PREFACE TO CHAPTER 9

The ability of *Taraxacum officinale* to tolerate and to adapt to a wide range of biotic and abiotic factors is well documented. In response to biotic and abiotic stress, certain plants including dandelion change their pattern of reproduction as a survival mechanism (Welham & Setter 1998). Plant life history models suggest that diseases should accelerate reproductive cycles (Agnew et al. 2000). In spring 2003, at the peak of flowering of the dandelion population and within four days after I did my first field application of *S. minor*, flowering accelerated to fruiting. This phenomenon was not previously discovered for dandelion. In Chapter 9, the developmental response of common dandelion to *S. minor* infection and subsequent seed dispersal and germination potential was examined.

Chapter 9 was recently published in manuscript form in *Biocontrol Science and Technology*, December 2005; 15(8):815-825. The manuscript was co-authored by Professor Alan K. Watson, my supervisor and Jerome Bernier, a summer student. I am the one who recorded the phenomenon, designed the experimental set-up, performed the experiments and the statistical analysis and wrote the manuscript. Jerome Bernier helped me in seed collection, in laboratory work related to seed germination experiment and isolating seed microflora. Professor Watson supervised the work, provided financial and technical resources, and corrected the manuscript. Jerome Bernier has granted permission to use the content of this manuscript in the present thesis (E-mail received on January 9 2006, Appendix-6).

CHAPTER 9

Sclerotinia minor advances fruiting and reduces germination in dandelion (Taraxacum officinale)

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9.1. Abstract

Sclerotinia minor Jagger is a promising biocontrol agent for dandelion in turfgrass. When a flowering dandelion population was treated with *S. minor*, flowering accelerated to the fruiting stage within four days. This developmental response was 4-5 days earlier than in the control, untreated plants and was not observed in herbicide treated plants. Seeds obtained from the fungal treated plants were smaller, lighter and their germination rate was reduced by 48.4% and 47.3% for spring and fall applications, respectively. *S. minor* was not detected in dandelion seeds from the fungal treated plants. In addition to effective control of mature (flowering) dandelions, seeds dispersed by dying plants have reduced germination and are not transferring *S. minor* off target.

Key words: fructification; *Sclerotinia minor*; *Taraxacum officinale*; turfgrass; bioherbicide; biocontrol.

9.2. Introduction

Taraxacum officinale Weber (dandelion) is a common, worldwide, herbaceous perennial weed, that infests parks, gardens, pastures, orchards, roadside, vegetable gardens, agricultural and horticultural crops (Stewart-Wade et al. 2002b). It interrupts the uniformity and limits the density of turfgrass when infesting home lawns, golf courses, athletic fields and roadside green spaces. The strong competitive and dispersal abilities of dandelion are partly due to high seed production and germination rates. The seed bank population in the top 13 cm of soil in a grassland area in the UK as estimated at 1.6×10^6 seeds ha^{-1} (Champness & Morris 1948), and up to 6×10^8 seeds ha^{-1} were reported to be produced in a heavily infested area of Canada (Roberts 1936). Mature seeds lack primary dormancy and are able to germinate almost as soon as they leave the plant (Martinková & Honék 1997). Reported germination capacity of dandelion seeds varies (Stewart-Wade et al. 2002b), but germination is generally 80-90% (Falkowski et al. 1989).

Turfgrass production is a major industry in North America and Europe. In the USA alone, there is an estimated 93 million dwellings and more than 18 million ha of turf (Monaco et al. 2002). Current methods for dandelion control in turfgrass include proper management practices and chemical control with three-way mixtures of phenoxy herbicides including 2,4-dichlorophenoxy acetic acid (2,4-D). However, chemical herbicides have received considerable negative publicity worldwide and many people are opposed to their use. *Sclerotinia minor* Jagger is an ascomycete plant pathogen that has biocontrol potential for dandelion in turfgrass (Ciotola et al. 1991; Riddle et al. 1991; Schnick et al. 2002; Stewart-Wade et al. 2002a).

Recently, *Arabidopsis* plants have been reported to have accelerated reproductive development in response to three pathogenic species (Korves & Bergelson 2003). Apparently, developmental response has not been previously reported for dandelion under a pathogen stress. The objective of this study was to examine the developmental response of common dandelion to *S. minor* infection and subsequent seed dispersal and germination potential.

9.3. Materials and methods

9.3.1. Experiment A

During the conduct of a field experiment designed to study the effect of *S. minor* on population dynamics of dandelion in turfgrass (Chapter 7), the dandelion population was observed to respond to *S. minor* inoculation by accelerating the change from flowering to fruiting. The experiment was conducted in a turfgrass field on the Macdonald Campus of McGill University in Ste-Anne-de-Bellevue, Quebec. The turf was mainly Kentucky bluegrass with 10% red fescue and was heavily infested with common dandelion (50-110 dandelion plants m^{-2}). A completely randomized design two factor experiment with six replicates was established in May 2003. The first factor was application time; either spring (May), fall (September), or both spring and fall. The second factor was application of one of three treatments: (1) broadcast foliar application of Killex® herbicide [2,4-D, mecoprop ((±)-2-(4-chloro-2-methylphenoxy) propanoic acid) and dicamba (3,6-dichloro-2-methoxybenzoic acid)] at 1.7 kg a.i. ha^{-1}; (2) broadcast granular formulation of *S. minor* (see Chapter 5) at 120 g m^{-2}, (3) untreated control. On the day of spring application, 70-80% of dandelions in all experimental plots were in the flowering stage

while, approximately 10% were observed on the day of fall application. Progression from flowering to fruiting was monitored from 4 to 10 days after treatment by visual estimation of the percentage of dandelions that changed from flowering to fruiting in each plot.

9.3.2. Experiment B

In the fall (September 2003) a second field experiment was conducted to test the effect of spot application of *S. minor* on the flowering response of individual dandelion plants. A total of 150 dandelions naturally occurring in a Kentucky bluegrass field on the Macdonald Campus of McGill University in Ste-Anne-de-Bellevue, Quebec, were selected and marked using wooden sticks. All the inflorescences (heads) of the selected plants were in the flowering stage on the day of treatment. Fifty percent of marked dandelion plants (75) were randomly selected and spot applied with 0.2 g per plant of a granular formulation of *S. minor* on the center of the rosette. The remaining 75 plants were not treated. The numbers of flowering and fruiting heads for each plant were recorded 4 days after application.

9.3.3. Seed collection and experiments

Six days after treatment in Experiment A, 30 random samples of dandelion fruiting heads were collected into paper bags from all replicates of all treatments. A 0.1 g sample of freshly collected seeds was removed and used to test for pathogen contamination (see 9.3.3.3). The remaining fruits were air-dried at 21 ± 2^{o}C for one month and then stored in

the refrigerator (4^{o}C). Similar procedures were used to collect seeds from the fall (September 2003) treated plots of the same experiment.

9.3.3.1. Seed Size and Morphology

Some seeds within each treatment were taken from all replicate samples, mixed and then 20 100-seed samples were used to determine average seed weight within each treatment. A 100-seed sample within each treatment was also used to determine the mean pappus diameter and stalk length of dandelion seeds (Appendix-5) using a measuring caliper.

9.3.3.2. Seed Germination

Four Whatman No. 1 filter papers were placed into 15-cm-diameter Petri plates, papers were saturated with distilled water, and excess water was decanted. Five replicates of 50 dandelion seeds from each treatment were placed on the moistened filter paper and the plates were sealed with parafilm. Plates were incubated at 26 ± 2^{o}C under two 34 W cool white fluorescent lights on a 16 h day 8 h night cycle. Germinated seeds were counted and removed every day for 21 days. Filter papers were kept moist through periodic spraying with distilled water. Total germination percentages, days to 50% of final germination (T_{50}) and germination span in number of days between 10% and 90% germination (T_{90}-T_{10}) were calculated (Furutani et al. 1985).

9.3.3.3. Seed Microflora

To detect the presence of *S. minor* in or on dandelion seeds, a random sample of 0.1 g of freshly collected seeds (~200 seeds) from each of the control and fungal treated samples

(a total of 6 samples from each treatment; see seed collection) were squashed separately using a sterile mortar and pestle. The squashed seeds were transferred aseptically into 20 ml culture tubes containing 9 ml of potato dextrose broth (PDB) and left to stand for 30 min. Serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} were prepared and 0.2 ml from each dilution and from the original PDB tube were transferred onto four potato dextrose agar (PDA) plates and four rose Bengal agar (RBA) plates. Plates were incubated at 20 ± 1^{o}C and were checked for fungal growth. Individual fungal colonies were subcultered on separate PDA plates, incubated for 5-10 days and identified. This method to detect fungal contamination was repeated for seeds collected in the fall of 2003 (Experiment B). To verify the above procedure to detect *S. minor* in or on seeds, a positive control was prepared by mixing a 1 cm diameter agar disc containing *S. minor* mycelium with 0.1 g of seeds and then processed similarly as the test samples above.

9.3.4. Data analysis

Experiments were conducted in a completely random design. Data were subjected to one-way analysis of variance (ANOVA) procedures using the Statistical Analysis System (SAS) (SAS Institute Inc 2001). Data were compared using the F-test at alpha 0.01 and 0.05 and Tukey's test was used to separate means.

9.4. Results

9.4.1. Developmental response to inoculation by *S. minor*

When treated with a bioherbicide formulation of *S. minor*, flowering dandelion populations in the spring and fall responded by suddenly changing to the fruiting stage

(Figure 9.1A). Four days after application, > 90% of the flowering dandelions in the spring fungal-treated plots were fruiting (Figure 9.1B). Natural progression to fruiting occurred 4 to 5 days later in the untreated control plots. This advanced fruiting response did not occur in the herbicide-treated plots (Figure 9.1C). White plumose pappi and downward bending scapes were observed in response to *S. minor* treatment (Figure 9.1D). In the fall (September 2003) treatment plots, flowering dandelion plants were estimated to be 10% of the population in each plot, and the same advanced fruiting response for about 90% of the flowering dandelion was observed 4-days post application in the fungal treated plots. Only 20-30% of the untreated flowering dandelions in the control plots changed to fruiting after the 4-day period. Since the percentage of flowering dandelions in the fall of experiment-A was very low compared to spring application, a second experiment (B) of spot applications of the bioherbicide to individual dandelions was conducted to quantify the effects of the fungus.

The fungus had similar effects when applied as a spot treatment in the fall (Table 9.1). Four days post application, the differences in number of flowering or fruiting heads were highly significant ($P < 0.001$) between the treated and untreated plants. About 80% of dandelion flowering heads were changed to fruiting under the fungus inoculation while 29.6% were obtained under natural development of untreated flowers. Eighty three percent of the 75 treated plants (~62 plants) were able to develop one or more fruiting heads compared with ~34 untreated plants (Table 9.1).

9.4.2. Seed size and morphology

Seed weight was significantly decreased by *S. minor* and by the herbicide compared to the control. Mean seed weight of seeds from the untreated plants was 0.73 ± 0.03 mg, while seeds from *S. minor* treated plants and herbicide treated plants were 0.34 ± 0.02 mg and were 0.32 ± 0.03 mg, respectively. In addition to smaller seeds, the seeds from *S. minor* treated plants had significantly longer beak length and smaller pappus diameter than seeds from untreated plants (Table 9.2). Seeds collected from herbicide treated plants had the longest beaks and the greatest pappus diameter (Table 9.2).

9.4.3. Seed viability

Both *S. minor* and the herbicide significantly ($P \leq 0.001$) reduced seed germination (Figure 9.2) in both spring and fall trials. Germination was the lowest in the seeds from the herbicide-treated plants, 4.8% in the spring and 18.4% in the fall compared to 38.4 and 35.2% germination of seeds from *S. minor* treated plants.

S. minor also altered the germination pattern of the seeds in the spring by retarding the T_{50} by 2 days, and by increasing the span of germination (T_{90}-T_{10}) and mean germination time by 5 and 2 days, respectively (Table 9.3). This relationship was not observed in the fall because all seeds germinated at a faster rate.

9.4.4. Seed contamination

There was no indication of *S. minor* seed infection or infestation from the PDA and RBA plate bioassays. In the positive control, the mycelium of *S. minor* was readily detected on all PDA plates and on some of RBA plates after 48 h of incubation. *Cladosporium*

oxysporum, *Fusarium oxysporum*, *Alternaria chlamydospora*, *Mucor* sp. and different yeast spp. were the common microflora recovered from seeds of both the *S. minor* treated and the untreated plants.

Table 9.1. Response of individual flowering dandelion plants to spot application of *S. minor* bioherbicide during the fall of 2003.

	Untreated (n=123)	Fungus treated [1] (n=101)
Measured parameters	4-days post	4-days post
Total # flowering heads	87	21** [2]
Total # fruiting heads	36	80**
% Plants with ≥1 fruiting heads	45.3	82.7**
% of fruiting heads	29.6	79.2**

[1] 0.2 g/plant of a granular formulation of *S. minor* was applied to individual plants.

[2] ** Significant difference between untreated and fungal treated dandelions at $P < 0.001$.

Table 9.2. Morphological response of spring collected dandelion seed to bioherbicide and chemical herbicide treatments.

Measured parameters	Untreated	Fungus treated [(1)]	Herbicide treated [(2)]
Mean stalk length (mm)	7.3 c [(3)]	7.9 b	9.3 a
Mean pappus diameter (mm)	6.6 b	4.9 c	7.2 a
Mean seed weight (mg)	0.73 a	0.34 b	0.32 b

[(1)]: broadcast application of a granular formulation of *Sclerotinia minor* at 120 g m^{-2}.

[(2)]: broadcast foliar application of Killex® herbicide (2,4-D, mecoprop and dicamba) at 1.7 kg a.i. ha^{-1}.

[(3)]: values in a row sharing a letter are not significantly different at 5% level according to Tukey's test

Table 9.3. Effect of *S. minor* application on dandelion seed germination.

Treatment	T50 [1]	T90-T10 [2]	Mean germination time [3]
	Days	Days	Days
Untreated - spring	4.8 ab [5]	8.2 b	6.6 b
Fungus treated [4] – spring	6.6 a	12.8 a	8.6 a
Untreated – fall	4.0 b	2.4 c	4.1 c
Fungus treated [4] – fall	4.0b	2.2 c	3.8 c

[1]: Days to 50% of final germination.

[2]: Days between 10% and 90% germination.

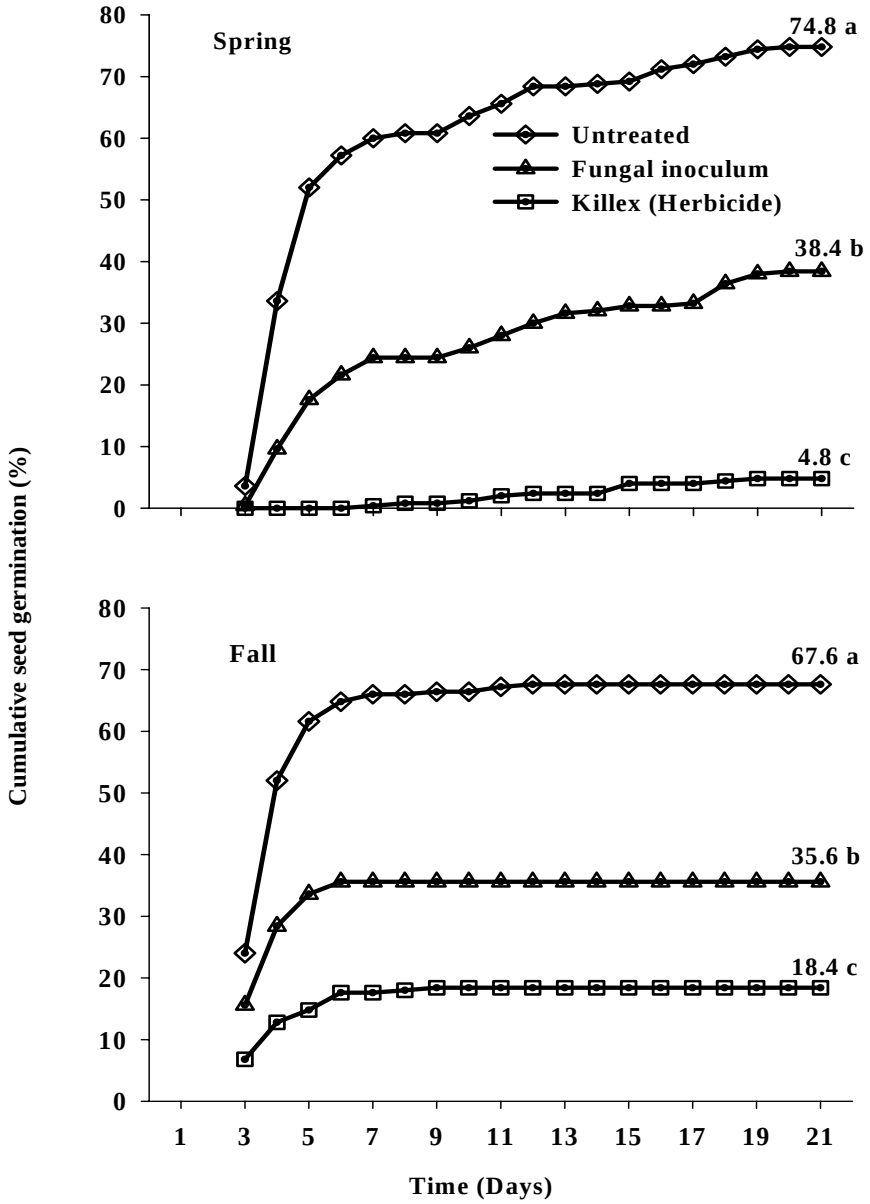
[3]: MGT= Σ (n x d)/N, where n is number of seeds germinated on day d and N is the total number of germinated seeds.

[4]: broadcast application of a granular formulation of *S. minor* at 120 g m^{-2}.

[5]: values in a column sharing a letter are not significantly different at 5% level according to Tukey's test.

Figure 9.1. Typical dandelions in the flowering and fruiting stages (A). Dandelions that changed from flowering to fruiting within 4 days after being treated with *S. minor* (B). Representative epinastic response of dandelion to 4 days after phenoxy based chemical herbicide treatment. Note, flowers treated with the herbicide remain in the flowering stage (C). *S. minor* infection causes the flowering scape to bend downward, the pappi to whiten and become plumose, and ultimate plant death (D).

Figure 9.2. Germination progression of dandelion seeds collected after spring (May) and fall (September) treatment applications. Final mean values sharing the same letter in the spring or fall application times are not significantly different at the 5% level according to Tukey's test.



9.5. Discussion

Dandelions flower throughout the year, with a primary peak in April and secondary one in September or October (Gray et al. 1973). The time required from blooming until seed ripening and bract opening to release seeds is reported to be 8-13 days (Gray et al. 1973). The mean time to fruiting among nine North American dandelion clones was significantly different and ranged between 11.5 to 17.0 days (Colier & Rogstad 2004) Under greenhouse conditions (24 ± 1^{o}C; 15 h of light/day at photon flux density of 350 ± 50 µmol m^{-2} s^{-1}) dandelion required a 10.3 ± 0.7 day period from blooming to seed release (M.H. Abu-Dieyeh & A. K. Watson, unpublished data). In response to *S. minor* inoculation, flowering dandelion plants in spring and fall turned to the fruiting stage within 4 days of inoculation (Figure 9.1 & Table 9.1). The shift from flowering to fruiting response of inoculated plants was 4 to 5 days faster than that of the non-inoculated plants and was never observed in plants treated with the chemical herbicide. Dandelions treated with *S. minor* were killed within 14 days. Therefore, the 40-50% earlier fruiting in dandelion apparently is a direct consequence of the fungal infection. This type of response for dandelion to a pathogenic infection has not been previously reported. Various biotic and abiotic stresses to dandelion including chemical herbicides, human manipulations, herbivory, or other microorganisms have not been reported to cause such a response (Stewart-Wade et al. 2002b). The ability of dandelion to tolerate and to adapt to a wide range of biotic and abiotic factors is well documented and explained by its common gene pool combined with high phenotypic plasticity (Solbrig 1971; Baker 1991), and by its ability to manipulate its internal resources (Wilson et al. 2001).

Some plants flower faster in response to shade, overcrowding, low nutrients, drought, heat and low light quality (Levy & Dean 1998), and plant life history models suggest that diseases should accelerate reproductive cycles (Agnew et al. 2000). In response to biotic and abiotic stress, certain plants including dandelion change their pattern of reproduction as a survival mechanism (Welham & Setter 1998). Dandelion in a disturbed habitat (alfalfa field) had higher reproduction than the undisturbed population. Welham & Setter (1998) hypothesized that dandelion has different reproductive efforts when mortality rates varied with degree of disturbance and neighbour density. Soybeans that are subjected to drought stress switch from vegetative to reproductive phases, shortening each reproductive phase leading to accelerated senescence (Desclauk & Roumet 1996). High temperature stresses during flowering results in reduced seed yield in both monocotyledonous and dicotyledonous plants (Young et al. 2004). Recently, Korves & Bergelson (2003) reported that the susceptible *Arabidopsis* plants accelerate their reproductive development by reducing time to flowering and the number of aerial branches on the primary inflorescence in response to two biotrophic bacterial pathogens and a biotrophic oomycete. The change in infected plant development could be an active response of plants to infection or the pathogen could be manipulating plant growth by inducing hormonal changes (Agrios 1997). Auxin, ethylene, and jasmonic acid which have active roles in plant development are elevated in infected plants (Wang et al. 2002).

S. minor is a necrotrophic fungus, causing rapid wilting, collapse and death of host plants (Melzer et al. 1997). The pathogenesis by *S. minor* involves the production of endo- and exo pectinases, cellulases, hemicellulases and proteases (Lumsden 1979). The accelerated reproductive cycle in dandelion may be a consequence of a systemic

signaling mechanism in dandelion or due to the manipulation of host growth by the fungus. A predominant 18-kDa protein in dandelion roots is known to serve as a vegetative storage protein (VSP) to tolerate abiotic stresses (Cyr & Bewley 1990). However, Xu et al. (2000) found that this protein is unlikely to play a role in cold stress tolerance and more likely belongs to allergen and pathogenesis related proteins (PR). Presently, the nature and the mechanism underlying the acceleration of dandelion reproductive cycle in response to *S. minor* is unknown and needs further investigation.

The germination capacity of dandelion seeds reported in the literature is variable and is highly correlated with experimental conditions. Martinková & Honék (1997) reported 94% germination on moist filter paper 28 days after the collection of the seeds. Collins (2000) obtained 85-94% germination under variable temperature and light regimes, and Cross (1931) reported 75-76% germination in alternating temperatures of $30^{o}C/20^{o}C$, while only 60-65% germinated at a constant temperature of $18\text{-}20^{o}C$. In our study, there was no significant difference in the germination of seeds collected from untreated plants in May or September (Figure 9.2). However, there were significant differences in total germination between the control seeds and seeds collected from the herbicide or the *S. minor* treated plants. The lowest germination was obtained for seeds from herbicide treatment with 4.8% in spring and 18.4% in the fall. This could be explained by different abilities of uptake and translocation of phenoxy herbicides by dandelions at different phenological stages. In the spring (May), > 80% of the dandelion population was in the flowering stage compared with 10% scattered flowering in the fall (September). In spring more resources are allocated for flowering and vegetative growth of dandelion (Cyr & Bewley 1990), while nitrogen reserves are restored in the roots at

the end of the summer (Rutherford & Deacon 1974). The systemic distribution of foliage-applied herbicides follows a source-to-sink pattern (Crafts 1962). Therefore, more translocation of herbicide is expected towards the fruiting heads in the spring and towards the roots in the fall, and this may explain the lower seed germination in the spring than in the fall. Although no fruiting response of dandelion was observed under the herbicide treatment, a significant seed weight reduction was recorded.

Dandelions are known to respond to 2,4-D by increasing uptake of water, decreasing carbohydrate reserves, increasing respiration rate, increasing total soluble protein, and increasing hydrolase activity (Deacon & Rutherford 1972; Wilson & Michiels 2003). Thus, the decrease in seed weight and the increase in pappus diameter and stalk length (Table 9.2) could be due to the auxin induced effects of 2,4-D leading to seed non-viability or lower viability in response to the herbicide and this may also explain the lowest germination potential obtained for these seeds.

Seeds from dandelion fruits from plants infected with *S. minor* have about 50% less germination than control seeds. This decrease in seed germination may be due to the high frequency of impaired seeds as a result of accelerating the dandelion life cycle by 4-5 days. By responding in this way, dandelions can retain 50% of their reproductive abilities instead of losing them completely to a rapidly advancing necrotrophic pathogen like *S. minor*. The impaired seed suggestion is supported by: (1) *S. minor* was not isolated from the seeds of *S. minor* treated plants; (2) significant seed weight reduction was obtained for seed samples from fungal treated plants; (3) the pappi of the fruits were visibly altered (Figure 9.1B), they are fluffier and whiter in colour than the normal fruits. The measurements taken for the stalk length and pappus diameter (Table 9.2) indicated longer

stalks (7.9 ± 0.66 mm) and lower pappus diameter (4.9 ± 0.76 mm) than the control seeds (7.3 ± 0.76 and 6.6 ± 0.81 mm, respectively). During flowering, an abiotic stress like high temperature was found to reduce the seed weight significantly, and affect the fruit and seed development in *Brassica napus* (Young et al. 2004).

The progress of seed germination in spring collected seed was also affected under *S. minor* infection as T50 was slower by 2 days, and the span of germination (T90-T10) and mean emergence time were significantly longer by 5 and 2 days, respectively (Table 9.3). However, differences in these parameters of seed germination were not observed between the fall treatments as both seeds germinated at a faster rate. In this study, seeds collected in fall from untreated plants were significantly lighter than in spring with an average of 0.56 ± 0.04 mg (data not presented in Table 9.2). Germination performance in dandelion seeds was highly correlated with achene weight; the heaviest achenes showed the best germination (Tweney & Mogie 1999) and rate of seed maturation (Collins 2000). Both factors may explain the difference in germination between the fall and spring treatments in this study (Table 9.3).

S. minor has consistently provided good control of dandelion in greenhouse and field experiments (see previous Chapters) but *S. minor* also reduces dandelion's reproductive and dispersal abilities by about 50%. The absence of *S. minor* from seeds of dandelion obtained from fungal-treated plots indicates that it is not transmitting this fungus off site to non-target species or crops like lettuce. This is an important fact because *S. minor* is not a host-specific fungus as it infects a wide range of host plants (Melzer et al. 1997). Finally, Korves & Bergelson (2003) proposed that the developmental response of *Arabidopsis* to biotrophic pathogens by reducing time to

flowering might affect tolerance of and or resistance to the disease. This may not apply on our model, as *S. minor* is a necrotrophic fungus that kills dandelion within 1-2 weeks of infection, and also seeds in apomictic dandelion are known to develop without fertilization (Roberts 1936).

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