PREFACE TO CHAPTER 3

Biotic and abiotic interactions are important components of any biocontrol system. Host plant age, within population genetic variability of the host, and turfgrass competition are interconnected and determine the efficacy and performance of a biological control agent. *Taraxacum officinale* inter-population variation is well documented in the literature and in Chapter 3, 14 accessions of *Taraxacum officinale* collected from different regions in Canada, USA and Europe were evaluated for their susceptibility to *S. minor*. In addition, the effect of plant age (growth stage) in the presence and absence of grass competition on susceptibility of dandelion to *S. minor* was also studied.

The results of this chapter have been submitted to Weed Research (submitted 29 Nov 2005) and the manuscript is presently in the peer review process. The manuscript is co-authored with my supervisor, Professor Alan K. Watson. 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 3

Efficacy of *Sclerotinia minor* for dandelion control: Effect of dandelion accession, age, and grass competition

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3.1. Abstract

Control of *Taraxacum officinale* (common dandelion) and other broadleaf weeds in temperate turfgrass has been readily achieved with phenoxy and like herbicides. The herbicide option has been revoked through municipal and provincial legislation in many regions of Canada, necessitating the need for alternative approaches. We examined the effects of dandelion accessions, age, and grass competition on the performance of *Sclerotinia minor* (IMI 344141) as a biological control for dandelion in turfgrass. The fungus showed identical symptoms on 14 different accessions of dandelion and reduced above ground and below ground biomass by 94% and 96%, respectively with no difference among accessions. Foliar damage and dandelion mortality caused by *S. minor* was affected by plant age and the presence of grass competition. Dandelions of all ages were more severely affected by *S. minor* treatment in the presence of grass competition. Grass competition had greater impact on foliar biomass, whereas the fungus had a greater impact on root biomass of newly established dandelions. In addition to competition for resources, we hypothesized that the grass sward provides a microenvironment favouring the success of *S. minor* as a biological control agent of dandelion. This suggests that proper management of the turfgrass environment improves the efficacy of *S. minor* as a biocontrol for dandelions.

Keywords: age, biocontrol, bioherbicide, accessions, competition, dandelion, *Sclerotinia minor,* synergy, *Taraxacum officinale,* turfgrass.

3.2. Introduction

The fungus *Sclerotinia minor* Jagger (IMI 344141) is being studied as a biological control for dandelion and other broadleaf weeds in turfgrass environments (Ciotola et al. 1991; Riddle et al. 1991; Brière et al. 1992; Schnick et al. 2002; Stewart-Wade et al. 2002a). Recent greenhouse and long-term field studies have demonstrated effective control even in low-maintained turf with very high weed infestation levels dominated by common dandelion (Abu-Dieyeh et al. 2005; Abu-Dieyeh & Watson 2006: Chapter 5). *S. minor* is an asporogenic ascomycete and when applied to turfgrass, rarely produces sclerotia (melanized survival structures), and these sclerotia do not survive over winter (Stewart-Wade et al. 2002a). Field and greenhouse studies confirmed that turfgrass species are not susceptible to *S. minor* IMI 344141 (Chapter 8). Independent human and environmental toxicological studies have established that *S. minor* IMI 344141 is neither toxic nor pathogenic to non-target organisms.

A biocontrol pathosystem involves several biotic and abiotic interactions. Understanding the components of a plant-pathogen system is required to maximize the biocontrol success rate (Cousens & Croft 2000). A major limiting factor can be the host plant age, as weed populations are often a mixture of individuals in various functional stages interacting in relationships with each other, with other populations and with the environment (Radosevich et al. 1997). Within population, genetic diversity of the host is important in biocontrol situations and should be matched with pathogen specificity (Cousens & Croft 2000). Generally, crop interspecific competition favours biocontrol success by increasing competitive stresses on weed growth and infestation (Kennedy & Kremer 1996; Cousens & Croft 2000)

Turf is maintained as a vegetation cover and acts as a cover crop affecting weed survival and exerts more below and aboveground competition than many other crops. Dense healthy turfgrass stands are the best defence against weed colonization (McCarty et al. 2001; Busey 2003), but quantitative data are not available to discern the relative contribution exerted by grass competition towards the performance of a biological control agent.

Taraxacum officinale G.H. Weber ex Wiggers (common dandelion) is a perennial weed that overwinters in the soil as seeds or as perennial roots which can resprout the following spring (Cyr et al. 1990). May and September are the peak months of a nearly year-round emergence of dandelion (Chepil 1946) and the plant may perenniate up to 10-13 years (Roberts 1936). Therefore, an established dandelion infestation in turf is a population of mixed ages. The susceptibility of a target weed to a bioherbicide may change with plant age. For example, younger stages were more susceptible to a biological control agent (Boyette & Walker 1985; Léger et al. 2001) while in others, the seedling stage was less susceptible (Makowski 1993; Hoke & Drager 2004). The detrimental effect of plant age could be overcome by increasing inoculum concentration (Masangkay et al. 1999) or by modifying the biotic and abiotic environments (Kennedy & Kremer 1996). Interspecific competition by planting a cover crop, improved the effectiveness of a biocontrol agent on hedge bindweed (Guntli et al. 1999). Moreover, grass vegetation cover has been shown to reduce many growth features of certain weeds (Müller-Schärer 1991; Story et al. 2000) and could be combined with a biological control agent as a complement for control (Dhileepan et al. 2000).

Biotic interactions between grass competition and dandelion age and biotypes are expected in turfgrass environments. But it is important to have a clear understanding of the magnitude of the contribution of each factor and the combinations of factors on dandelion control efficacy as influenced by *S. minor*. The objectives of this research were to assess the susceptibility of different dandelion accessions and different ages of dandelion to *S. minor* (IMI 344141) and to quantify the relative importance of turfgrass competition and the biological stress of *S. minor* on dandelion survival and biomass reduction.

3.3. Materials and methods

3.3.1. Phenotypic variations of dandelion accessions

During the summer of 2004, fruiting heads of individual dandelion plants were collected from university campuses or botanical garden lawns from different locations in the USA, Canada, and Europe. Seeds of each accession 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 40 x 30 x 8 cm trays in the greenhouse (24 ± 2^{o}C with 15 hr of light/day at photon flux density minimum of 350 ± 50 µmol m^{-2} s^{-1}). Ten 2-wk-old seedlings with uniform vigour from each accession were individually transplanted to a 15-cm-diameter pot containing mixed potting soil (as described above). These potted seedlings were arranged in a completely randomized design on a greenhouse bench and maintained under conditions described above, with programmed drip irrigation of 20 ml/pot three times a day. Eight weeks after transplanting (10-wk-old plants) a morphological study was accomplished to assess the variations among accessions (Appendix-3).

3.3.2. Efficacy of the granular *S. minor* bioherbicide on 14 dandelion accessions

Sclerotinia minor (IMI 344141) was isolated from diseased lettuce plants (*Lactuca sativa* L.) from southwestern Quebec and the stock culture was maintained as sclerotia at 4^{o}C. The mycelia of the germinated sclerotia were used to inoculate autoclaved barley grits (1.4-2.0 mm diameter) as described in chapter 5 (Abu-Dieyeh and Watson 2006). The *S. minor* granular formulation was freshly prepared two weeks prior to treatment applications. Viability and virulence of the fungal inoculum were assessed prior to use on PDA plates and excised dandelion leaves (see chapter 5).

Seven 2-wk-old seedlings with uniform vigour from each of the 14 accessions were individually transplanted into 15 cm diameter pots containing mixed potting soil (as above). Pots were placed randomly on a greenhouse bench and maintained under conditions described above. Eight weeks after transplanting, plants were misted with water and five plants of each accession were selected at random and each was inoculated with 0.2 g of the *S. minor* granular formulation. Two plants of each accession remained as untreated controls. Two min. duration of mist was applied daily over all the pots for one week. Symptoms of damage to dandelions were recorded weekly for two weeks after application using a 0 to 10 visual scale, where 0 = no or less than 10% damage in aboveground biomass compared to the control of the same accession, 1 = 11-20% ….9 = 91-99% and 10 = 100% collapse of the aboveground biomass. Data were converted back to a percentage (after Schnick et al. 2002). The experiment was repeated.

3.3.3. Effect of the *S. minor* bioherbicide on above and below ground biomass of dandelion

Fifty 2-wk-old seedlings for each of seven accessions (two from Canada, three from USA, and two from Europe) were transplanted equidistantly into drained, plastic containers (40 x 32 x 20 cm, 19 L capacity) (Sterilite Inc., Montreal QC) containing mixed potting soil. There were 10 containers with five seedlings for each of the seven accessions. The containers were placed in a randomized complete block design in the greenhouse and maintained under conditions described above. The plant containers received a programmed drip irrigation of 50 ml five times a day. Two weeks after transplanting, 2.5 g of a commercial grass seed mixture [30% Kentucky bluegrass (*Poa pratensis* L), 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 uniformly over the soil surface in each container.

Just prior to weed control treatment application (8 weeks after transplanting), the plants were misted with water. Three out of five plants in each pot were selected at random and treated with 0.2 g of the *S. minor* granular formulation. The other two plants were left untreated as controls. Symptoms of damage to dandelions were recorded weekly for four weeks after application using the 0 to 10 visual scale. Plant survival was recorded weekly for six weeks. 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 container. Damage estimates of the three treated plants in each container were averaged and analyzed as one experimental measure. Six weeks after application, all dandelion plants were carefully removed from the soil, the roots were thoroughly washed and dissected above the crown, separating above ground and below ground biomass. Treated and control plant materials (leaves or roots) from each container were separately bulked, placed in paper bags, oven dried at 80^{o}C for 72 h, and then weighed.

3.3.4. Interactions among the *S. minor* bioherbicide, dandelion age, and grass competition

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 mixed potting soil (as described above) in plastic containers (40 x 32 x 20 cm, 19 L capacity). Dandelion seeds were sown at timed intervals to attain plant material of desired plant age states (4, 6, 8, 10 and 13 weeks) at the time of treatment. Extra seeds were sown for each plant age to have replacement plants if required. One week after germination, seedlings were thinned to four equidistant seedlings per container. Two weeks after germination of dandelion seeds, 2.5 g of a commercial grass seed mixture (C.I.L.® Golfgreen™, Brantford, ON) was scattered over the entire surface of one-half of the containers designated for a certain plant age group. The remaining containers of each plant age were not sown with grass. Once established, the grass was clipped with hedge shears (PlantSmart, Wal*Mart, Montreal, QC), each week to maintain approximately an 8 cm grass height, but clipping of dandelion leaves was avoided.

The experiment was split-split-plot design with four replications of each plant age and conducted twice through time, in March 2003 and 2004. Main plots were weed control treatments, untreated or treated with a spot application of 0.2 g plant^{-1} granular formulation of *S. minor*. The subplots were the presence or absence of grass competition and the sub-sub-plots were the different dandelion ages of 4, 6, 8, 10 and 13 weeks of age. Table 3.1 provides the average number of leaves for each group of plant ages at the time of application. Prior to fungal application, all plants were misted lightly with water to aid granule adhesion to dandelion leaves. Plants were grown in a greenhouse at 20 ± 2^{o}C with 15 h of light per day at a photon flux density minimum of 350 ± 50 µ mol m^{-2} s^{-1}. Plant containers received programmed drip irrigation of 150 ml three times per day.

Treatment efficacy was visually recorded weekly for six weeks after application using a 0-10 scale (as described above). Damage estimates of all 4 plants in each container were averaged and treated as one experimental unit. Dandelion survival and regrowth after 100% damage of above ground biomass were recorded weekly for six weeks. Six weeks after application, dandelion plants were harvested as described above.

3.3.5. Determining the efficacy of the *S. minor* bioherbicide on dandelion at flowering stage

Dandelion rarely flowered under greenhouse conditions and the effect of *S. minor* on the flowering stage of dandelion was delayed until a sufficient number of plants came into flower. A separate experiment was conducted under similar greenhouse conditions as described above with 10 plants in the presence of grass [16- to 18-wk-old; 20 leaves (18-24) leaves/plant] and another 10 plants without grass [14- to 16-wk-old; 16 leaves (14-20 leaves/plant] all in the reproductive phase (at least one flowering scape). The *S. minor* granular formulation was applied (0.2 g/plant) on five plants of each group; the other five remained as untreated controls. The plants were misted with water prior to inoculation and daily for a week after inoculation. One month after application, the aboveground damage, above and belowground biomass and survival rate were assessed as mentioned above.

3.3.6. Data Analysis

Morphological phenotypic differences among different accessions were analyzed with Kruskall-Wallis test for one way analysis of variance and the means of each character were separated using Tukey's test at $P \geq 0.05$ (data not presented). Damage through time data for each time period, dry matter biomass data and the number of survived plants from the two experimental repeats on plant age were analyzed separately showing similar results. The data from both repeats were pooled then subjected to Levene test of SAS (SAS Institute Inc., Cary, NC, 2002) to test for homogeneity of error variances. Data from both repeats were combined as error variances were homogeneous. SAS GLM procedure of repeated measures was used to determine effects on aboveground damage through time. Above ground damage data at 2-wk-post application for the 14 accessions study, % survival of plants and the above and below ground biomass data of each experiment were subjected to ANOVA of SAS procedure and the means were separated using Tukey's test at $P \geq 0.05$ (SAS 2002).

3.4. Results

3.4.1. Vegetative phenotypic variation of dandelion accessions

Dandelion accessions were differentiated by several characters, most of them related to leaf morphology (M.H. Abu-Dieyeh & A.K. Watson, unpublished, see Appendix-3). Despite the intra-population variation within each accession, within each single character there was significant variation among the accessions. The rosettes of dandelion could be with erect, in between or have flat leaves. The tap root length ranged from 98 to 133.1 mm. Leaf and root biomass variations were also recorded among the different accessions of dandelion.

3.4.2. Susceptibility of dandelion accessions to *S. minor*

There were no significant ($P = 0.731$) differences in above ground damage among the 14 accessions two weeks after *S. minor* application in a preliminary experiment (data not shown). Damage ranged from 82 to 96%. There were no significant differences in aboveground damage (79.4% to 96%) among seven accessions four weeks after application.

Biomass reduction of treated plants was highly significant ($P \leq 0.01$) compared to untreated plants. The average percentage reduction in leaf and root biomass was 94% and 96%, respectively (Figure 3.1).

Some plants were not completely defoliated and survived the treatment. Other plants resprouted from the root crown even after complete above ground collapse (Figure 3.2). There was no significant differences amongst accessions in either the percentage of plants survived ($P = 0.654$) or the number of resprouted plants ($P = 0.967$) after *S. minor* treatment. The surviving foliage and regrowth was very weak and lacked vigour.

3.4.3. Combined effects of grass competition and plant age on biocontrol efficacy

Highly significant effects ($P \leq 0.01$) were obtained from grass competition, plant age, and interactions of the two factors on above ground damage of dandelion caused by *S. minor*. In the presence of grass competition, 4- and 6-wk-old-treated plants completely collapsed without any recovery, whereas older plants, after almost 100% damage two weeks after application, showed some degree of recovery, positively correlated to plant age (Figure 3.3). For all ages up to 10-wk-old-treated plants, the fungus caused severe cumulative damage of approximately 90% and only the 13-wk-old-treated plants were able to recover partially with 50% damage six weeks after application.

When grown in grass free environment culture, 4-wk-old-treated dandelions were highly susceptible with 100% collapse of all tested plants (Figure 3.3). Older plants were also heavily damaged (80-95% above ground damage) two weeks after application. The level of damage decreased with corresponding less damage with older plants. Incomplete damage of plant leaves and/or vegetative regrowth was the cause of decreasing damage values. There was significantly less damage to the 13-wk-old-treated plants than to younger plants one week after application. The 6-wk-old-treated plants responded similarly to the 8- and 10-wk-old-treated plants from the first to the fifth week after application. In the presence or absence of grass competition, there was no significant difference in biocontrol efficacy on 4-wk-old-treated plants. However, differences were significant ($P \leq 0.01$) on the 6- and 13-wk-old-treated plants. For the 8- and 10-wk-old-treated plants there was no significant difference between the two grass treatments up to two weeks after application, but afterwards the differences were significant (Figure 3.3).

Except for the well established roots of the 13-wk- old dandelion, competition due to grass significantly reduced the survival rate in all plant ages. Survival was due to regrowth after a partial or complete (100%) foliar damage of the treated plants. The aboveground damage was always 100% whenever the grass was present and the regrowth, if any, was very frail tiny sprouts from damaged crowns of some plants. The survival rate was correlated with plant age in the presence or absence of grass competition. Survival was significantly lower in the presence of grass competition than in the absence of grass competition in all aged plants except for the 4- and 13-wk-old-treated plants. In the presence of grass, no regrowth occurred for the 4- and 6-wk-old-treated plants. Meanwhile in the absence of grass, only the youngest age did not regrow (Figure 3.4).

The biomass of leaves and roots was severely diminished by combining grass competition and *S. minor* treatments. The mean differences were significantly ($P \leq 0.01$) less than any other treatment combination for both aboveground and root biomass (Figure 3.5). The aboveground biomass for untreated dandelions without grass competition were similar for the 8-, 10- and 13-wk-old-treated plants (Figure 3.5A) while the 6-wk-old-treated plants had the same root biomass as all older ages (Figure 3.5B). The fungus alone or the presence of grass alone had similar effects on the leaf dry matter of the 4-, 6- and 13-wk-old-treated plants while the grass factor alone caused significantly more reduction than the fungus alone on the 8- and 10-wk-old-treated plants (Figure 3.5A). However, there was no significant difference in root dry matter reduction between fungus or grass presence alone, except in the 6-wk-old-treated plants where the fungus without grass was significantly more effective in reducing root biomass than grass competition alone (Figure 3.5B).

The greatest biomass gain in the presence or absence of grass competition occurred when dandelions were from 4 to 6 wk of age (Figure 3.5). The root biomass gain was significantly higher than the gains during all other growth periods in the absence of grass, while not higher than the 10- to 13-wk growth period under the presence of grass. Except for the oldest plant age, the fungus caused greater reduction in root biomass than grass competition did (68-100% compared with 56-89%), whereas grass competition caused greater reduction in leaf biomass, except for the two youngest ages (38-72% compared with 34-44%).

Whereas in the presence of grass competition, flowering dandelions were highly susceptible to *S. minor* showing significant above ground damage and biomass reduction (Table 3.2). Grass competition resulted in biomass reductions similar to those caused by the fungus alone. One month after application, only one out of five *S. minor* treated flowering dandelions was able to survive in the presence of grass with very weak regrowth, as compared to three out of five with some regrowth in monoculture.

Table 3.1. Average (± standard deviation) number of dandelion leaves of different age groups at the time of application of the *S. minor* granular formulation. Average of 16 plant replicates.

Grass planting	Dandelion Age (weeks) 4	6	8	10	13
present	4.8 ± 0.83	6 ± 0.82	8.3 ± 0.75	11 ± 1.1	13.7 ± 1.0
absent	5.6 ± 0.63	7.2 ± 0.83	9.2 ± 1.6	12.1 ± 1.3	16.2 ± 1.7

Table 3.2. Influence of grass competition on the efficacy of *S. minor* to cause damage and biomass reduction after one month of application on dandelion at flowering stage. Average of five plant replicates.

			Biomass (g)	
S. minor	Grass competition	Aboveground Damage %	Leaf	Root
Treated	present	94 a(1)	0.28 c	0.38 c
	absent	62 b	1.55 b	1.76 b
Untreated	present	6.0 c	1.68 b	1.98 b
	absent	4.0 c	3.83 a	3.64 a

(1) Means in a column sharing the same letter are not significantly different according to Tukey's test at *P* = 0.05.

Figure 3.1. Effect of *S. minor* (IMI 344141) on shoot (A) and root (B) biomass of different dandelion accessions six weeks after spot application with 0.2 g/plant of *S. minor* granules. Within each graph, bars with a common letter are not significantly (*P* = 0.05) different according to Tukey's test. Dandelion accessions: 1. Ste-Anne-de-Bellevue, Québec, Canada; 2. Cornwall, Ontario, Canada; 3. Tuscaloosa, Alabama, USA; 4. Anchorage, Alaska, USA; 5. San Marino, California, USA; 6. Cory Lodge, Cambridge, UK; 7. Utrecht, The Netherlands;

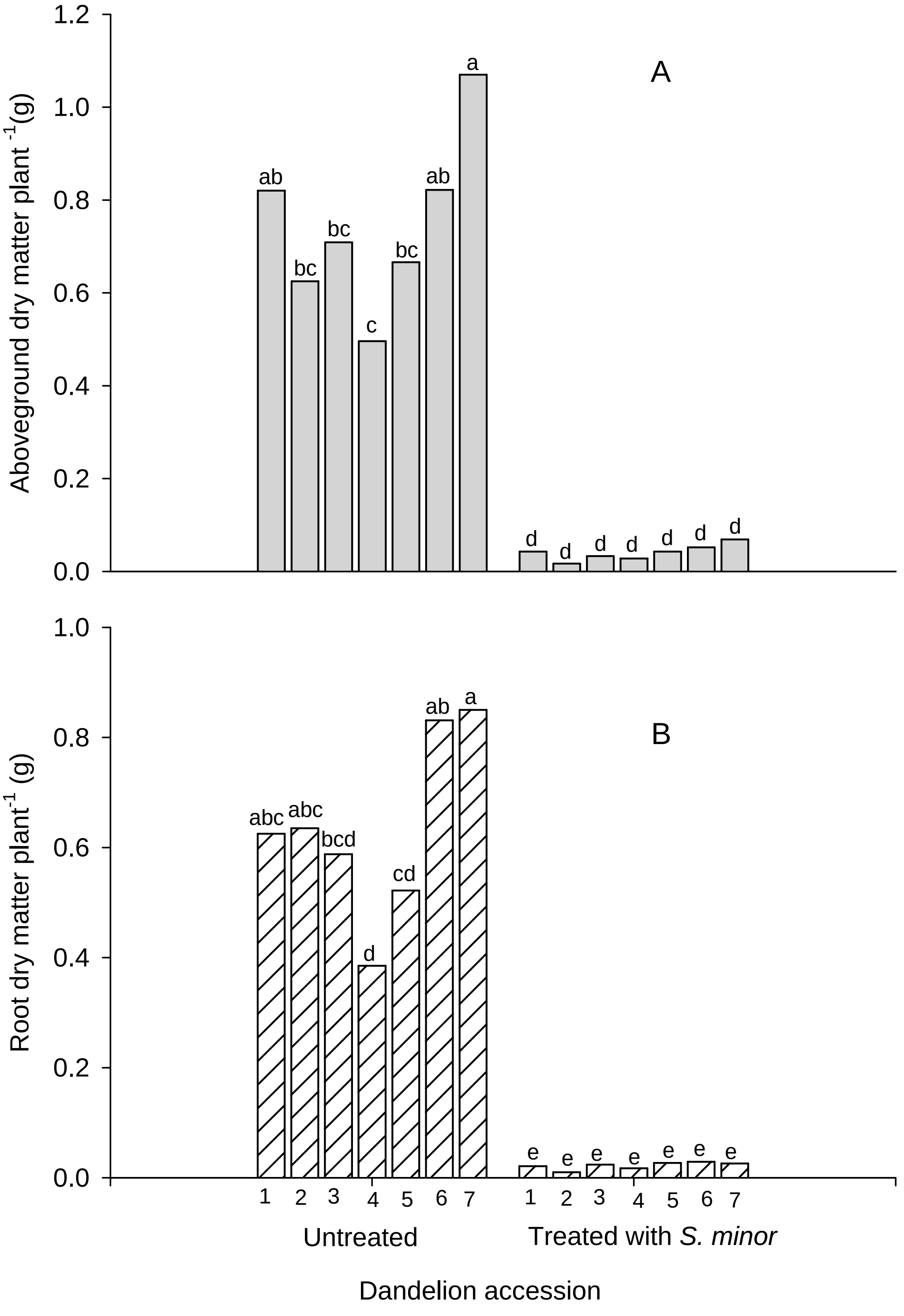

A
B
Aboveground dry matter plant $^{-1}$(g)
Root dry matter plant^{-1} (g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ab
bc
bc
c
bc
ab
a
d
d
d
d
d
d
d
abc
abc
bcd
d
cd
ab
a
e
e
e
e
e
e
e
1
2
3
4
5
6
7
Untreated
Treated with *S. minor*
Dandelion accession

Figure 3.2. Survival and regrowth of dandelion accessions after spot application (0.2 g per plant) of *S. minor* granules. Error bars represent standard errors of total survival means. Values are the means of 30 replicates. Dandelion accessions: 1. Ste-Anne-de-Bellevue, Québec, Canada; 2. Cornwall, Ontario, Canada; 3. Tuscaloosa, Alabama, USA; 4. Anchorage, Alaska, USA; 5. San Marino, California, USA; 6. Cory Lodge, Cambridge, UK; 7. Utrecht, The Netherlands.

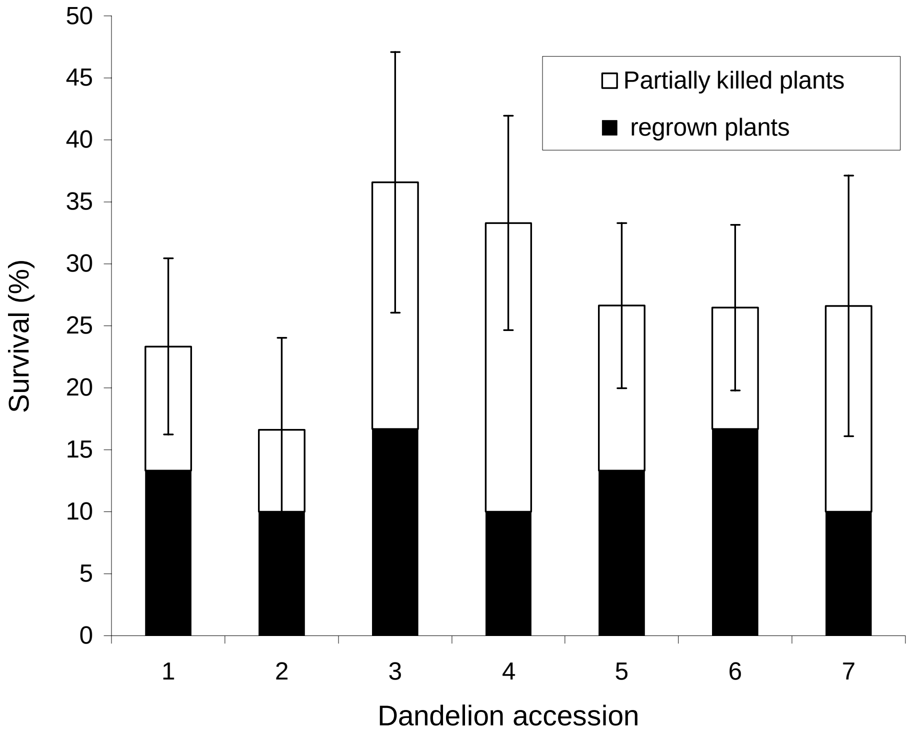


Figure 3.3. Effects of plant age and grass competition on the control of dandelion using *S. minor*. The means were separated using Tukey's test at $P = 0.05$, within each graph, and at any time post application, values with a common letter are not significantly different. (A) grass absent (B) grass present.

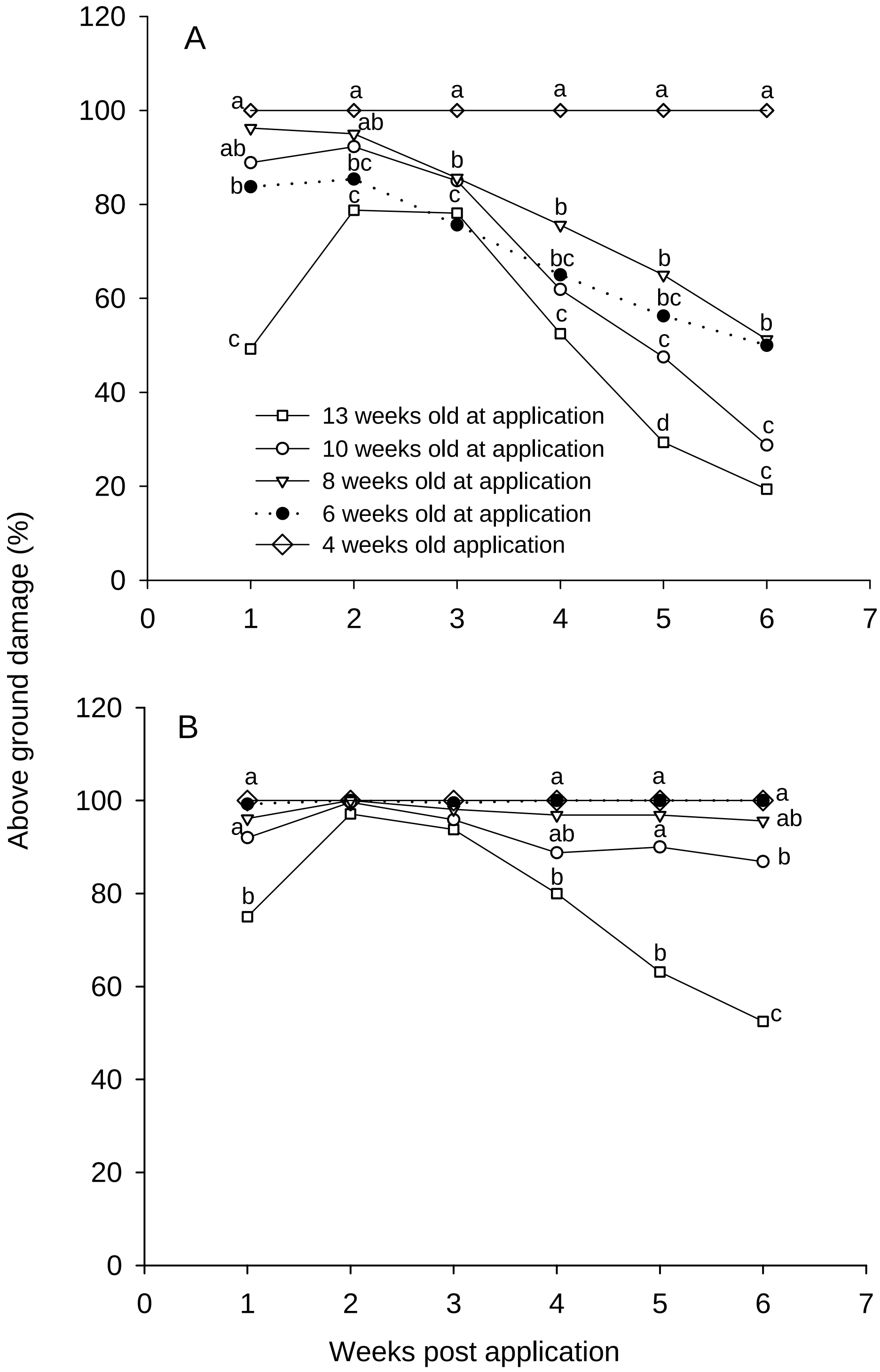

A
B
Above ground damage (%)
Weeks post application
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7
13 weeks old at application
10 weeks old at application
8 weeks old at application
6 weeks old at application
4 weeks old application
a
ab
b
bc
c
d

Figure 3.4. The effect of plant age and presence of grass on percentage survival of dandelions, six weeks after *S. minor* application. Values with a common letter are not significantly different according to Tukey's test at *P* = 0.05.

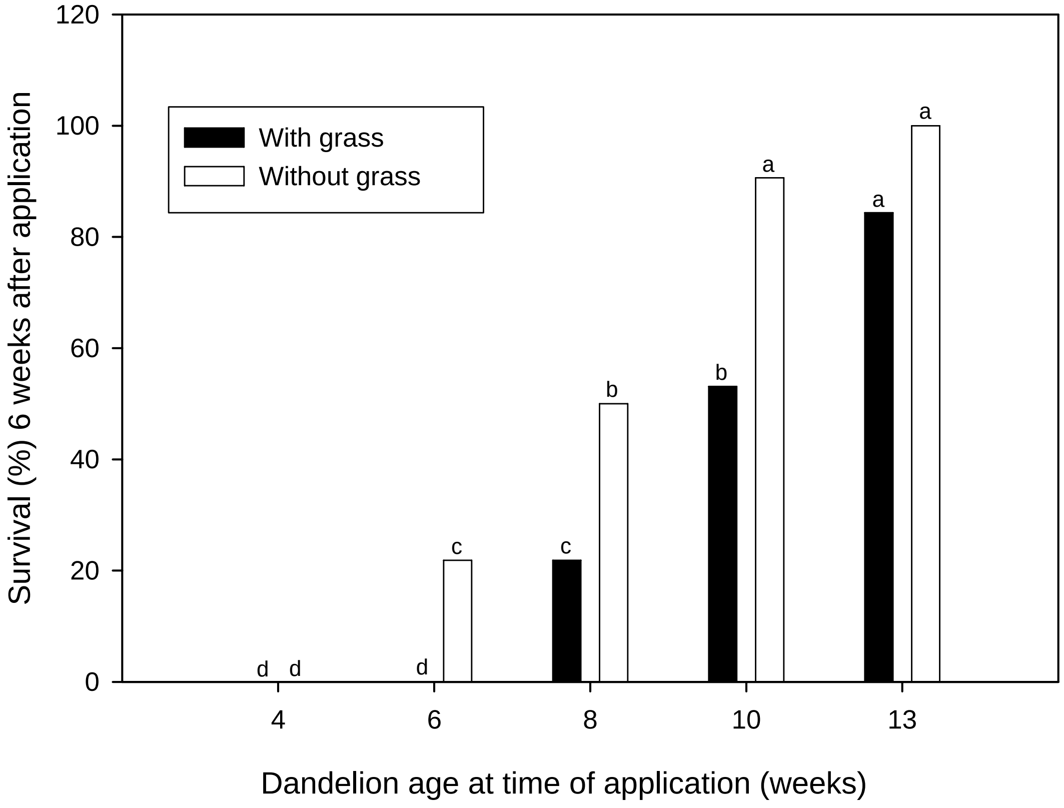
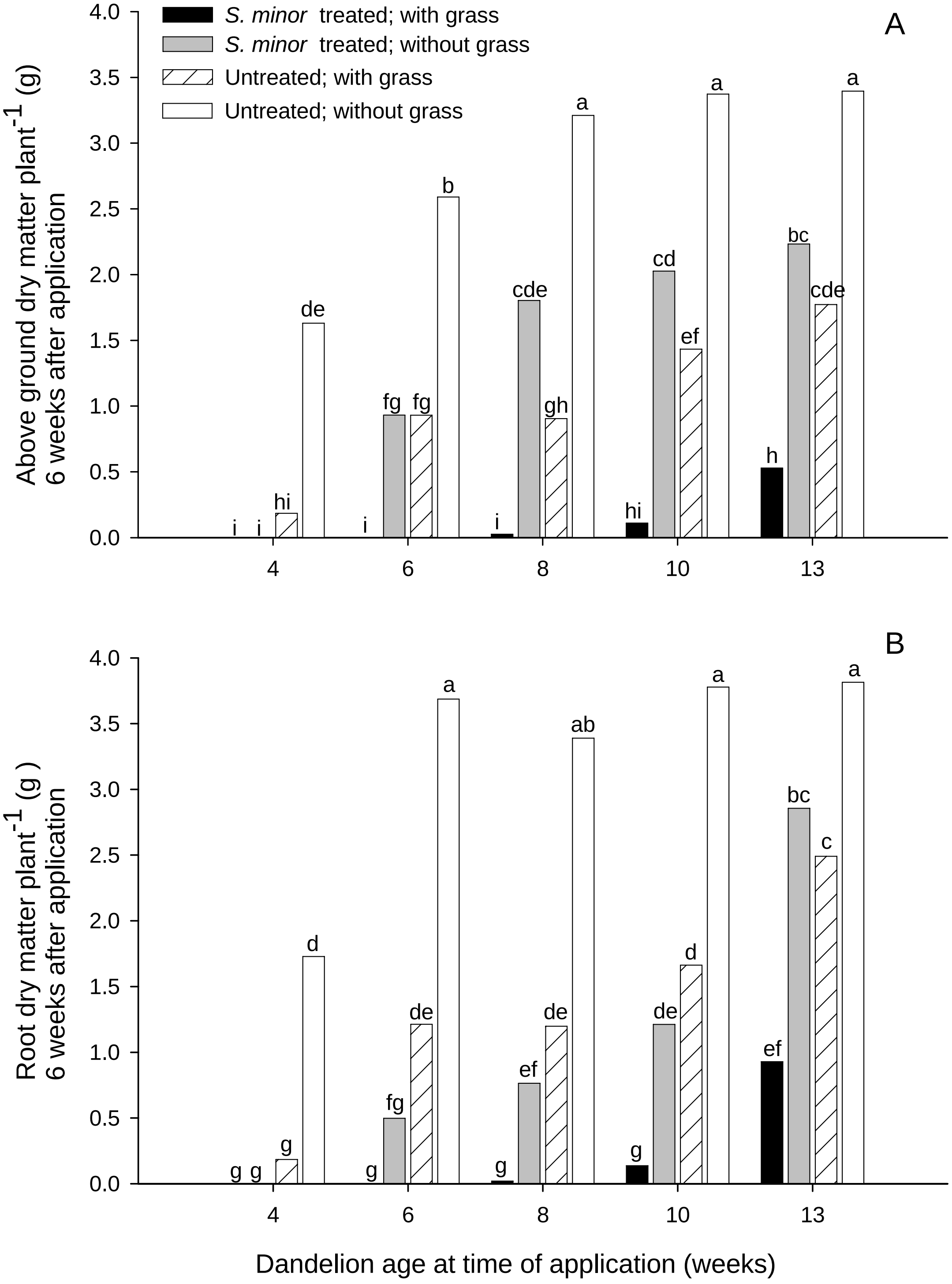


Figure 3.5. The effect of plant age and grass competition on aboveground (A) and root biomass (B) of dandelions six weeks after application of *S. minor* granules. Values in a figure with a common letter are not significantly different according to Tukey's test at *P* = 0.05.


A
S. minor treated; with grass
S. minor treated; without grass
Untreated; with grass
Untreated; without grass
Above ground dry matter plant^{-1} (g)
6 weeks after application
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
i
i
hi
de
i
fg
fg
b
i
cde
gh
a
hi
cd
ef
a
h
bc
cde
a
4
6
8
10
13
B
Root dry matter plant^{-1} (g)
6 weeks after application
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
g
g
g
d
g
fg
de
a
g
ef
de
ab
g
de
d
a
ef
bc
c
a
4
6
8
10
13
Dandelion age at time of application (weeks)

3.5. Discussion

Despite obligate apomixis, intraspecific variation in *T. officinale* is well documented in the literature (as reviewed by Stewart-Wade et al. 2002b) by isozyme analysis (Solbrig 1970; Lyman & Ellstrand 1984), microsatellite DNA analysis (Falque et al. 1998) and meristic and morphologic analysis (Collier & Rogstad 2004). The high intraspecific morphological variation within dandelion populations has also been attributed to phenotypic plasticity (Richards 1973) Dandelion is a highly adapted perennial (Stewart-Wade et al. 2000b), with high phenotypic plasticity occurring in genotypically diversified populations within the same field (Solbrig 1970).

Significant morphologic and meristic variations were found in our 14 dandelion accessions grown under constant controlled environmental conditions. Differences in floral stage timing and reproductive efforts among the accessions were also recorded (M.H. Abu-Dieyeh & A.K. Watson, unpublished data, see Appendix-3). Similar to the findings of Collier & Rogstad (2004), the variations among our dandelion accessions were due to genotypic variations rather than phenotypic plasticity. In plant: pathogen systems, phenotypic variations can be assessed using virulence bioassays rather than isozymes or molecular techniques (Cousens & Craft 2000).

Sclerotinia minor is a necrotrophic, broad host range plant pathogen that can cause economic damage to many crops particularly lettuce and peanut (Abawi & Grogan 1979; Melzer et al. 1997). Newtown and Sequeira (1972) identified lettuce lines that were highly resistant to *S. sclerotiorum* and attributed this to their upright growth habit. However, subsequent evaluation of these lines by Abawi et al. (1980) and Subbaro (1998) indicated that there was no association between growth habit and resistance and none of the screened accessions possessed resistance to *S. minor*. Despite the significant morphologic and meristic differences among the 14 accessions collected from different locations in North America and Europe, all the accessions were similarly susceptible to the *S. minor* granular formulation.

The regrowth from the root crown of *S. minor* treated plants could be an expression of tolerance. However, this regrowth was very weak and may make dandelions more vulnerable to interspecific competition, season long mowing, or winter injury. Several studies have indicated that the regrowth after a biotic stress may be more constitutively resistant to some pathogens and insects than older leaves (Karban & Baldwin 1997; Green & Bailey 2000; Hatcher & Melander 2003), but this is not the case in our pathosystem as the regrown dandelion leaves have shown a high degree of susceptibility to a second *S. minor* application (M.H. Abu-Dieyeh & A.K. Watson, unpublished data).

Although, there were no significant differences in survival and regrowth among the 14 accessions, the highest percentage regrowth occurred in accessions no. 3 (Alabama) and no. 4 (Alaska) (Figure 3.2). Interestingly, these accessions had the longer taproots, but not the highest root biomass. It is known that competitive, well-established dandelions possess deep tap roots that can extend below the level of competition of grass roots (Loomis et al. 1938: cited in Stewart-Wade et al. 2002b).

In this study, dandelions of all ages were susceptible to *S. minor* in the presence or absence of grass competition. However, grass competition significantly increased the aboveground damage, increased root and leaf biomass reduction, and reduced the survival rate of treated dandelions. Grass competition may promote *S. minor* to exert faster and greater disease development and consequential less chance for the plant to capture resources and regenerate from the roots.

The susceptibility of dandelions to *S. minor* decreased with plant age as has been shown for other pathosystems including those studied for biocontrol (Boyette & Walker 1985; Lèger et al. 2001; Riddle et al. 1991; Neuman & Boland 2002), although some plants are less susceptible at the seedling stage (Makowski 1993; Hoke & Drager 2004). Decreasing susceptibility with age is common even with herbicides, and dandelion seedlings are susceptible to 2,4-D, while established dandelions are intermediate in susceptibility due to the sorption capacity of the cuticular membrane (Baker & Bukovac 1971).

The effect of grass competition on dandelion establishment and development was determined by examining the dry matter data (presented in Figure 3.5) for untreated plants as they progressed in age. During the 6 wk period from treatment to harvest, dandelion plants gained the greatest biomass during the 4-wk to 6-wk period under both environments, but 2-fold higher root biomass (1.96 vs 1.03 g plant $^{-1}$) in the absence of grass compared with the presence of grass. In the absence of grass competition, root biomass was maximized in the 6-wk-old plants and shoot biomass maximized in the 8-wk-old plants. These results may explain why the efficacy of *S. minor* on the 6-wk-old-treated dandelion was similar to the efficacy on older plants in the grass free environment.

In a competitive environment we hypothesized that, dandelions will allocate resource differently, this allocation could occur gradually and should favour root resources in later growth stages, consequently resulting in root extension deeper than grass roots. Although the final leaf or root biomass accumulation (of the 4 wk- to 13-wk-old plants) was proportionally similar in the presence or absence of grass competition, it was attained by the 8- and 6-wk-old-treated plants, respectively in grass-free environment, while both leaf and root maximal biomass occurred in the 13-wk-old plants in the presence of grass. In the presence of grass, root biomass gain was ~ 2-fold greater than the leaf biomass from the 8- to 13-wk-old plants (1.29 and 0.71 g plant^{-1}, respectively) Therefore the 13-wk-old-treated plants were better established in the presence of grass, enabled them to regenerate after *S. minor* application as reflected in enhanced survival compared with younger plants.

Except for the 4-wk-old-treated and 13-wk-old-treated plants, grass competition combined with *S. minor* reduced the survival of dandelion significantly compared with no grass competition. In the case of the grass free environment, the survival was more due to the incomplete damage of the aboveground biomass, while it was due to root regrowth after a complete aboveground damage in the presence of grass competition. This regrowth was very weak since the reduction in root biomass was very severe, thus these plants may have likely soon succumb if the experiment was extended longer. Our data indicated a decrease in survival of regrown dandelions after eight weeks compared to six weeks post application of *S. minor* in greenhouse conditions (Chapter 5). Surprisingly, with the 8- and 10-wk-old-treated plants, the grass competition alone exerted more significant reduction in aboveground biomass than the fungus with no grass competition while the fungus alone caused more significant reduction on root biomass than grass competition on the 6-wk-old-treated dandelions. Four-wk-old-treated dandelions were extremely susceptible to *S. minor* and killed in both grass environments and importantly it was the age with the greatest gain of leaf and root biomass in both grass environments hence no significant effect was obtained from grass factor on the efficacy of control. Earlier studies suggested that the establishment of dandelion seedlings was strongly inhibited in areas of dense grass cover due to insufficient open ground and light penetration (Molgaard 1977). Chances of seedling establishment are decreased 23 times in areas with lush grass vegetation compared with open areas (Ford 1981).

Grass competition significantly enhanced the efficacy of *S. minor* on flowering dandelion. Although the flowering plants were older than 13 weeks, there was more aboveground damage, more biomass reduction, and less survival in the presence of grass competition. Similar results were obtained with *Phoma glomerata* on dandelion (Neuman and Boland 2002). Survival of dandelion treated in the spring (about 90% in the flowering stage) with *S. minor* was significantly lower than that in the fall treatment (Abu-Dieyeh & Watson 2006: Chapters 5 & 7). In the absence of control, the survival of 2 cm long dandelion roots collected in May, at the time of maximum flowering, was less than that for the roots obtained from June to September of the same year (Mann & Cavers 1979). In the spring, dandelions are known to allocate more resources for flowering and vegetative growth than for root growth (Cyr et al. 1990), and this may explain the reduced survival and regrowth of flowering dandelions after *S. minor* infection.

Our results address the importance of grass competition to control dandelion with *S. minor* in turfgrass systems. From our field experiments, greater survival of treated dandelions occurred in plots with low grass density and/or bare ground than in high density grass plots (Chapter 7). Indeed, dandelion populations in field environments are expected to be of mixed ages and mixed genotypes and consequently, survival and regrowth after *S. minor* application could be explained by several factors and interactions rather than grass competition alone. Additional factors, such as competition duration, degree of grass establishment, intraspecific competition within a dandelion population, and interspecific competition with other weeds, may all affect the competitiveness between dandelion and turfgrass, and consequently the performance of *S. minor*.

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