PREFACE TO CHAPTER 5

Mowing is a necessary cultural management practice for turf maintenance and mowing height could be a variable affecting a biocontrol system. Different mowing heights would be expected to create different microenvironments for *S. minor* colonization and growth. Periodic mowing at close heights could add more stress, not only on weeds, but also on turfgrass. In general perennial plants have seasonal variations for allocations of carbohydrate reserves depending on their phenological stage and environmental conditions. Based on the above facts, the objective of Chapter 5 was to investigate the performance of *S. minor* under different mowing heights in both greenhouse and field conditions. In greenhouse and field experiments, the interaction of mowing height with *S. minor* efficacy on dandelion was studied by estimating aboveground damage, measuring leaf and root biomass and counting the number of dandelions recovered after *S. minor* application.

A manuscript of the results of Chapter 5 has been accepted for publication in Biocontrol Science and Technology (submitted August 1, 2005 accepted for publication November 7, 2005). The manuscript is co-authored by Professor Alan K. Watson, my supervisor. I designed the experimental set-up, performed the experiments and the statistical analysis, and wrote the manuscript. Professor Watson supervised the work, provided financial and technical resources, and corrected the manuscript.

CHAPTER 5

Effect of turfgrass mowing height on biocontrol of dandelion with *Sclerotinia minor*

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A running title: Mowing effect on performance of a turf bioherbicide

Biocontrol Science & Technology – submitted August 1, 2005 accepted for publication
November 7, 2005

5.1. Abstract

The fungus *Sclerotinia minor* Jagger is under development as a bioherbicide for control of dandelion and many broadleaf weeds in turfgrass environments. The effect of *S. minor* on dandelion survival was evaluated under different mowing heights and compared with Killex™ the commonly used herbicide. In the greenhouse, the onset of symptoms was more rapid, foliar damage was more severe, and the reduction of aboveground biomass and root biomass was greater for the bioherbicide than the herbicide. The bioherbicide reduced root biomass ≥ 10 fold compared with untreated plants. Under high weed infestation levels in the field, *S. minor* caused a greater initial reduction of dandelion density than did the herbicide during the two-weeks-post application period, although reductions were greater in herbicide treated plots by six weeks after application. Over the growing season, *S. minor* and the herbicide had similar suppressive effects on dandelion density except under the closest mowing height (3-5 cm). After treatment, close mowing favoured dandelion seedling recruitment and the biocontrol had no residual activity. Survival of dandelion roots was significantly less after spring than fall treatment of *S. minor* and season long mowing at the close height significantly reduce root survival. Close mowing may be detrimental for *S. minor* applications on heavily infested domestic lawns and amenity grassland areas.

Keywords: Bioherbicide; biological weed control; clipping; fungus; mowing; *Sclerotinia minor*; *Taraxacum officinale*; turfgrass; weed control

5.2. Introduction

Broadleaf weeds reduce the quality and quantity of turfgrass and disrupt its visual uniformity (McCarty et al. 2001). Dandelion, *Taraxacum officinale* Weber, is a strong competitive perennial weed that infests turfgrass environments and is common in home lawns, turfgrass swards, pastures, forages, golf courses, athletic fields, wooded areas, and roadside verges (Stewart-Wade et al. 2002b). It is an undesirable plant, causing aesthetic problems during flowering and seed production. In 1992, a survey of turfgrass specialists estimated that there were 7.3 million hectares of lawn in the United States (Elmore 1994). The lawn care industry in North America has been expanding at a rate of 5-8% per year (United States Environmental Protection Agency 1999). The need for regular management and maintenance of these areas creates a massive turfgrass industry in North America and Europe. In 2001, 78 million households in the U.S. used home and garden pesticides (U.S. Environmental Protection Agency 2004). Herbicides accounted for the highest usage of pesticides in the home and garden sector with users spending 632 million dollars for over 36 million kilograms applied on lawns and gardens. 2,4-D (2,4-dichlorophenoxy acetic acid) was the most widely used pesticide in the home and garden sector.

Repeated applications of phenoxy herbicides such as 2,4-D and mecoprop ((±)-2-(4-chloro-2-methylphenoxy) propanoic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid), or combination products such as Killex™ have been widely used for dandelion control (Anonymous 1997). Environmental and public health concerns about pesticides, especially the use of chemical herbicides in turfgrass for aesthetics, have lead to the banning or severe restrictions on the use of pesticides in many regions of Canada (Cisar

2004). Consequently, alternatives to chemical herbicides are being sought (Neumann & Boland 2002; Stewart-Wade et al. 2002a; Zhou et al. 2004).

Sclerotinia minor Jagger is an asporogenic ascomycete plant pathogen that has biocontrol potential for dandelion in turfgrass, and several formulations of *S. minor* have been shown to have biological control activity (Ciotola et al. 1991; Riddle et al. 1991; Brière et al. 1992; Stewart-Wade et al. 2002a; Abu-Dieyeh & Watson 2006: Chapter 5). *S. minor* mycelium in sodium alginate granules (Brière et al. 1992) and mycelial-colonized barley grits (Stewart-Wade et al. 2002a) effectively controlled dandelion and broadleaf plantain (*Plantago major* L.) without damage to turfgrass species. When applied to turfgrass, *S. minor* IMI 344141 rarely produces sclerotia (melanized survival structures), and these sclerotia do not survive over winter. Mycelia of *S. minor* IMI 344141 does not survive beyond 10 days in the turfgrass environment (M.H. Abu-Dieyeh & A.K. Watson, unpublished data). Field and greenhouse studies confirmed that turfgrass species are not susceptible to *S. minor* IMI 344141. Independent toxicological studies have established that *S. minor* IMI 344141 is neither toxic nor pathogenic to humans, birds, fish, daphnia, honey bees, earthworms, or wild animals (A.K. Watson personal communication).

Extension recommendations for turfgrass often indicate that a dense, healthy turfgrass stand is the best defence against weed colonization and can be managed by proper mowing, watering, and fertilization (McCarty et al. 2001; Busey 2003). A sensible recommendation but the methods are often stated in generalities and are not based on scientific research (as stated by Busey 2003). Mowing is a major cultural practice for turf maintenance. Lower mowing heights can cause additional stress for certain grass species and/or broadleaf weeds, especially in summer (as reviewed by Busey 2003; Hatcher & Melander 2003), and higher canopies may maintain higher humidity levels close to the soil surface for longer time periods, which can be advantageous for pathogen activity (Gielser et al. 2000). When a fungal pathogen was combined with simulated mowing, weed suppression was greater than the fungus alone (Green et al. 1998; Kluth et al. 2003). In this research, the effect of turfgrass mowing height on the efficacy of a granular formulation of *S. minor* to suppress dandelion in a suburban lawn environment was evaluated.

5.3. Materials and methods

5.3.1. Production and formulation of *Sclerotinia minor*

Sclerotinia minor (IMI 344141) was isolated from diseased lettuce plants (*Lactuca sativa* L.) from southwestern Quebec in 1988 and stock cultures maintained as sclerotia at 4^{o}C. When required, sclerotia from a stock culture were washed twice in sterile distilled water, placed in 70% ethanol for 40 s, transferred to 1% hypochlorite solution for 3 min, rinsed twice with sterile distilled water, and set to dry on sterilized filter paper. The surface sterilized sclerotia were transferred aseptically onto potato dextrose agar (PDA, DIFCO Laboratories, Detroit, MI) plates and incubated for 4-5 days at 20 ± 1^{o}C. Five agar plugs (5-mm diameter), from the actively growing margin of colonies on PDA were transferred to 100 ml of a modified Richard's solution (MRS) having the following constituents per litre: 10 g of sucrose, 10 g of KNO_3, 5.0 g of KH_2PO_4, 2.5 g of $MgSO_4{\cdot}7H_2O$, 0.02 g $FeCl_3{\cdot}6H_2O$, and 150 mL V-8 juice (Campbell Soup Company Inc.) in 250 mL Erlenmeyer flasks. Cultures were incubated for 5 days on a rotary shaker at 60 rpm at room temperature (20 ± 1°C). The grown mycelium was collected into a sterilized blender cup (Waring Commercial, Torrington, CT) and homogenized gently with two 20 s bursts and then inoculated onto autoclaved barley (*Hordeum vulgare* L.) grits. For this, whole barley grains were ground and sieved to 1.4-2.0 mm diameter grits. Three hundred g of barley grits were transferred into autoclavable bags with a breathable patch 44 x 20.5 cm, 0.02 µm filter: 24 mm (SunBag, transparent, SIGMA-ALDRICH, Montreal, QC). Two hundred and ten mL of distilled water was placed into each of the bags and autoclaved at 121^{o}C for 20 min. After autoclaving the bags were allowed to cool and a 15 mL aliquot of the liquid *S. minor* mycelial culture was transferred aseptically into each bag. Inoculated bags were incubated at 20 ± 1^{o}C in the dark and shaken on the 3rd to 6th days of incubation. The contents of each bag were then dried separately by spreading the colonized barley grits onto mesh trays for 12 hr under a laminar flow. The dried inocula (a_w. 0.4) were placed in plastic bags (PolyBags, 17.5 x 40 x 7.5 cm, Gerrity Corrugated Paper Products, Concord, ON) and the bags were sealed, and stored at 4^{o}C. These *S. minor* granular formulations were used in our experiments after two to four weeks of storage. The viability of the *S. minor* preparations was verified by incubating ten granules from each bag on PDA plates. Colony diameters were measured after 24 and 48 h of incubation at 20 ± 1^{o}C in the dark. Additionally, ten granules from each bag were placed onto excised dandelion leaves maintained on moist sterile filter papers in Petri dishes and incubated at 20^{o}C in the dark; one granule per leaf. The diameter of the lesions caused by the fungus was measured after 24 and 48 h of incubation. Previous unpublished quality control studies indicated viable batches to have colony diameter of 14-30 mm after 24 h and 40-70 mm after 48 h and virulent batches to have an average lesion diameter >15 mm after 48 h of incubation (Appendix-4). *S. minor* preparations were only used in our experiments if they met these criteria.

5.3.2. Effect of *S. minor* and mowing height on dandelion control in the greenhouse

Dandelion seeds collected in spring 2002 from lawns on the Macdonald campus, McGill University, Ste-Anne-de-Bellevue, QC and stored at 4^{o}C were sown onto potting soil (2/3 black pasteurized soil and 1/3 Pro-mix (Premier Promix, Premier Horticulture Ltee, Riviere-du-Loup, QC) in plastic containers (40L x 32W x 20D cm, 19 L capacity; Sterilite Inc., Montreal, QC). One week after germination, seedlings were thinned to four equidistant seedlings per container. Two weeks after germination of the dandelion seeds, 2.5 g of a commercial grass seed mixture [30% Kentucky bluegrass (*Poa pratensis*), 40% creeping red fescue (*Festuca rubra* L. var. *rubra*) and 30% turf type perennial ryegrass (*Lolium perenne* L.), C.I.L.® Golfgreen™, Brantford, ON] was scattered over the surface of each container.

Grass was cut weekly with hedge shears (PlantSmart, Wal*Mart, Canada), commencing three weeks after grass sowing to a height of 5, 10, 15 or 20 cm. Four weed control treatments were imposed: (1) untreated, (2) a spot application of 0.2 g plant^{-1} non-colonized autoclaved barley grits, (3) a spot application of 0.2 g plant^{-1} barley granular formulation of *S. minor*, and (4) a broadcast foliar application of Killex™ at 1.7 kg a.i ha^{-1} (25 ml of the 0.6% original concentration per container). The herbicide was applied with a 1.18 L vacuum sprayer (Home and Garden sprayer. Model no 1998. RLF10-Master Premium. Root-Lowell Manufacturing Co, Lowell, MI). The weed control treatments were applied three weeks after initiation of grass cutting heights and thus dandelion plants were eight weeks old. Prior to weed control treatment applications, all plants were misted lightly with water for two min. duration to aid barley grit adhesion with dandelion leaves.

The plants were grown in the greenhouse at 20 ± 2^{o}C with 15 h of light per day at a minimum photon flux density of 350 ± 50 µmol m^{-2} s^{-1}. Plant containers received programmed drip irrigation of 150 mL 3 x day^{-1}. To enhance the establishment of the plants, a 15:15:30 N-P_2O_5-K_2O with micronutrients (Plantex®, Plant Product Co, Brampton, ON) was applied at 3.5 g L^{-1} when the dandelions were five weeks old.

Symptoms of damage to dandelions were visually estimated weekly for four weeks after application using a 0 to 10 visual scale compared to the control within the same mowing height and the same block, where 0 = < 9%, 1 = 10-19% ….9 = 90-99% and 10 = 100% collapse of aboveground biomass. Data were converted back to a percentage for analysis and presentation (after Schnick et al. 2002). Plant regrowth was measured as a reduction in % damage by estimating the biomass of new leaves produced post inoculation compared to the control within the same mowing height and the same block. Damage estimates of the four plants in each container were averaged and analyzed as one experimental measure. The number of post inoculation dandelion plants that survived was recorded weekly for six weeks. Six weeks after application, all of the dandelion plants were carefully removed from the soil to extract their entire tap root. The roots were thoroughly washed and dissected above the crown, separating above ground and below ground biomass. All leaf or root biomass from each container was bulked, placed in paper bags, oven dried at 80^{o}C for 72 h, and then weighed.

The experiment was a split-plot design with five replications and was conducted twice through time (Jan 2003 and Jan 2004). Main plots were weed control treatments and subplots were grass heights. In the repeat trial in 2004, the non-colonized autoclaved barley grits treatment was omitted and excluded from the pooled analysis as no significant differences were obtained for any of the studied parameters when compared with the untreated treatment in the first trial. Data for each parameter from the two experimental trials were subjected to the Bartlett test of SAS (SAS Institute Inc., Cary, NC, 2002) to test for homogeneity of variances. Data for all parameters were homogeneous, thus they were pooled. The main effects of weed control treatments, mowing heights, and their interaction were determined using ANOVA of SAS, and the effects of time on aboveground damage, data were analyzed using SAS GLM procedure of repeated measures. The means were separated using the Tukey test at $P = 0.05$ (SAS Institute Inc., Cary, NC, 2002).

5.3.3. Effect of *S. minor* and mowing height on dandelion control in the field

Two field experiments were conducted, one in each of two different sites on the Macdonald Campus of McGill University, in Ste-Anne-de Bellevue, QC (45^{o}25'N latitude, 73^{o}55'W longitude, 39.00 m elevation). The climate data for the two years of study (2003 and 2004) are summarized in Table 5.1. Study Site-1 (2003 experiment) was approximately 600 m^2 on a loamy sand soil (coarse sand = 9%, fine sand 73%, silt= 12%, clay= 6%) with a pH of 7.15 and 8% organic matter. The lawn was established in 1973 and received low maintenance management throughout its history except for repeated mowing during the growing season (May to October). The grass sward was approximately 60% Kentucky blue grass, 30% perennial ryegrass, patches of timothy

(*Phleum pratense* L.), and rare occurrences of annual blue grass (*Poa annua* L.). The lawn flora was highly diversified with broadleaf weeds (17 species were observed throughout the study period) and the dominant weed species was dandelion (*Taraxacum officinale*) with 50-60 plants m^{-2} recorded prior to the spring treatment application.

Study Site-2 (2004 experiment) was located in an open lawn area of approximately 600 m^2 with low human disturbance, established in 1980. The turf at site -2 was in better visual quality than in Site-1 with mainly Kentucky blue grass (~90%) and 10% red fescue (*Festuca rubra* L.). Herbicides had not been applied for the past 10 years and the major broadleaf species were dandelion (60-70 plants m^{-2}), white clover (*Trifolium repens* L.) and broadleaf plantain (*Plantago major* L.). The soil was loamy sand (12% coarse sand, 75% fine sand, 7% silt and 6% clay) with a pH of 7.2 and 6.6% organic matter.

Both field experiments were established in May and maintained until the end of October. Study sites (20 x 30 m) were marked with metal posts and plastic ropes and the corners of each plot were permanently marked by wooden sticks to maintain plot integrity for the duration of the study. The area of the experimental unit (plot) was one meter square with 0.8 m alleys between plots. The distance between any two blocks was 2-3 m. The experimental design was a split plot with four replications. Mowing heights were the main plots and weed control treatments were the subplots. The three levels of mowing heights, 3-5 cm, 7-10 cm, and 12-15 cm were initiated two weeks prior to weed control treatment applications. Plots were mowed weekly, except during the two-weeks-post weed control treatment period, with a gas powered rotary push mower. Grass clippings were returned during July and August to act as a source of nitrogen (Kopp &

Guillard 2002), but removed during other months during the six-weeks-post treatment periods to avoid cross contamination between blocks.

Four levels of weed control were imposed on 15-May and on 15-September at each study site: 1) untreated control, 2) a broadcast foliar application of Killex™ at 1.7 kg a.i. ha^{-1} (200 ml m^{-2} of 0.6% of original concentration), 3) a broadcast application of 60 g m^{-2} granular formulation of *S. minor*, and 4) a broadcast application of 120 g m^{-2} granular formulation of *S. minor*. The herbicide was applied onto the grass surface using a 1.18 L vacuum sprayer. The *S. minor* formulation was applied using a 200 ml plastic bottle fitted with a perforated lid (~10 mm diameter) with suitable openings to pass the barley grits. If there was no rainfall on the day of application or the grass was not wet, the entire field was sprinkler irrigated for two hours prior to late afternoon treatment applications. No additional irrigation or fertilization management was applied during the course of the study.

The number of dandelions was counted in each plot the day before weed control treatments, and on a single day in the last week of each month thereafter. In order to monitor post treatment recovery of dandelions, 10 dandelion plants in each of the fungal treated plots were randomly marked using white coloured pins prior to treatment applications. In Site-2, the number of dandelion seedling recruits versus mature plants in each plot was counted every two weeks starting two weeks after application (30-May) and continuing to the end of June.

Dandelion density data were adjusted using the "Before-After, Control-Impact (BACI) equation" (Green 1979) to overcome spatial heterogeneity data differences from pretreated time (15 May) and also to overcome the effect of progression in time.

BACI value = (At / Bt) / (Ac/Bc) x 100 (equation 1)

Where, B = dandelion density at the time of treatment (before the impact), A = dandelion density after treatment (after the impact), t = treatment, and c = control. The control used for comparison was the untreated plot at the same mowing height in the same block. To realize the effect of mowing heights on dandelion in untreated plots, the value obtained from the three mowing heights within the same block were averaged and used as control in equation 1.

Data for each time period from the two years (two study sites) were subjected to Levene procedure (SAS Institute Inc., Cary, NC, 2002) for testing homogeneity of variances. Because of differences in environmental conditions between years and/or locations, monthly data were heterogeneous except for the spring pre-treatment application (mid of May); two-weeks-post application (end of May); six-weeks-post application (end of June), and for post control season average. Therefore, the season average, the two-weeks- and six-weeks-post application data from the two experiments were pooled and treated as one experiment. The 120 g m^{-2} *S. minor* treatment was excluded from the analysis as no significant differences were obtained in comparison with the 60 g m^{-2} treatment for all studied parameters. Normality for each parameter was tested on model residuals using the Shapiro-Wilk test (SAS Institute Inc., Cary, NC, 2002). Data were analyzed using ANOVA to determine the significant interactions among mowing heights and different weed control treatments. Differences in treatment means were determined using Tukey's test (SAS) at $P = 0.05$.

5.4. Results

5.4.1. Effect of *S. minor* and mowing height on dandelion control in the greenhouse

Eight-week-old dandelions (6-8 leaves) were highly susceptible to the *S. minor* application. The above ground biomass in all treated plants collapsed during the first week after application (Figure 5.1). Symptom expression was more rapid with *S. minor* and at 1-month-post application it was more effective than the herbicide (Killex™). The herbicide and the *S. minor* treatments caused highly significant ($P \leq 0.01$) above ground biomass damage to the dandelion plants. *S. minor* caused significantly more damage than the herbicide over the entire study period (Figure 5.1).

Mowing height had no significant effect on dandelion above ground damage in any of the weed control treatments (Figure 5.1), but the above ground and root biomass of untreated dandelion were significantly reduced under the 5-10 cm compared with 15-20 cm mowing heights (Figure 5.2A & B). At six-weeks-post application, both *S. minor* and the herbicide caused highly significant ($P \leq 0.01$) above ground and root biomass reduction compared with untreated plants. Biomass reductions were greater for *S. minor* (Figure 5.2A & B). The plants surviving the initial *S. minor* treatment had short, weak roots and few leaves sprouted from the crown (Figure 5.3). Regrowth following *S. minor* treatments was least when combined with the closest mowing height (5 cm) (Figure 5.4).

5.4.2. Effect of *S. minor* and mowing height on dandelion control in the field

The greatest effect of *S. minor* was observed two weeks after application when dandelion densities declined by 65 to 95%. Under the 7-10 and 12-15 cm mowing heights, the effect of the *S. minor* (60 g m^{-2}) was significantly greater than that of the herbicide, but at the 3-5 cm mowing height, the effect of *S. minor* and the herbicide were similar two weeks after application (Figure 5.5A).

Six weeks after application, dandelion densities within untreated plots were reduced by mowing at 3-5 cm compared to mowing at 7-10 or 12-15 cm (Figure 5.5B). Dandelion population densities were reduced more by the herbicide than by *S. minor* at all mowing heights six weeks after application (Figure 5.5B). Under close mowing (3-5 cm), dandelion densities in *S. minor*-treated plots increased from 35 to 80% between two and six weeks post application (Figure 5.5B).

The BACI post application average of dandelion population densities over the entire growing season (Figure 5.5C) illustrates the recovery ability of the dandelion population and also the accumulated affect of mowing. While the effect of mowing heights was concealed under the strong effect of the chemical herbicide treatment, it was apparently able to interact significantly with seasonal average dandelion density in untreated and fungal treated plots (Figure 5.5C).

Prior to the spring treatment, approximately 50% of the dandelion plants in all plots were new seedling recruits (Figure 5.6). In untreated plots, these recruits decreased gradually to approximately 10-20% of the population by six weeks after application (Figure 5.6) mostly due to rise in temperature (Table 5.1). In herbicide treated plots, no seedlings were observed after two- and four-weeks-post application and very few at six weeks after application. In *S. minor* treated plots, the ratio of recruitments to mature plants was highly variable according to time and mowing factors. Within the first two-weeks-post application, no seedlings were observed under the 7-10 and 12-15 cm mowing heights but a small number (less than 5 m^{-2}) was reported under the closest mowing height (Figure 5.6). After four weeks, the large recruitment of seedlings under the closest mowing height resulted in significantly more mature plants after six weeks compared with the other mowing heights (Figure 5.6).

Two weeks after *S. minor* treatment, the percentage of dandelion plants that regenerated after complete foliar damage was significantly less in the spring (up to 15%) than in the fall (up to 32%) treatments. Although the mowing height did not affect the percentage of root regrowth after the spring treatment of *S. minor*, there was significantly less regrowth under the closest mowing height (22%) compared with the two higher heights (33%) after the fall treatment (Figure 5.7).

Table 5.1. Weather data for Ste-Anne-de-Bellevue, Quebec during the two years of study 2003 and 2004. Environment Canada Meteorological Data. Ste-Anne-de-Bellevue Station.

	Temperature (^oC)			Relative humidity (%)			Rainfall (mm)
Month / year	Average	Min	Max	Average	Min	Max	Total monthly
May-03	13	2	28	70	15	100	136
May-04	7	-1	29	68	22	100	123
June-03	17	7	32	70	27	100	124
June-04	17	4	29	65	26	99	226
July03	20	11	30	73	27	100	357
July-04	20	11	31	75	30	99	574
August-03	22	14	29	79	35	100	53
August-04	19	8	29	78	45	100	182
September-03	17	5	29	77	31	100	416
September-04	16	5	26	79	41	100	96
October-03	7	-2	29	80	31	100	222
October-04	9	2	25	77	33	100	85

Figure 5.1. Effect of mowing heights and weed control treatments on above ground damage (%) of dandelion in a grass planting. Within each post application time, the means of the three weed control treatments are significantly different at the 1% level according to Tukey test.

(1): Spot application of a granular formulation of *S. minor* at 0.2 g plant^{-1}.

(2): Broadcast foliar application of Killex™ herbicide (2,4-D, mecoprop and dicamba) at a rate of 1.7 kg a.i. ha^{-1}

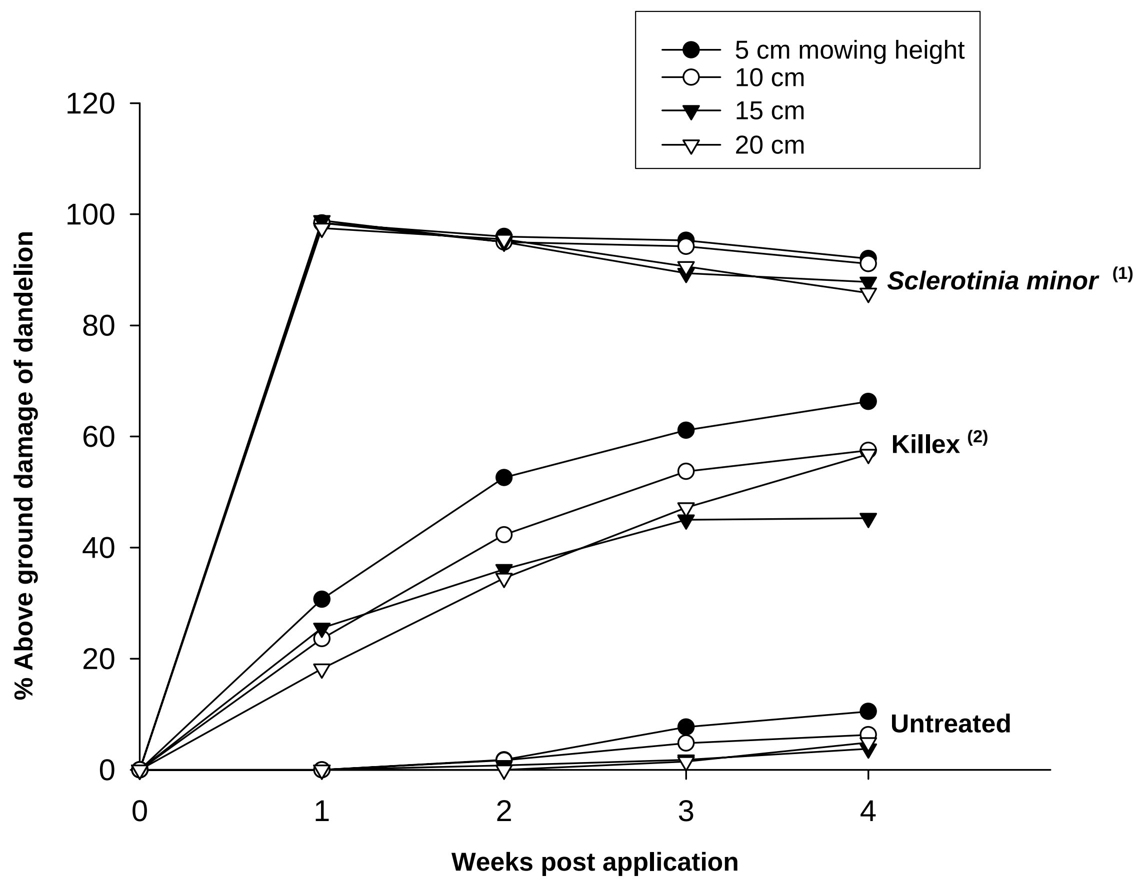
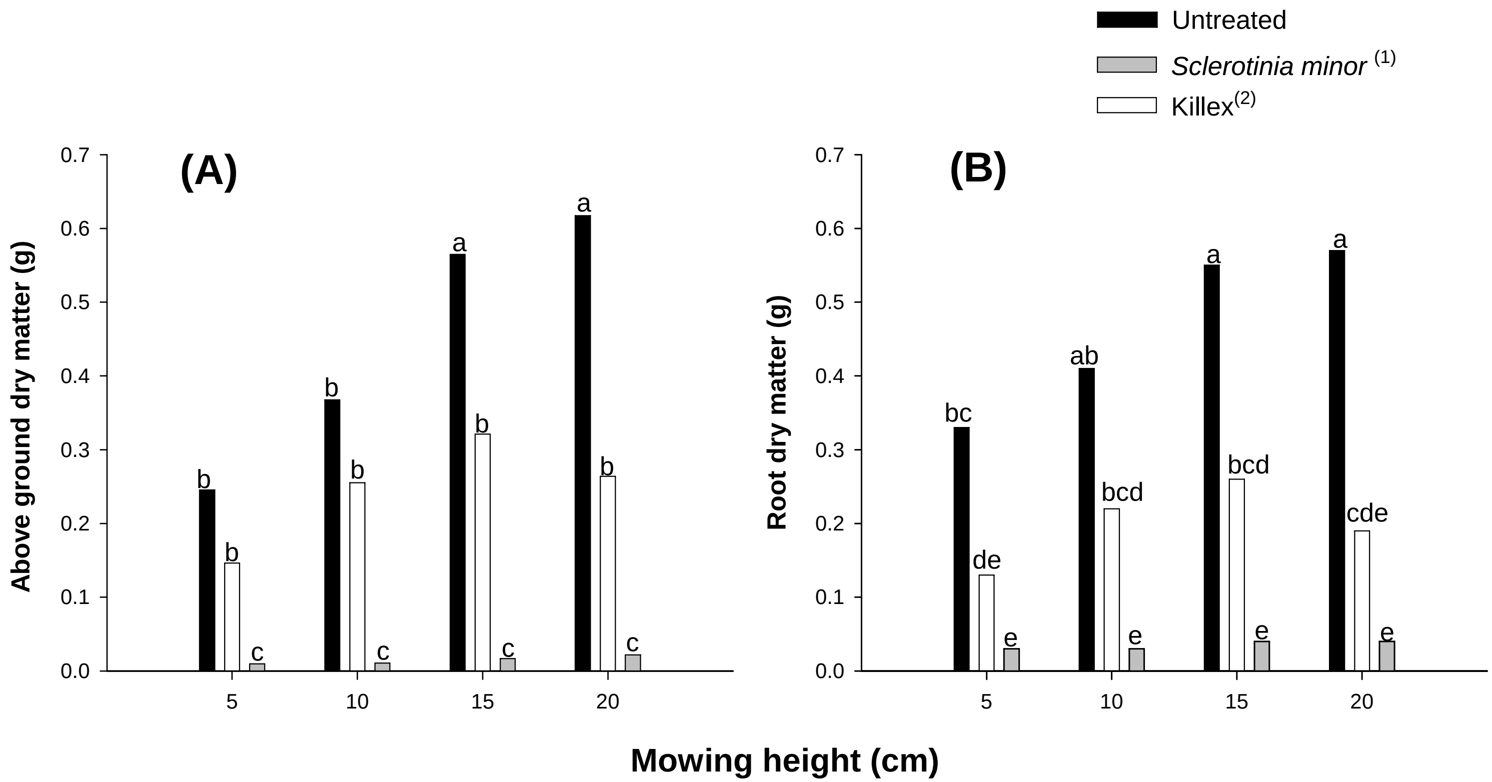


Figure 5.2. Effect of mowing heights and weed control treatments on aboveground (A) and root (B) biomass of dandelion, six weeks after treatment application. Mowing heights were initiated one month prior to treatment application and maintained throughout the study period. Within the same graph, bars with a common letter are not significantly different at the 5% level according to Tukey's test.

(1): Spot application of a granular formulation of *S. minor* at 0.2 g plant^{-1}.

(2): Broadcast foliar application of Killex™ herbicide, at a rate of 1.7 kg a.i. ha^{-1}.



Untreated
Sclerotinia minor (1)
Killex(2)
(A)
(B)
Above ground dry matter (g)
Root dry matter (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
5
10
15
20
b
b
c
b
b
c
a
b
c
a
b
c
bc
de
e
ab
bcd
e
a
bcd
e
a
cde
e
Mowing height (cm)

Figure 5.3. A comparison of the above and below ground biomass between untreated dandelion (two plants above) and dandelion regrown six weeks after inoculation with *Sclerotinia minor* (three plants below).

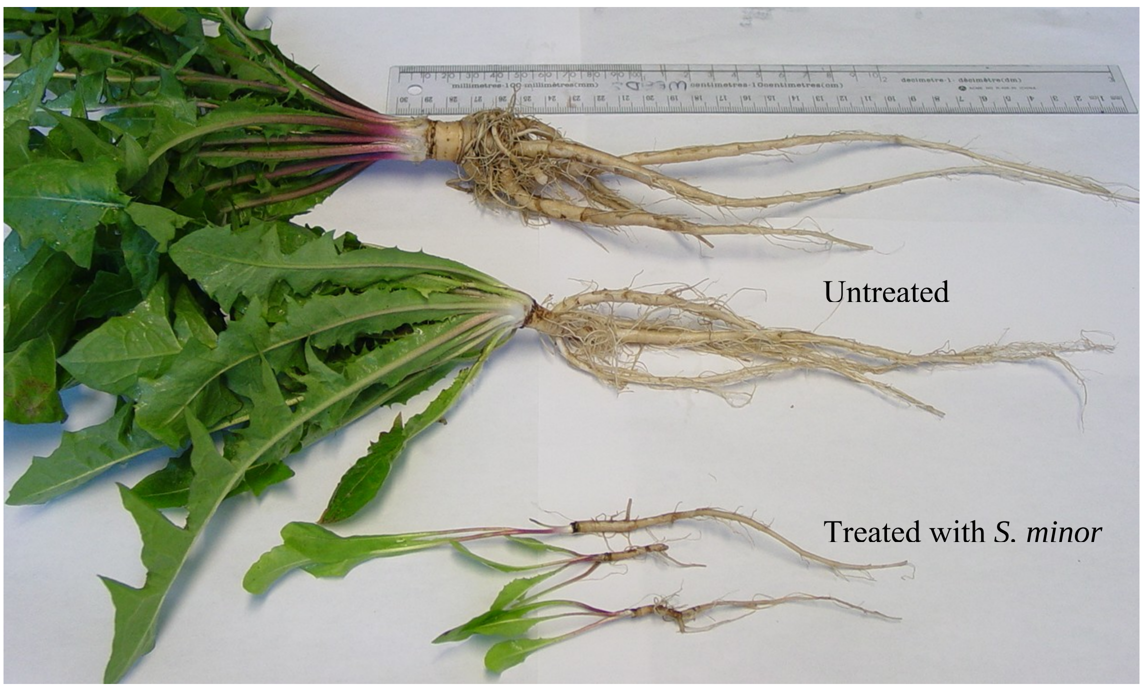


Figure 5.4. Effect of mowing height on dandelion root regrowth, six and eight weeks after *S. minor* inoculation. Within each post application time, bars with a common letter are not significantly different at the 5% level according to Tukey's test.

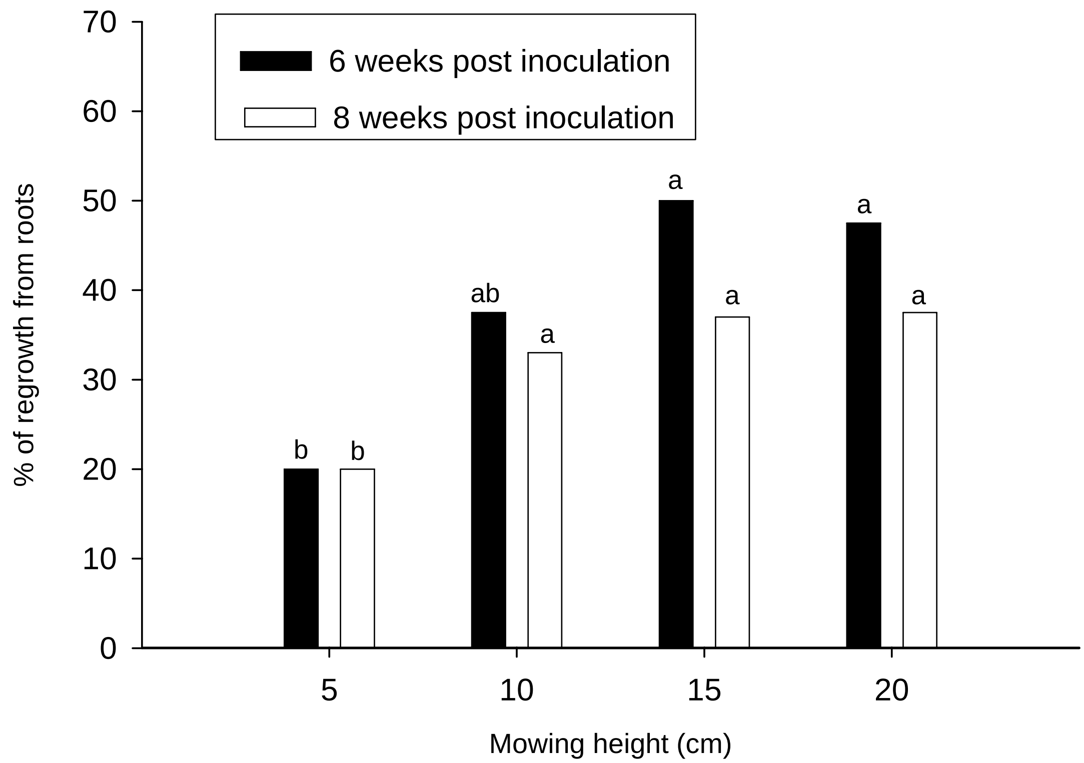


Figure 5.5. Effect of mowing heights and weed control treatments on post application dandelion density after two weeks (A), six weeks (B), and season average (C). Mowing heights were initiated two weeks prior to spring application and maintained throughout the experiment. Within the same graph, bars with a common letter are not significantly different at the 5% level according to Tukey test.

(1): Broadcast foliar application of Killex™ herbicide, at a rate of 1.7 kg a.i. ha^{-1}.

(2): Broadcast of a granular formulation of *S. minor* at 60 g m^{-2}.

(3): Before-After, control-Impact = (At / Bt) / (Ac /Bc) x 100,   B = density before treatment; A= density after treatment; t = treated and c = control.

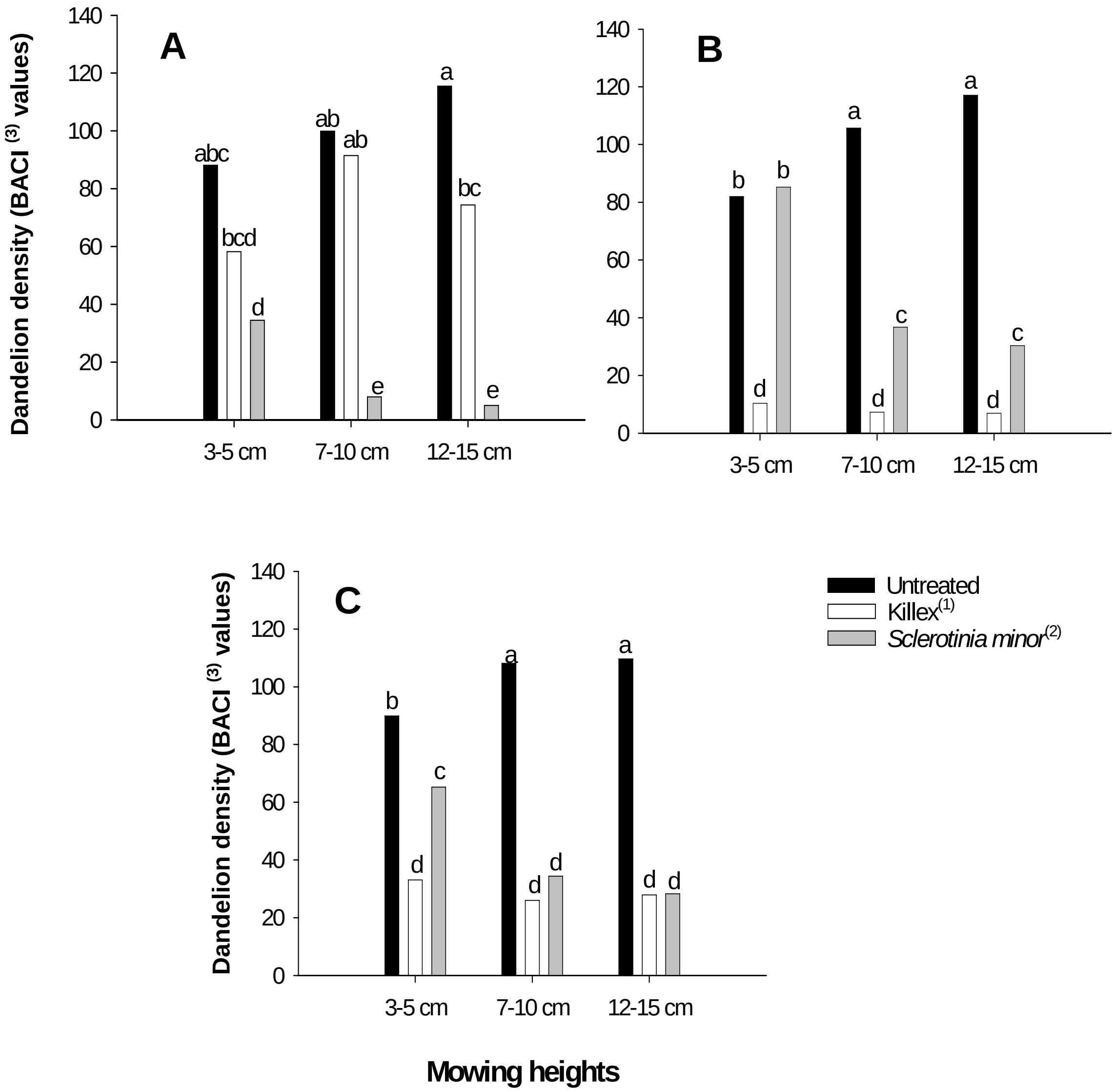

A
B
C
Dandelion density (BACI (3) values)
0
20
40
60
80
100
120
140
3-5 cm
7-10 cm
12-15 cm
abc
bcd
d
ab
ab
e
a
bc
e
b
d
b
a
d
c
a
d
c
b
d
c
a
d
d
a
d
d
Untreated
Killex(1)
Sclerotinia minor(2)
Mowing heights

Figure 5.6. Effect of mowing heights and weed control treatments on seedling and mature plant densities of dandelion after spring application (15 May 2004). *S. minor* rate = 60 g m^{-2}; Killex™ herbicide rate = 1.7 Kg a.i./ha. Within a plant stage in each graph, bars labelled with a common letter are not significantly different at $P = 0.05$ according to Tukey's test.

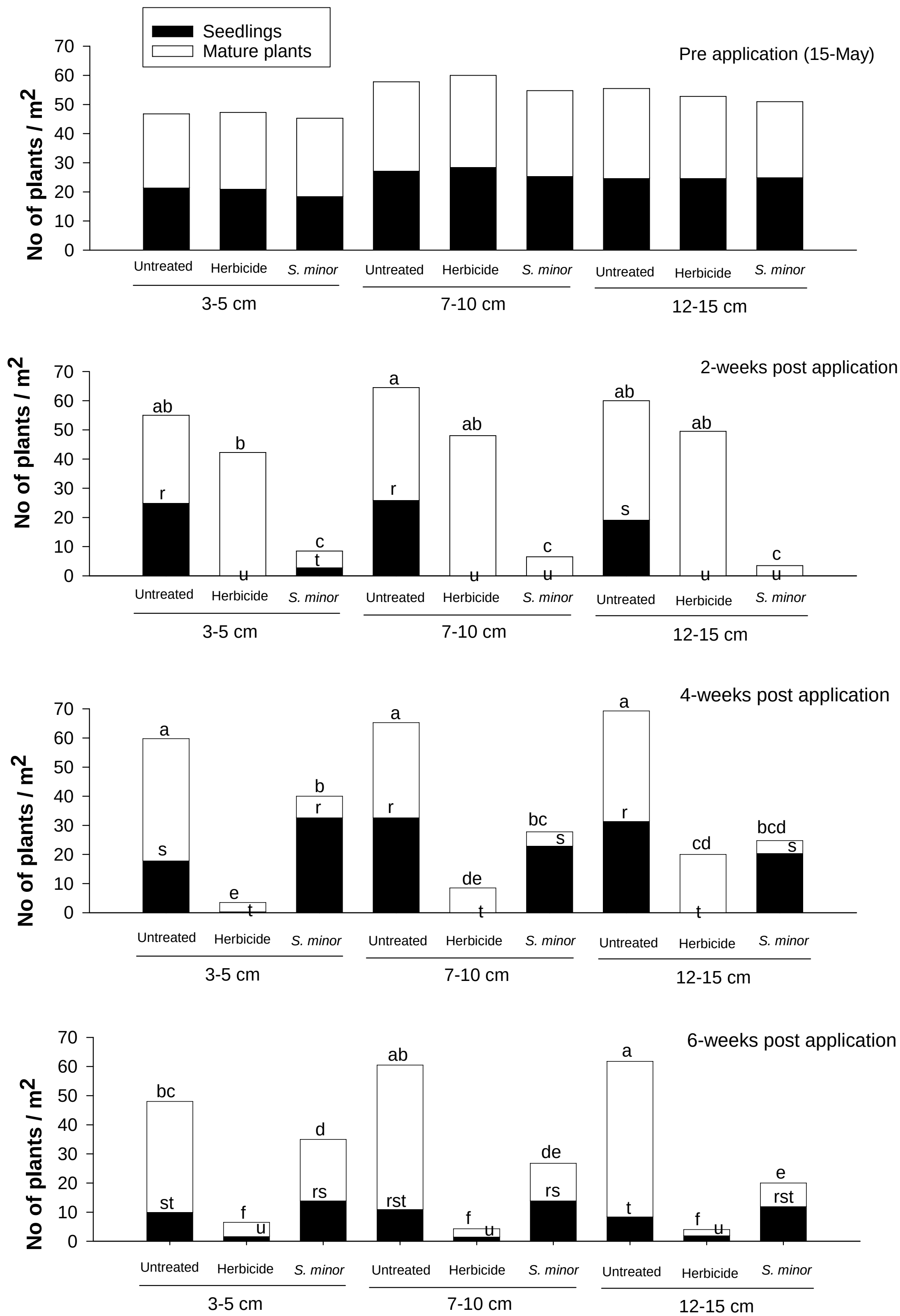
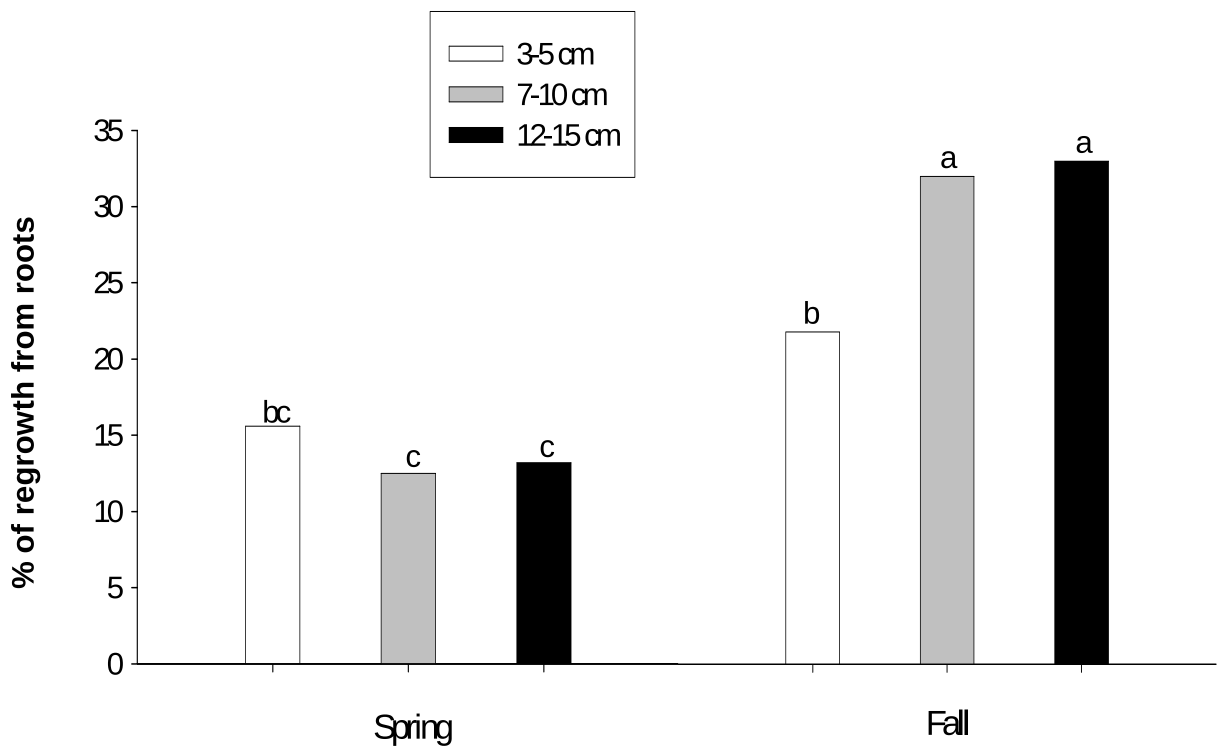

Seedlings
Mature plants
Pre application (15-May)
2-weeks post application
4-weeks post application
6-weeks post application
No of plants / m2
Untreated
Herbicide
S. minor
3-5 cm
7-10 cm
12-15 cm

Figure 5.7. Effect of mowing heights on regrowth of dandelion roots, three-weeks-post treatment application. Mowing heights were initiated two weeks prior to spring application and maintained throughout the experiment. Bars with a common letter are not significantly different at the 5% level according to Tukey's test.



5.5. Discussion

Previous studies on the virulence and efficacy of *S. minor* on dandelion have demonstrated its biocontrol potential (Ciotola et al. 1991; Riddle et al. 1991; Brière et al. 1992; and Stewart-Wade et al. 2002a). Our results support those studies and indicate the importance of correct mowing regimes on dandelion survival rates as influenced by *S. minor*.

In the absence of weed control, periodic mowing at any of the studied levels was not effective in controlling dandelion. However compared to other heights, periodic mowing at ≤ 5cm caused significant reduction in root biomass and field population density of dandelion, but close mowing height caused a significant increase in broadleaf groundcover percentage and diversity (Chapter 6). In another study, mowing every two weeks eliminated field bindweed but did not prevent dandelion colonization (Timmons 1950). The high regenerative capacity of dandelion roots (Stewart-Wade et al. 2002b) is the main cause of recolonization.

The level of mowing height capable of exerting significant stress on plants is highly variable and depends mainly on the plant species, the surrounding environment, the time of the year, and the frequency of mowing (Zanoni et al. 1969; Meyer & Schmid 1999; Liu & Huang 2002 and Narra et al. 2004). Although close mowing may be harmful to weeds, it may also be harmful to turfgrass species resulting in increased weed infestation by tipping the competitive balance in favor of the weeds (Busey 2003). The major turfgrass species in our fields were Kentucky bluegrass and perennial ryegrass, and in well maintained turf, both species are recommended to be mowed at a medium height, ~5-7 cm (Turgeon 1985; Fry & Huang 2004). Therefore, our grass mowing regime at ≤ 5 cm may have impacted the growth and survival of dandelions resulting in increased colonization by competitive species with more resilience and tolerance to close mowing.

Under greenhouse conditions, the severe effect of the fungus on above and below ground biomass of dandelion was apparent on the third day after application and was complete within two weeks. Mowing height had no effect on the efficacy of *S. minor* in the greenhouse experiment. This could be explained by the rapid destructive nature of *S. minor*, a necrotrophic fungus causing wilting, collapse and death of the infected plant parts (Abawi & Grogan 1979; Melzer et al. 1997). This rapid destruction is achieved in high moisture conditions over a wide range of temperature (5-25^{o}C) (Melzer & Boland, 1994). Therefore, the variation among the microenvironments within these mowing regimes likely did not affect *S. minor* performance.

In our field experiments, two dates were chosen, 15 May and 15 September, to synchronize two factors; 1) suitable climatic condition for *S. minor* to cause disease, and 2) high abundance of dandelion during periods of recruitment. Average daily mean temperature, relative humidity, and dew point for the two-weeks-post application period were 15^{o}C, 78% RH and 10.6^{o}C, respectively. Under these conditions the fungus needs only 2-3 days to germinate, invade and colonize dandelion plants. The maximum effect of the fungus occurs within 10-14 days and then the fungus dies and disintegrates on the soil surface (Appendix-2). Thus, the maximum effect of *S. minor* on dandelion density was obtained two weeks after application. Foliar turfgrass pathogens can cause more disease in microenvironments with higher canopies (Fagerness & Yelverton 2001; Martin et al. 2001), but disease incidence of lettuce, a major host of *S. minor*, was not affected by different microclimates of the crop canopy (Melzer & Boland 1994).

By two weeks after application in the field, the fungus destroyed most of the dandelion population in all treated plots without being influenced by changes in the microenvironment under the different mowing heights. In the 3-5 cm mowing height plots, bare soil was exposed within the thin grass canopy allowing greater sunlight interception at the soil surface. Consequently, high dandelion seedling recruitment occurred after the fungus lost viability and died. Full light is a major requirement for germination of dandelion seeds (Letchamo & Gosselin 1996) and buried seeds are unable to germinate (Noronha et al. 1997).

After the effect of *S. minor* declined, dandelion seeds started to germinate and seedling emergence continued until the end of June, and then germination declined. At mid June, significantly greater seedling emergence occurred in plots with the 3-5 cm mowing height compared with the 7-10 and 12-15 cm mowing heights. Therefore, at six-weeks-post application, population density of dandelion increased significantly in the 3-5 cm mowing height plots compared with other mowing heights and became similar to untreated plots. In plots treated with *S. minor*, dandelion re-established from seeds and survived better under the 3-5 cm mowing height than under the 7-10 cm and the 12-15 cm mowing heights. Consequently, dandelion biocontrol with the fungus at the two higher mowing heights was as effective as the chemical herbicide.

The initial effect of the herbicide on dandelion density was much slower than that of the fungus, but by six-weeks-post application the density was reduced by 90%, significantly lower than *S. minor* effect. Mowing height did not interact with the herbicide effect. Seedling recruitment after the herbicide treatment was very low, due to persistence of the herbicide in the soil with a half-life of 2-269 days reported (Cox 1999).

Treating dandelion at the reproductive stage with Killex™ reduced the germination potential down to 4.8% and 18.4% after spring and fall treatments, respectively (Abu-Dieyeh et al. 2005). Moreover the herbicide treatment significantly reduced dandelion seed bank (10 cm depth) compared with the untreated, control plots (Chapter 7).

Root regeneration of dandelion is one of its competitive features and makes control a difficult task (Stewart-Wade et al. 2002b) since a small section of root can propagate a new plant when covered by 5-10 cm of soil (Falkowski et al. 1989). Our greenhouse results indicated that *S. minor* caused a highly significant reduction of root biomass, but 20-50% of the treated dandelions had resprouted from the roots at six-weeks-post application. Resprouted plants were very weak lacking vigour and characterized by tiny roots and tiny leaf shoots. Thus *S. minor* is not only attacking aboveground biomass of dandelion but also affecting the roots, leaving them less likely to survive grass competition and more prone to other biotic and abiotic stresses, especially winter frost. Under greenhouse conditions, the closest mowing height caused significant reduction of sprout percentage compared with the two higher mowing heights (15 and 20 cm). There was no difference in the number of resprouted dandelions obtained at eight-weeks- compared with six-weeks-post application. No signs of disease development by *S. minor* were observed on these new shoots, but the weakness of the root caused by the direct effect of the fungus on the mother plants may diminish its survival under the additional stress of grass competition. The extensive defoliation stress caused by repeated mowing at the 5 cm level reduced the root biomass significantly in untreated plants and this may explain the reduction of regrowth under this mowing height. Similarly in other perennial plants, repeated defoliation reduced regrowth ability of *Ranunculus acris* L., after infection by the fungus *S. sclerotiorum* (Green et al. 1998) and repeated cutting integrated with a rust fungus, *Puccinia punctiformis* exerted synergistic control effects on growth rate and reproductive success of *Cirsium arvense* (Kluth et al. 2003).

Mowing heights were initiated two weeks prior to the spring application, hence stress on the roots would not be strong, whereas the accumulative effect of defoliation stress by mowing at the 5 cm level over the growing season prior to the fall treatment resulted in significantly less root regrowth compared with other heights. Dandelion, allocates more resources for flowering and vegetative growth in the spring (Cyr et al. 1990) while nitrogen resources are restored in the roots at the end of summer (Rutherford & Deacon 1974) which may explain the increase in the percentage of regrowth in the fall. The maximum regrowth percentage reported in the field is lower than greenhouse experiment which indicates that field environments exert more ecological stress on dandelion leading to improved performance of *S. minor*.

In conclusion, in a low-maintained cool-season turf environment, integrating *S. minor* with appropriate mowing could be as effective as a herbicide. Dandelion suppression was the least under close mowing due to the new opened environment which induced more germination from the soil seed bank. Extensive periodic defoliation by mowing and application of *S. minor* on flowering dandelion (spring application) might be the cause of decreasing dandelion root survival through exhaustion of the root carbohydrate and nitrogen reserves. Understanding these physiological changes in dandelion roots under different mowing heights will support successful deployment of *S. minor* for dandelion control. Additionally, monthly monitoring of the weed species dynamics and turfgrass quality provides a good portrayal of the biocontrol system.

5.6. Acknowledgments

The authors thank Philippe Seguin for his valuable help in statistical analysis and also Miron Teshler for his help in field and technical work. The financial support from Hashemite University, Zerka, Jordan and from the Natural Sciences and Engineering Research Council of Canada (NSERC) Idea to Innovation (I2I) grant are gratefully acknowledged.