PREFACE TO CHAPTER 7

Dandelion has been recognized as a significant weed with competitive and adaptable abilities. Dandelion has two major competitive features: the high seed production and the strong deep taproot that can regenerate. Dandelion physiology, phenology and reproductive ability fluctuate within the growing season. Application time is often the key to success with any control option. Timings that match the seasonal climate conditions for superior efficacy of *S. minor* and the weakest stage in the life cycle of the host plant are paramount to successful weed control. Moreover a successful biocontrol agent should control both the aboveground plants and belowground reproductive parts. In Chapter 7 the population dynamics of dandelion, including the dandelion seedbank and root regrowth and dynamics of other broadleaf species were investigated in a three-year field study. The timing of two weed control methods, the *S. minor* biocontrol and the standard chemical herbicide were compared.

The results of Chapter 7 have been prepared in manuscript form to be submitted to Weed Science. 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 7

Population dynamics of *Taraxacum officinale* and other broadleaf weeds in turfgrass as influenced by chemical and biological control methods and different seasonal applications

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7.1. Abstract

A three year field study was conducted to determine the effect of a biological control agent, *Sclerotinia minor* and a common herbicide on the population dynamics of dandelion, on the dandelion seedbank, and on the population dynamics of other broadleaf species. Treatments were applied as one spring, one fall, or one spring plus one fall treatment per year. The response of the dandelion population to a spring herbicide treatment was similar to two applications (spring & fall) per year. Significantly less dandelion control occurred after the first fall application. A similar reduction in the dandelion population density was obtained two weeks after *S. minor* application with either spring or fall treatments, indicating no effect of seasonal climate variation on efficacy of *S. minor* and any progressive effect on the dandelion population was more likely to be explained by the physiology and phenology of the plant. Compared to the herbicide treatment effect, *S. minor* had similar effects on the dandelion population size starting from the second year under the two applications of *S. minor* per year treatment and a delay up to the third year under the one spring application of *S. minor*. One fall application of *S. minor* was similar to the herbicide across the study period, but it was the least effective during the second year compared with spring and spring & fall applications. The *S. minor* treatments significantly reduced the dandelion seedbank and this effect was not significantly different from Killex™ herbicide treatment effect. The rate, frequency and seasonal timing of application had no effect on the dandelion seedbank size, but terminating the application would gradually replenish the seedbank. Populations of *Trifolium repens*, *Plantago major*, *Lotus corniculatus* and *Ambrosia artemisifolia* were similarly suppressed by either the *S. minor* or the herbicide treatments.

Oxalis stricta significantly increased after one year of herbicide treatment compared with the *S. minor* and untreated control treatments, indicating the risk of a species shift toward a more herbicide tolerant weed species. Turf quality was improved due to herbicide and *S. minor* treatments, but grass injury and crabgrass invasion were recorded in three (out of 18) herbicide treated plots.

Keywords: 2,4-D, biological control, population dynamics, *Oxalis stricta*, *Sclerotinia minor*, seedbank, *Taraxacum officinale*, turfgrass

7.2. Introduction

Turfgrass is a vegetative ground cover that prevents soil erosion and offers recreational and aesthetic benefits for the society (Beard & Green 1994). Most turfgrass environments have weed problems and require a degree of management to be functional and aesthetically pleasing (Monaco et al. 2002). The need for regular management and maintenance of these areas creates a massive turfgrass industry in North America and Europe. The annual expenditure was estimated to be 25-45 $ billion in the United States (Beard & Green 1994) and the lawn care industry in North America is expanding at a rate of 5-8% per year (United States Environmental Protection Agency 1999).

Dandelion, a strong colonizing and competitive plant, has been recognized as a significant weed of lawns and other turfgrass environments (Stewart-Wade et al. 2002b). It overwinters in the soil as seeds or perennial roots which can resprout in the following spring (Cyr et al. 1990). May and September are peaking months of a nearly year-round emergence of dandelion (Chepil 1946).

Repeated applications of dicamba (3,6-dichloro-2-methoxybenzoic acid), phenoxy herbicides such as 2,4-D (2,4-dichlorophenoxy acetic acid) and mecoprop ((±)-2-(4-chloro-2-methylphenoxy) propanoic acid), or combination products such as Killex™ have been widely used for dandelion and other broadleaf weed control (Anonymous 1997). Despite the success of the above mentioned herbicides in controlling broadleaf weeds (Emmons 1995) their use on lawns has come under severe pressure from environmental and public health perspectives resulting in various levels of government enacting legislation inhibiting or banning the use of pesticides in urban areas (Cisar 2004). Over reliance on successive annual herbicide applications in turfgrass is ecologically inevitable. There are cases of weeds with no known selective herbicide treatment, herbicide resistance, and shifts in weed population towards more tolerant species (Busey 2003). Consequently, research onto alternative approaches of cultural and biological methods of control has been intensified (Kennedy & Kremer 1996; Hatcher & Melander 2003; Laresen et. al. 2004).

Sclerotinia minor has been studied as a possible biocontrol agent for dandelion (Ciotola et al. 1991; Riddle et al. 1991; Brière et al. 1992; Schnick et al. 2002; Stewart-Wade et al. 2002a). Studies have been conducted with a barley based formulation of *S. minor* (IMI 344141) to evaluate its effectiveness on broadleaf weeds and quantify possible ecological consequences in turfgrass environments. Field studies have confirmed the efficacy of *S. minor* in controlling dandelion and reducing broadleaf weed ground cover (Abu-Dieyeh & Watson 2006: Chapter 5). More over its effectiveness on dandelion seeds and seedling establishment without negative impacts on turfgrass was also explored (Abu-Dieyeh et al. 2005: Chapter 9; Chapter 8).

It is a difficult task to achieve a successful weed control due to continual changes in weed populations (Hartzler 2000). In general, any weed control method removes or suppresses target species, but this activity may result in modification or disruption of the habitat of other organisms (Radosevich et al. 1997). Basically an ecological approach broadens management options and decreases the probability of failure (Booth et al. 2003). Researchers emphasized the importance of studying the dynamics of weed populations or communities to broaden the function of cultural and biological control options (Burpee 1990; Kennedy & Kremer 1996; Radosevich et al. 1997; Cousens & Croft 2000; Headrick & Goeden 2001; Busey 2003).

The bioherbicide approach has had limited commercial or practical success due to problems with mass production, formulation and commercialization, and persistence under harsh environmental conditions (Kennedy & Kremer 1996; McFadyen 1998; Charudattan & Dinoor 2000; Hallett 2005). Biocontrol is successful when the biotic components and the environment interact in such a manner that weed control or suppression occurs (Kennedy & Kremer 1996). Application timing is a key factor in bioherbicide performance, not only because of the need to match the proper meteorological conditions for microbial growth but also the need to match the weakest eco-physiological time of the target plant species. The proper time is determined by interacting factors of pathogen, host and environment. Given the high costs of such programs, the success rate should be maximized and this task cannot be achieved without understanding the population dynamics of the host plant (Cousens & Croft 2000).

Generally a few studies have been done on population dynamics of dandelion under the effect of control or management practice (Darwent & Elliott 1979; Blackshaw et al.

1994; Harker et al. 2000; Carlassare & Karsten 2003; Froese & Van Acker 2003). The ecology and population dynamics of dandelion in turfgrass systems under the influence of a biocontrol agent have never been investigated. In this study, we investigate the effect of a *S. minor* (IMI 344141) barley based formulation on the population dynamics of dandelion and the consequences on associated broadleaf turf species with respect to seasonal timing and in comparison with a standard chemical treatment.

7.3. Materials and Methods

7.3.1. Fungus formulation

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 Abu-Dieyeh and Watson (2006: Chapter 5). 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 potato dextrose agar (PDA) plates and on excised dandelion leaves. The diameter of colonies and lesions caused by the fungus were measured after 24 and 48 h of incubation. Previous unpublished quality control studies indicate viable batches to have colony diameters 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.

7.3.2. Site description

The study site was represented by a lawn area of around 900 m^2 located in the Macdonald Campus of McGill University, in Ste-Anne-de-Bellevue, QC (45°25'N latitude, 73°55'W longitude, 39.00 m elevation). The field was permanently demarked with metal posts and plastic ropes for a period of three growing seasons (2003 to 2005). The selected lawn area was on a loamy sand soil (coarse sand = 9%, fine sand = 82%, silt= 5%, clay= 4%), with a pH of 6.6 and 6.3% organic matter. The lawn had received minimal maintenance management throughout its history except for repeated mowing during the growing season (May to October). The grass sward was approximately 90% Kentucky blue grass (*Poa pratensis* L.) and about 10% red fescue (*Festuca rubra* L.). The lawn flora was highly diversified with 18 broadleaf weed observed throughout the study period and the dominant weed species was dandelion (*Taraxacum officinale* Weber ex Wiggers). The level of infestation ranged from medium (40 to 60 dandelion plants per m^2 and 30 to 60% grass ground cover) to severe (80-120 dandelion plants per m^2 and 10 to 20% grass ground).

7.3.3. Experimental design

The experiment was a randomized complete block design with six replications and two factors. The first factor was time of application with three levels; (a) spring (15 May); (b) fall (15 September) and (c) spring & fall applications per year. The second factor was weed control treatment with four levels; (a) untreated control, (b) a broadcast foliar application of KILLEX™ [2,4-D (95g/L); Mecoprop (isomer-d 50g/L); Dicamba (9 g/L) all present as amines. The Solaris group, Ontario Canada] herbicide at 1.7 kg a.i. ha^{-1}

(200 ml m^{-2} of 0.6% of original concentration), (c) a broadcast application of granular formulation of *S. minor* at 60g m^{-2}, and (d) the *S. minor* formulation at 120g m^{-2}. The herbicide was broadcast applied onto the grass surface using a 1.18 L vacuum sprayer (Home and Garden sprayer. Model no 1998. RLF10-Master Premium. Root-Lowell Manufacturing Co, Lowell, MI). The *S. minor* formulation was broadcast 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.

The treatments were applied during 2003 and 2004, but in 2005 the treatments were modified to 40g m^{-2} of the *S. minor* formulation instead of either 60g or 120g m^{-2} and no herbicide was applied. The rationale of these changes in the third year were to evaluate a lower bioherbicide rate after dandelion suppression to more normal levels encountered in weedy home lawns (20 to 40 plants m^{-2}), while cessation of the herbicide application was to evaluate the re-colonization of weeds after two years of chemical treatment.

The experimental unit (plot) was 1.0 m^2 with 0.8m alleys between any two plots. The distance between any two blocks was 4 to 5m. The corners of each plot were permanently marked to maintain plot integrity for the duration of the study. Plots were mowed regularly as needed at a medium cutting height of 7 to 10cm 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 prevent contamination of adjacent plots. The field received one fertilizer (C-I-L® Golfgreen Lawn Fertilizer 28-3-6, Nu-Gro IP Inc., Brantford, ON, Canada) application per year (at the beginning of October) at 7 kg per 400 m^2 as recommended by the manufacturing company.

The numbers of dandelions and other broadleaf weed species were counted and the total percentage ground cover of broadleaf weeds were estimated in each plot the day before the weed control treatments and the last week of each month thereafter. For *Trifolium repens L.* (white clover), the number was estimated by measuring how many 10 cm diameter patches of white clover covered the ground of a plot. In order to monitor post treatment recovery of dandelions, 10 dandelion plants in each of the fungal treated plots were randomly marked using white colored pins prior to treatment applications. In each monthly assessment survey, turfgrass quality was visually assessed using a growth rating of 0 to100 based on combinations of colour and density where 0 = no growth and 100 = completely uniform turf (Johnson and Murphy 1992). As the study sites are low-maintained turf, the acceptable visual quality according to the scale used is 50%.

No mowing was done in 2005 prior to the 15th of May to avoid cutting the floral scapes of dandelion. The number of flowering scapes in each plot was counted to investigate the extent of the impact of the past two years of treatment on the pre-dispersed reproductive efforts of dandelions.

7.3.4. Effect of *S. minor* treatment on dandelion seed bank

To investigate the effect of weed control treatments on the dandelion seed bank, Soil sampling was done in the middle of August 2004 and 2005. This was to avoid peak periods of dandelion seed production, which are expected mainly during May to June and infrequently in September with very rare scattered fruits formed in the summer (July to

August). Ten soil core samples per plot (a total of 72 plots) were taken randomly with a 2 cm diameter auger down to a10 cm depth. Soil samples were spread in trays and left to dry at room temperature for a one week period then concentrated by sieving through a course (2 mm mesh size) and a fine (0.355 mm mesh size) sieve, to remove root and vegetative parts and needless coarse and fine soil materials (Ter Heerdt et al. 1996). This methodology retrieved all dandelion seeds which were previously mixed with soil in a positive control treatment. Each of the concentrated samples was then spread out into a layer of approximately 5 mm thickness onto trays (45 x 25 x 8 cm) filled with a 2 cm depth of moistened pro-mix soil (Premier Promix, Premier Horticulture Ltee, Riviere-du-Loup, QC). The trays were completely randomized and left under greenhouse conditions at 24 ±2^{o}C with 15 hr of light/day at a minimum photon flux density of 350 ±50 μ mol m^{-2} s^{-1}. The soil was maintained moist by regularly misting water over the soil. Four control trays containing only the pro-mix potting soil were distributed randomly to test for possible soil contamination by dandelion seeds (data not presented since no seedlings germinated). To assess for the effect of the soil and experiment conditions on seed germination, 25 dandelion seeds were sown in each of four positive control trays containing the pro-mix potting soil. Eighty to 90% germination was obtained from the positive control trays and this rate is within the normal germination rate reported for dandelion seeds from several studies as reviewed by Stewart-Wade et al. (2002b).

The trays were checked daily and emerged dandelion seedlings were counted and removed. The experiment lasted for one month as no further seedlings emergence was recorded. The whole experiment was repeated for soil samples collected from the same plots in August 2005.

7.3.5. Statistical data analysis

Statistical analyses were conducted using the SAS statistical package (SAS Institute Inc., Cary, NC, USA, 2002). To overcome the differences in the dandelion density across blocks, data were adjusted as a percentage of pre treatment data collected on 15 May 2003. Normality for each parameter was tested on model residuals using the Shapiro-Wilk test. Data of the three-year-monthly assessments for weed counts and plot diversity were analyzed using GLM procedure of repeated measures to determine the significant interactions among treatment factors through time. White clover density data were transformed as ($\log_{10}$ + 10) to achieve normality. Dandelion regrowth and floral scape count data were analyzed using ANOVA for a randomized block design. The seedbank density data were square root transformed to achieve normality, then main effects of application time and weed control treatments were tested using ANOVA of SAS. A paired t-test was used to compare the seedbank density data between the two years for each treatment combination. Tukey's test (SAS) at $P = 0.05$ was used to separate the means for all analysis with significant effects (SAS Institute Inc., Cary, NC, USA, 2002).

7.4. Results and discussion

7.4.1. Dynamics of dandelion population under no weed control treatment

Meteorological data of the study sites revealed a slight monthly variation in temperature and relative humidity between the three studied years. The total amount of rainfall from May to October was 1308, 1286 and 1523mm for 2003, 2004 and 2005 respectively. However the monthly rainfall varied greatly between the three years (Table 7.1). July and August were the months of highest temperature with a minimum temperature of 7.7^{o}C, a maximum of 32.1^{o}C and an average ranged between 19.3 to 22.2^{o}C. However the high and repeated precipitation in those two months buffered the average relative humidity to around 70%. The high ranges of temperature and relative humidity prevailed within each of all studied months (Table 7.1).

The dandelion population remained relatively stable across the three years with two major peaks in May and September (Figure 7.1). In 2005, there were no frosts in April (average monthly temperature was 7.5^{o}C) so dandelions emerged earlier from the over wintered perennial roots than in the previous years.

In April 2005 the dandelion population size was similar to what was recorded in July and November 2004 and around 50% of the population recovered after the winter. In May the population was composed of well established plants and seedlings. Seedling recruits were the highest in May followed by early June (Abu-Dieyeh & Watson 2006: Chapter 5) and this increased the population dramatically during May forming the first peak. As temperature increased in July the population was reduced by approximately 40 to 60%, subsequently the population increased gradually to form another peak in September. In September dandelions were more robust than in other months and the population was of mixed ages and it was difficult to distinguish newly established dandelions from old plants. A gradual decrease in the population occurred in October followed by a prominent reduction in November due to the cold (average temperature was 2.5^{o}C for Nov 2005). While the low population size in November and April is mainly due to cold temperatures, the population reduction in July and early August could not only be explained by high temperatures. In 2005, a small reduction in dandelion density occurred in July (approximately 20%) compared with the same month in the previous years. This reduction could be attributed to the high precipitation reported in June 2005 (Table 7.1) which encouraged new seedling recruits into the population. Mature dandelion seeds can germinate and produce seedlings throughout the year (Chepil 1946; Martinková & Honĕk 1997; Collins 2000). Dandelion seeds germinate over a wide range of temperature from 5 to 35^{o}C (Mezynski & Cole 1974; Hoya et al. 2004) with best germination under alternating temperatures and light (Stewart-Wade et al. 2002b). The survival of dandelion seedlings decreased with high temperatures with 30% survival at 31^{o}C and 20% at 36^{o}C (Hoya et al. 2004). Even though the high temperature in July and August could be harmful to the dandelion seedling population, frequent high rainfall events during the season may interact positively favouring dandelion germination and seedling establishment.

Vegetative reproduction from tap root fragments can also contribute to the increase in dandelion populations (Mann & Cavers 1979). In cropping systems that involve soil manipulation practices, root fragmentation can significantly increase dandelion populations. Separation of ramets occurs naturally after decay of the tissues that connect them and the longevity of connections between ramets determines the success of persistence of the genet (Booth et al. 2003). Vegetative reproduction of dandelion from tap roots in turfgrass environment may aid in dandelion persistence as a result of mowing, manual eradication of tap roots, and frost.

Other studies reported dandelion emergence nearly year-round with two peaks in May and September (Chepil 1946), but the rate of increase for an entire population was the highest in fall (Vavrek et al. 1997). Our results from three years data revealed that dandelion densities were similar in May and September regardless of abiotic or biotic factors. This implies that the vulnerability of the population to environmental selective pressures is very low and even thought the population fluctuated within the year it was maintained across the three years. No significant reduction in the dandelion population occurred under two successive growing seasons of regular mowing at different heights (Chapter 6). Genetic variability within dandelion populations was found to be an effective tool in regulating the population through different degrees of competitiveness and survival in response to pressures from interspecific competition (Vavrek 1998) and disturbances (Solbrig & Simpson 1974).

7.4.2. Effect of chemical herbicide treatments on the dandelion population

The spring herbicide treatment had an immediate direct effect in the first year, while the fall treatment reduced the population dramatically in October, but about 70% of dandelion population recovered in the following spring (May 2004) (Figure 7.1). The result of the second fall application was effective and not significantly different from other herbicide treatments (Figure 7.1). Importantly, after the second fall application, the population size increased in mid May of the following year (2005) and then diminished at the end of the month without any weed control treatment. This mortality was more likely to be explained by persistence of the herbicide residuals from last fall treatment. The response of the dandelion population to two applications per year (spring & fall) of Killex™ was almost similar to one spring application (Figure 7.1).

Applications of 2,4-D cause several metabolic changes in common dandelion root tissues particularly the depletion of carbohydrate reserves (Deacon & Rutherford 1972) Perennials rely on these reserves to overwinter (Wilson & Michiel 2003) and dandelion allocates more resources for flowering and vegetative growth in the spring (Cyr et al. 1990). Seasonal changes in carbohydrates of dandelion roots have been reported by Wilson et al. (2001) with more monosaccharides as percentage of total sugar in spring and more fructan polymers as the season progressed towards the fall. However, freezing soil temperatures are associated with increased fructose and decreased fructan as a percentage of total sugar (Wilson et al. 2001). The availability of carbohydrate reserves in dandelion roots limited the response of these roots to 2,4-D throughout the season, therefore optimum control of dandelion in lawns was achieved by spring application of 2,4-D combined with spudding (Mann 1981).

Our results indicated that more successful chemical control of dandelion could be achieved by spring than fall application. The seasonal changes of carbohydrates in the root system is a reasonable explanation but other facts could also be involved such as younger plants translocate herbicides faster than do older plants (Crafts 1961), and the response of plants to 2,4-D depends critically on the stage of development which determines the rate of penetration and translocation of the herbicide (Tomkins & Grant 1974). Unfortunately the age structure of the population was not investigated, but similar to what was reported by Roberts & Nelson (1981), more seedlings constituted the population in May and June than in later months and this supports the effectiveness of spring application. Wilson & Michiel (2003) suggested a control strategy for dandelion and Canada thistle by applying herbicides, like dicamba and 2,4-D in late fall, 10 days after the first frost which was accompanied by reduced quantities of low degree-polymerizing fructans and consequently achieved better control than when dicamba was applied 11 days before the frost. Even though this application time is not comparable to our study, the results we obtained in the third year support the importance of the accumulative effects of herbicide residuals and frost on well established dandelions.

7.4.3. Effect of *S. minor* treatments on dandelion population

The meteorological data for the 14 days after treatment application are summarized in Table 7.2. The differences between spring and fall treatments in temperature, relative humidity (RH), dew point, and even rainfall did not cause significant differences on the efficacy of dandelion control two weeks after application of *S. minor*. The rainfall was the most variable factor, but the field was irrigated when there was no rainfall on the day of application and two days later. In general, moisture is an important requirement for many pathogens and is known to enhance the efficacy of *S. minor* (Melzer & Boland 1994). Thus according to our climate data, the *S. minor* formulation was effective in field conditions with mean daily temperatures from 9 to 20^{o}C; mean daily RH above 58%, and a dew point range from 3.6 to 17.4^{o}C. Unfortunately, soil moisture was not monitored in this study, but plots were irrigated during dry periods to avoid moisture stress. In the fall of 2003 and 2005, heavy rainfall events occurred four to five days after applications and there was no effect on the efficacy of *S. minor* as by that time the infection was already established. The biocontrol product was rainfast and was not moved off target.

In the first year, the spring Killex™ treatment was significantly more effective than the *S. minor* treatment (Figure 7.2). Unlike the herbicide, the fungus has no residual activity and has mostly disintegrated within 10 days after application. Therefore clearing aboveground population two weeks after application induced seedling recruitment from the soil seedbank (Abu-Dieyeh & Watson 2006: Chapter 5). A percentage of vegetative regrowth from the non killed roots could also contribute to population size. The recruitment size was around 40 to 50% of the pre-treated population size and diminished during the summer (Figure 7.2). Establishment of dandelion seedlings decreased when temperatures increased to 30^{o}C or more (Hoya et al. 2004) and this temperature was encountered in many days during July and August. Interestingly August, instead of September, was the peak month and then the population size diminished even though there was no *S. minor* fall application. This reduction was more likely due to mortality as a result of dry conditions that prevailed in August 2003 (53 mm total rainfall) or improvement of grass vigour after clearing most of the well established competitive dandelions by spring *S. minor* application. Dandelion seedlings are less competitive in superior grass quality turf (Molgaard 1977; Ford 1981).

In the second year and prior to the spring application of *S. minor*, seedling recruitment was expected but a second application resulted in a level of suppression that was not significantly different from the herbicide and prevailed across 2004 and 2005 (Figure 7.2). The effect of the fall treatment started within two weeks after the September application and significantly reduced the dandelion population compared with the untreated control (Figures 7.1 & 7.2). In the second year, a significant level of population suppression (approximately 60%), similar to the Killex™ treatment, occurred from May to July, but in August the suppression was minimal (Figures 7.1 & 7.2). However, the second fall application was effective into the following year (2005) and was not significantly different from the herbicide treatment. Clearly, spring & fall treatment per year of *S. minor* appears necessary initially for the high level of infestation encountered in the studied field, but subsequent applications could be minimized in frequency or rate to control future recruited population.

In another study examining the effect of application time on the efficacy of *S. sclerotiorum* on *Cirsium arvense* (Hurrell et al. 2001), less effective control occurred with late summer and autumn applications than with spring applications. The authors suggested that free moisture in the autumn promoted efficacy, but the intense rainfall after treatment reduced efficacy through wash off (Hurrell et al. 2001). In our study as the *S. minor* had similar efficacy on dandelion 2-weeks post spring and fall applications thus any progress difference in dandelion population could be attributed to the physiology and phenology of dandelion rather than seasonal climate variations.

Our results illustrated that some dandelions had the ability to regrow after a complete aboveground damage and the extent of regrowth was significantly lower after spring (approximately 10% of the population) than fall application (Figure 7.3). While no significant reduction on regrowth percentage was recorded across the years for spring treatments, the reduction was significant after fall treatment in 2005 and reduced to 10% compared with the past two years (2003 & 2004) when the regrowth was 20 to 25%. However, variations among plots of the same treatment were observed to be important and the regrowth percentage was highly determined by grass quality and density, thus we separated the first year regrowth (%) data according to first year average turf quality (%) values to two sets of data, visually accepted turf quality (≥ 50%) and poor turf quality (30 to 40%). In superior grass quality plots the regeneration of dandelion roots reduced significantly in either of spring or fall treatment application (Figure 7.4).

Previously, we presented the impact of grass competitive environment compared to grass free environment on root regrowth of dandelion after *S. minor* infection under greenhouse conditions (Chapter 3) and also the effect of season-long mowing at the close height (3-5 cm) followed by *S. minor* treatment on reducing root regrowth even though this treatment was accompanied by a significant increase of dandelion recruitment. Supporting our results, dandelion root fragments derived from source plants at the time of flowering in May, had very little survival (Mann & Cavers 1979). Fall treatment application seems to interact with *S. minor* efficacy in a similar manner to what was explained for 2,4-D efficacy and the previously discussed seasonal changes of root resource allocations of carbohydrate reserves were the major explanation (Mann 1981; Cyr et al. 1990; Wilson et al. 2001). Our results are consistent with Green et al. (1998) who studied the regenerative capacities of four phenological stages of *Ranunculus acris,* giant buttercup (perennial weed) crowns after *S. sclerotiorum* infection and found that regeneration was least after treatment at pre-flowering stage and greatest at 32 to 38 weeks post onset of flowering. The authors correlated the lowest regrowth of pre-flowering stage to depletion of carbohydrate reserves in the crown (Green et al. 1998). In greenhouse conditions, we reported less survival of flowering plants than vegetative 13-week-old plants after *S. minor* spot application even though the tested flowering plants were older (Chapter 3) and similar results were obtained with *Phoma glomerata* on dandelion (Neuman & Boland 2002).

Regrown plants after *S. minor* treatment have shown to be weak with short tap roots (Abu-Dieyeh & Watson 2006: Chapter 5) and accompanied by severe reduction in leaf and root biomass even for 13-week-old plants (Chapter 3). Our results stress the need of manipulating interspecific competition to favour turfgrass over dandelion and consequently increase the competitive pressure on dandelion roots pre and post *S. minor* infection. This could be achieved by increasing grass density and choosing highly competitive turf species or cultivars. In general, an additional control measure may be needed to increase stress on weeds using bioherbicide-based control (Hasan & Ayres 1990; Hatcher & Melander 2003). However, regrowth should be investigated with respect to anatomical and biochemical changes in the crown and root of dandelion due to *S. minor* infection.

7.4.4. Effect of *S. minor* application rate

The preliminary unpublished data about the recommended field rate of *S. minor* barley-based formulation was 40 to 60g m^{-2} depending on the level of the dandelion infestation. The infestation in our field ranged from medium, 40 to 60, to severe, 80 to 120 dandelion plants per 1.0 m^2 and so we applied 60g as a reliable rate for extremely infested lawns with a history of low-maintenance. The current popularity of inundative biological control may result in problems, as an increasing number of activities will be executed by untrained people (van Lenteren et al. 2003). Due to the above recommendation we introduced 120g m^{-2} to evaluate any consequences for misusing the formulation. No important significant differences were obtained in any of the studied parameters by using 120 instead of 60g m^{-2} (Figure 7.2). No impacts on turfgrass or consequences on weed species composition were observed due to the 120g m^{-2} treatment. Moreover, applying the lower rate (40g m^{-2}) in the third year on all *S. minor* treated plots, after the population has been suppressed to around 20 dandelions m^{-2}, had similar effects to what was obtained with the higher concentrations in the past two years (Figure 7.1). Our suggestion is that, the field recommended rate of *S. minor* is highly determined by the level of dandelion infestation and turfgrass quality and although 60g is an effective rate in the worst turf quality, the minimum optimum rate is difficult to predict but should be lower.

7.4.5. Consequences of treatments on population dynamics of other weeds

July was the month of the highest diversity of broadleaf weeds with a range of 3 to 8 species m^{-2} and an average of 4 to 5 species m^{-2} (Figure 7.5). Relatively high temperature and rainfall were recorded during July and these factors could be involved in flourishing other broadleaf weeds particularly after the competitive pressure had been released from dandelion as a dominant species during this month. Even though *Trifolium repens* and *Plantago major* were less abundant than dandelion, they still had high temporal and spatial frequency even when dandelion population size was in its peak (Figure 7.6). These two species were shown to exert competitive responses and competitive effects with dandelion and may be classified as important competitors due to similar rosette growth form of plantain and tap root of white clover to dandelion (Vavrek 1998).

Both *S. minor* and herbicide treatments significantly reduced the diversity of broadleaf weeds (Figure 7.5), particularly in the third year and under the spring & fall per year treatment. However, a tendency of increasing diversity was observed in August 2005 in the Killex™ treatment. It is important to note that there was no application of Killex™ in 2005 and so the date from last spring treatment was 14 months and from the fall treatment was 10 months. The reported data for 2,4-D half-life persistence in soil is variable and ranged between 2 to 269 days (Cox 1999). The increased plot diversity of herbicide treated plots was mainly due to gradual disintegration of herbicide residuals which may encouraged other weed species to germinate under a new environment of no or limited abundance of dandelion.

S. minor and herbicide treatments were highly effective, not only in suppressing dandelion population, but also in significantly reducing other perennial weeds like *Trifolium repens*, *Plantago major*, *Lotus corniculatus* and *Ambrosia artemisifolia* (Figure 7.6). The suppression of the above mentioned weeds could be attributed to the direct effect of the treatment or to the new improved grass quality or more likely to both factors. However, all species were recorded to be susceptible to spot application of *S. minor* (Chapter 4). Other broadleaf species like *Medicago lupulina* (black medic), *Pyrrhopappus carolinianus* (Carolina false dandelion), *Cerastium fontanum* (mouseear chickweed), and *Vicia sativa* (comman vetch), were suppressed under both *S. minor* and Killex™ treatments, but the rare spatial or temporal abundance of these species made their data not allowable for statistical analysis.

Importantly, the abundance of *Oxalis stricta* significantly increased under the herbicide treatment (Figure 7.6). We reported earlier from another study site the significant increase of *Malva neglecta* density and the increase in mean values of *Oxalis stricta* and *Polygonum aviculare* under herbicide treatment (Chapter 6). A shift in weed population under herbicide treatment in turfgrass was reported by Johnson (1982) who found minor weeds changed to major weeds due to successive annual herbicide treatment. The frequent use of 2,4-D as early as 1950 had shifted cropping systems from broadleaf weed to annual grass domination (Aldrich 1984). Weed shift occurs when weed management practices are not equally effective and so certain biotypes or species survive, however factors that affect germination, emergence, seedling survivorship and seed production could be contributed to weed shift mechanism (Hilgenfeld et al. 2004). Even though weed shift after a chemical management practice had received more concerns due to the possibility of weed resistance or tolerance, however weed shift could occur as a result of periodic cultural management practices like mowing and fertilization in turfgrass (Busey 2003) and tillage in other cropping systems (Ball 1992).

Oxalis stricta is a prominent herb with underground perennial rhizomes and an erect habit (Doust et al. 1985). Combination of the same herbicides as in Killex™ was found to be ineffective in controlling *Oxalis stricta* in turfgrass (Bing & O'Knefski 1980). This evidence may explain the increased abundance of this weed in the herbicide treated plots and indicate a risk of weed shifts due to repeated Killex™ applications.

7.4.6. Consequences of treatments on turfgrass

Within each of the three treatments: spring, fall and spring & fall, *S. minor* and Killex™ applications improved the turfgrass quality in a similar manner (Figure 7.7). With the spring and spring & fall treatments, significant improvements of turf quality occurred directly six weeks after application while the fall treatment was delayed to September 2004 (Figure 7.7). Turf quality improvement was more likely to be correlated with magnitude of suppression of dandelion population rather than application timing, frequency, or *S. minor* concentration. Basically, vegetative turfgrass growth abilities increase after weeds have been controlled (Turgeon 1985). Grass injuries due to Killex™ application were observed in two plots (out of six) after spring & fall treatment and in another plot after fall treatment. These plots were also invaded by *Digitaria ischaemum*, (smooth crabgrass) in 2004 and 2005. The grass injury was more likely to appear in plots with low grass quality, during summer months and after the second application. It is important to note that the studied turf is a low-maintained stand and the grass injury was more due to stressful effect of excessive use (two applications per year) on poor quality grass. Grass injury was not observed in any of *S. minor* treated plots across the study period.

7.4.7. Consequences of treatments on dandelion floral scapes and seed bank

At the onset of flowering in May 2005 and prior to any treatment application or mowing practice, dandelion floral scapes were found to be significantly reduced in all *S. minor* and herbicide treated plots compared with the untreated control (Figure 7.8). No further flowering across the season was observed in the treated plots. However scattered flowers, with more frequency in September, were observed in the untreated plots. Dandelion has a major peak of seed production and dispersal in spring and with infrequent and scattered flowers over the entire season, but mainly in September (Gray et al. 1973; Stewart-Wade et al. 2002b). The low numbers of floral scapes was due to population size reduction and particularly to the low proportion of well established plants as recorded in the April 2005 assessment (Figure 7.1 & 7.2). The results may indicate a shift in the surviving population towards a younger population due the treatments over the past two year. Newly emerged dandelions bloom in the spring of the following season and some plants can flower in their first year under optimum conditions (as reviewed by Stewart-Wade et al. 2002b). The reproductive stage of dandelion is a major contributor in its weediness and the most undesirable part of dandelion life cycle because of aesthetic problems in lawns (Holm et al. 1997).

A severe depletion in the dandelion seed bank in the upper 10 cm soil layer occurred after *S. minor* applications (Figure 7.9). In 2004, the average seedbank density in untreated plots was 2737 seeds per 1.0 m^2 while the seedbank in *S. minor* treated plots was significantly reduced ($P = 0.001$) compared with untreated plots with an average reduction of 73%. Generally, within *S. minor* treatments, no significant differences of seedbank densities were obtained due to the two inoculum concentrations (60 and 120 g m^{-2}) or due to time or frequency of *S. minor* application (Figure 7.9). The variation in seed bank densities within *S. minor* treatments was mainly due to inter-block variations in infestation level. The chemical herbicide exerted the strongest impact on seedbank density with an average reduction of 88% compared to untreated plots. There were no differences due to different application times. In most treatments, no significant differences were reported between herbicide and fungal treatments (Figure 7.9). However removal of the herbicide treatment in 2005 led to significantly higher seedbank densities than 2004, although it was still significantly less than untreated plots.

The treatment applications were started in spring 2003 thus treated plots received either one, two or three applications but no significant differences were obtained due to the number of applications. Under the *S. minor* treatments, there was no further significant decrease in seed bank size in 2005 compared with 2004. We concluded that one application of *S. minor* was enough to reduce dandelion seedbank, but to maintain long-term reduction successive annual applications should be done.

2,4-D is the main effective ingredient of Killex™ and is known to persist for several months in the soil (Ross & Lembi 1999) with a half-life ranging from 2 to 269 days (Cox 1999). Since no herbicide treatment was applied in 2005, the duration from last 2004 treatment was 14 and 10 months for spring and fall, respectively. The significant higher seedbank obtained under spring than under fall treatment was more likely to be due to differences in the duration from last treatment. Spring is the season of dandelion seed rain and two seasons had passed after the spring treatment while only one season had passed after the fall treatment.

Dandelion seed bank was estimated to be 1,575,000 seeds per ha in the top 13 cm of a grassland area and 2,350,000 seeds per ha in the top 18 cm of an arable field (Champness and Morris 1948). Germination of dandelion seeds was faster and more in light conditions than in the dark (Letchamo & Gosselin 1996). The conditions of buried seeds are unfavourable to seedling development and the light is necessary to induce their germination (Noronha et al. 1997). The establishment success was negatively correlated with depth of seed burial (Bostock & Benton 1979) and 1 to 6% of seeds remained viable after four years of burial in soil (Chepil 1946).

The soil seedbank is a soil reservoir opened for both processes of deposits and withdrawals (Harper 1977). Seedbank deposits occur through seed production and dispersal while withdrawals occur through germination, predation, senescence and death (Radosevich et al. 1997). Reducing dandelion densities in treated plots may lead to less seed production but this could do minor and temporary effects on seedbank density since dandelion seeds are wind-dispersed and compensation from close areas could shortly occur and replenish minor withdrawals.

We suggested that *S. minor* may kill seeds present in the soil surface layer and so reduced further deposits in the seedbank. This suggestion was presented in other studies (Abu-Dieyeh et al. 2005; Chapters 8 & 9). However seedbank depletion could also occur from extensive repeated withdrawals due to recruitment from dandelion seedbank after *S. minor* treatment.

In conclusion, *S. minor* has no residual activity and one month after application dandelion population has the ability to persist in a suppressed level mainly due to seedling recruitment. Root regrowth was a minor compensative for population size after *S. minor* treatment with significantly less values in superior turf quality plots, after spring than fall treatment, and in the third year. Our findings demonstrated the effectiveness of *S. minor* treatment on dandelion seed output which may assist in reducing its establishment through building strong perennial tap roots. Therefore substantial biocontrol benefit can arise over a long term after reducing old dandelion population through repeated application of *S. minor*, subsequent improved turfgrass competition and continuous exhausting of seedbank.

Table 7.1. Weather data for Ste-Anne-de-Bellevue, Quebec during the years of study 2003, 2004, and 2005. Environment Canada Meteorological Data. Ste-Anne-de-Bellevue Station.

	Temperature °C			Relative humidity (%)			Rainfall (mm)
Month / year	Average	Min	Max	Average	Min	Max	Total monthly
May-03	12.8	2.1	28.2	70.1	15.1	100.0	136.2
May-04	7.2	-0.7	29.0	68.3	22.1	100.0	123.0
May-05	11.5	-1.4	26.1	68.1	20.8	100.0	62.6
June-03	17.9	6.6	32.4	69.7	26.5	100.0	123.9
June-04	17.2	4.4	29.0	65.2	25.7	99.1	226.2
June-05	21.1	8.31	32.8	72.5	23.2	100.0	708.6
July-03	20.4	11.2	30.3	73.2	26.5	100.0	357.4
July-04	20.5	10.5	31.0	74.6	30.1	99.0	574.2
July-05	22.2	16.8	31.7	73.7	36.2	97.8	93.4
August-03	22.0	14.1	29.1	78.9	34.6	100.0	53.0
August-04	19.3	7.7	29.1	77.6	44.7	99.5	181.9
August-05	21.4	7.8	32.1	73.3	26.8	100	199.1
September-03	16.8	5.3	28.9	76.6	30.9	100.0	415.7
September-04	15.9	4.7	26.2	78.6	41.5	100.0	96.0
September-05	17.3	3.7	29.1	77.0	39.6	100.0	243.1
October-03	7.4	-1.8	28.9	80.1	30.9	100.0	221.9
October-04	8.9	-2.3	24.6	76.8	32.9	100.0	84.6
October-05	10.8	-2.8	26.5	83.0	36.5	100.0	217.6

Table 7.2. Meteorological summary during the 14 days after spring and fall applications (averaged across the three years 2003, 2004 and 2005).

	Temperature (^oC)		Relative humidity (%)		Dew point (^oC)		Total Rainfall (mm)	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Average	14.3	16.0	78.9	81.6	10.3	12.6	39.9	97.2
Min	9.2	10.9	58.8	71.9	3.6	6.9	13.2	15.3
Max	19.4	20.4	95.1	100	15.3	17.4	57.6	201.5

Figure 7.1. Effect of seasonal herbicide and bioherbicide applications on dandelion population dynamics. Within each graph, means with a common letter at each time are not significantly different at $P = 0.05$ according to Tukey's test. Asterisks (*) refer to the pre-treatment assessment in the middle of May.

$^{(1)}$ Killex™ rate = 1.7 kg a.i. ha^{-1}. $^{(2)}$*S. minor* rate = 60 g m^{-2}

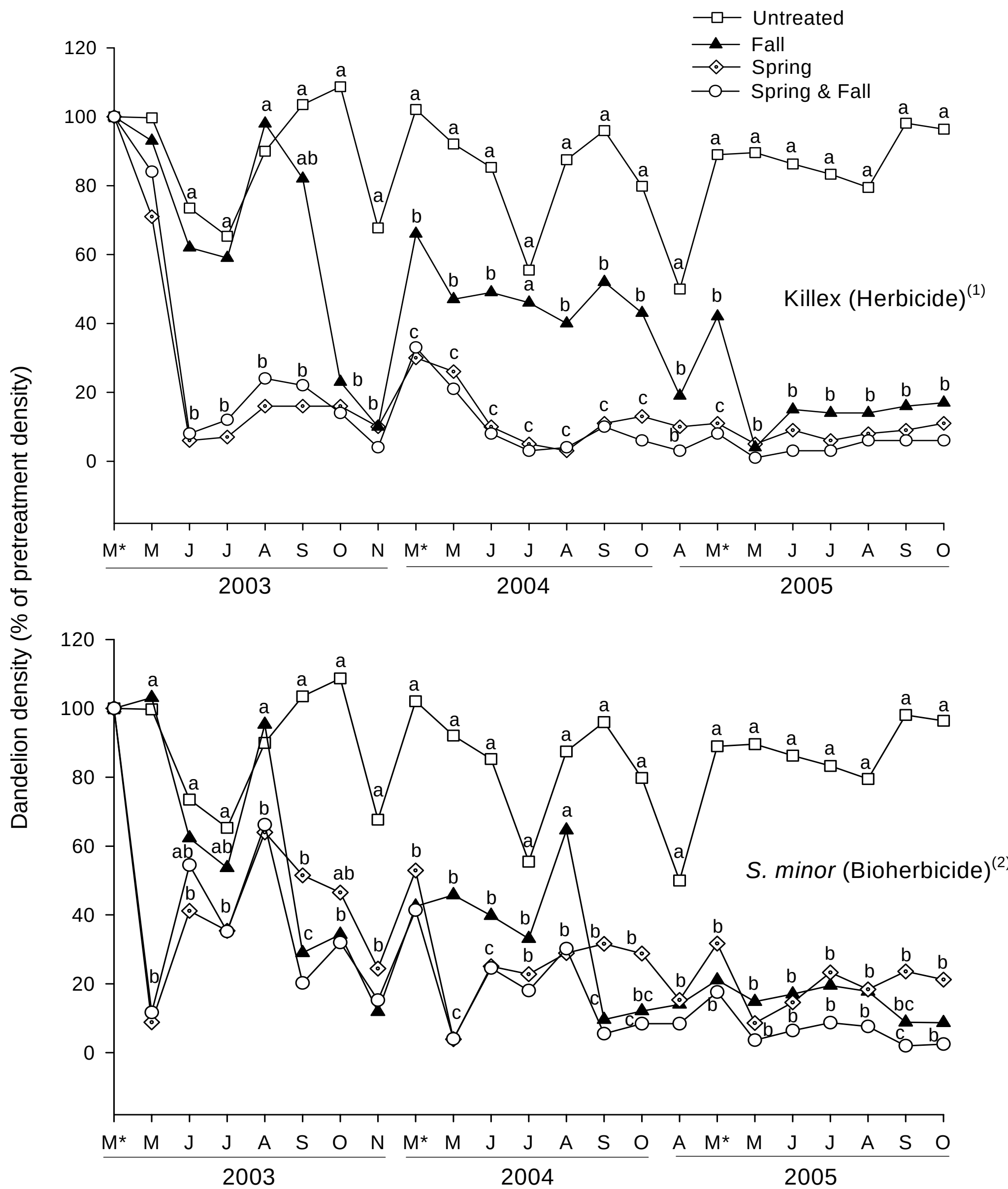


Figure 7.2. Effect of spring versus fall weed control treatments on dandelion population dynamics. (A) spring (B) fall and (C) spring & fall per year. Within each graph, means with a common letter at each time are not significantly different at P = 0.05 according to Tukey's test. Asterisks (*) refer to the pre-treatment assessment in the middle of May.

(1) Killex™ rate = 1.7 kg a.i. ha^{-1}.

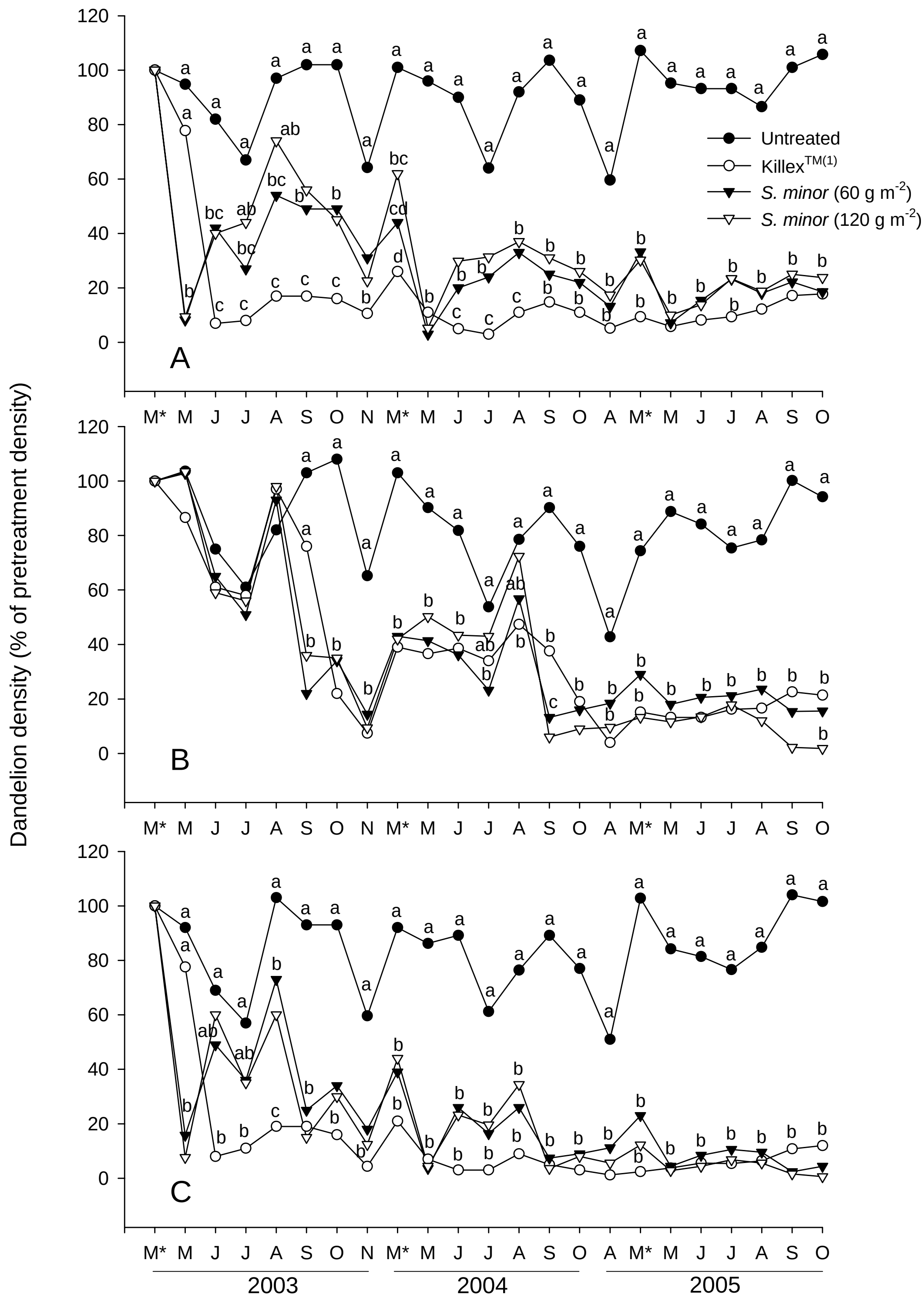

Dandelion density (% of pretreatment density)
Untreated
Killex™(1)
S. minor (60 g m-2)
S. minor (120 g m-2)
A
B
C
M* M J J A S O N M* M J J A S O A M* M J J A S O
2003
2004
2005

Figure 7.3. Regrowth capacities of dandelion roots after spring and fall applications *Sclerotinia minor*. Error bars refer to standard deviations of the means. Values are the means of 12 plot replicates. Bars with common letters are not significantly different at $P = 0.05$ according to Tukey's test.

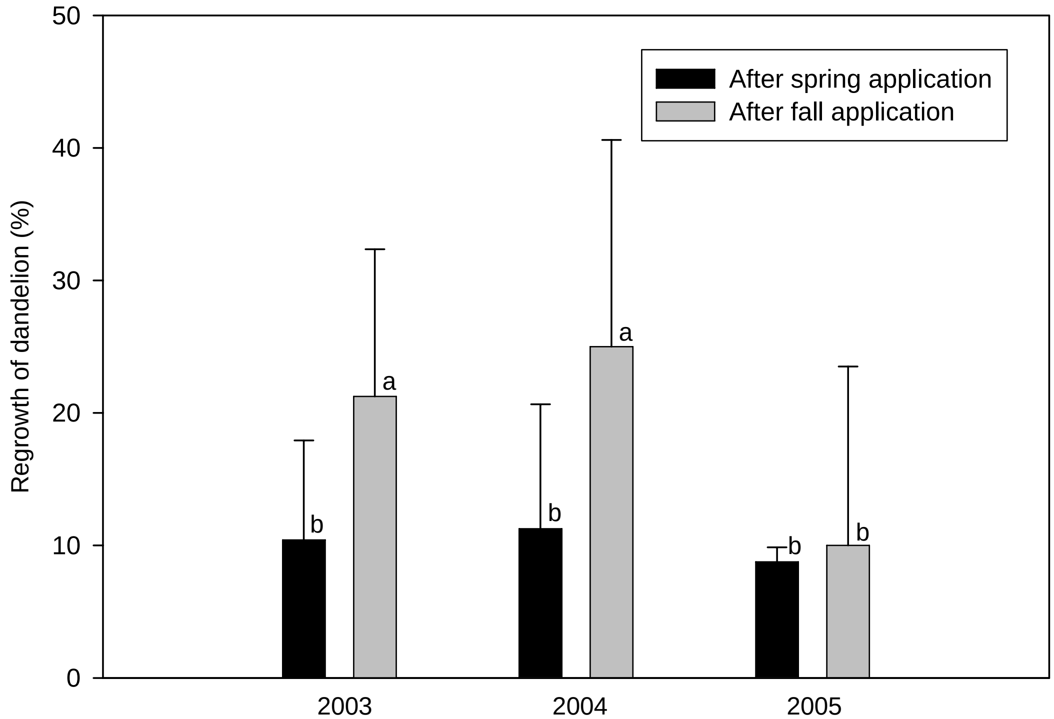


Figure 7.4. Regrowth capacities of dandelion roots according to plot grass quality. Error bars refer to standard deviations of the means. Values are the means of 5 plot replicates. Within a seasonal application time, bars with common letters are not significantly different at P = 0.05 according to Tukey’s test.

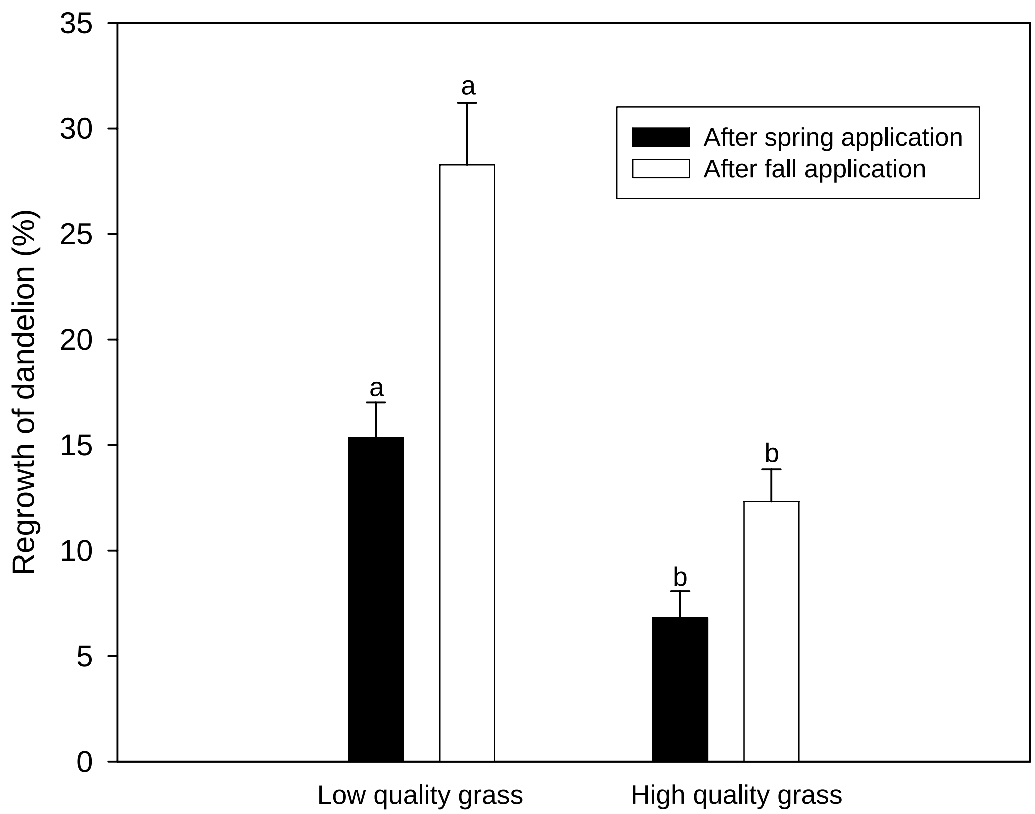
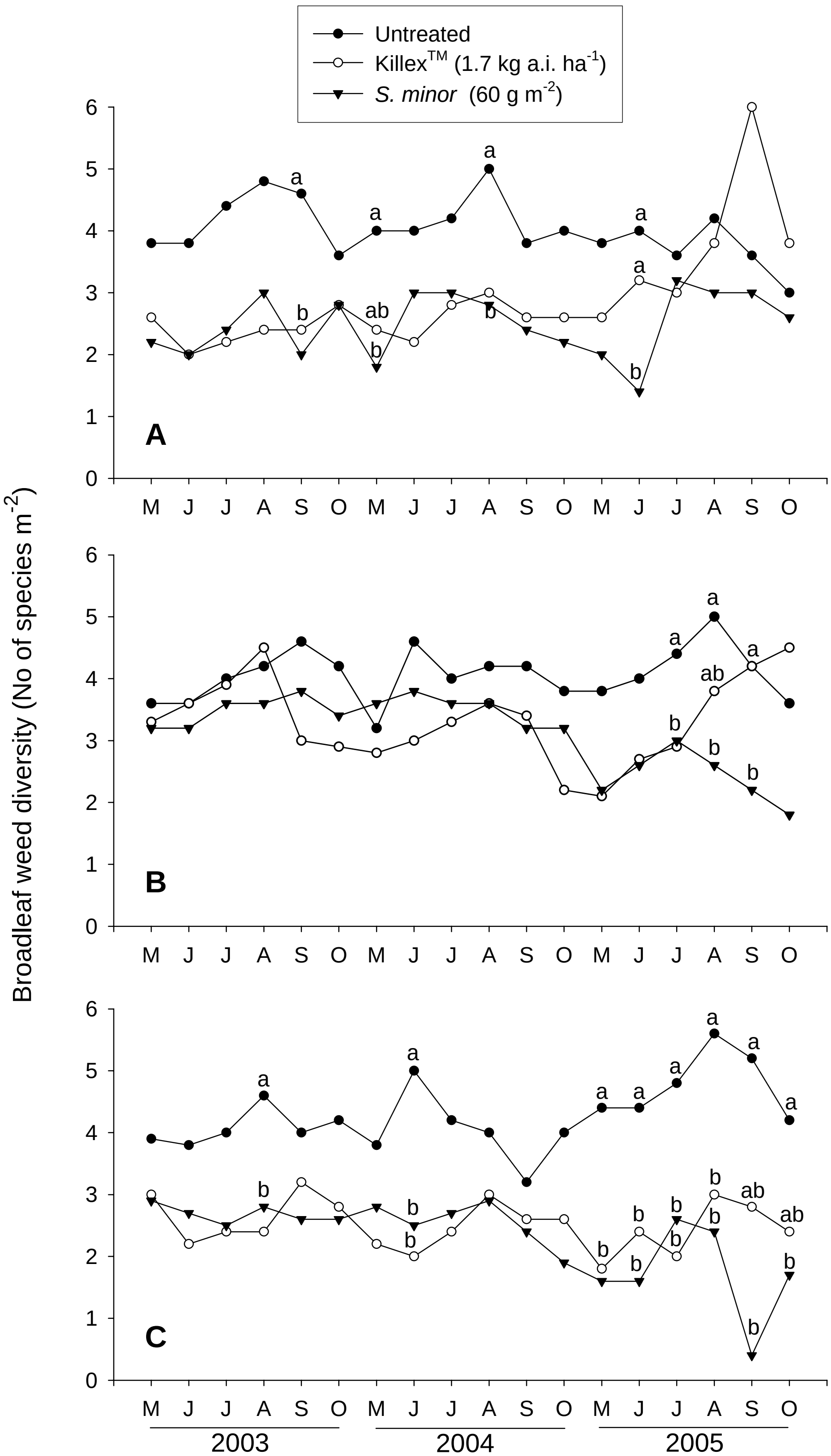


Figure 7.5. Effect of seasonal herbicide and bioherbicide applications on broadleaf weed species diversity. (A) spring, (B) fall and (C) spring & fall application (s).

Within each graph, means with a common letter at each time are not significantly different at $P = 0.05$ according to Tukey's test.



Untreated
Killex™ (1.7 kg a.i. ha-1)
S. minor (60 g m-2)
Broadleaf weed diversity (No of species m-2)
A
B
C
M J J A S O M J J A S O M J J A S O
2003
2004
2005

Figure 7.6. Effect of herbicide and bioherbicide applications on the population dynamics of selected broadleaf species. Within each graph, means with a common letter at each time are not significantly different at $P = 0.05$ according to Tukey's test.

[(1)]density was estimated by measuring how many 10 cm diameter patches of white clover covered the ground of a plot.

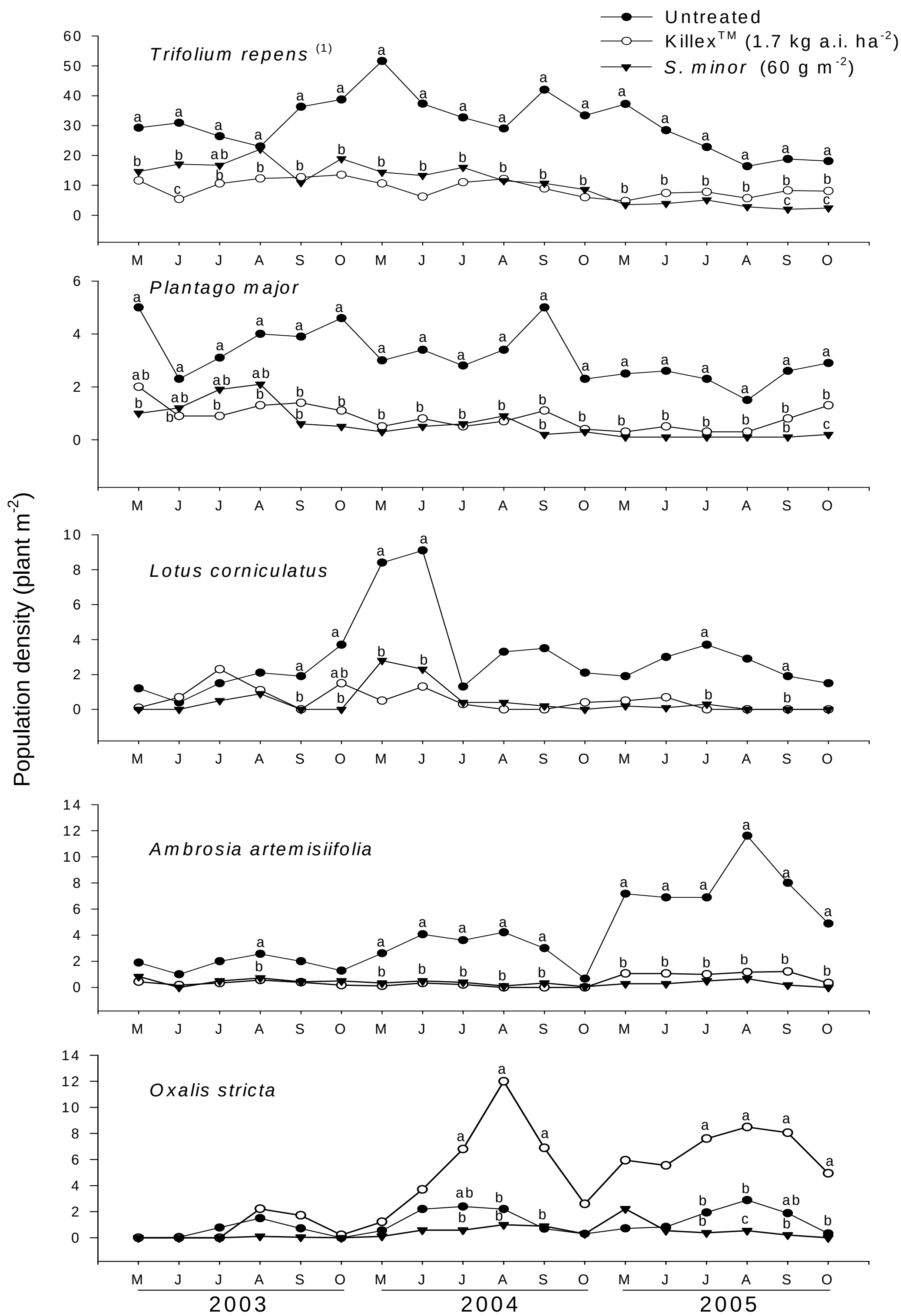
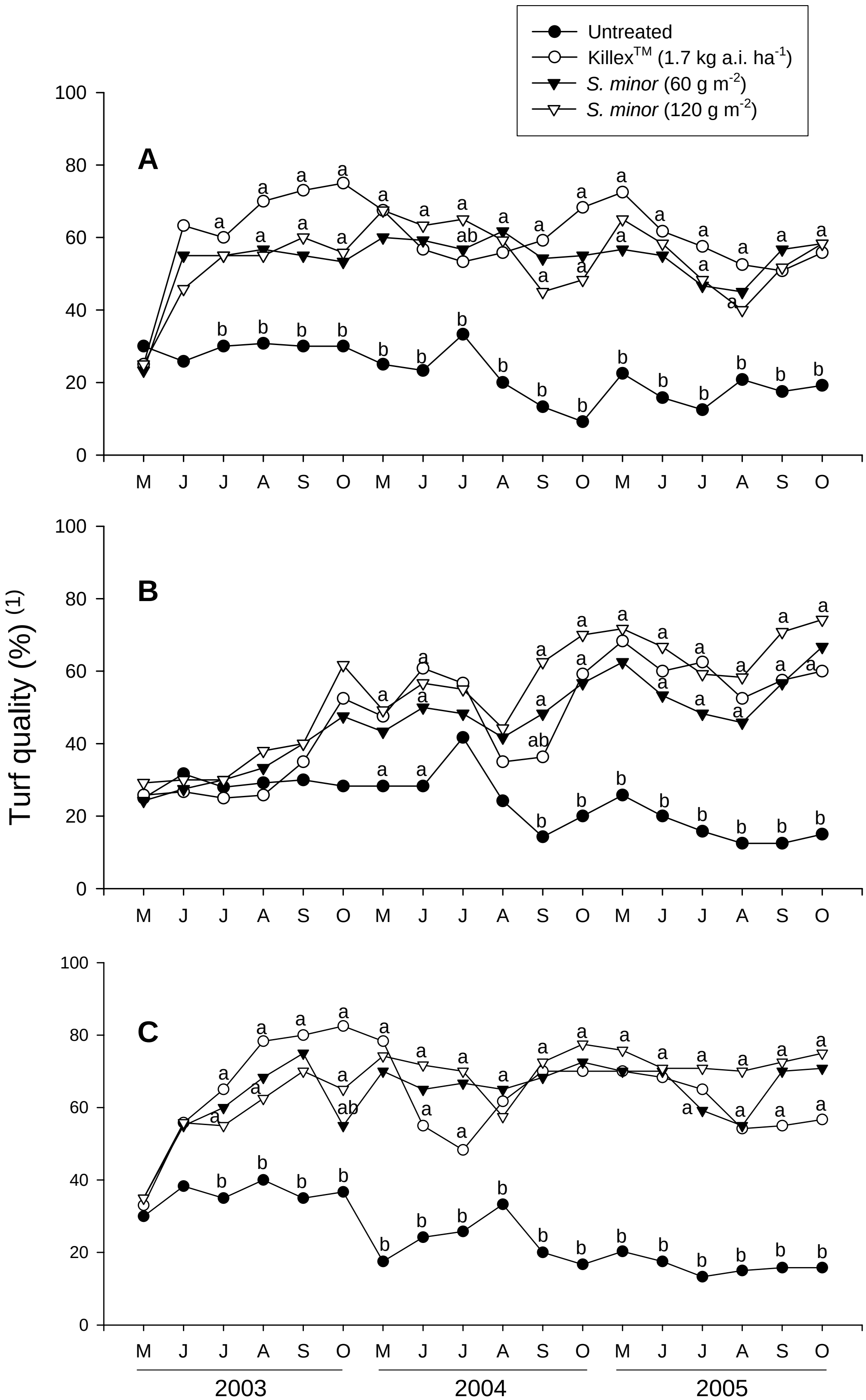

Untreated
Killex™ (1.7 kg a.i. ha-2)
S. minor (60 g m-2)
Trifolium repens (1)
Plantago major
Lotus corniculatus
Ambrosia artemisiifolia
Oxalis stricta
Population density (plant m-2)
M J J A S O M J J A S O M J J A S O
2003
2004
2005

Figure 7.7. Effect of seasonal herbicide and bioherbicide applications on turfgrass quality. (A) spring, (B) fall and (C) spring & fall application (s). Within each graph, means with a common letter at each time are not significantly different at $P = 0.05$ according to Tukey's test.

[(1)]a visual rank scale of 0 to 100% was used with 0% for no grass and 100% for optimum grass quality.



Untreated
Killex™ (1.7 kg a.i. ha⁻¹)
S. minor (60 g m⁻²)
S. minor (120 g m⁻²)
A
B
C
Turf quality (%) (1)
M J J A S O M J J A S O M J J A S O
2003
2004
2005

Figure 7.8. Number of dandelion floral scapes encountered after two years of weed control treatment (H = herbicide; B1 = 60g m^{-2}, and B2 = 120g m^{-2} *S. minor* bioherbicide) and different seasonal applications (S = spring; F = fall, and S & F= spring and fall per year). Average of six replications. Within each year, means followed with the same letter do not significantly different (*P* = 0.05) by Tukey's test.

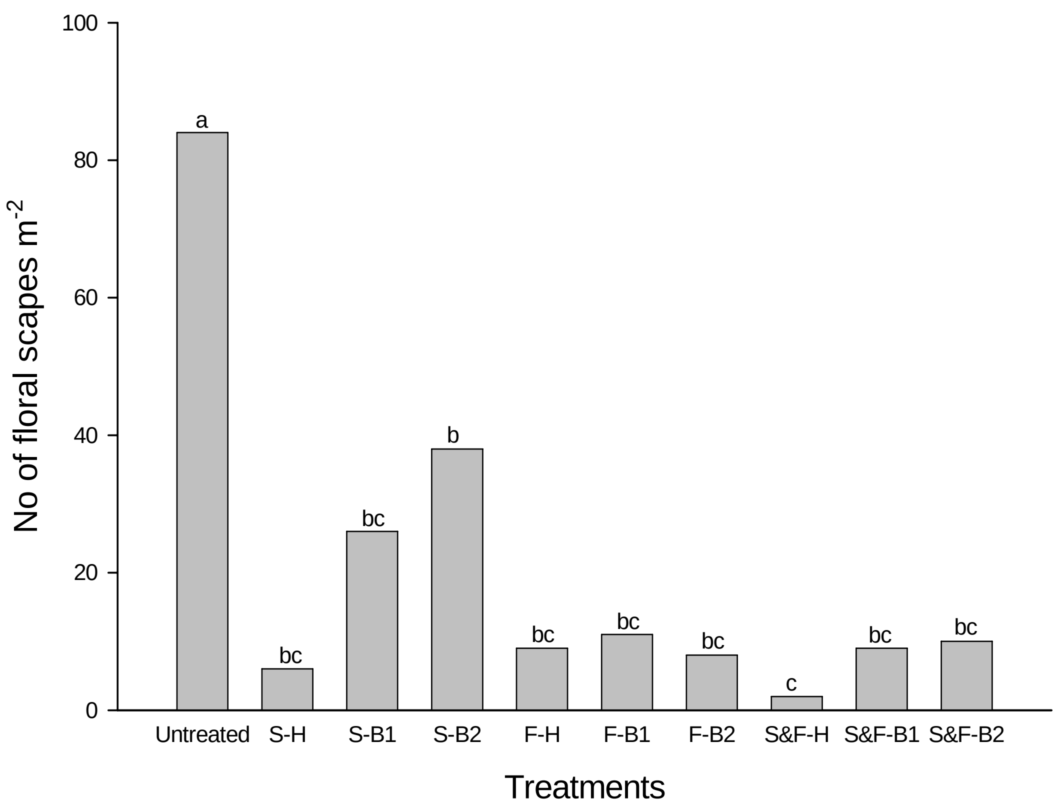
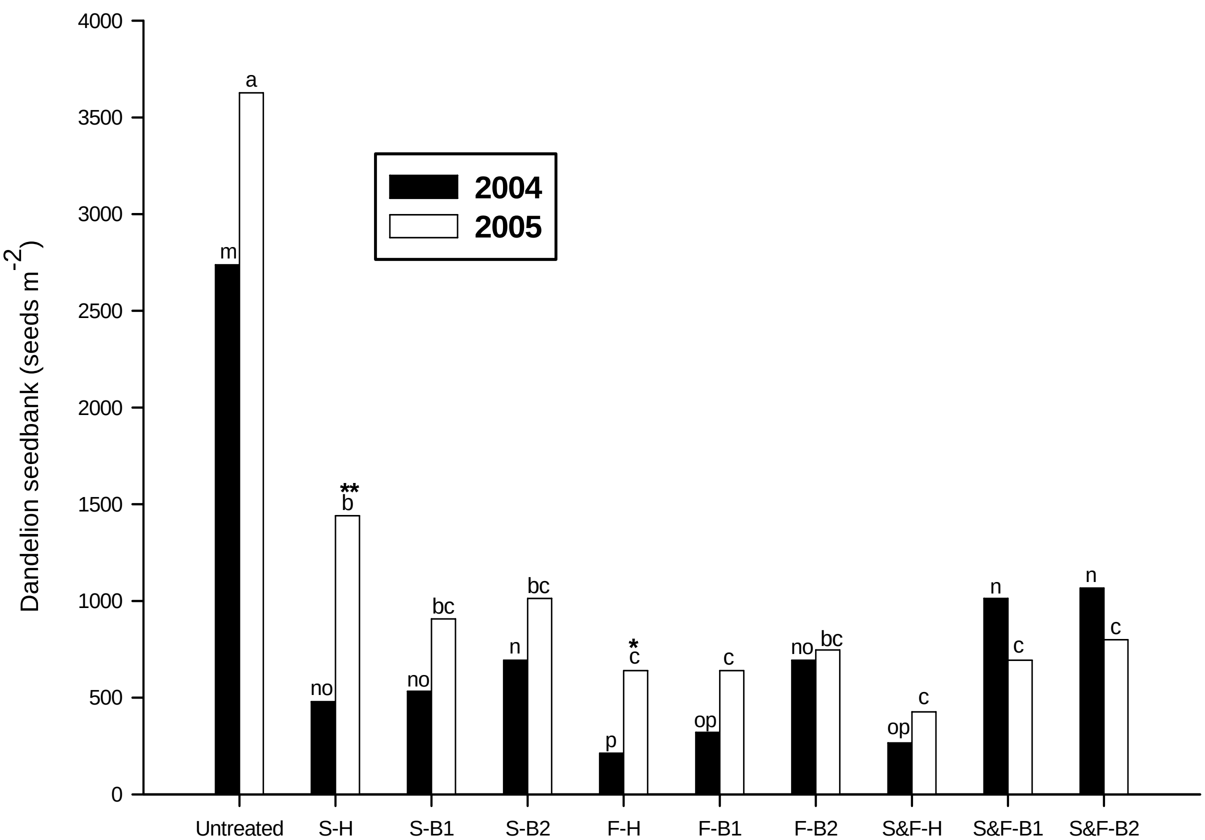


Figure 7.9. Effect of herbicide and bioherbicide applications on the dandelion seed bank in the upper 10 cm soil layer in 2004 and 2005. (S = spring; F = fall and S&F= spring and fall application time) and different weed control treatments (H = herbicide; B1 = 60g m^{-2} and B2 = 120g m^{-2} *S. minor* bioherbicide). Average of six replications. Within each year, means followed with the same letter do not significantly different (P = 0.05) by Tukey's test. Data between 2004 and 2005 are (*) significantly different at P = 0.05 and (**) at P = 0.001.



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