Journal of Applied Ecology 2007
44, 115–124

Grass overseeding and a fungus combine to control *Taraxacum officinale*

MOHAMMED H. ABU-DIEYEH and ALAN K. WATSON
Department of Plant Science, McGill University, 21 111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9

Summary

1. Common dandelion *Taraxacum officinale* is the most abundant and frequent weed within turfgrass in temperate climates. With increasing legislation banning herbicide & non-chemical means of control are needed replace phenoxy herbicides. The weediness of *T. officinale* is mainly the result of high seed production, dispersal and germination potential. A successful long-term weed control strategy should suppress established plants, exert negative effects on seed production and prevent seedling establishment.
2. The potential of *Sclerotinia minor* to cause *T. officinale* seed mortality and reduce seedling emergence without impact on turfgrass species was evaluated in greenhouse and field experiments.
3. A pre-emergence application of *S. minor* at the time of seeding and a post-emergence application at 10 days after seeding significantly reduced *T. officinale* emergence to 17% and 2%, respectively, compared with 70–80% germination in the untreated control. There were no adverse effects of direct *S. minor* contact on turfgrass seed germination, seedling emergence or seedling establishment.
4. Grass overseeding alone did not improve grass quality or reduce *T. officinale* population densities in a low-maintained lawn environment. When *S. minor* was combined with grass overseeding, at application or 10 days after application, a 70–80% reduction of the *T. officinale* population occurred in the first year, increasing to 95% in the following year in the absence of further treatments. Turfgrass appearance and quality significantly and continuously improved up to 80%, compared with 10–20% in the control plots. Densities of other weeds, white clover *Trifolium repens* and field bindweed *Convolvulus arvensis*, were also significantly reduced when *S. minor* was applied with grass overseeding compared with the bioherbicide alone.
5. *Synthesis and applications*. We have demonstrated that *S. minor* reduces seed numbers, seedlings and establishment of *T. officinale* and, when combined with grass overseeding, the grass sward flourishes and weed emergence and colonization are significantly reduced. Other broadleaf weeds are susceptible to *S. minor* and thus this bioherbicide could have utility in no-till maize, cereal grain and grass seed production systems, where producers are searching for non-chemical weed control.

Key-words: biological weed control, common dandelion, competition, overseeding, *Sclerotinia minor*, seedling survivorship, turfgrass

Journal of Applied Ecology (2007) **44**, 115–124
doi: 10.1111/j.1365-2664.2006.01247.x

Introduction

Dandelion *Taraxacum officinale* Weber is a common weed infesting home lawns, pastures, forage crops, roadside verges, golf courses and athletic fields (Stewart-Wade *et al*. 2002a). The species colonizes numerous different habitats, grows in a diversity of soil types (von Hofsten 1954), resists drought (von Hofsten 1954) and adapts to a wide range of light and shade intensities (Longyear 1918). Although *T. officinale* is described as an *r*-strategist, emphasizing seed production and favouring colonization (Gadgil & Solbrig 1972), it is also considered a *k*-strategist under different environmental conditions (Solbrig 1971; Ford 1981).

Correspondence: Alan Watson, Department of Plant Science, McGill University, 21 111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9 (fax +1 514 398 7897; e-mail alan.watson@mcgill.ca).

Colonization of an unoccupied area by a plant species generally relies on reproduction by seeds (Hoya *et al*. 2004). The high seed potential and dispersal abilities of *T. officinale* are major features that lead to its prevalence in turfgrass environments. Variations in *T. officinale* reproductive ecology have been attributed to the size and vigour of the plant (Longyear 1918), intrapopulation biotype variations (Solbrig & Simpson 1974), the habitat of occurrence (Ford 1981) and the density of neighbours (Welham & Setter 1998).

Increasing public concerns about pesticide use in urban environments have prompted research in alternative approaches (Hatcher & Melander 2003; Larsen, Kristoffersen & Fischer 2004). Although cultural management techniques can reduce dependency on chemical herbicides and fertilizers, they do not provide reliable broadleaf weed control (Busey 2003). Many biological weed control programmes have focused on seed-attacking insects (Kremer 2000) and limited research has been placed on the micro-organisms that reduce seed viability and seedling emergence (Kremer 2000; Medd & Campbell 2005). As the natural reproduction and dispersal of *T. officinale* depend exclusively on seeds, a successful bioherbicide should adversely affect seed viability, seedling emergence and/or survival.

Sclerotinia minor Jagger (Ascomycete) (IMI 344141) has been evaluated as a potential bioherbicide for *T. officinale* (Ciotola, Wymore & Watson 1991; Stewart-Wade *et al*. 2002b). Recent greenhouse and field results have shown *S. minor* to significantly reduce population densities as well as the above- and below-ground biomass of *T. officinale* (Abu-Dieyeh & Watson 2006). When combined with regular mowing at *c.* 7 cm, the *S. minor* treatment was as effective as the herbicide Killex™ (The Solaris Group, Mississauga, ON) for broadleaf weed suppression. The potential of *S. minor* to cause seed mortality, reduce the *T. officinale* seed bank and seedling emergence, and to interfere with grass seed germination and establishment have not been examined (Abu-Dieyeh & Watson 2005, 2006).

Weed infestations in turfgrass most probably reflect the low competitive ability of the grass (Larsen, Kristoffersen & Fischer 2004). Therefore minimizing the development of opportunities for weed encroachment is a key component of non-pesticide control (Larsen, Kristoffersen & Fischer 2004). Overseeding grass is a common management practice in turf renovation (Turgeon 1985; Larsen, Kristoffersen & Fischer 2004). Can grass overseeding and consequently grass establishment improve the success of *S. minor* to control *T. officinale* and other broadleaf weeds? We hypothesized that the challenge for *T. officinale* control using *S. minor* is to manipulate grass competition by reducing vegetation gaps and encouraging growth of dense turfgrass as soon as *S. minor* has been applied. Thus, the objectives of this study were to: (i) assess the effect of *S. minor* on *T. officinale* seed viability and seedling emergence; (ii) determine the susceptibility of turfgrass species to *S. minor*; and (iii) determine if overseeding can enhance weed control and turf vigour.

Methods

FUNGUS FORMULATION

Sclerotinia minor (IMI 344141) was isolated from diseased lettuce plants *Lactuca sativa* L. from south-western Quebec, Canda, and the stock culture was maintained as sclerotia at 4 °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 & Watson (2006). The *S. minor* granular formulations were freshly prepared 2 weeks prior to application. Viability and virulence of the fungal inoculum were assessed prior to use on potato dextrose agar (PDA) plates and on excised dandelion leaves.

EFFECT OF *S. MINOR* ON THE EMERGENCE OF *T. OFFICINALE* AND A TURFGRASS COMMERCIAL SEED MIXTURE

Fruiting heads of individual *T. officinale* plants were collected in spring 2003 from naturalized plants in lawns on the Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada, and stored at 4 °C until use. The grass seeds used in this experiment were from a commercial grass seed mixture (30% Kentucky bluegrass *Poa pratensis* L., 40% creeping red fescue *Festuca rubra* L. var. *rubra s.l.* and turf type perennial ryegrass 30% *Lolium perenne* L.; CIL® Golfgreen™, Brantford, Canada).

Potting trays (45 × 25 × 8 cm) were filled with a mixture of 2/3 pasteurized black soil and 1/3 pro-mix (Premier Promix, Premier Horticulture Ltee, Riviere-du-Loup, Canada) and divided into two equal parts (20 × 25 cm = 0·05 m^2), each representing an experimental unit, one for the treatment with the barley-based formulation of *S. minor* and the other as the untreated control. A separation zone of 5 × 25 cm was left bare for all the treatments.

The experiment was arranged in a completely randomized design, with four replications and two factors. The first factor was seeds sown at three levels, dandelion seeds alone (25 seeds per treatment area), grass seeds alone (1 g treatment $area^{-1}$), and a mixture of 25 dandelion seeds plus 1 g grass seeds treatment $area^{-1}$. The second factor was time of *S. minor* application, with two levels: at sowing or 10 days after sowing (after emergence of both dandelion and grass seedlings). The *S. minor* colonized granules (1·4–2·0 mm diameter) were applied at 3 g treatment $area^{-1}$ (equal to 60 g m^{-2}) on the surface of the pre-moistened soil. For 3 days after *S. minor* application, the trays were covered with clear plastic covers to maintain a moist soil surface. The trays were placed in a greenhouse at 24 ± 2 °C with 15 h of light/day at a photon flux density minimum of 350 ± 50 µmol m^{-2} s^{-1}. The trays were checked daily and misted with water whenever needed. The experiment was conducted in March 2005 and repeated in May 2005.

The ability of *S. minor* to colonize *T. officinale* and turfgrass seeds was determined daily. The numbers of emerged *T. officinale* seedlings and survived seedlings were recorded daily for 4 weeks and the total emergence was calculated as a percentage of the total number of seeds sown ($n = 25$). Treatment effects on grass emergence were recorded each week for 4 weeks as a percentage value in relation to the untreated control in the same tray. Any growth retardation, disease symptoms and damage on the grass species was recorded.

Data for each parameter from the two experimental trials were subjected to the Bartlett test for the homogeneity of variances (SAS Institute Inc., Cary, NC). Data for all measured parameters were homogeneous, thus the two experiments were pooled and analysed as one with eight replications. The main effects of *S. minor* treatments and grass planting on the emergence of *T. officinale* were determined separately using the log-analysis of the categorical modelling procedure (CATMOD; SAS Institute Inc.).

EFFECT OF *S. MINOR* ON GERMINATION POTENTIAL AND EMERGENCE OF FIVE TURFGRASS SPECIES

To determine the effect of *S. minor* on individual grass species, five common cool-season temperate turfgrass species, *Poa pratensis*, *F. rubra* var. *rubra*, *L. perenne*, *Festuca rubra* L. ssp. *commutata* and *Agrostis palustris* Huds seeds, were sown (0·1 g pot^{-1}) into a pre-sterilized potting mix consisting of 2/3 sand and 1/3 vermiculite in 5 × 5 × 8-cm pots (area = 0·0025 m^2). The seeding rate was equal to 40 g m^{-2}, which simulated the commercial seeding rate (30–40 g m^{-2}). The experiment was conducted between June and August 2005 and treatments were arranged in a completely randomized design with five treatment combinations: (i) untreated control; (ii) and (iii) 0·2 g pot^{-1} of autoclaved non-colonized barley, applied either at the time of or 10 days after sowing; (iv) and (v) 0·2 g pot^{-1} of *S. minor*-colonized barley (at a rate equal to 80 g per m^2, which was twice the recommended application rate), applied either at the time of or 10 days after sowing. There were six replicate pots of each treatment combination.

Plants were grown in a greenhouse at 20 ± 2 °C with 15 h of light per day at a minimum photon flux density of 350 ± 50 µmol m^{-2} s^{-1}. The pots were placed in trays (40 × 30 × 8 cm) and 1 cm of water was maintained in the trays. Mist water was applied for 5 min prior to treatment application and repeated daily until the end of the experiment. The pots were covered with clear plastic for 3 days after inoculum application, to maintain the moist conditions necessary for fungal growth. The experiment was repeated under similar conditions except that the soil substrate was changed to an autoclaved (40 min at 121 °C) 1 : 1 mixture of black soil and sand.

Treatment effects were recorded weekly for a period of 4 weeks as a percentage of the untreated controls. Any disease symptoms or damage on the grass species were also recorded. Four weeks after the first treatment application, the entire plants were removed and soil residues adhering to the roots carefully washed. The total plant biomass of each pot was bulked, placed in paper bags, oven dried at 80 °C for 72 h, and then weighed. The main treatment effects on total biomass were determined separately for each grass species using ANOVA and the means were separated using Tukey's test at $P = 0{\cdot}05$. Turfgrass quality data were subjected to Kruskal–Wallis one-way ANOVA on ranks and the means were tested for significance vs. the 100% value assigned for untreated control at $P = 0{\cdot}05$ using Dunnett's method. All analyses were undertaken in SAS (SAS Institute Inc.).

FIELD STUDY: COMBINING *S. MINOR* WITH GRASS OVERSEEDING

Two field studies were conducted during 2004 and 2005. The first study was initiated in May 2004 (spring trial) and continued until the end of September 2005, while the second study was started in September 2004 (autumn trial) and continued until September 2005. Both trials were adjacent to each other on a lawn on the Macdonald Campus of McGill University (45°25′N latitude, 73°55′W longitude, 39·00 m a.s.l.). The soil was a loamy sand (9% coarse sand, 80% fine sand, 5% silt, 6% clay), with a pH of 6·7 and 7% organic matter. The lawn received low-maintenance management throughout its history except for regular mowing during the growing season (May–October). Grass groundcover was 40–60%, with approximately 95% *P. pratensis* and 5% *L. perenne*. Broadleaf weed diversity was low, as only five broadleaf weeds were observed, with *T. officinale* the dominant weed species (60–80 dandelion plants m^{-2}). White clover *Trifolium repens* L. was the second most prevalent weed species, followed by field bindweed *Convolvulus arvensis* L.

A randomized complete block design with three replications was used in both field experiments. The treatments were: (i) granular formulation of *S. minor* at 40 g m^{-2}; (ii) grass overseeding at 15 g m^{-2}; (iii) granular formulation of *S. minor* (40 g m^{-2}) combined with grass overseeding (15 g m^{-2}) on the same day; (iv) granular formulation of *S. minor* (40 g m^{-2}) combined with grass overseeding (15 g m^{-2}) at 10 days after *S. minor* application; (v) granular formulation of *S. minor* (40 g m^{-2}) combined with grass overseeding (15 g m^{-2}) at 20 days after *S. minor* application; and (vi) untreated and not oversown control. All treatments were applied twice, in the spring and autumn of 2004 for the spring trial, and in autumn 2004 and spring 2005 for the autumn trial.

The *S. minor* granular formulation was broadcast applied using a 200-mL plastic bottle fitted with a perforated lid (*c.* ~10 mm diameter) with suitable openings to pass the granules. If there was no rainfall on the day of application or the grass was not wet, the entire field was sprinkler irrigated for 2 h prior to late afternoon

treatment applications. The commercial grass seed mixture described above was used in this experiment. The grass seeds were manually spread over the plot area, wherein bare ground and gaps within the grass canopy received more seeds. Then the area was manually rolled over to ensure proper incorporation of seeds in the soil surface. If there was no rainfall, the whole field was sprinkler irrigated for 2 h daily for up to 2 weeks after sowing and then watered whenever needed.

The experimental unit (plot) was 1·0 m^2 with 0·6-m alleys between any two plots. The distance between any two blocks was 2–3 m. Plots were permanently marked to maintain plot integrity for the duration of the study. The entire field was mowed regularly at 7–10 cm 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 were removed during the 6 weeks post-fungal treatment periods to prevent possible cross-contamination between plots.

Pre-application data were collected 1 or 2 days prior to the spring or autumn applications in the middle of May and September, respectively. Post-application data were collected in the last week of each month. The data included the number of individuals of each broadleaf weed species per plot, an estimate of broadleaf ground cover and an estimate of the turfgrass quality. *Trifolium repens* density was estimated by measuring how many 10-cm diameter patches of *Trifolium repens* covered the surface of a plot. Turfgrass quality was rated visually based on a combination of colour, density and uniformity of turf on a 1–100 scale, where 1 represents no growth or brown and dry, dead turf, and 100 represents completely green uniform turf (Wiecko 2000). A rating of 50% indicates minimum acceptable turf quality for the studied low-maintained fields.

Data from the two field trials could not be combined because of seasonal and application timing variations. However, adjusting the data using the before–after, control–impact (BACI) equation (Green 1979) led to similar trend effects and so only the spring trial results are presented.

$$\text{BACI value} = (At/Bt)/(Ac/Bc) \times 100 \qquad \text{eqn 1}$$

where B is the *T. officinale* density at the time of treatment (before the impact), A is the *T. officinale* density after treatment (after the impact), t is the treatment and c is the control. The control used for comparison was the untreated plot in the same block.

Normality for each parameter was tested on model residuals using the Shapiro–Wilk test. The 2-year population density and turfgrass quality data of the spring study were analysed using the GLM procedure of repeated measures to determine the significant interactions among different treatments with the time factor. The average population density data of *Trifolium repens* and *C. arvensis* were transformed to $[\log(x + 1)]$ then analysed using the two-way ANOVA for a complete block design by considering treatment combinations as one factor and the year as a second factor. Differences in treatment means for all analyses were determined using Tukey's test at $P = 0{\cdot}05$. All statistical analyses were conducted using the SAS statistical package (SAS Institute Inc.).

Results

EFFECT OF *S. MINOR* ON THE EMERGENCE OF *T. OFFICINALE* AND A TURFGRASS COMMERCIAL SEED MIXTURE

Dandelion seeds and seedlings were highly susceptible to *S. minor* and completely colonized (*c.* 100%) by the fungus if in direct contact with the mycelia emerging from the granules. The pre-emergence application of *S. minor* (at sowing) significantly ($P = 0{\cdot}01$) diminished emergence of *T. officinale*, from 70–80% total germination in the control to 17% in the treated areas. Moreover, the post-emergence application (10 days after sowing) reduced *T. officinale* seedling survival to 2% ($P < 0{\cdot}0001$) (Fig. 1). In a mixture of *T. officinale* and grass seeds without the fungus, a 10% non-significant ($P = 0{\cdot}915$) decrease in emergence was observed compared with *T. officinale* alone (Fig. 1). Regardless of the grass factor, *T. officinale* emergence through time was significantly affected by the timing of the *S. minor* application. Application at sowing reduced emergence to 7·5% 1 week after application, but emergence gradually increased and reached 16·5% 3 weeks after application. This emergence escape when *S. minor* was applied at sowing was significantly higher than the treatment at 10 days after sowing, which remained at only 2% emergence (Fig. 1).

When *S. minor* was applied at sowing, grass germination and seedling establishment were temporarily subdued from days 7 to 10. However, by the third and

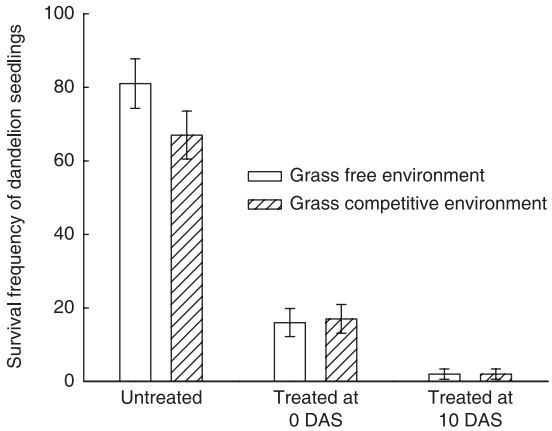

Fig. 1. Effect of *Sclerotinia minor* application (a rate equal to 60 g m^{-2}) on seedling survival frequency of *Taraxacum officinale*. Values are means of eight replications, vertical lines represent standard errors. DAS, days after sowing. Log-linear analysis; the maximum likelihood analysis of variance for inoculum treatment, $\chi^2 = 104{\cdot}63$, $P < 0{\cdot}0001$; grass factor, $\chi^2 = 0{\cdot}01$, $P = 0{\cdot}915$; inoculum × grass, $\chi^2 = 0{\cdot}39$, $P = 0{\cdot}822$.

Table 1. Effect of treatments on grass establishment estimated as a percentage in comparison with untreated control (100%). +, positive effect; –, negative effect; 0, no change compared with untreated control. Kruskal–Wallis one-way ANOVA on ranks was applied. Within each row, mean values with * are significant vs. the 100% values assigned for untreated control at $P = 0{\cdot}05$ according to Dunnett's method. Average of six replications.

		Treatment†			
	Weeks post-application	*S. minor* barley grits		Non-colonized barley	
		0 DAS‡	10 DAS	0 DAS	10 DAS
Creeping bentgrass	1	–32*	0	–10	0
Agrostis palustris	2	0	0	–3	–5
	3	0	–3	0	–2
	4	+13	+17*	+4	0
Chewing's fescue	1	–35*	0	–10	0
Festuca rubra	2	0	0	–3	–7
ssp. *commutata*	3	0	0	–1	–3
	4	+15*	+18*	+2	0
Kentucky bluegrass	1	–31*	0	–10*	0
Poa pratensis	2	0	0	–3	–5
	3	0	0	–3	–2
	4	+13*	+22*	+2	0
Perennial ryegrass	1	–26*	0	–12	0
Lolium perenne	2	0	0	–5	–5
	3	0	+5	0	–2
	4	+10*	+18*	+1	0
Creeping red fescue	1	–25	0	–10	0
Festuca rubra	2	0	0	–5	–4
ssp. *rubra*	3	0	+5	–3	–4
	4	+10*	+16*	0	0

†A rate equal to 80 g m^{-2}.
‡Days after sowing.

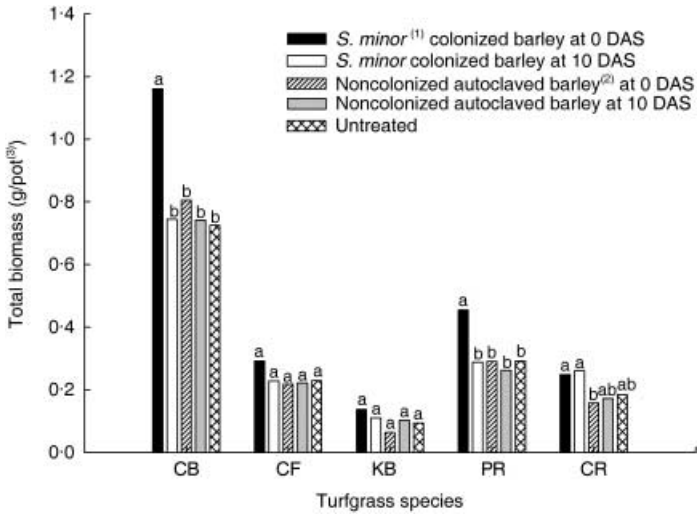

Fig. 2. Effect of *Sclerotinia minor* and inoculation time on total dry matter biomass of five turfgrass species. Average of six replications. DAS, days after sowing; CB, creeping bentgrass ($F_{4,29} = 14{\cdot}8$, $P < 0{\cdot}001$); CF, chewing's fescue ($F_{4,29} = 2{\cdot}1$, $P = 0{\cdot}119$); KB, Kentucky bluegrass ($F_{4,29} = 2{\cdot}1$, $P = 0{\cdot}113$); PR, perennial ryegrass ($F_{4,29} = 5{\cdot}9$, $P = 0{\cdot}002$); CR, creeping red fescue ($F_{4,29} = 4{\cdot}7$, $P = 0{\cdot}006$). Within the same grass species, bars with similar letters are not significantly different at $P = 0{\cdot}05$ according to Tukey's test. (1) *S. minor* application rate was 80 g m^{-2}. (2) Autoclaved non-colonized barley application rate was 80 g m^{-2}. (3) Each pot had an area of 0·0025 m^2 and was sown with 0·1 g grass seeds.

fourth week of the experiment there were no differences in grass quantity and quality among the treatments. Fungal growth was rarely (*c.* 10%) observed on the grass seeds and colonization of grass seeds was very rare (less than 5%). Applying *S. minor* 10 days after sowing had no adverse effect on grass seedlings. Although turfgrass quality was not assessed in this experiment, 1 month after grass sowing treated grass generally had healthy and better visual appearance and vigour than the untreated control (data not presented). These observations were quantitatively verified by the results of the following experiment.

EFFECT OF *S. MINOR* ON THE GERMINATION, EMERGENCE AND ESTABLISHMENT OF FIVE TURFGRASS SPECIES

One week after application, the vigour of turfgrass seedling emergence was slightly reduced by the *S. minor* treatment, but by 4 weeks after application superior grass ratings were recorded for the *S. minor* treatments compared with the untreated and autoclaved non-colonized barley treatments (Table 1). There were no signs of damage on the grass species as a result of *S. minor* except when applied 10 days after sowing on *A. palustris*. The damage was observed in a few areas where spreading mycelia from clumps of granules formed a thick mat preventing normal growth of the slender *A. palustris* seedlings. Damage was temporary, as ultimately the total biomass of *L. perenne* and *A. palustris* was significantly increased when *S. minor* granules were applied at sowing compared with the control treatments (Fig. 2). In the repeat of this experiment, the soil substrate was changed to more accurately simulate field conditions and there were no significant treatment effects on total biomass of the five grass species.

COMBINING GRASS OVERSEEDING WITH *S. MINOR* APPLICATION

Data from the two field studies, spring and autumn trials, showed similar trends in dandelion population densities, hence only spring trial data are presented (Fig. 3). The effectiveness of combining *S. minor* with grass overseeding to suppress the *T. officinale* population over time was highly significant (Table 2 and Fig. 3). Grass overseeding alone had no suppressive effect on *T. officinale* densities. After the first application, *S. minor* alone, or with grass overseeding, significantly decreased the *T. officinale* population compared with the control.

Seeding at 0 or 10 days after application of *S. minor* resulted in suppression of the *T. officinale* population down to 2–6% of its original size compared with 13–17%, 33–39% and 65–95% for *S. minor* plus seeding at 20 days after application, *S. minor* alone and seeding alone, respectively (Fig. 3). In the second year and without additional seeding or *S. minor* treatments,

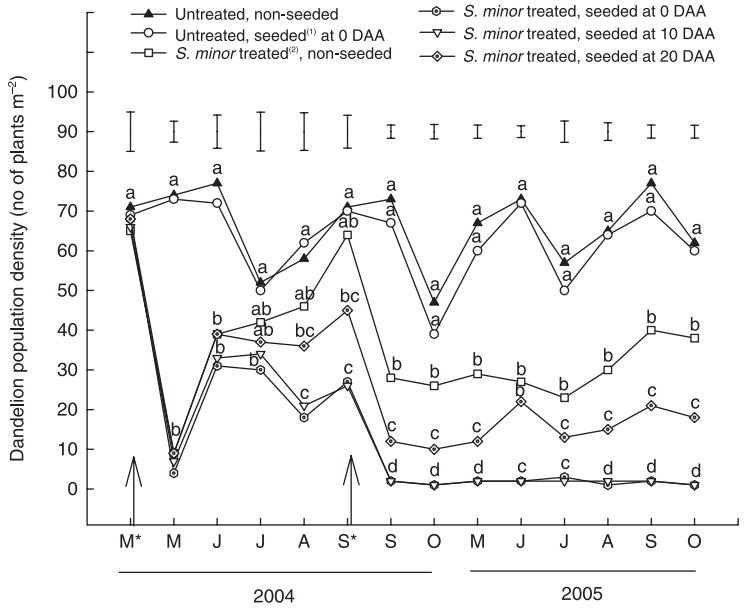

Fig. 3. Effect of combining grass overseeding with *Sclerotinia minor* on *Taraxacum officinale* population dynamics in turfgrass. DAA, number of days after fungus application. Vertical lines represent one standard error value for each time. Arrows indicate dates of *S. minor* application. Asterisks refer to the assessment conducted at the middle of the indicated month. Within each time assessment, means with a common letter are not significantly different at $P = 0{\cdot}05$ according to Tukey's test. (1) A commercial grass mixture was used to over seed plots at 15 g m^{-2}. (2) The *S. minor* treatments were applied at 40 g m^{-2}.

T. officinale was reduced to 2–5 plants m^{-2} over the entire season in plots seeded at 0 or 10 days after *S. minor* application (Fig. 3). Overseeding at 20 days after *S. minor* application had significantly less effect on *T. officinale* than overseeding at 0 or 10 days after *S. minor* application. While the previous year's treatment of *S. minor* reduced the *T. officinale* population by approximately 50%, in the second year seeding at 0 or 10 days after *S. minor* application reduced the population to approximately 5% of its original density (Fig. 3).

Gradual and progressive improvement of the turf was observed in all grass overseeding treatments, with significantly higher values in plots seeded at 0 and 10 days after *S. minor* application than those seeded at 20 days after *S. minor* application (Table 2 and Fig. 4). Sowing grass seed without the fungal treatment had higher, but non-significant, turf quality than the untreated, non-seeded control. Turf quality was improved by *S. minor* treatment alone, with significantly higher values by September of the first year and from June to September of the second year (Fig. 4).

The density of *Trifolium repens* was significantly reduced in the first year under all *S. minor* and seeding treatments. In the second year, the density declined in all plots, even in those untreated, but the greatest reduction occurred in the plots seeded at 0 and 10 days after *S. minor* application (Fig. 5). *Convolvulus arvensis* population densities were not affected in the first year, but in the second year densities were significantly reduced in the plots overseeded at 0 and 10 days after *S. minor* application. Bindweed density significantly increased in the control, the overseeded without *S. minor* and the *S. minor* alone treatments in the second year compared with the first year (Fig. 5).

Discussion

A successful bioherbicide for perennial weeds should not only target plant vigour and density but also attack seeds and reduce seedling establishment (Kremer 2000; Medd & Campbell 2005). Our earlier results from greenhouse and field experiments demonstrated the considerable effectiveness of *S. minor* in reducing above- and below-ground biomass of potted *T. officinale* and also reducing *T. officinale* population densities in field environments (Abu-Dieyeh & Watson 2006). Four weeks post-application, the onset of symptoms was more rapid and the foliar and root biomass reduction was greater for the bioherbicide than the herbicide Killex™.

Innate reproduction and dispersal of *T. officinale* depends exclusively on seeds and *T. officinale* is described basically as an *r*-strategist, emphasizing seed production and favouring colonization of new habitats (Gadgil & Solbrig 1972; Bostock & Benton 1979). Although

Table 2. *F*-values$_{(\text{degree of freedom})}$ and significance probabilities from repeated-measures ANOVA for the influence of combining grass overseeding and *Sclerotinia minor* on *Taraxacum officinale* population density and turfgrass visual quality over the course of 2 years in low-maintained turfgrass lawn

	T. officinale density				Turfgrass quality			
			Modified ANOVA†				Modified ANOVA	
	F-values	ANOVA*	G-G	H-F	*F*-values	ANOVA	G-G	H-F
Treatment	$296{\cdot}32_{(5,12)}$	< 0·0001			$275{\cdot}4_{(5,12)}$	< 0·0001		
Time	$140{\cdot}89_{(14,168)}$	< 0·0001	< 0·0001	< 0·0001	$75{\cdot}65_{(11,132)}$	< 0·0001	< 0·0001	< 0·0001
Treatment × time	$15{\cdot}75_{(70,168)}$	< 0·0001	< 0·0001	< 0·0001	$10{\cdot}96_{(55,132)}$	< 0·0001	< 0·0001	< 0·0001
Epsilon			0·311	0·714			0·377	0·842

*ANOVA = univariate analysis for testing between-subject effects (combinations of grass overseeding and *S. minor* treatments).
†The within-subject effects (monthly data and treatment monthly data interaction) tested using Greenhouse & Geisser's (G-G) and Huynh & Feldt's (H-F) adjusted values (SAS Institute Inc.).

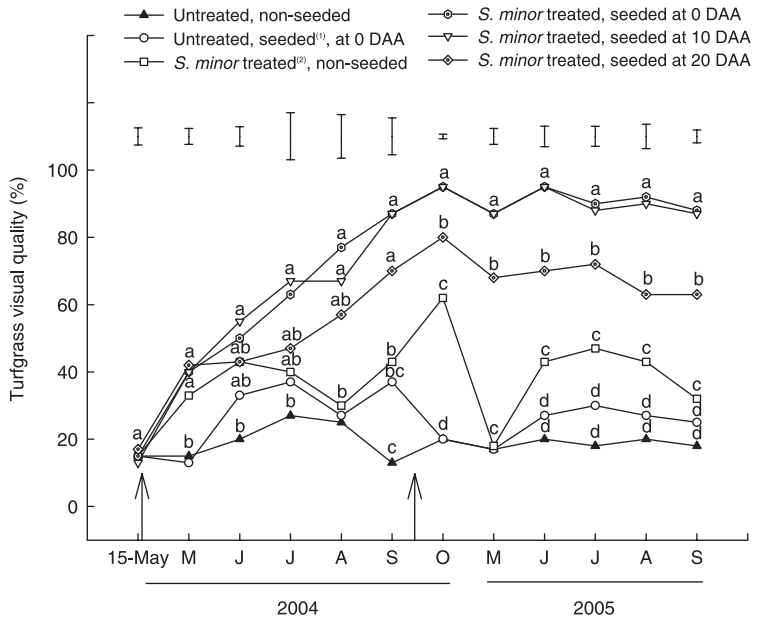

Fig. 4. The effects of combining grass overseeding with *Sclerotinia minor* on turfgrass visual quality. DAA, number of days after fungus application. Vertical lines represent one standard error value for each time. Arrows indicate dates of *S. minor* application. Within each time assessment, means with a common letter are not significantly different at $P = 0{\cdot}05$ according to Tukey's test. (1) A commercial grass mixture was used to over seed plots at 15 g m^{-2}. (2) The *S. minor* treatments were applied at 40 g m^{-2}.

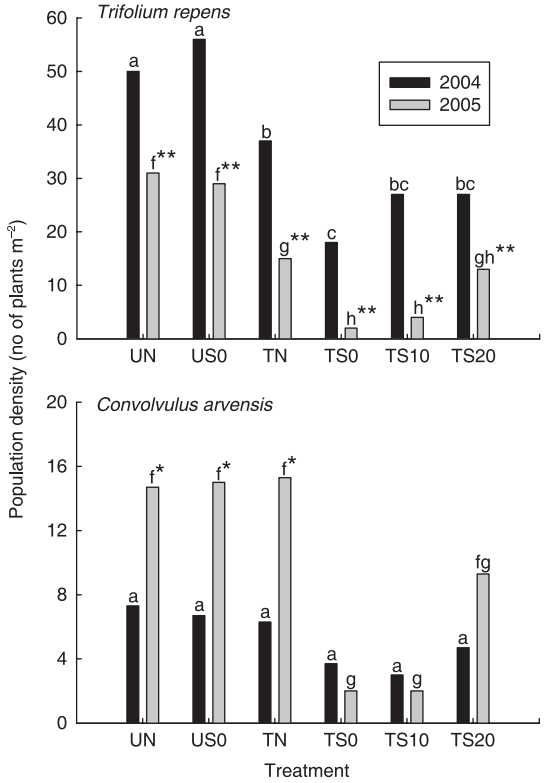

Fig. 5. Effect of combining grass overseeding with *Sclerotinia minor* application on white clover *Trifolium repens* and field bindweed *Convolvulus arvensis* densities. UN, untreated and non-seeded; US0, untreated and seeded; TN, *S. minor*-treated and non-seeded; TS0, *S. minor*-treated and seeded on day of the *S. minor* application; TS10, *S. minor*-treated and seeded 10 days after *S. minor* application; TS20, *S. minor*-treated and seeded at 20 days after *S. minor* application. For *Trifolium repens*, $F_{5,35}$ (treatment) = 70·6, $P < 0{\cdot}0001$; $F_{1,35}$ (year) = 243, $P < 0{\cdot}0001$; $F_{5,35}$ (treatment × year) = 2·4, $P = 0{\cdot}1$). For *Convolvulus arvensis*, $F_{5,35}$ (treatment) = 17·7, $P < 0{\cdot}0001$; $F_{1,35}$ (year) = 32·2, $P < 0{\cdot}0001$; $F_{5,35}$ (treatment × year) = 6·0, $P < 0{\cdot}005$). Within the same graph, bars with similar letters are not significantly different at $P = 0{\cdot}05$ according to Tukey's test. Significant difference at $P = 0{\cdot}05$ (*) and at $P = 0{\cdot}01$ (**) between the 2 years. Seeding treatment was applied once in spring 2004 using a commercial grass mixture at 15 g m^{-2}. *Sclerotinia minor* treatment was applied twice (spring and autumn of 2004) at 40 g m^{-2}.

differences in the reproductive effort are reported in the literature (Stewart-Wade *et al*. 2002a), 20 000 mature seeds plant^{-1} season^{-1} (Dunn & Moyer 1999) with a general germination capacity of 80–90% (Falkowski, Kukulka & Kozlowski 1989) are major contributing factors to the weediness and invasiveness of this species. Therefore, prospective control strategies for *T. officinale* should consider seed production, dispersal, germination, establishment and seed bank deposition. Indeed, preventing seedlings from developing into perennial plants would be central to a long-term control strategy.

When applied on a flowering population, *S. minor* advanced fruiting and caused an approximately 50% reduction in the germination potential of pre-dispersed *T. officinale* seeds (Abu-Dieyeh, Bernier & Watson 2005). The reduction was not the result of fungal infection of seeds but biotic stress. The present study has demonstrated the high degree of susceptibility of *T. officinale* seeds to *S. minor*. When the fungus was applied at sowing and the granules were close enough (within 0·5–1·0 cm) to *T. officinale* seeds, the growing mycelia from the granules colonized the seeds and consequently reduced germination to 17%. Buried seed and seeds not in direct mycelial contact escaped. Post-emergence application of *S. minor* (10 days after sowing) killed all seedlings contacted with the fungal mycelia and reduced seedling survivorship to only 2%. The turfgrass was not adversely affected by *S. minor* application.

Grass overseeding is a common management practice in turfgrass environments (Turgeon 1985) and successful biological control should not harm the turfgrass seeds and/or prevent new seedling establishment. The application of *S. minor* granules did not adversely affect five turfgrass species. In our study, intact grass seeds were not infected and the few seeds that became infected were probably already dead or damaged and then colonized by the fungus.

When *S. minor* was applied at the time of sowing, germination was delayed because of physical factors. The fungus mycelia formed a thin layer covering the soil surface and prevented the grass seeds from starting or achieving proper germination in the first week, but 1 week later (as the *S. minor* mycelia disintegrated) all grass species were as vigorous as their controls. Gaps in the grass canopy of *A. palustris* (only in two replicates out of six) occurred when treated with the fungus

10 days after sowing. Seedling damping-off resulted from a physical barrier because of the bulky growing mycelia on granules, which prevented the tiny grass seedlings from growing. Neither seedling damping-off nor signs of damage were observed whenever the fungus spread smoothly on the soil surface under the grass canopy. *Agrostis palustris* growth habit differs from other grasses by developing a dense canopy with fine-textured leaves and stolons (Turgeon 1985) and this may explain why it was the only species affected.

Interestingly, 1 month after applying *S. minor* at sowing, the fungal-treated grass had higher total biomass values than the untreated control, and the biomass was significantly superior for *L. perenne* and *A. palustris*, in spite of the earlier canopy gaps of the latter. In the second trial, total biomass values were again higher with the *S. minor* treatment but were not significantly different from the controls. The increase in biomass cannot be related to the extra organic matter and other nutrients from granules, because the autoclaved non-colonized barley in the treated controls did not improve the total biomass or grass quality more than the untreated control. These studies have demonstrated that *S. minor* inundation has no adverse effects on grass germination or establishment but further studies are required to confirm the apparent growth promotion of grass seedlings from *S. minor* and investigate the mechanisms involved.

When much of the established *T. officinale* (the dominant species) population was removed by a spring application of *S. minor*, the opened areas were re-colonized by one or more flushes of *T. officinale* seedlings (Abu-Dieyeh & Watson 2006). Mature *T. officinale* seeds lack primary dormancy and can germinate any time if conditions permit (Martinková & Honék 1997; Stewart-Wade *et al*. 2002a) and full light is a major requirement for seed germination (Letchamo & Gosselin 1995). Buried seeds are unable to germinate (Noronha, Andersson & Milberg 1997). The ability of *S. minor* to colonize *T. officinale* seeds could be exploited to lower the seed bank of *T. officinale* each season.

It is likely that the mortality of the parent population will enhance the competitiveness of established turfgrass through vegetative means of reproduction; however, the newly created gaps will change community interactions because of changes in habitat structure and function (Booth, Murphy & Swanton 2003). Species respond in different ways to disturbance regimes, and factors such as gap size, seed input and seed size have profound influences on the performance of gap specialists (McConnaughay & Bazzaz 1987; Klinkhamer & De Jong 1988). McConnaughay & Bazzaz (1987) suggested that survivorship was greater in larger gaps for small-seeded species, while Klinkhamer & De Jong (1988) reported the importance of small-scale disturbance and seed input on *Cirsium vulgare* colonization.

We had hypothesized that grass overseeding on bare ground and/or in grass canopy gaps left after depletion of the *T. officinale* population will initiate competition in the upper soil layer between new seedlings of grass and *T. officinale*, and overseeding the grass at the time of *S. minor* application will exert more competitive pressure on new *T. officinale* recruits. In practice, relying on cultural methods for perennial weed suppression is generally not feasible; it is a slow gradual process, with weed suppression sometimes occurring after many years (Busey 2003; Hatcher & Melander 2003; Larsen, Kristoffersen & Fischer 2004).

Overseeding alone did not suppress *T. officinale* or other broadleaf weeds and did not significantly improve turf quality. Similarly, intraseeding a new cultivar of creeping bentgrass into an established golf putting green had limited success of establishment because of intraspecific competition (Kendrick & Danneberger 2002). However, combining grass overseeding with the *S. minor* bioherbicide led to excellent control of *T. officinale* and associated broadleaf weeds, and also increased turf quality over time to the maximum possible level expected for the turf type. The grass seedlings emerged 1 week after sowing regardless of *S. minor* presence, while dandelion emergence started later. The *S. minor* treatment created a new environment and its subsequent suppressive effects on the weed population could be attributed to the improved grass competition.

Autumn seeding of cool-season turfgrass is often recommended over spring seeding because of superior establishment (Reicher & Throssell 2005). Suitable weather conditions for *S. minor* infection occurred during both the spring- and autumn-treatment periods and seeding in the middle of May or the middle of September made no difference to the *T. officinale* population. However, higher temperatures in late June and colder temperatures in late October may have been responsible for less grass establishment when overseeding was delayed to 20 days after *S. minor* application. Additionally, this 20-day delay of overseeding grass might favour *T. officinale* seedling emergence and establishment rather than the grasses. According to Radosevich, Hott & Ghersa (1997), disturbing the area by direct control targeting the abundant weed species basically encourages weed seed germination and the seed bank should be considered along with above-ground vegetation in any weed management programme. Grass overseeding at 0 or 10 days after *S. minor* application is a favourable ecological management approach. Strong biocontrol effects on established *T. officinale* were obtained, grass competition was improved and, with this management strategy, *T. officinale* seeds would probably not colonize the superficial soil surface layer.

Other turfgrass weeds, such as *Trifolium repens* and *Convolvulua arvensis*, were both susceptible to *S. minor* application and the reduction in their population densities could be attributed to strong established grass competition. *Trifolium repens* was more vulnerable and its density was reduced under all treatments; this could be attributed to regular mowing (M. H. Abu-Dieyeh & A. K. Watson, unpublished data).

Established *C. arvensis* plants are very difficult to eradicate using mechanical and chemical control methods because of vigorous regrowth from the root buds and rhizomes after destruction of the shoots (Weaver & Riley 1982). Significant control of *C. arvensis* was only obtained in the second year with *S. minor* followed by overseeding at 0 or 10 days later. In all other treatments the *C. arvensis* infestations increased in the second year, indicating the importance of grass competition and repetitive application of *S. minor* to control this persistent weed.

Competitiveness of grasses on weed invasion is determined by grass species and cultivars (Ferrel *et al*. 1998), timing of turf establishment (Borman, Krueger & Johnson 1991), grass seeding rate and turf cultural management (Busey 2003). Several competitive features have been reported for *T. officinale* in turfgrass, while the deep tap root can extend below the level of competing grass roots, the phenotypic variability of the rosette growth form allows *T. officinale* to compete above-ground for light (Stewart-Wade *et al*. 2002a). Grasses deplete available nutrients in the upper layer of soil and increasing grass density may reduce growth of *T. officinale* seedlings (Vavrek 1998). Molgaard (1977) reported *T. officinale* seedling establishment was strongly inhibited in areas of dense grass cover because of insufficient open ground and light penetration. The chances of seedling establishment are decreased 23 times in areas with lush grass vegetation compared with open areas (Ford 1981). In a competitive environment, the growth of all five *T. officinale* genotypes was reduced more with *Poa pratensis* relative to the other competitors *Plantago major* and *Trifolium pratense* (Vavrek 1998). All ages of *T. officinale* (4–13 weeks) were more susceptible to *S. minor* in the presence of grass competition rather than in the absence of competition (M. H. Abu-Dieyeh & A. K. Watson, unpublished data). Thus, minimizing the creation of ecological niches required for weed encroachment is a key issue of non-pesticide control (Larsen, Kristoffersen & Fischer 2004).

In conclusion, boosting grass competition by grass overseeding after controlling the established *T. officinale* population with applications of *S. minor* led to reduced recruitment from the seed bank and to closure of the turf canopy preventing further weed colonization. The high susceptibility of *T. officinale* seeds to *S. minor* widens the potential effectiveness of *S. minor* as a bioherbicide for long-term reduction of the seed bank. Indeed the vulnerability of *T. officinale* seedlings to *S. minor* stresses the need for selecting the proper application time to prevent seedlings from establishing into strong perennials. As Poaceae species are not susceptible to *S. minor* (Melzer, Smith & Boland 1997), this bioherbicide could be applicable not only to turfgrass but also to other grass-cropping systems to control broadleaf weeds. Reduced tillage systems have increased *T. officinale* and other broadleaf weed populations (Buhler *et al*. 1994), while certified grass seed producers and organic producers are searching for non-chemical weed control.

Acknowledgements

The authors would like to thank Inaam Shaheen, Miron Teshler, Boris Touvykine, Simona Teshler, Caroline Poirier and Sebastien Alex for their assistance in the experimental work. The financial support from Hashemite University, Zerka, Jordan and from the Natural Sciences and Engineering Research Council of Canada (NSERC) Idea to Innovation (I2I) and Discovery grants are gratefully acknowledged.

References

Abu-Dieyeh, M.H. & Watson, A.K. (2006) Effect of turfgrass mowing height on biocontrol of dandelion with *Sclerotinia minor*. *Biocontrol Science and Technology*, **16**, 509–524.

Abu-Dieyeh, M.H., Bernier, J. & Watson, A.K. (2005) *Sclerotinia minor* advances fruiting and reduces germination in dandelion (*Taraxacum officinale*). *Biocontrol Science and Technology*, **15**, 815–825.

Booth, B.D., Murphy, S.D. & Swanton, C.J. (2003) *Weed Ecology in Natural and Agricultural Systems*. CABI Publishing, Cambridge, MA.

Borman, M.M., Krueger, W.C. & Johnson, D.E. (1991) Effect of established perennial grasses on yields of associated annual weeds. *Journal of Range Management*, **44**, 318–322.

Bostock, S.J. & Benton, R.A. (1979) The reproductive strategies of five perennial Compositae. *Journal of Ecology*, **67**, 91–107.

Buhler, D.G., Stoltenberg, D.E., Becker, R.L. & Gunsolus, J.L. (1994) Perennial weed populations after 14 years of variable tillage and cropping practices. *Weed Science*, **42**, 205–209.

Busey, P. (2003) Cultural management of weeds in turfgrass: a review. *Crop Science*, **43**, 1899–1911.

Ciotola, M., Wymore, L. & Watson, A.K. (1991) *Sclerotinia, A Potential Mycoherbicide for Lawns.* WSSA Abstracts 30. Weed Science Society of America, Louisville, KY.

Dunn, R. & Moyer, J. (1999) *Dandelion Control in Direct Seeding Systems.* http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex2511. Accessed 10 December 2005.

Falkowski, M., Kukulka, I. & Kozlowski, S. (1989) Characterization of biological properties and fodder value of dandelion *Taraxacum officinale* Web. *Proceedings of the XVI International Grassland Congress 1989* (ed. P. Desroches), pp. 775–776. Nice, France. Association Français Productears Fourrageur, Parris.

Ferrel, M.A., Whitson, T.D., Koch, D.W. & Gade, A.E. (1998) Leafy spurge (*Euphorbia esula*) control with several grass species. *Weed Technology*, **12**, 374–380.

Ford, H. (1981) The demography of three populations of *Taraxacum officinale*. *Biological Journal of Linnean Society*, **15**, 1–11.

Gadgil, M. & Solbrig, O.T. (1972) The concept of r- and k-selection: evidence from wild flowers and some theoretical considerations. *American Naturalists*, **106**, 14–31.

Green, R.H. (1979) *Sampling Design and Statistical Methods for Environmental Biologists*. John Wiley, New York, NY.

Hatcher, P.E. & Melander, B. (2003) Combining physical, cultural and biological methods: prospects for integrated non-chemical weed management strategies. *Weed Research*, **43**, 303–322.

von Hofsten, C.G. (1954) *Studies on the genus Taraxacum with special reference to the group Vulgaria DT in Scandinavia*. LTs Förlag, Stockholm.

Hoya, A., Shibaike, H., Morita, T. & Motomi, I. (2004) Germination and seedling survivorship characteristics of hybrids between native and alien species of dandelion (*Taraxacum*). *Plant Species Biology*, **19**, 81–90.

Kendrick, D.L. & Danneberger, T.K. (2002) Lack of competitive success of an intraseeded creeping bentgrass cultivar into an established putting green. *Crop Science*, **42**, 1615–1620.

Klinkhamer, P.G.L. & De Jong, T.J. (1988) The importance of small-scale disturbance for seedling establishment in *Cirsium vulgare* and *Cynoglossum officinale*. *Journal of Ecology*, **76**, 383–392.

Kopp, K.L. & Guillard, K. (2002) Clipping management and nitrogen fertilization of turfgrass: growth, nitrogen utilization and quality. *Crop Science*, **42**, 1225–1231.

Kremer, R.J. (2000) Combinations of microbial and insect biocontrol agents for management of weeds. *Proceedings of the X International Symposium on Biological Control of Weeds* (ed. N.R. Spencer), pp. 931–940. Montana State University, Boseman, MT.

Larsen, S.U., Kristoffersen, P. & Fischer, J. (2004) Turfgrass management and weed control without pesticides on football pitches in Denmark. *Pest Management Science*, **50**, 579–587.

Letchamo, W. & Gosselin, A. (1995) Light, temperature and duration of storage govern the germination and emergence of *Taraxacum officinale* seed. *Journal of Horticultural Science*, **71**, 373–377.

Longyear, B.O. (1918) The dandelion in Colorado. *Agricultural Experimental Station Agriculture College of Colorado Bulletin*, **236**, 1–35.

McConnaughay, K.D.M. & Bazzaz, F.A. (1987) The relationship between gap size and performance of several colonizing annuals. *Ecology*, **68**, 411–416.

Martinková, Z. & Honék, A. (1997) Germination and seed viability in a dandelion (*Taraxacum officinale*) Agg. *Ochrana Rostlin*, **33**, 125–133.

Medd, R.W. & Campbell, M.A. (2005) Grass seed infection following inundation with *Pyrenophora semeniperda*. *Biocontrol Science and Technology*, **15**, 21–36.

Melzer, M., Smith, E. & Boland, G. (1997) Index of plant hosts of *Sclerotinia minor*. *Canadian Journal of Plant Pathology*, **19**, 272–280.

Molgaard, P. (1977) Competitive effects of grass on establishment and performance of *Taraxacum officinale*. *Oikos*, **29**, 376–382.

Noronha, A., Andersson, L. & Milberg, P. (1997) Rate of change in dormancy level and light requirement in weed seeds during stratification. *Annals of Botany*, **80**, 795–801.

Radosevich, S., Holt, J. & Ghersa, C. (1997) *Weed Ecology Implications for Vegetation Management*. John Wiley & Sons, New York.

Reicher, Z. & Throssell, C. (2005) *Establishing Turfgrass Areas from Seeds*. Purdue University, West Lafayette, Indiana. www.agry.urdue.edu/turf. Accessed 15 December 2005.

Solbrig, O.T. (1971) The population biology of *T. officinale*. *American Scientific*, **59**, 686–694.

Solbrig, O. & Simpson, B. (1974) Components of regulation of a population of dandelions in Michigan. *Journal of Ecology*, **62**, 473–486.

Stewart-Wade, S.M., Green, S., Boland, G., Teshler, M., Teshler, I., Watson, A.K., Sampson, K., DiTommaso, A. & Dupont, S. (2002b) *Taraxacum officinale* Weber, dandelion (Asteraceae). *Biological Control Programs in Canada, 1981–2000* (eds P.G. Mason & J.T. Huber). CABI Publishing, New York, NY.

Stewart-Wade, S.M., Neumann, S., Collins, L. & Boland, G.J. (2002a) The biology of Canadian weeds. 117. *Taraxacum officinale* G.H. Weber ex Wiggers. *Canadian Journal of Plant Science*, **82**, 825–853.

Turgeon, A.J. (1985) *Turfgrass Management*. Reston Publishing Co. Inc., Virginia.

Vavrek, M.C. (1998) Within-population genetic diversity *of Taraxacum officinale* (Asteraceae): differential genotype response and effect on interspecific competition. *American Journal of Botany*, **85**, 947–954,.

Weaver, S.E. & Riley, W.R. (1982) The biology of Canadian weeds. 53. *Convolvulus arvensis* L. *Canadian Journal of Plant Science*, **62**, 461–472.

Welham, C.V. & Setter, R.A. (1998) Comparison of a size-dependent reproductive effort in two dandelion populations. *Canadian Journal of Botany*, **76**, 166–173.

Wiecko, G. (2000) Sequential herbicide treatments for goosegrass (*Eleusine indica*) control in Bermudagrass (*Cynodon dactylon*) turf. *Weed Technology*, **14**, 686–691.

Received 14 March 2006; final copy received 9 August 2006
Editor: Phil Hulme