.

Pesticides used in Québec and the golf course component

In 2003, 3 660 622 kg of pesticide active ingredients were sold in Québec with 190 346 kg of theses pesticides being sold for green space management, including golf courses, municipalities and enterprises. Data on pesticides used only by golf courses was first presented in June 2007 (Laverdière et al. 2007). An average of 39 382 kg of active ingredients are being used annually. In reality, golf course used 1% of the total pesticides used in Québec (taking the amount of pesticides sold before the Code de gestion des pesticides in 2003).

Even if golf courses are not a major player in the overall pesticides used in Québec, public policy still target golf courses to reduce their pesticides used. The playing area of 263 golf courses is 7578 hectares and 39 382 kg of active ingredients are used each year by golf courses. (Gorse and Dion, 2007; Laverdière et al., 2007).

However, it is important to note that no studies show a correlation between any human disease and golf course pesticide use. Potential impact of pesticides on human health is of public concern since many chemical substances have been evaluated as being potentially harmful for human health.

Chemical pesticides have been evaluated by several organisations including the international agency or research on cancer (IARC), environmental protection agency of United State (EPA), the national toxicology program (NTP) each of these organizations had built their own scale to classify pesticides according to their possible effect on human health. According to the IARC, chlorophenoxy herbicides like 2,4-D, mecoprop, and MCPA, are classified as being possibly carcinogenic for humans, meaning that there is evidence indicating that these substances are potentially carcinogenic for animals, but effects on humans have not been sufficiently demonstrated (Anonymous 1999 in Anonymous, 2003). Both 2,4-D and mecoprop can be used to control prostrate knotweed.

Main target weed: *Polygonum aviculare* L.

The major problematic weeds in the three golf courses in short grass area are prostrate knotweed, white clover (*Trifolium repens* L.), broadleaf plantain (*Plantago major* L.) and dandelion. The last three weeds species are also found in the rough areas and are susceptible to the bioherbicide.

The main target weed in this study is prostrate knotweed, an annual member of the Polygonaceae family, subfamily Polygoneae. The seedling is composed of a short hypocotyl (0.5 to 3 cm) with lanceolate cotyledons (10-15 mm by 1-1.5 mm) (Costea and Tardif, 2004). The first leaves are lanceolate in shape and the mature leaves are alternate with a short petiole. The blade is narrowly elliptic, lanceolate or obovate with an entire margin. These

characteristics make it difficult to detect at younger stages in a turfgrass system. The root system can go as deep as 70 cm. The first 15-25 cm of the soil consists of a dense layer of secondary root growth (less than 1mm in diameter) which grow horizontally (Costea and Tardif, 2004). The main root is a tap root of 0.3-0.6 mm in diameter (Kutschera and Sobotic 1992, in Costea and Tardif, 2004). The flowers are perfect with short pedicels. The primary inflorescences are auxiliary cymes with 2 to 8 flowers.

Prostrate knotweed possesses a unique form of heterocarpy: a plant can produce two types of achenes at different stages: autumn achenes and summer achenes (Costea and Tardif, 2004). Summer achenes develop from early flowers, are smaller, and their pericarp is dark brown. Autumn achenes have a green pericarp and are about three times longer than summer achenes. Autumn achenes develop from late summer flowers to autumn flowers. This feature increases the reproductive potential of the plant.

Prostrate knotweed is one of the first summer annuals to germinate (Quast and Qtto, 2004). Prostrate knotweed germination is triggered by light and requires a temperature of 17^{o}C for germination (Batlla and Benech-Arnold, 2003). In Québec, seeds become non dormant in March-April and germinates between March and May in a single flush (Costea and Tardif, 2004).

Habitats colonized by prostrate knotweed are very wide. The weed is common in all provinces of Canada except Nunavut. The climatic condition required varies from subtropical to subarctic. Prostrate knotweed thrives in

compacted soil, poorly aerated, nutrient rich and nutrient poor substrates. Prostrate knotweed has been found in all types and textures of soil (Costea and Tardif, 2004). This ability to grow in almost any condition, make it more competitive than grass in specific situations in golf courses. Prostrate knotweed flourishes in depressions where water accumulates.

Prostrate knotweed is a challenge to control, it produces a tremendous amount of seeds and it can sustain water lodging. Preliminary results show better weed control when the bioherbicide is use in combination with a jute cover.

Impediment for the bioherbicide

As stated earlier, mowing is an important component of golf courses environment. In an experiment conducted under field conditions on residential turfgrass, some of the problematic weeds infesting golf course (prostrate knotweed and chickweed) have been shown to increase when mowing height is 3-5 cm, compared to 7-10 and 12-15 cm (Abu-Dieyeh and Watson, 2006). Also, the effect of the bioherbicide decreased with the mowing height. The number of prostrate knotweed plants per meter square is higher at 3-5 cm: 12.0 plants has been reported at 3-5 cm, compared with 3.2 and 12-15 plants for 7-10 cm and 12-15 cm, for the first year. The second year after application the number of plants is higher in the lowest mowing height (Abu-Dieyeh and Watson, 2006). According to above results, the low mowing height of the golf course is an advantage for prostrate knotweed, thus a challenge for the bioherbicide. To counterbalance these difficulties, jute cover will be used to favour weed control.

Covers

In midsummer, climatic conditions (high temperatures and drought) impede the bioherbicide's performance. Also some weed species, like prostrate knotweed, are more difficult to control. To address these constraints, jute covering has been tried as a technical tool to modify the environmental conditions and thus promote weed control (Abu-Dieyeh, personal communication).

Jute is the only material that has been tested in combination with the bioherbicide before this research project. Other material used in the agricultural and horticultural sectors might be beneficial for the bioherbicide. The two important parameters for the bioherbicide are relative humidity and temperature. Experiments have been done to characterize the effect of different covers as far as those two factors are concerned. Four different types of cover, used in the agricultural or horticultural sectors, have been tested to determine their specific effect on the environment surrounding the bioherbicide. The types of covers are jute (Terra Tex, manufacturer: Les industries Lenrod LTD., Ville St-Laurent, Québec, Canada), black geo textile (Platinum geo-textile series 400, manufacturer: Quest home and Garden, Mississauga, Ontario, Canada), white row cover (polypropylene, product number AGRYP2216X100, manufacturer: Dubois agrinovation Saint-Rémi, Québec, Canada) and black plastic mulch (polyethylene, product number PAPEN364011, manufacturer: Dubois agrinovation Saint-Rémi, Québec, Canada). Covers have been tested under extremely hot climatic condition for Montreal area summer (32^{o}C). Covers are also used to control hard to control weeds like prostrate knotweed.

Fungicides and golf courses

In the associated golf courses, dollar spot is the most problematic disease. It is important to note that the two fungi, *S. minor* and *S. homoeocarpa*, are not closely related, but *S. homoeocarpa* has not yet been reclassified (Bolton et al., 2006).

In Québec, fungicides are the pesticide the most used. 29 885 kg of fungicide active ingredients have been used in golf courses. This represents 75.9% of the total amount of pesticide applied. Herbicides are the second pesticide with 7 252 kg of active ingredients that account for 18.4% of the total. Finally, 2 221 kg of active ingredients are used as insecticides. Small amounts of pesticide used are rodenticides and growth regulators for 14 kg and 9.5 kg of active ingredients respectively.

Fungicides recommended to control dollar spot are Banner Maxx and Daconil (Syngenta). They contain 14.3% propiconazole and 82.5% chlorothalonil respectively. Both products are registered in Canada to control dollar spot in golf courses. Bayfidan (Bayer) containing 250g/l triadimenol is also registered to control dollar spot. On golf courses, the higher rates of Banner and Daconil for curative purposes are Banner 51 ml/100m^2, Daconil 212g/100m^2 and for Daconil and Banner applied together 115g/100m^2 and 30ml/100m^2. Banner preventive rates are 26-51 ml/m^2 and can be applied every 21-28 days for a maximum of six applications per year. For Daconil, application rates are from 58 to 115 g/m^2, 115-212 g/m^2 lower rates for preventive treatment and higher for curative treatment. Application can be made every seven to 14 days.

The bioherbicide is a fungus and it can be affected by fungicide applications. *S. minor* and *S. sclerotiorum* caused same symptoms, same types of disease and they have common hosts. They can be found together at an infection site. Products developed to control *Sclerotinia* disease should be able to control both pathogens. Many active ingredients have an effect on *Sclerotinia* sp.: iprodione, vinclozolin, boscalid, fluazinam, fenhexamid, fludioxonil metham-sodium and dicloran (Matheron and Porchas, 2000; Matheron and Porchas, 2004; Reilly and Lamoureux, 1981; Ryley et al., 2000). Iprodione can be used in greenhouses to control sclerotiniose (Anonymous 2006). Fluazinam and fenhexamid are registered to be used to control *Sclerotinia* diseases on lettuce in fields (Anonymous, 2008). It is interesting to note that *S. minor* and *S. sclerotiorum* do not always respond similarly to all fungicide treatments (Matheron and Porchas, 2004).

Propiconazole and triadimenol are both in the triazole chemical family. These fungicides inhibit the demethylation, more specifically at the sterol biosynthesis step (Waterfield and Sisler, 1989). Chlorothalonil is in the family of the chloronitrile. This fungicide cause the depletion of glutathione and the inhibition of essential cellular enzymes (Tillman et al. 1973)

Resistance to chlorothalonil has not been reported (McDonald et al., 2006). Hsiang (2006) observed a S. *homeoecarpa* sensitive population containing members with reduced sensibility to demethylation-inhibiting (DMI) fungicides including propiconazole, myclobutanil, fenarimol and tebuconazole.

In summary, golf course environments are very different from residential turfgrass. Prostrate knotweed control in golf course environment with the bioherbicide presents a new challenge. Short grass modifies the bioherbicide environment. Covers might compensate for environmental difficulties. Fungicides used in golf courses to control dollar spot might interfere with the bioherbicide activity on prostrate knotweed.

Chapter 3 - Methodology

Experiments conducted in golf course environments in 2006

In all experiments conducted in golf courses, square plots of $0.1m^2$ (0.33m*0.33m) were marked with paint in fairways, primarily infested by prostrate knotweed. Other weeds were white clover, plantain and dandelion. Experiments focused on prostrate knotweed. Weed density percentages were visually estimated at 0, 7 and 14 days post application (DPA) (method explained p. 33).

Determination of the optimal application rate to control prostrate knotweed

To determine the best application rate, an experiment was conducted at Royal Montreal golf club located in Ile Bizard, Québec, Canada (Latitude: 45^{o} 50" N, longitude 73^{o}88" W) in 2006. The experiment was a completely randomized design (CRD) with five treatments and three replicates. The treatments were 0 (untreated control), 20, 30, 40 and 60 grams of inoculum per meter square. The bioherbicide treatments were broadcast applied on the treated area. The experiment was conducted only one time in 2006 and was not been repeated in 2007 because of the loss of inoculum virulence. Prostrate knotweed infestation levels on experimental plots were 35% to 80% at bioherbicide application time. First mowing were effectuated 14 DPA, and the areas were protected from players.

Effect of jute covering combined with the bioherbicide on prostrate knotweed control

To verify the effect of jute covering combined with bioherbicide rates on weed control, the two experiments were conducted at two locations in 2006: Beaconsfield golf, Québec, Canada (latitude 45° 45" N, longitude 73°81" W) and Royal Montreal golf courses Québec, Canada. The experiment was a split-plot design with three replications. Main plots were bioherbicide application rates: 0, 20, 30, 40 and 60 g/m^2 and the sub-plots (0.33m*0.33m) were covered with jute and uncovered. Bioherbicide treatments were broadcast applied. The cover was applied right after bioherbicide application and was removed three days later. Prostrate knotweed densities in the plots at the beginning of the experiment were 30 to 85 %. Sub-plots were blocked according to prostrate knotweed densities.

Experiment conducted in golf course environments in 2007

Effect of jute cover and bioherbicide rate on prostrate knotweed density in golf course environments in 2007.

To determine the effect of jute covering and bioherbicide rates on prostrate knotweed control, the same experiment was conducted at two different locations in 2007: Ile Perrot Golf and Country Club and Royal Montreal, Québec, Canada (latitude 45°38" N, longitude 73°96" W).

The experiment was a split-plot design with three replicates. Main plots were cover and no cover and the sub-plots (0.33m*0.33m) were rates of

application: 0, 20, 30, and 40 g/m^2. At the beginning of the experiment, visual assessment of prostrate knotweed densities varied from 25 to 90%. The main plots were blocked according to prostrate knotweed densities

Statistical analysis of experiments conducted in golf course in 2006 and 2007

For all experiments executed in this research statistical analyses were conducted using the SAS statistical software (SAS Institute Inc., Cary, NC, 2002-2004). For both experiments conducted in golf courses in 2006 and the one in 2007 data at 7 DPA and 14 DPA were subjected to Levene procedure (Gomez and Gomez, 1984) for testing homogeneity of variances at both times (7 DPA and 14 DPA). Data analyzed are the percentage of pretreatment of each plots. For experiments on cover (2006 and 2007) homogeneity was tested separately both for times and treatments (cover and uncover). Since cover treatment does not have significant effect on prostrate knotweed density, regression analysis has been conducted to explain the relation between knotweed density and bioherbicide rates. For the three experiments, data were subjected to log transformation to achieve normality and were analyzed using ANOVA to determine treatment effects. Normality was tested on the transformed residual variance using the Shapiro-Wilk test. Significant difference between bioherbicide rates were determined using the Tukey's test at $P = 0.05$.

Comparison among four cover types and their specific effect when combined with the bioherbicide.

Experimental site

The experiment was conducted at Sainte-Anne-de-Bellevue on the Macdonald campus of McGill University (latitude 45^{o}25" N, longitude 73^{o}55" W). The grass was heavily infested with weeds. Turfgrass maintenance program only contain mowing at 7 cm. The experiment was repeated on the Macdonald campus in the same area. Grass composition was dominated by Kentucky bluegrass (90%) and red fescue (*Festuca rubra* L.) (10%). Weed species included white clover, dandelion, plantain, and prostrate knotweed.

Methodology

To test effects of different type of covers and bioherbicide rates, a split-plot experiment with four replicates (blocks) was established. For each block, the four types of cover: jute, black non-woven geo-textile (100% polypropylene fibers, mechanically interlocked by heat setting), white row cover (polypropylene) and black polyethylene, were randomly assigned to main plots, and treatments (0, 20, 40 and 60 g/m^2) were randomly assigned to subplots(0.33m*0.33m) of each main plots. Irrigation was provided twice a day for two hours on the first three days. Climatic data including relative humidity, temperature and dew point, were recorded under each cover type and in the uncovered plots. Data were recorded using a data logger [model: *Hobo®* *HO8-032-08* *Pro series weatherproof data logger*, manufacturer: *Onset Computer Corporation* Pocasset, Massachusetts (*USA*)]. Relative humidity data and temperature was recorded each hour during 72 hours period. Data were combined in block of four hours. Each time interval in

figure 5 and 6 represents the mean of four data recorded hourly starting July 31, 2007 at 6h00pm for 72 hours. Weed densities for each plot were visually estimated at 0, 7, 14 and 21 DPA. Light intensity passing through each cover type was measured using a light meter [model*: LI-250A*, manufacturer: *LI-COR INC.*]. This light meter measures the illuminance in lux. Ten light measurements were recorded for each cover type at noon. At 3 DPA, when covers were removed, grass damage and mycelia growth of the bioherbicide active ingredient were evaluated. Grass damage was visually ranked from 1 to 10, where 1 corresponded to no grass damage and 10 for grass death. Mycelia growth was visually evaluated with a scale of 5: 0 for no growth and 5 for high growth. High growth means that the sub-plot is entirely covered by mycelia.

Statistical analysis

Data analyzed were the percentage of the pretreament weed density for each plot. To attain requirements for the analysis of variance (normality and homogeneity), one block of each repetition of the experiment was removed. Weed density data were Log transformed. Normality of the data were tested using the Shapiro-Wilk test (P = 0.0765). Data at each time were subjected to the Levene procedure and data were homogenous. Blocks did not have an effect, so it was removed from the model. An ANOVA and Tukey's test were used to determined difference between treatment means.

Effect of Banner and Daconil on bioherbicide efficiency

Two active ingredients, propiconazole [Banner ® MAXX fungicide (14%)] and chlorothalonil [Daconil ® Ultrex (82.5%) and Daconil ® 2787 (40.4%)] were used in two experiments to determine their effect on bioherbicide performance. These three fungicides were chosen because they are used by the associated golf courses to control dollar spot disease, the major fungal disease for golf course fairways.

Effect of propiconazole (Banner) and chlorothalonil (Daconil) on bioherbicide viability

The laboratory experiment evaluated the effect of different concentrations of the two fungicide active ingredients on the bioherbicide growth on potato dextrose agar (PDA, DIFCO Laboratories, Detroit, MI). PDA was amended with four different concentrations of propiconazole: 0.1; 0.01; 0.001; 0.0001ug/ml, and eight concentrations of chlorothalonil [Daconil ® 2787(40.4%)]: 1000, 100, 10, 1; 0.1; 0.01; 0.001; 0.0001 ug/ml. Higher concentrations of chlorothalonil were tested to determine the lethal dose because preliminary results indicated that the fungus was still viable at 0.1 ug/ml. Control treatment was PDA without any fungicide treatment. Bioherbicide grits (barley based formulation of *S. minor*) were sieved and only 1.4 to 2 mm grits were used. One grit per plate was applied and the plates were kept for 48 hours in a growth chamber at 20 ± 1^{o}C in the dark. Each concentration was replicated 10 times. Bioherbicide mycelium growth diameter was recorded at 48 hours. The experiment was performed twice.

Effect of fungicide treatments and time interval between fungicide application and bioherbicide application on weed control.

The goal of this experiment was to evaluate the effect of three common fungicide treatments used in golf courses on bioherbicide performance and the effect of the time interval between fungicide and bioherbicide applications. Plots of 0.1 m^2 were established at Macdonald campus of McGill University. Weeds present were of large diversity. Plots containing mainly white clover were chosen, but others weed species were present: prostrate knotweed, dandelion, plantain, wild strawberry (*Fragaria vesca* L.), chicory (*Cichorium intybus* L.), sulphur cinquefoil (*Potentilla recta* L.), common mallow (*Malva neglecta* Wallroth), orange hawkweed **(***Hieracium aurantiacum* L), and European wood-sorrel (*Oxalis stricta* L.).

The experiment used was a factorial design with two factors: 1. different time interval (number of days) between fungicide and bioherbicide applications (0, 4, 7, 14, 21 days); 2. three fungicide treatments applied. For fungicide treatments, the highest application rate recommended in Québec was used. Fungicide treatments used were: 1 Banner (14% propoconazole) at 51 ml/100 m^2, 2 Daconil ® Ultrex (82.5% chlorothalonil) at 212g/100m^2 and 3 Daconil and Banner at 115g/100m^2 and 30ml/100m^2 respectively. Treatment combinations were randomly assigned to every plot in a factorial design with two factors. The bioherbicide was applied the same day on every plot. Each combination was presented three times in both repetitions of the experiment. On each plot a jute cover were applied for three days, because inoculum caused considerable plant

infection/control under field conditions when the jute cover was used. During the first three days, irrigation was provided if needed once a day (if no precipitation occurred). White clover density was visually estimated at 0 and 7 days post application (DPA).

Statistical analysis

For each plate, mycelium growth diameter was recorded at 48 hours. Mycelium diameter for every concentration of each active ingredient was statistically analyzed. The two repetitions of the experiment were combined because the combined data was normal and homogenous. To achieve normality, data from chlorothalonil (Daconil) experiment were root square transformed and data from propiconazole (Banner) experiment were log transformed. Normality and homogeneity were tested for each concentration. Data were analyzed using ANOVA to determine the effect of each concentration. Contrast statements were used to determine differences between control and active ingredients concentrations.

The experiment on fungicide treatments under field conditions was conducted twice, but data could not be combined due to lack of normality and homogeneity. Data analyzed were the percentage of pretreatment calculated for each plot. Data of both repetitions were tested for normality and homogeneity using the Shapiro-Wilk test for normality and the Levene procedure for homogeneity. Data of the first repetition were log transformed, to reach normality. Even with the transformation, the residual were not clearly normal (P=0.0022), but they were homogenous. For the second experiment data were log transformed

and the residual was normally distributed (P=0.4947). Data were subjected to ANOVA to determine the effect of each factor and the effect of the interaction. Tukey's test at *P*=0.05 was used for multiple comparison within factors.

Weed density data

Weed density corresponds, in this research, to the percentage of ground covered by weeds. Percentage of weed cover was visually estimated. Plots of 0.1 m^2 (0.33m* 0.33m) were attentively examined and percentage of the area covered by weeds was estimated as follows: 100% - no grass can be seen in the plots, so plots were totally invaded by weeds; 0% - no weeds were present in the plot. To insure the precision of the data, weed density of 10 pictured plots were evaluated using Adobe Photoshop software. Each picture was crop to the border of the plot and weeds were painted in blue. Following this a ratio of pixels (pixels painted in blue on the overall number of pixels) was done. Statistical T test effectuated on the difference demonstrated no statistical difference between visual observation and computer weed density assessment. Following this conclusion, only visual observations were recorded and analyzed.

Bioherbicide viability and virulence

The inoculums were made using the protocol described in detail by Abu-Dieyeh 2006. Sclerotia of the fungus *S. minor* isolate IMI 1344141 were grown on potato dextrose agar (PDA). The active parts of the mycelium, the extremities, were used to inoculate modified Richard's solution (MRS) in 500 ml Erlenmeyer flasks. Flasks were placed on a rotary shaker and mycelia were allowed to grow for six to

seven days. Autoclave ground barley grits in breathable bags were inoculated with the *S. minor* colonised MRS. *S. minor* grew on and into the barley for five to six days and then the inoculum was dried to 0.4 water activity.

Viability and virulence are two quality control tests. Viability represents the ability of the inoculum to grow on PDA, and virulence is the ability of the inoculum to infect detached dandelion leaves. A viable batch produces a colony of 14-30 mm after 24h and 40-70 mm after 48h of incubation in a growth chamber at 20±1^{o}C in the dark. A virulent batch causes a lesion of more than 15 mm after 48h of incubation in the same conditions (Abu-Dieyeh, 2006). Inoculum is sieved and only inoculated barley grits of 1.4 to 2.0 mm are used in golf courses experiments and for fungicide experiments executed in laboratory. For experiments on cover types and the experiment on fungicides conducted under field conditions, grits were between 1.4 to 2.5 mm.

The laboratory experienced a loss of virulence during the summer 2007. We used a wider range in grits size because of the lost of virulence. Good quality inoculum was limited in quantity. We used inoculum produced in 2006. This material was stored in the freezer at -14^{o}C in the dark. Viability and virulence of the inoculum used were recorded at 48 h (Table1). Each test was effectuated using 10 inoculated barley grits.

Table 1. Viability and virulence of inoculum used in four different experiments. Colony growth was recorded after 48 hours of incubation at 20±1C in the dark. A viable batch produces a colony of 40-70 mm and a virulent batch causes a lesion of more than 15 mm (Abu-Dieyeh, 2006).

Experiment	Viability (mm)	Virulence (mm)
Golf 2006	53	20
Golf 2007	32	14
Cover 2007	47	23
Fungicide field	39	22

Weather data

Weather data for all experiments were obtained from Environment Canada meteorological data from the Dorval Station for experiment conducted in golf courses and from the Sainte-Anne-de-Bellevue station for experiment conducted at Macdonald campus of McGill University, Sainte-Anne-de-Bellevue (Appendix 1). Weather data are reported daily. Each repetition of experiment, when it was repeated on time, was conducted when environmental conditions (temperature and relative humidity) data were similar. Experiments were planned according to weather forecast. All experiments on the golf courses were conducted during cloudy and rainy periods.

Chapter 4 - Results

Experiments conducted in golf course environments in 2006

Determination of the optimal application rate to control prostrate knotweed

Prostrate knotweed density was affected by bioherbicide rates at seven days post application (7 DPA) (P=0.0002) and at 14 DPA (P=0.0001) (Figure 1). At both 7 and 14 DPA, all rates (20 g/m^2, 30 g/m^2, 40 g/m^2, 60 g/m^2) were significantly different from the control treatment (0 g/m^2) according to Tukey's test at P=0.05. However, no difference on prostrate knotweed density was detected between rates. At 7 DPA, prostrate knotweed percentage of pretreatment density was as low as 47%, 29%, 34% and 23% for the rates 20, 30, 40, 60 g/m^2, respectively. At 14 DPA prostrate knotweed density reached 21%, 13%, 12%, and 9% for the same rates (Figure 1). Repeated measure analysis demonstrated significant difference between times (P=0.0013). This means that the effects of the bioherbicide persisted over two weeks. Visual observation confirms the presence of living mycelium in the plots two weeks after application. More experiments should be done to determine the optimal application rate.

Effect of jute covering combined with the bioherbicide on prostrate knotweed control

In this experiment the effect of jute cover and bioherbicide rates on prostrate knotweed density were evaluated. No statistical difference has been detected between cover and no cover plots at 7 DPA and 14 DPA (P= 0.3751; P=0.1160).

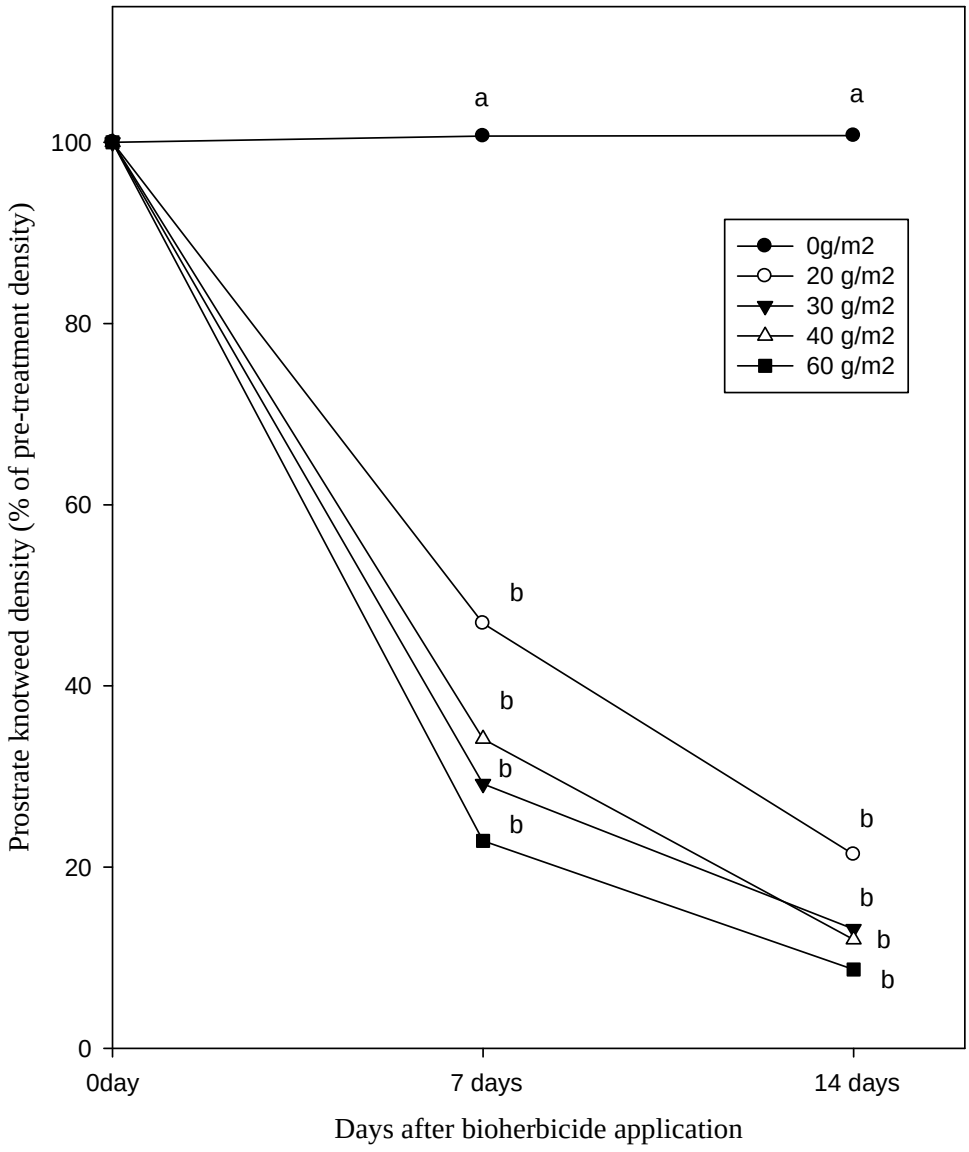

Figure 1. Effect of bioherbicide rate on prostrate knotweed density. Lines with a common letter at each time are not significantly different at *P*=0.05 according to Tukey's test for both times. Location: Royal Montreal, 2006.

Regression analyses do explain for both treatments (cover and no cover) and at both times (7 DPA and 14 DPA) the effect of bioherbicide rates on prostrate knotweed density. Covered plots show more mycelium development than uncovered plots at 3 DPA (Plate 1A and B). It appeared that cover fasten the infection process and also reduced variability between plots. The cover usage increases infection caused by the bioherbicide at lower application rates (Figure 2). Repeated measures analysis showed statistical difference between 7 DPA and 14 DPA. At 7 DPA in uncovered plots, 20 g/m^2 and 30 g/m^2 application rates were not significantly different (0.05 level) from the control treatment (0 g/m^2) (P=0.8835 and P=0.0927) with 74% and 50% of knotweed density . 40 g/m^2 and 60 g/m^2 achieved good control with 36% and 23% of prostrate knotweed density. At 14 DPA in uncovered plots, only 20 g/m^2 was not different from the control (P=0.4097) with 62% of knotweed density. The others rates 30 g/m^2, 40 g/m^2, 60 g/m^2 reduced prostrate knotweed density to 32%, 21% and 22% respectively. In covered plots more weed control was achieved at lower rates. At 7 DPA, only 20 g/m^2 is not significantly different from the control treatment (P=0.0539) with 52% of prostrate knotweed density. While 30 g/m^2, 40 g/m^2 and 60 g/m^2 reached 38%, 35% and 25% weed density. At 14 DPA in covered plots, all rates are statically different from 0 g/m^2 at the 0.05 level. 20 g/m^2 reached 31% weed density. The others rates, 30 g/m^2, 40 g/m^2 and 60 g/m^2, achieved a weed percentage lower than 20% (16%, 19% and 15%).

A

B

Plate 1. Mycelium growth in uncovered plots three days post application (DPA) of the *Sclerotinia minor* based bioherbicide (A). Mycelium growth in covered plots with jute three DPA of the *Sclerotinia minor* based bioherbicide (B). Location: Royal Montreal, 2006.

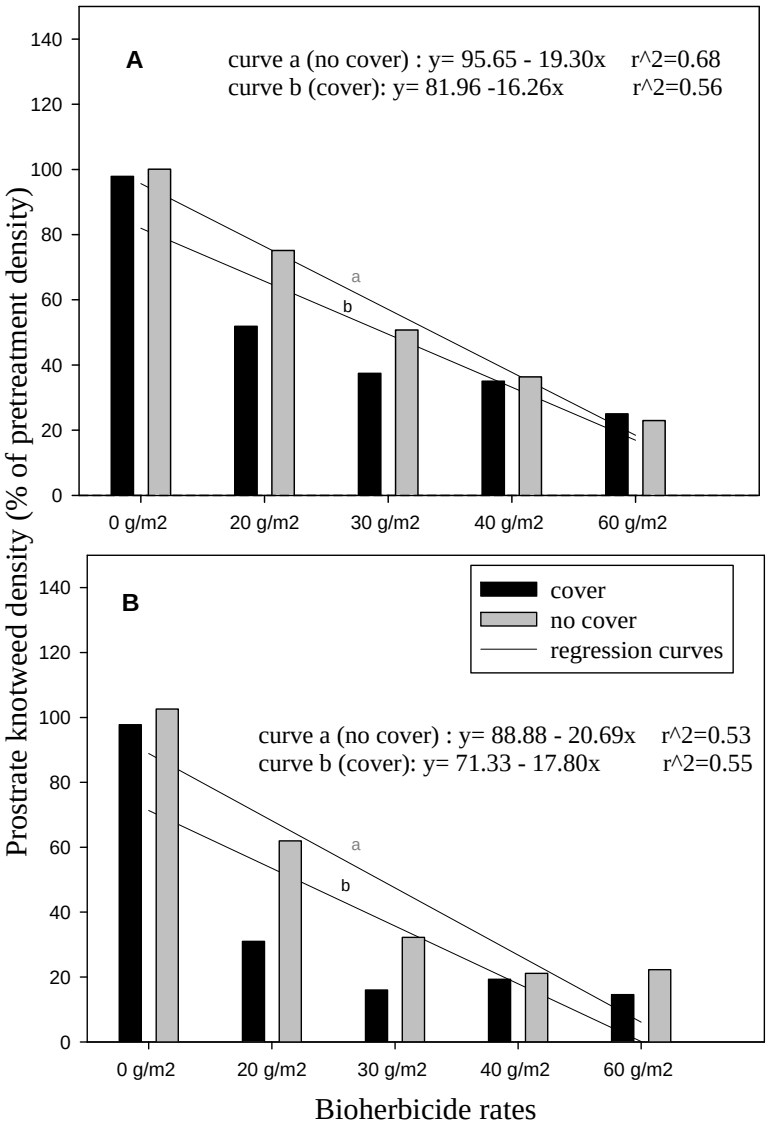

Figure 2. Effect of bioherbicide rate and jute cover on prostrate knotweed density at seven days post application (7 DPA) (A) and 14 DPA (B). Relationship between cover treatment and bioherbicide rates and no cover treatment and bioherbicide rates at both times are showed by regression curves. Cover was applied for three days following the bioherbicide application. Location: Royal Montreal and Beaconsfield, 2006

Experiment conducted in golf course environments in 2007

Effect of jute cover and bioherbicide rates on prostrate knotweed density in golf course environments in 2007.

Similarly to experiments conducted in 2006, this experiment aimed to evaluate effects of jute cover and bioherbicide rates on prostrate knotweed density. Results are different from experiments conducted in 2006 because of the quality of the inoculums (Table 1). Significant effects of jute cover and bioherbicide rates have been detected at the 0.05 level. Jute cover increased bioherbicide prostrate knotweed control under golf course environments (Figure 3). Time has no distinct effect (P=0.0501**).** At 7 DPA and 14 DPA cover and bioherbicide rates resulted in significant prostrate knotweed density reduction (P = 0.0029; P = 0.0019 and P = 0.0001; P = 0.0049). At both time intervals, jute cover enhanced the bioherbicide activity, thus increased weed control. At 7 DPA and 14 DPA in covered plots, all rates were different from the control at the 0.05 level, but no difference was detected between rates at the same level. At 7 DPA 20 g/m^2, 30 g/m^2 and 40 g/m^2 achieved 45%, 48% and 50% prostate knotweed density respectively and at 14 DPA same rates reached 56%, 40% and 48% prostrate knotweed density. In uncovered plots at 7 DPA, 30 g/m^2 and 40 g/m^2 are different from the control at the 0.05 level with 74% and 66% prostrate knotweed density, but at 14 DPA only 30 g/m^2 is different from the control at the same level with 78% prostrate knotweed density.

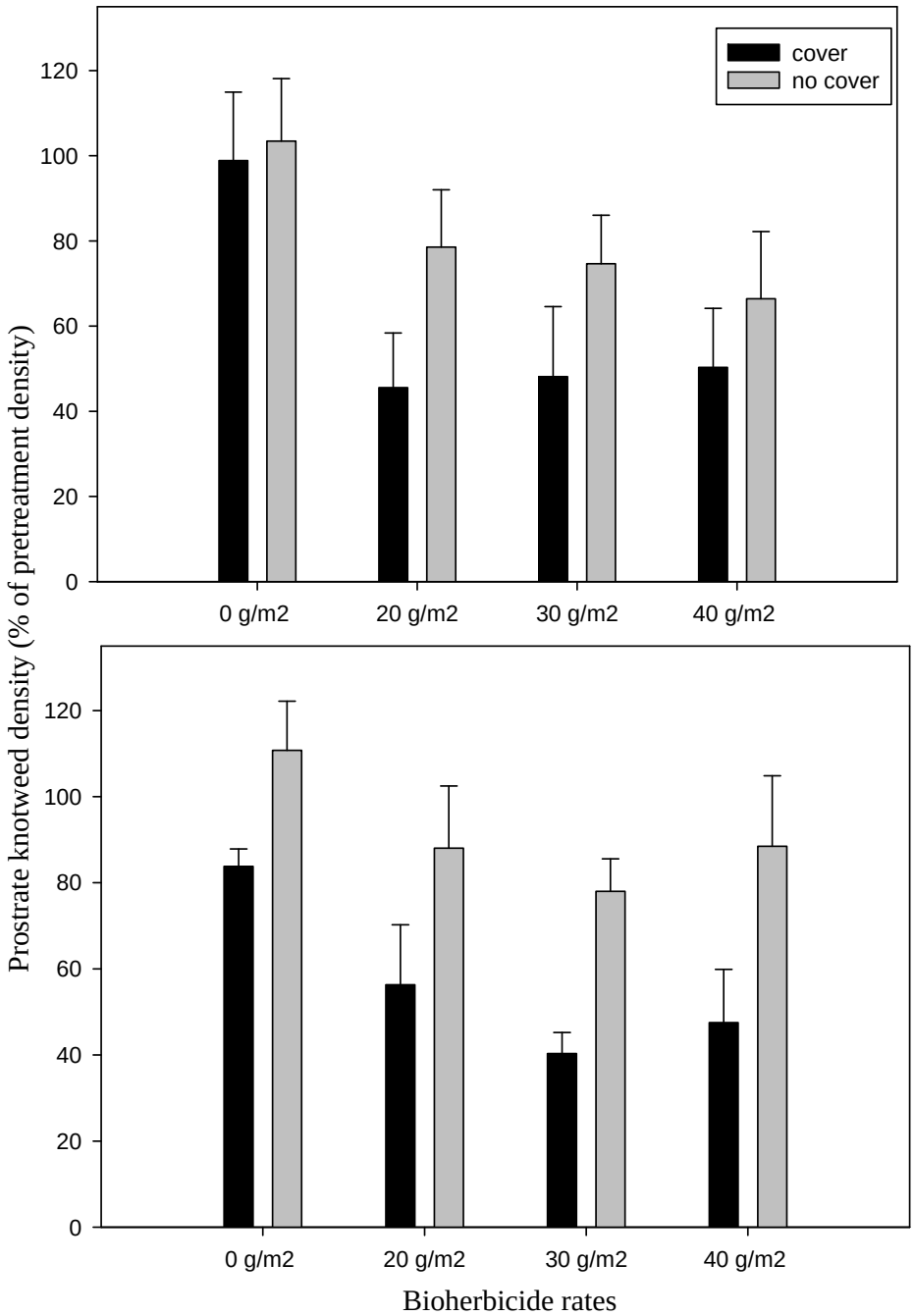

Figure 3. Effect of jute cover and bioherbicide rate on prostrate knotweed control at seven days post application (7 DPA) (A) and 14 DPA (B). Cover was applied for three days following the bioherbicide application. Error bars are standard error. Location: Royal Montreal and Beaconsfield, 2007.

Comparison between four cover types and their specific effect when combined with the bioherbicide

Impact of the covers: temperature, humidity, mycelium growth, light transmission, grass damage

To determine the best cover to use, environmental conditions under each type of cover were monitored Biggest temperature difference between covers and no cover happened when the sun is the strongest (between 10 a.m. and 2 p.m.). Jute plot differed from no cover plot of 7^{o}C, compare to 10^{o}C and 13^{o}C and 17^{o}C for polypropylene, geo textile and polyethylene respectively (Figure 4). Jute cover is the one that produces temperature the most similar to the uncovered plots. The two black covers (geo textile and polyethylene) greatly increased temperature during the day. This experiment has been conducted in extreme conditions for the bioherbicide: 11 days of 14 had a maximum of over 25^{o}C (appendix 1). During the repetition of the experiment, temperature was lower, five days of 14 reached more than 25^{o}C (appendix 1).

Relative humidity was also recorded under each cover type. All covers have the capacity to retain water. Most of the time relative humidity level recorded under each cover was higher than 90% (Figure 5). Irrigation was done every between 10h00 to 12h00. Relative humidity dropped between 2h00 to 18h00 due to high temperature. At these periods, the lowest relative humidity recorded was 69% for polypropylene, 73% of jute cover, 74% for geo textile and 80% for polyethylene compare to 58% for uncovered plots.

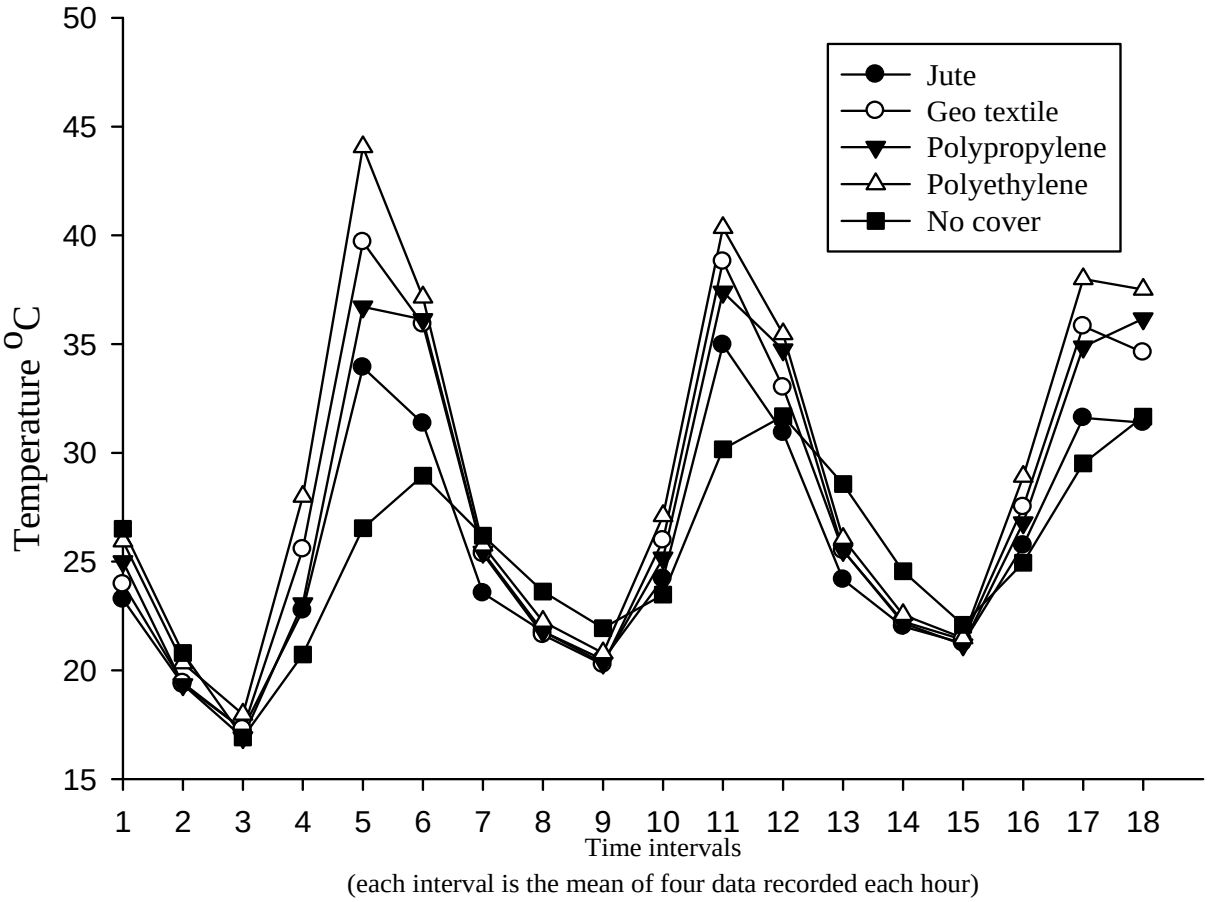

Figure 4. Effect of row covers on the air temperature. July 31, 2007 started 6h00 pm for a 72 hours period.

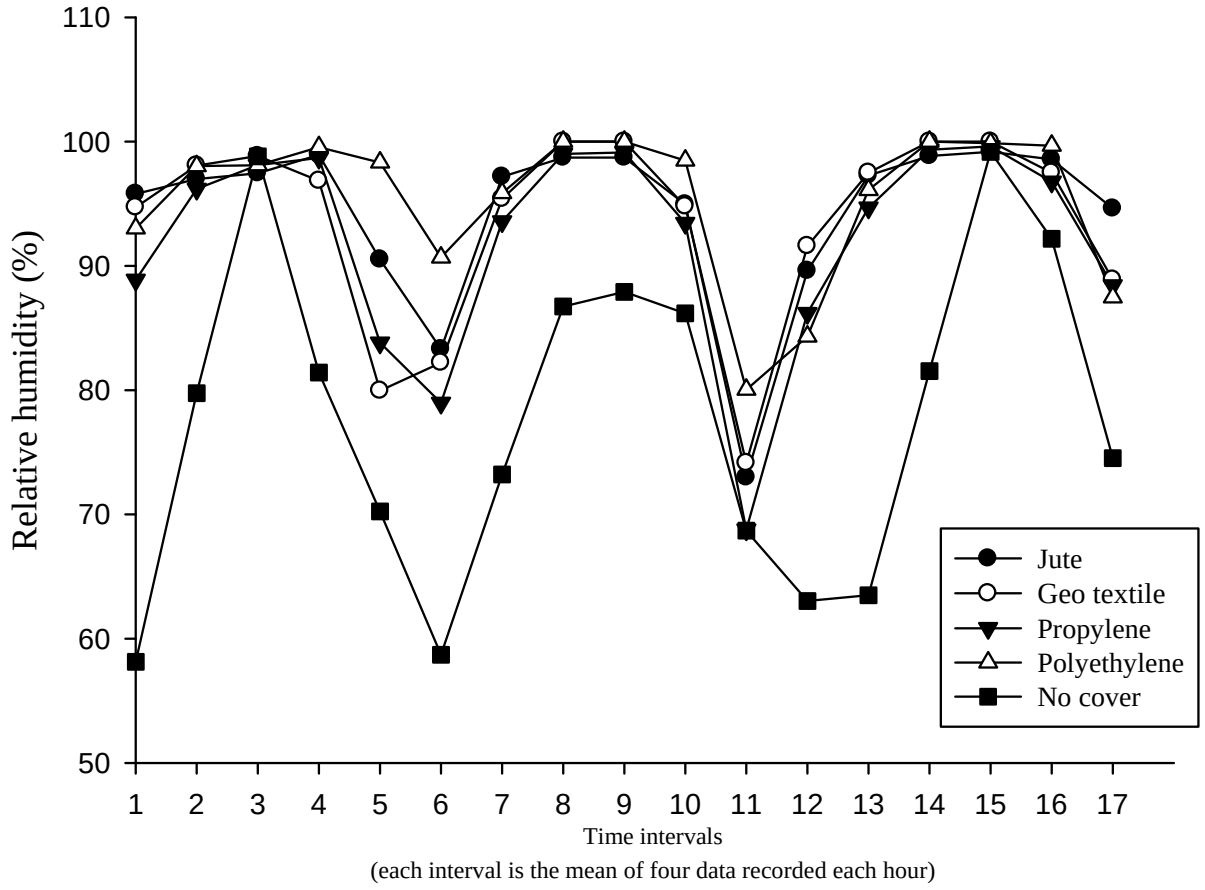

Figure 5. Effect of row covers on air relative humidity. July 31, 2007 started at 6h00 pm for a 72 hours period

Mycelia growth recorded at 3 DPA shows significant difference between covers. The jute cover promotes the most mycelia growth with a visual ranking of 5 of 5 (Figure 6). All plots covered by jute allowed good mycelium growth Geo textile is closest to the jute cover with a 3.9/5 and the polyethylene cover also allowed good mycelium growth with 3.2/5. Even when temperature becomes very high under polyethylene cover, the bioherbicide germinated and produced good mycelia.

To evaluate light intensity passing through the covers, illuminance was recorded with the light meter (Figure 6). The polyethylene cover does not allow any light to pass through. The white row cover (polypropylene) allows light to reach the ground (998 lux) and it is the one that is the most similar to the uncovered plots (1305 lux). Jute cover and the geo textile material decrease significantly the amount of light that reaches the ground with 114 lux and 86 lux respectively.

For efficient weed control, it is important to favour grass growth and not damage it. Polyethylene greatly damage grass with 9.5 on 10. Geo textile damage only the tip of the grass leaf 2 on 10. Others tested covers did not have any detrimental effects on grass (Figure 6). Some covers tested had detrimental effect on grass (Plate 2a). For example, the polyethylene black cover greatly damaged grass and the geo textile cover burned leaf tips (Plate 2b). The jute cover and the row cover (polypropylene) did not negatively affect the turf grass.

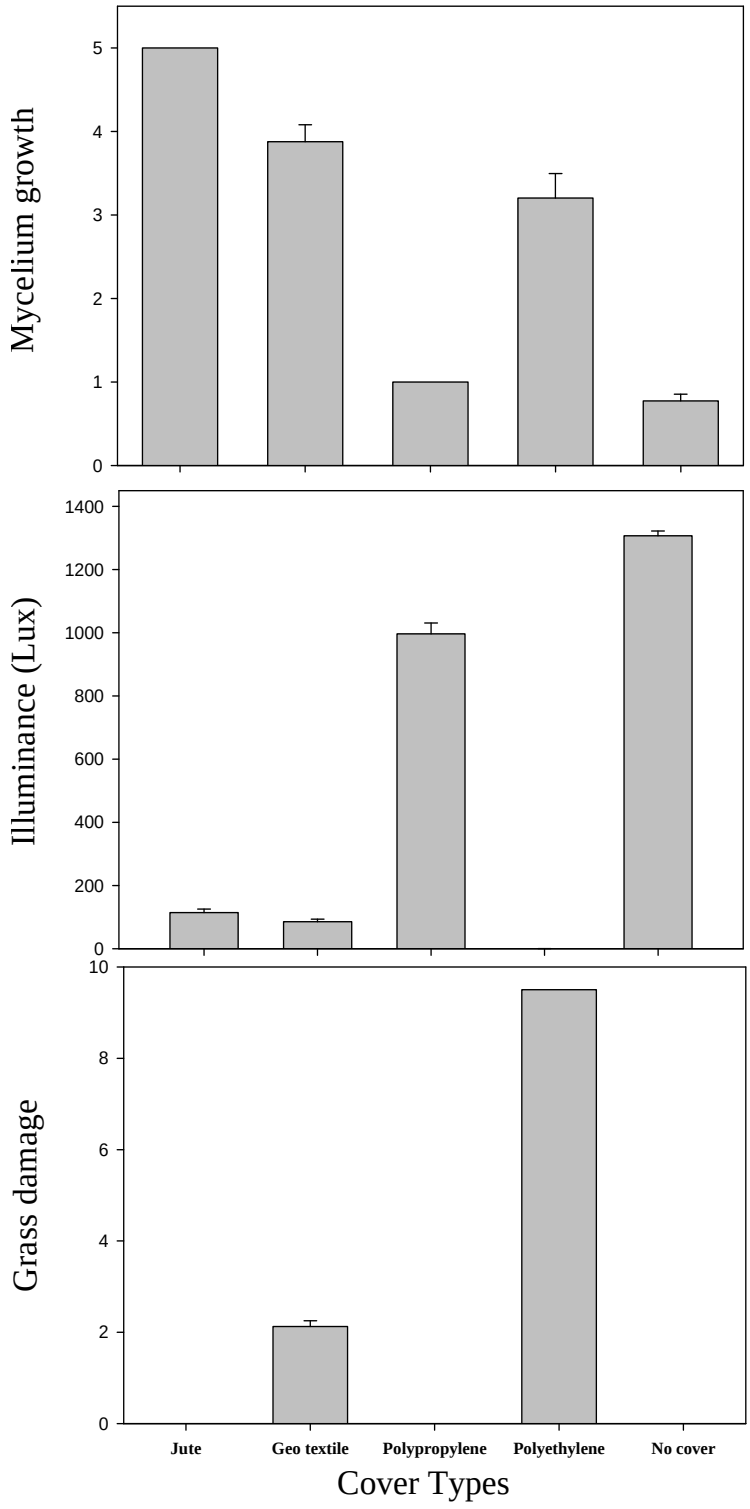

Figure 6. Effect of different covers on mycelium growth, light illuminance, and grass damage. Error bars are standard error. Location: Macdonald campus of McGill University, Sainte-Anne-de-Bellevue, 2007.

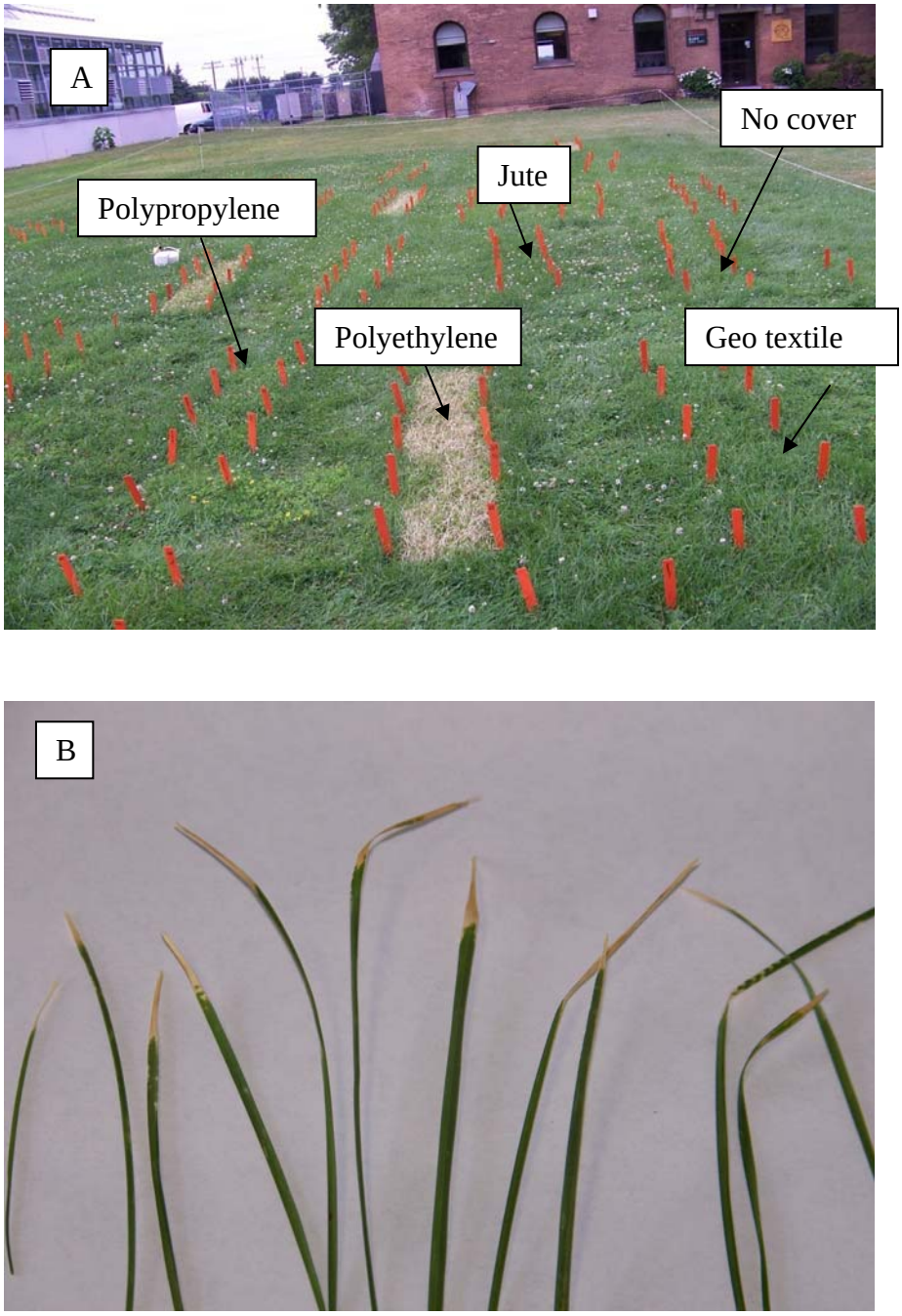

Plate 2. Grass damage caused by four different cover types three days after application of the *Sclerotinia minor* based bioherbicide. Dead grass is found under the polyethylene cover (A), Grass damage under the geo textile three days after application of the *Sclerotinia minor* based bioherbicide (B). Location: Macdonald campus of McGill University, Sainte-Anne-de-Bellevue, 2007

Effect of cover type and bioherbicide rate on white clover density.

Blocks did not have any effect, and cover types and rates both had a significant effect on white clover density. The interaction between cover type and bioherbicide rates had a significant effect on white clover density at the three times (7 DPA *P*= 0.0002, 14 DPA *P*= 0.0027, 21 DPA *P*= 0.0001). Repeated measure analysis demonstrated a significant effect of time and a significant effect of the interaction between time and cover types. The bioherbicide applied in combination with the jute cover provided better white clover control than all others covers at any time excluding polyethylene (Figure 7). At 40 g/m^2, the jute cover reached 15% and 23% at 14 and 21 DPA. At 60 g/m^2, the jute cover reduced white clover density to 7% and 10 % for 14 and 21 DPA respectively. The polyethylene material killed the weeds without the bioherbicide and no statistical difference could be detected between rates. The polypropylene row cover greatly increased weed density when the bioherbicide is not applied and at 20 g/m^2, 40 g/m^2 and 60 g/m^2 white clover density is not different from uncovered plots at 7, 14 and 21 DPA; almost no control is achieved. Geo textile in combination with the bioherbicide showed some white clover control, but the jute cover achieved more white clover control. At 21 DPA for 40 g/m^2 and 60 g/m^2, jute cover controlled and geo textile controlled are statistically different. The jute cover reached 24% and 9% of white clover density compare to 79% and 66% for the geo textile cover.

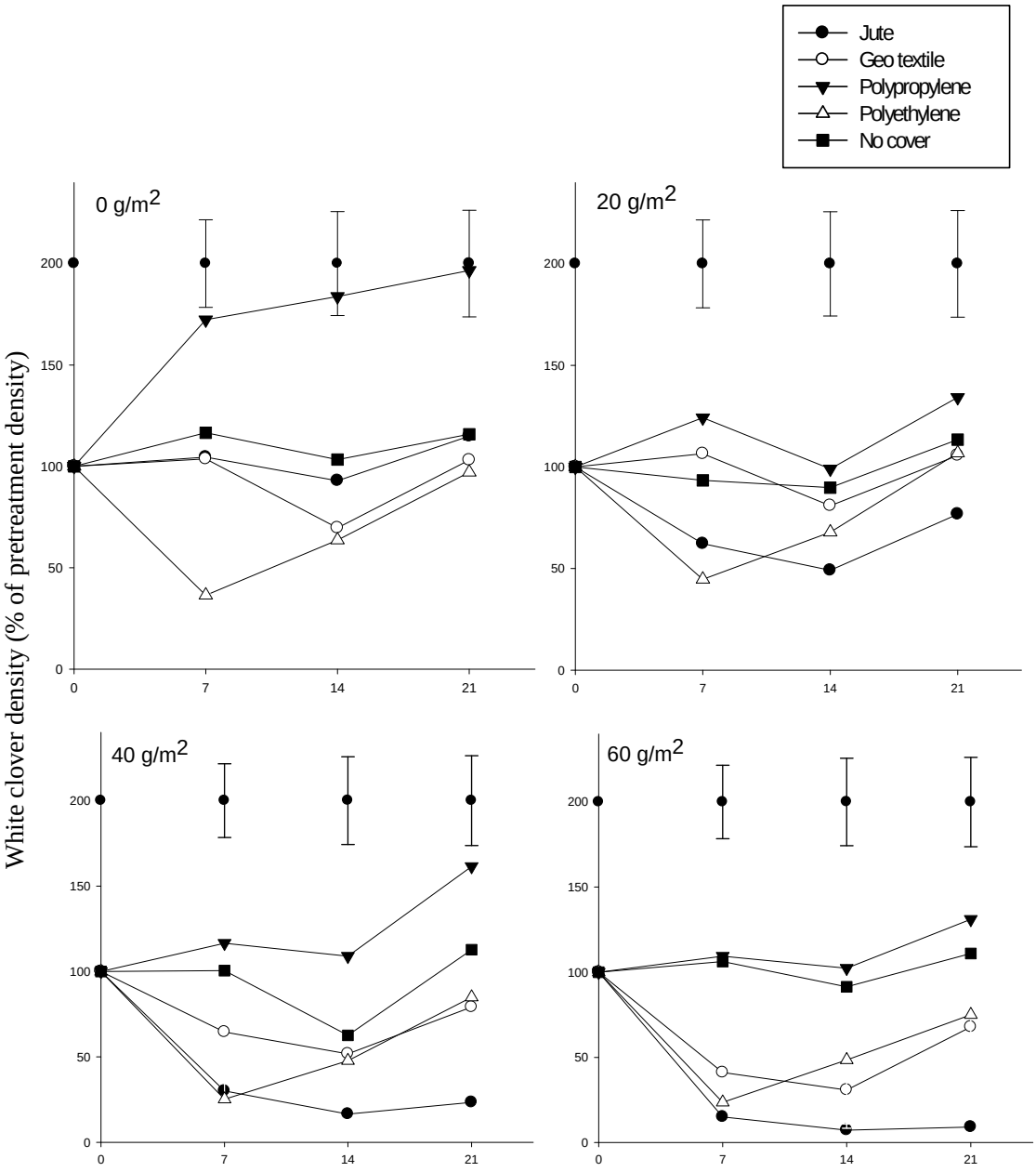

Figure 7. Effect of different cover treatments and bioherbicide rate on white clover density. Covers were placed immediately after treatment and removed after four days. White clover density are significantly different at P=0.05 according to Fisher's least significant differences (LSD) test. LSD value is indicated by LSD bar for each time. Location: Macdonald campus of McGill University, Sainte-Anne-de-Bellevue, 2007.

Effect of Banner and Daconil on bioherbicide efficiency.

Effect of propiconazole (Banner) and chlorothalonil (Daconil) on bioherbicide viability

For chlorothalonil (Daconil), a significant effect was only detected with rates over 10 ug/ml. Active ingredient rates above 10 ug/ml (1; 0.1; 0.01; 0.001; 0.0001 ug/ml) did not significantly affect bioherbicide growth on PDA compared to bioherbicide growth on PDA without fungicide (Figure 8). For propiconazole (Banner), 0.1 ug/ml rate totally inhibited bioherbicide growth and the 0.01 and 0.001 ug/ml rates also significantly reduced bioherbicide growth compared to the control. Lower concentrations, 0.0001 and 0.00001 ug/ml, did not affect bioherbicide growth on PDA.

Effect of fungicide treatments and time interval between fungicide application and bioherbicide application on weed control.

To determine the numbers of days that a golf manager needs to wait between a fungicide and a bioherbicide applications and to determine the effect of different fungicide treatments on the bioherbicide performance a factorial experiment was conducted. Factor 1 was number of days between fungicide application and bioherbicide application (0, 4, 7, 14, 21 days); factor 2 was the fungicide treatments (Banner, Daconil and their mixture).

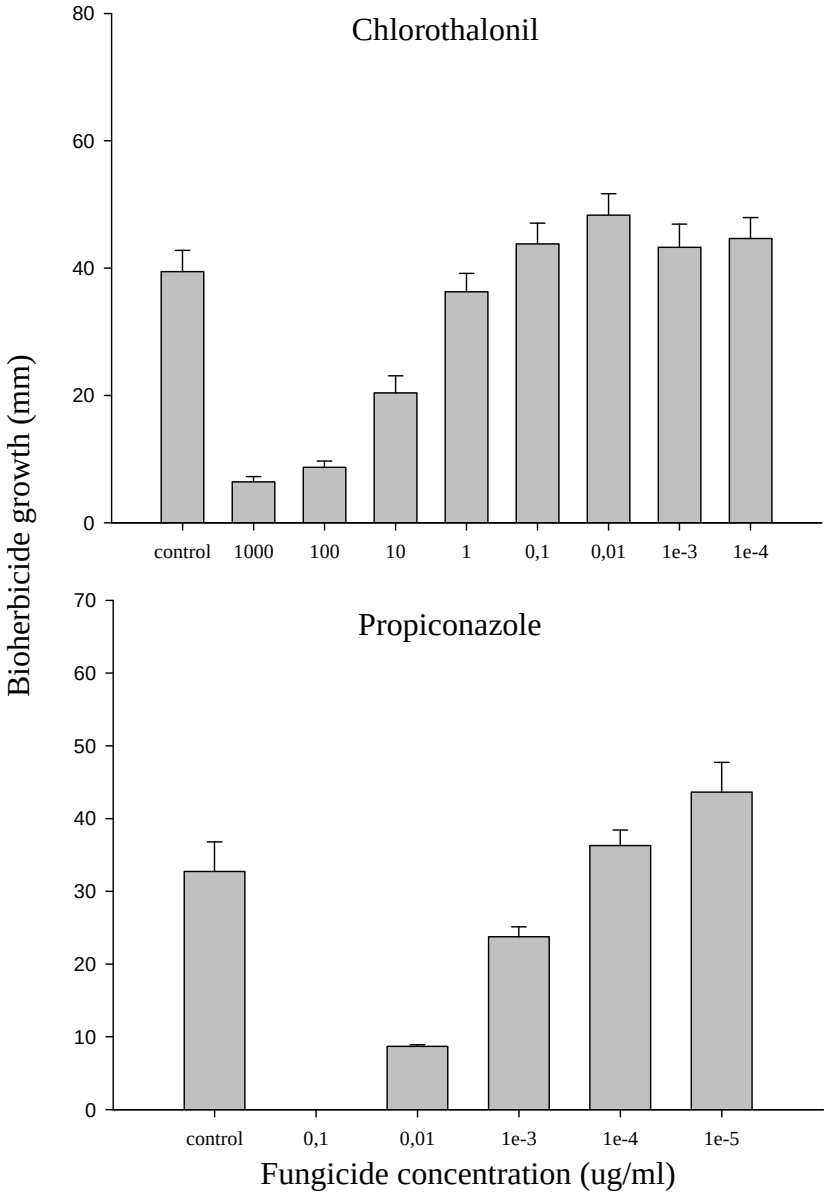

Figure 8. Effect of chlorothalonil and propiconazole active ingredients on bioherbicide viability on potato dextrose agar. Data recorded after 48 hours. Error bars are standard error.

For the first trial, statistical analysis revealed that the numbers of days had no effect on the bioherbicide activity (P=0.9993), but fungicide treatment had a significant effect (P=0.0424) (Figure 9a). The interaction between the two factors was not significant.

For the second trial, there was a significant effect of the fungicides (P=0.0101) and the numbers of days between fungicide and bioherbicide application (P=0.0353) on bioherbicide weed control. The interaction between factors was not significant. Only the bioherbicide applied 14 days after fungicide applications was statistically different from day 0 (P=0.0281) (Figure 9b). All other intervals, including 21 days, were equal to day 0.

For the fungicide treatments, Banner alone (treatment A) and Banner and Daconil together (treatment C) were statistically different P=0.0298 and P=0.0116, in comparison with the control (no fungicide).

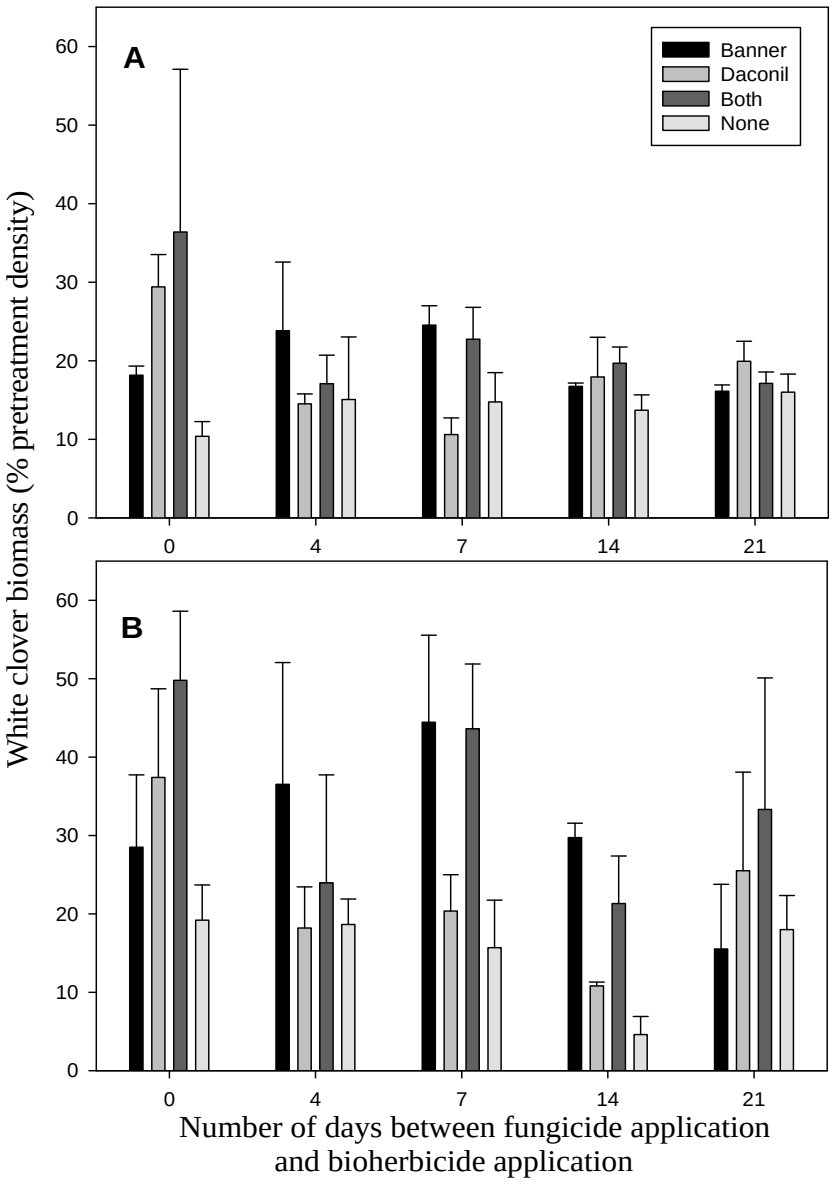

Figure 9. Effect of fungicide treatment and length of time between fungicide application and bioherbicide application on bioherbicide activity. All the plots were covered with jute for four days after bioherbicide application. Fungicide rates used were the highest rates for curative purposes: Banner at 51 ml/100m^2; Daconil at 212g/100m^2; Daconil and Banner 115g/100m^2 and 30ml/100m^2. Data were recorded seven days after bioherbicide application. (A) is the first repetition and (B) is the second repetition. Error bars are standard error. Location: Macdonald campus of McGill University, Sainte-Anne-de-Bellevue, 2007

Chapter 5 - Discussion

Optimal bioherbicide rate

In this research, the higher rates had greater effect on prostrate knotweed control. However, no statistical difference was detected between 30 g/m^2, 40 g/m^2 and 60 g/m^2 for all experiments executed in golf courses environments in 2006. Schnick et al., (2002) found similar results for dandelion control: 40 g/m^2 and 60 g/m^2 successfully controlled dandelion, but 60 g/m^2 achieved only slightly higher results. 40 g/m^2 demonstrated good weed control in previous studies in residential turfgrass (Abu-Dieyeh and Watson, 2007). Accordingly, 40 g/m^2 to 60 g/m^2 was the optimal application rate under optimal conditions for golf course environments. Prostrate knotweed density was 12% and 9% respectively for both rates (without cover). This conclusion is based on trials conducted without mowing. During this experiment environmental factors were favourable: temperature was around 20^{o}C and relative humidity was high (over 85% max, for eight days on 14) (Appendix 1). Higher rates could be used to fasten the spread of the disease or to compensate for inadequate environmental conditions, including intensive mowing. Further experiments should be conducted to define the optimal bioherbicide rate for golf courses.

Best cover to promote weed control with the bioherbicide

It is well known that covers modify environmental conditions (Horowitz et al., 1983; Ibarra et al., 2001; Perring et al., 1989; Qureshi et al., 2007). Several cover types are employed in agriculture and their utility varies. Row covers were first used to increase resistance to frost (Perring et al., 1989). Row covers increase air temperature surrounding

the crop (Ibarra et al., 2001). They also effectively prevent damage from insect pests (Qureshi et al., 2007) and can protect crops from bad weather (heavy rain, wind) (Dubois Agrinove website). Black plastic mulch is applied on the soil surface to control weeds and increase temperature. Transparent plastic mulch increases temperature to a higher degree than black plastic, but both can kill weeds by solarization (Horowitz et al., 1983). The geo textile material controls weeds in landscape management. It is thick and fibrous, and undesirable plants should not be able to break through. In the experiment on cover types, the objective was to find the cover material most favourable to the bioherbicide growth and activities on weeds. The four covers had different effects on weed control with the bioherbicide. Plots covered with the jute cover got superior control compared to all other covered and uncovered plots at 40 g/m^2 and 60 g/m^2 at 21 DPA. The jute cover appears to be the most appropriate one; it does not damage grass, it retains water, and allows some light penetration.

High temperature and low humidity decrease survival of *S. minor* (Adams, 1987). Temperature and humidity are very important factors for disease development processes. Temperatures higher than 30^oC greatly reduce *S. minor* infection (Adams, 1987; Adams and Wong, 1991). One of the hypotheses of this thesis was that jute cover should decrease extreme temperatures. The experiment on cover type was conducted when the temperature was high (Tables 4 and 5). However, jute cover did not reduce the temperature compared to uncovered plots, but it reduced temperature fluctuation compared to other tested covers. Temperature under the jute cover was the nearest to the uncovered plots. Almost no weed control was accomplished in the uncovered plots, while in the jute covered plots, successful weed control was achieved (15% and 7% white

clover density for 40 g/m^2 and 60 g/m^2 at 14 DPA). Humidity level was much lower in uncovered plots compared to any tested cover, especially during intense sun radiation at around 2:00 PM. The recorded humidity level revealed that uncovered plots might have missed moisture since the required level is 95% (Watson, 2007). Low weed control in uncovered plots can be due to those periods of dryness that might have affected the survival of the fungus under these high temperatures. The irrigation schedule was similar to the one used in past experiments and adequate weeds control were achieved without any cover, but the temperature was lower (close to 20^{o}C) (Mohammed Abu-Dieyeh, personal communication). If the only difference between jute covered plots and the uncovered plots was the humidity level, better irrigation schedule should compensate for high temperature. This hypothesis has not been verified. However, humidity cannot compensate for temperature higher than 40^{o}C (temperature recorded under polyethylene cover). It is important to mention that jute alone does not have any effect on weeds, but it might have a positive effect on the grass.

Even in high humidity levels, the fungus had difficulty to survive temperatures over 35^{o}C. Only the jute cover and uncovered plots did not reached temperature over 35^{o}C, and at similar moisture level, all others covers, did not achieved satisfactory weed control probably because of extreme temperature. Temperature monitored under the two black cover (geo textile and polypropylene) are too high for the bioherbicide growth, especially the polyethylene one. Highest temperature was closed to 45^{o}C. Optimal temperature for *S. minor* mycelium germination around is between 18^{o}C and 25^{o}C (Hao et al., 2003).

All covers might have other beneficial impacts on the bioherbicide. It most probably increases contact between leaves and the bioherbicide. Contact is essential to disease development (Huang and Hoes, 1980). Light meter measurements demonstrate lower light quantity reaching the ground, thus the bioherbicide may have been protected from UV radiation by all covers except the polypropylene row cover. UV radiation is known to have no effect on the production of sclerotia, but it strongly inhibits mycelium growth of the related species S. *sclerotiorum* (Nagy and Fischi, 2002).

Another hypothesis is that the bioherbicide used was probably not of the best quality, notably because it had been kept frozen since 2006. Virulence and viability tests were passed (Table 1), but maybe the fungus lost some abilities to infect under field conditions, where it has to sustain climatic conditions, plant defence mechanisms, natural enemies, ultra-violet (UV light), etc. Jute cover slightly reduced the dew point, while other covers increased it compared to the uncovered plots.

The jute cover might be difficult to integrate in a highly managed system like golf courses. Also jute cover might enhance dollar spot disease. *S, homoeocarpa* since it shares common characteristics with *S. minor*; they are both cold climate fungus and they require dew to develop.

Fungicides and bioherbicide interactions

The type of fungicide and the numbers of days separating the two treatments (fungicide first and bioherbicide second) can both affect bioherbicide weed control. Many

fungicides have been reported to have some inhibitory effects on *S. minor*, but no reports mentioned chlorothalonil and propiconazole being used for any crop.

The two fungicides tested in this research had different effects on bioherbicide viability. The propiconazole active ingredient had greater inhibitory effect on bioherbicide mycelium growth on PDA than chlorothalonil active ingredient. It took over 10ug/ml of chlorothalonil to significantly inhibit mycelium growth while propiconazole required only 0.001ug/ml. For both active ingredients, when the highest registered application rates are applied, mycelium growth of *S. minor* is inhibited on potato dextrose agar (PDA) plates. The highest registered application rate Daconil (chlorothalonil) (350 ml/100m^2) equals approximately 0.027 ml per PDA and this amount represents between the tested 10 and 100ug chlorothalonil/ml rates. At this rate, mycelium growth is significantly reduced. For Banner (propiconazole), the highest registered application rate (51 ml/100m^2) is equal to 4.0 x 10^{-3} ml per PDA plate. This rate is far greater than the inhibitory rate of 0.1 ug propiconazole/ml (1.3 x 10^{-5} ml per plate). Banner has a greater inhibitory effect on *S. minor* mycelium growth than does Daconil.

In both repetitions of the field experiment, treatments containing Banner (propiconazole) (treatments A and C) impeded the bioherbicide activity on weed control. The two treatments are statistically different from the control independent of the number of days between the fungicide and the bioherbicide applications. Nonetheless, it is important to note that the effect of the bioherbicide was lowered by the fungicide application, but it was not totally inhibited. Weed control in plots containing Banner (propiconazole) varied from 10%-66%. Daconil (chlorothalonil) fungicide did not greatly

affect the bioherbicide efficacy. Daconil can probably be used in combination with the bioherbicide, but banner will most likely adversely affect bioherbicide efficacy during the first 14 days.

No statistical difference has been found between the numbers of days for the first repetition of the experiment. However greater inhibition was observed when the bioherbicide and a fungicide were applied together. Also mycelium growth at 3 DPA confirms the growth inhibition caused by fungicide treatment containing Banner (propiconazole) (Data not presented). The lack of statistical differences can be explained by the high variability within treatments and the small number of replicates (only three replicates). In the second repetition, the bioherbicide was also more affected when fungicides and bioherbicide were applied the same day, but the type of fungicide has much greater effect. Statistical analysis revealed differences between the number of days: Fungicide treatments applied 14 days before the bioherbicide application yielded different results compared to the three treatments applied the same day. According to this, and taking into account the large variability within treatments, fungicides tested do not have any significant effect on the bioherbicide after 14 days. In the first trial (Figure 9A), fungicide treatments applied seven days before the bioherbicide appeared to be the last treatment date to affect the bioherbicide. When the fungicide treatment was applied 14 days and 21 days before the bioherbicide, difference with the control (plots without fungicide at any numbers of days) was minimal, and this small difference could be due to high variability that often occurs in field experiments. In the second trial (Figure 9B), when the bioherbicide is applied seven days after fungicides treatment, weed control seems to be reduced. At 14 days, difference with the control (all plots without fungicide

treatments) is minimal. Other experiments should be conducted to test the effect of fungicide on bioherbicide weed control when fungicides are applied after the bioherbicide application.

Difference between both repetitions can be explained by the difference in temperature (Appendix 1). During the second repetition, temperature was lower at night (4.3 and 4.8^{o}C) (Appendix 1) and the bioherbicide grew more slowly. Three days after the bioherbicide application, minimal mycelium growth occurred. During the first repetition, good mycelium growth was recorded three days after the application. Cold temperature slows down the bioherbicide growth, infection and development.

Integrated pest management

Like any other tool or technique, to include the bioherbicide in an integrated pest management program for a golf course, some minor adjustments have to be done to attain the maximum benefit from this new tool. It is important to note that the effect of the bioherbicide will depend on climatic conditions, grass competitiveness, weed infestation level and weed age. Fortunately many adjustments needed to get the maximum benefits from the bioherbicide are good for the grass since bioherbicide aims at increasing grass competitiveness against weeds. Mowing height and frequency; fungicide applications and temperature and moisture are the major factors to manage to obtain satisfactory results.

In golf courses, grass on green and fairways is usually mowed each day or every other day. Bioherbicide needs between three to five days to control weeds depending on environmental factors. Mowing effect has never been studied with standard quality

inoculums in golf courses. In 2006, mowing was delayed for two weeks and in 2007 inoculum was inadequate. In 2007, control occurred on covered plots and the mowing was delayed for only four days. The mowing schedule can be delayed and mowing height can also be higher without any harm to grass. In fact, higher mowing height should decrease prostrate knotweed germination, because like many others weeds it needs light stimuli (Batlla and Benech-Arnold, 2005) and also grass should become more competitive against pests.

Daconil (chlorothalonil) can be used in combination with the bioherbicide, but both treatments should not be applied on the same day. Fortunately, it is not recommended to apply fungicide on rainy days, while it is strongly recommended to apply the bioherbicide under rain. If there is no rain, bioherbicide application requires irrigation, while fungicide treated areas should not be irrigated until fungicide residues are dried. Since a delay of four days is enough to reduce the effect of Daconil (chlorothalonil), no interaction is presumed if this methodology is followed.

Grass competitiveness has to be favoured to increase long term weed control. It was found that overseeding increases weed control in residential turfgrass (Abu-Dieyeh and Watson, 2007). Overseeding can be used in golf courses. Other techniques used to favour grass quality and competitiveness in golf courses, like core aeration and strip cutting, should be experimented in combination with the bioherbicide.

Prostrate knotweed age might have an impact on bioherbicide weed control efficacy. Weed susceptibility to the bioherbicide can vary with age. For example, dandelion is more affected when it is young. In a grass environment, 4- and 6-week-old

treated plants died without recovery, while older plant can recover (even after 100% above-ground damage). The 8- and 10-week-old treated plants showed 90 % damage six weeks after application. Only 13-week-old treated plants present 50% damage after six weeks (Abu-Dieyeh and Watson, 2005). Prostrate knotweed susceptibility related to its age has not been assessed, but good control was achieved in knotweed population when the bioherbicide was applied in early June (Mohammed Abu-Dieyeh, personal communication). Experiments effectuated in golf courses in 2006, demonstrated the ability of the bioherbicide to control older prostrate knotweed, since the bioherbicide application had been done in August, and prostrate knotweed is known to germinate in a single flush in May (Costea and Tardif, 2004).

Limits of the experiments

In 2006, grass was not mowed for two weeks on the course, thus the effect of intense mowing on bioherbicide efficacy remains unknown. In this research, the fungus infected knotweed in short grass (3 cm) in three to five days (depending on environmental factors), thus mowing height and frequency might not significantly affect bioherbicide performance, if the first mowing after bioherbicide application is done once the infection has started.

In the fungicide experiment, all plots were covered with jute for three days to ensure bioherbicide growth and irrigation was applied after bioherbicide applications. These two practices may have influenced fungicides' behaviour: cover might have reduced fungicide evaporation and irrigation might have increased leaching of the fungicides.

Chapter 6 Conclusions

In this research, the efficacy of the bioherbicide SARRITOR has been tested in golf course environments on prostrate knotweed. It has been demonstrated that prostrate knotweed can be partially control by the bioherbicide at a rate of $60g/m^2$. This weed is hard to control and others techniques should be used in combination with the bioherbicide to increase efficiency. Higher rate should also be tested.

The use of a jute cover for three days after bioherbicide application increases weed control, especially under extreme climatic conditions for the bioherbicide and when the quality of inoculum is compromised. The cover hastens the infection by increasing moisture and may protect the bioherbicide from the uv light since less light reach the ground.

Banner (propiconazole) and Daconil (chlorothalonil) are both fungicides used in golf courses to control dollar spot disease. Banner contains propiconazole and this active ingredient inhibits significantly the bioherbicide growth and mycelium development on DPA. Under field conditions, weed control was significantly reduced when Banner highest rate were applied. Daconil active ingredient is chlorothalonil and this ingredient does not significantly affect the bioherbicide growth and development under field condition when the bioherbicide is applied more than four days after Daconil treatment. At very high rates (10 ug/ml and over) it inhibited the growth of the bioherbicide on PDA.

Future research should focus on integration of this new tool in golf course environments. Technique already used in golf course can be combined to the bioherbicide

to increase weed control. Core aeration, strip cutting and overseeding are examples. Irrigation schedule should also be studied to increase efficiency of the bioherbicide.

Appendix 1

Weather data from the Environment Canada Meteorological Data, Dorval Station: for the time period August 22 –September 2 2006 (Golf courses experiments).

	Temperature °C			Relative Humidity %		
Day	Max.	Min.	Average	Max.	Min.	Average
August 22	25.1	14.2	19.7	82	46	67.9
August 23	20.5	11.1	15.8	83	42	61.4
August 24	21.1	9.2	15.2	86	47	66.2
August 25	20.1	9.8	15.0	82	42	58.8
August 26	22.9	8.9	15.9	84	35	58.1
August 27	17.9	14.5	16.2	96	74	90.0
August 28	22.0	14.7	18.4	98	65	82.6
August 29	24.4	15.2	19.8	96	58	77.4
August 30	20.2	10.2	15.2	75	45	59.4
August 31	20.8	7.9	14.4	88	56	72.2
Sept. 1	23.0	11.3	17.2	91	43	65.7
Sept. 2	21.4	12.8	17.1	82	49	64.3
Sept. 3	17.2	12.1	14.7	96	59	84.3
Sept. 4	19.1	15.1	17.1	97	82	89.3

Weather data from the Environment Canada meteorological data. Dorval station: for the time period June 20- July 4 2007 (golf courses experiments).

	Temperature °C			Relative Humidity %		
Day	Max.	Min.	Average	Max.	Min.	Average
June 20	23.8	15.8	19.8	97	52	73.4
June 21	20.3	13.9	17.1	90	62	74.3
June22	20.0	12.8	16.4	88	50	69.9
June 23	21.2	10.3	15.8	73	36	54.9
June 24	23.4	10.2	16.8	79	32	61.1
June 25	28.5	17.4	23.0	93	47	71.5
June 26	32.3	20.2	26.3	75	48	61.1
June 27	33.2	20.2	26.7	79	51	66.1
June 28	24.3	9.7	17.0	99	54	78.5
June 29	22.3	8.0	15.2	98	43	70.3
June 30	20.1	10.9	15.5	92	44	66.5
July 1	18.0	11.2	14.6	86	55	72.2
July 2	22.5	11.6	17.1	94	47	72.2
July 3	25.3	12.7	19.0	97	35	68.5
July 4	M	12.9E	M	98	68	81.6

Weather data from Environment Canada meteorological data. Sainte-Anne-de-Bellevue station: for the time period July 31-August 13 2007 (cover experiment. repetition 1).

	Temperature °C			Relative Humidity %		
Day	Max.	Min.	Average	Max.	Min.	Average
July 31	30.3	16.7	23.5	86	49	69.6
August 1	29.2	17.5	23.4	94	45	66.8
August 2	33.0	22.5	27.8	79	50	57.8
August 3	31.8	21.2	26.5	93	56	74.5
August 4	26.0	14.3	20.2	93	46	74.8
August 5	24.7	11.8	18.3	98	36	62.5
August 6	21.9	14.9	18.4	100	65	86.7
August 7	26.4	17.5	22.0	100	62	83.0
August 8	28.5	15.8	22.2	95	51	77.4
August 9	22.3	13.5	17.9	95	47	66.8
August 10	25.8	15.4	20.6	97	53	77.3
August 11	28.7	14.5	21.6	99	48	77.5
August 12	29.4	19.9	24.7	86	49	68.8
August 13	25.8	16.1	21.0	95	34	62.3

Weather data from Environment Canada meteorological data. Sainte-Anne-de-Bellevue station: for the time period August 22- September 4 2007. (cover experiment repetition 2).

	Temperature °C			Relative Humidity %		
Day	Max.	Min.	Average	Max.	Min.	Average
August 22	22.4	7.2	14.8	100	35	67.2
August 23	24.5	17.5	21.0	96	70	80.3
August 24	27.3	20.3	23.8	99	77	88.4
August 25	28.2	21.2	24.7	99	72	87.3
August 26	24.9	15.6	20.3	95	58	79.8
August 27	24.2	10.2	17.2	100	45	74.7
August 28	27.1	12.4	19.8	100	47	76.5
August 29	30.8	17.7	24.3	98	52	77.5
August 30	21.0	13.4	17.2	100	76	93.4
August 31	22.6	12.2	17.4	100	51	83.6
Sept. 1	20.7	9.9	15.3	92	42	66.8
Sept. 2	23.3	7.9	15.6	100	40	68.6
Sept. 3	26.6	15.2	20.9	75	48	62.8
Sept. 4	20.6	9.6	15.1	81	43	60.3

Weather data from Environment Canada meteorological data. Sainte-Anne-de-Bellevue station: for the time period August 14-August 27 2007 (fungicide experiment repetition 1)

	Temperature °C			Relative Humidity %		
Day	Max.	Min.	Average	Max.	Min.	Average
August 14	21.6	10.5	16.1	94	51	78.8
August 15	25.0	12.5	18.8	32	44	70.2
August 16	25.5	10.2	17.9	95	65	82.0
August 17	22.9	8.5	15.7	100	37	72.8
August 18	18.7	12.3	15.5	94	47	69.3
August 19	20.2	7.9	14.1	92	40	66.8
August 20	20.1	5.8	13.0	100	43	73.1
August 21	22.8	6.4	14.6	100	35	70.2
August 22	22.4	7.2	14.8	100	35	67.2
August 23	24.5	17.5	21.0	96	70	80.3
August 24	27.3	20.3	23.8	99	77	88.4
August 25	28.2	21.2	24.7	99	72	87.3
August 26	24.9	15.6	20.3	95	58	79.8
August 27	24.2	10.2	17.2	100	45	74.7

Weather data from Environment Canada meteorological data. Sainte-Anne-de-Bellevue station: for the time period September 26- October 9 2007 (fungicide experiment repetition 2)

	Temperature °C			Relative Humidity %		
Day	Max.	Min.	Average	Max.	Min.	Average
Sept. 26	25.8	16.8	21.3	95	65	81.8
Sept. 27	17.7	12.5	15.1	99	73	89.9
Sept. 28	20.3	10.8	15.6	99	73	89.8
Sept. 29	17.0	4.8	10.9	95	52	76.5
Sept. 30	17.5	4.3	10.9	99	56	77.6
Oct. 1	23.2	9.8	16.5	96	57	77.8
Oct. 2	20.7	12.6	16.7	93	67	81.6
Oct.3	26.0	16.2	21.1	85	55	72.2
Oct. 4	23.2	10.4	16.8	91	38	75.0
Oct. 5	25.6	9.3	17.5	97	53	76.4
Oct. 6	15.7	9.0	12.4	96	57	82.0
Oct.7	13.9	7.2	10.6	93	46	66.3
Oct. 8	12.5	6.5	9.5	99	75	93.0
Oct. 9	12.8	3.8	8.3	96	71	82.7

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