PREFACE TO CHAPTER 4

Sclerotinia minor Jagger is a necrotrophic fungus with a wide host range. A one pathogen-multiple host strategy has recently been highlighted as an approach to overcome the commercial limitations of the single target bioherbicide paradigm (Hallett 2005). *S. minor* is a widely distributed pathogen with a broad host range (Melzer et al. 1997; Hollowell et al. 2003); however the susceptibility of many turfgrass broadleaf weeds to *S. minor*, and particularly their susceptibility to the barley-based formulation of *S. minor*, are unknown. In Chapter 4 the susceptibility of several turfgrass broadleaf weeds to *S. minor* spot application was preliminary investigated during the fall of 2003 and 2004.

CHAPTER 4

Susceptibility of turfgrass broadleaf weeds to *Sclerotinia minor*

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4.1. Abstract

The fungus *Sclerotinia minor* is being developed as a biological control for dandelion in turfgrass environments. The susceptibility range of turfgrass broadleaf weeds to *S. minor* is not known. The efficacy of *S. minor* on broadleaf weeds, common to turfgrass, was preliminary evaluated under field conditions. 32 broadleaf weeds, from 13 different families, were found to be susceptible to spot application of *S. minor*. 23 of these species had not previously been recorded as hosts for *S. minor*. These results support the importance of a one pathogen multiple-weed strategy to overcome commercial limitations of a single target bioherbicide paradigm.

Key words: biological control, bioherbicide, *Sclerotinia minor,* selectivity, turfgrass; turf broadleaf weeds.

4.2. Introduction

Most turfgrass environments have weed problems and require a degree of management to be functional and aesthetically pleasing (Monaco et al. 2002). Broadleaf weeds disturb the visual turf uniformity due to different growth habits, different leaf shape and size or color contrast (McCarty et al. 2001). A vigorous sward monculture turf is a key goal and extremely challenging for turf managers (Cisar 2004). Broadleaf weeds compete with turf for light, soil nutrients, soil moisture and physical space and can replace weaken turf (Emmons 1995). 73 grass and grass-like and 145 broadleaf species are classified as weeds in turfgrass environments (McCarty et al. 2001). A combination of two or three herbicides is normally recommended to control a wide spectrum of broadleaf weeds (Emmons 1995). Repeated applications of dicamba or phenoxy herbicides such as 2,4-D

and mecoprop or a combination product such as "killex™" are extensively used for dandelion control (Anonymous 1997). As public concern increased around possible adverse health and environmental effects of lawn pesticides and led to ban or restrict their uses (Riddle et al. 1991; Cisar 2004), the search for biological control options intensified.

Sclerotinia minor Jagger (IMI 344141) is an ascomycete plant pathogen with biocontrol potential for dandelion control in turfgrass (Riddle et al. 1991; Ciotola et al. 1991; Brière et al. 1992). The mycelial-colonized barley grains formulations of *S. minor* have shown bioherbicidal activity on dandelion and plantain in turfgrass systems, without causing damage to turfgrass species (Stewart-Wade et al. 2002a). Turfgrass field studies have confirmed the efficacy of a granular barley-based formulation of *S. minor* in controlling dandelion and reducing broadleaf weed ground cover (Abu-Dieyeh & Watson 2006: Chapter 5).

The bioherbicide approach has had limited commercial or practical success due to problems in mass production, formulation and commercialization, limited acreage of the host weed, and pathogen-one-weed strategy (Kennedy & Kremer 1996; McFadyen, 1998; Charudattan & Dinoor 2000). A bioherbicide effect may be broaden using a multiple-pathogen strategy with a mixture of host specific pathogens, each one controlling a specific weed within a group of weeds (Chandramohan et al. 2002; Chandramohan & Charudattan 2003) or using a nonspecific plant pathogen like *Sclerotinia* or *Rhizoctonia* to control many weed species after considering the risk off-target effects (Hallett 2005).

Although the host range of *S. minor* as a natural pathogen has been documented (Melzer et al. 1997; Hollowell et al. 2003), the susceptibility of many broadleaf weeds of

turfgrass to *S. minor* (IMI 344141) are not known. This type of knowledge is not only required for efficacy evaluation of *S. minor* under field conditions but also a necessity for any further study on population dynamics of weeds after *S. minor* application. Therefore, experiments were conducted to determine the range of broadleaf weed species that are susceptible to *S. minor* (IMI 344141).

4.3. Materials and Methods

Sclerotinia minor (IMI 344141) was isolated from diseased lettuce plants (*Lactuca sativa* L.) from southwestern Quebec in 1988 and the stock culture was maintained as sclerotia at 4^{o}C. The mycelia of the germinated sclerotia were used to inoculate the autoclaved barley grits (1.4-2.0 mm diameter) as described in Abu-Dieyeh and Watson (2006: Chapter 5)

During the fall (September and October) of 2003 and 2004, a total of 32 broadleaf turfgrass weed species were tested for their susceptibility to the granular barley formulation of *S. minor*. The number of plants tested for each weed species varied (5-22 plants) depending on species occurrence and abundance in the study area. For each species, a cohort of similar age plants (whenever possible) was selected, individual treated plants were labelled and nearby individuals served as controls to aid in assessment of treatment effect. Applications were made on days after an extended rainfall period (2-4 hrs) to ensure adequate soil moisture; otherwise the site was irrigated using a lawn sprinkler. Based on species growth habit and size, the application rate was either 0.5 g per plant (for common burdock, chicory, Canada thistle and field bindweed) or 0.2 g per plant of *S. minor* formulation (for the other studied species). Depending on

the species growth habit, the product was applied on the center of the rosette or around the stem and/or beneath the leaves close to the soil surface. The above ground damage was assessed two weeks after application using 0 to 10 visual scale, where 0 = no or less than 10% damage in aboveground biomass compared to the nearest untreated neighbour plants, 1 = 11-20% ….9 = 91-99% and 10 = 100% collapse of the aboveground biomass. Data were converted back to a percentage (after Schnick et al. 2002). Mortality was assessed three weeks after application. Descriptive statistics (SigmaStat 2.03 statistical software, SPSS 2001) were applied for each species to reflect the adaptability of *S. minor* under field condition.

4.4. Results

Thirty-two species of weeds within 13 families were shown to be susceptible to *S. minor* treatment (Table 4.1). Eight of these species are annuals, three biennial and 21 are perennials. Mean foliar damage ranged from 16.7% for common lambsquarters (*Chenopodium album* L.) and 100% for yellow rocket (*Barbarea vulgaris* R.Br.) and lady's thumb (*Polygonum persicrium*). Three weeks after application, the survival rate of some weeds was 0%, of those: yellow rocket; common chickweed and lady's thumb. On the other side, species like Lamb's quarter, prostrate knotweed, alfalfa and narrowleaf plantain eventhough they are susceptible to *S. minor* infection they are still able to survive at higher rates than other species (Table 4.1).

Table 4.1. Above ground damage and mortality caused by spot treatment with a granular formulation of *Sclerotinia minor* to weeds encountered in turfgrass fields.

Plant species	Life cycle [1]	Treated n[2]	Above ground damage (%)				Survival	
			Mean	S.d.[3]	Min	Max	(n)	(%)
Asteraceae								
Achillea millefollium (common yarrow)	P	21	92.4	11.8	60	100	6	28.6
Ambrosia artemisiifolia (common ragweed)	A	16	75.6	27.1	20	100	6	37.5
Arctium minus (common burdock)	B	15	87.3	12.2	60	100	8	53.3
Chrysanthemum leucanthemum (oxeye daisy)	P	10	94.0	13.5	60	100	2	20.0
Cichorium intybus (chicory)	P	20	87.5	13.3	60	100	8	40.0
Cirsium arvense (Canada thistle)	P	10	74.0	26.7	40	100	5	50.0
Conzya Canadensis (Canada fleabane)	A	13	70.0	33.2	0	100	7	53.8
Erigeron annuus (annual fleabane)	P	5	98.0	4.5	90	100	1	20.0
Pyrrhopappus carolinianus (Carolina false dandelion)	B	10	82.0	17.5	40	100	5	50.0
Sonchus oleraceus (annual sow-thistle)	A	18	95.3	10.9	60	100	3	16.7
Taraxacum officinale (dandelion)	P	20	87.5	12.1	60	100	5	25.0
Brassicaceae								
Barbara vulgaris (yellow rocket)	B,P	11	100.0	0.0	100	100	0	0.0
Capsella bursa-pastoris (shepherd's-purse)	A	13	60.0	30.3	20	100	8	61.5
Caryophyllaceae								
Stellaria media (common chick weed)	A	10	100.0	0.0	100	100	0	0.0
Chenopodiaceae								
Chenopodium album (common lambsquarters)	A	6	16.7	12.1	1	30	5	83.0
Convolvulaceae								
Convolvulus arvensis (field bindweed)	P	14	70.0	30.9	20	100	7	50.0
Fabaceae								
Lotus corniculatus (birdsfoot trefoil)	P	18	90.6	10.6	70	100	5	28.0

Medicago lupine (black medic)	B	10	86.0	13.5	60	100	3	30.0
Medicago sativa (alfalfa)	P	6	23.3	23.4	0	60	6	100.0
Trifolium repens (white clover)	P	22	90.0	13.5	60	100	9	40.9
Vicia sativa (common vetch)	P	14	82.9	17.3	60	100	6	42.9
Lamiaceae								
Glechoma hederacea (ground ivy)	P	13	96.9	6.3	80	100	2	15.4
Malvaceae								
Malva neglecta (common mallow)	P	20	60.5	33.8	0	100	12	60.0
Oxalidaceae								
Oxalis stricta (yellow woodsorrel)	P	15	72.0	27.3	20	100	10	66.7
Plantaginaceae								
Plantago lanceolata (narrowleaf plantain)	P	5	44.0	11.4	30	60	5	100.0
Plantago major (buckhorn plantain)	P	15	86.0	15.0	60	100	6	40.0
Polygonaceae								
Polygonum aviculare (prostrate knotweed)	A	10	45.0	37.5	0	100	7	70.0
Polygonum persicaria (ladysthumb)	A	6	100.0	0.0	100	100	0	0.0
Rumex crispus (curled dock)	P	6	76.7	15.1	60	100	4	66.7
Rosaceae								
Duchesnea indica (Indian mock strawberry)	P	12	90.8	13.1	60	100	3	25.0
Potentilla recta (sulphur cinquefoil)	P	14	80.7	14.4	60	100	7	50.0
Scrophulariaceae								
Linaria vulgaris (yellow toadflax)	P	20	79.0	33.4	20	100	6	30.0

(1): A= annual; B= biennial and P= perennial

(2): n = number of plants treated

(3): s.d. = standard deviation

4.5. Discussion

Weed diversity is highly variable between different turfgrass fields and many factors could affect the diversity including the selection of turfgrass species or cultivar, the chemical and cultural management practices, and the age of turf since establishment (McCarty et al 2001; Busey 2003). Weed seeds could be introduced to the turf at any time by various means of dispersal, but a dense healthy grown turfgrass generally limits weed colonization (Monaco et al. 2002; Busey 2003).

A commercially promising biocontrol agent for turf broadleaf weeds should control the dominant species and suppress associated species. All field sites in the present study were clearly dominated by dandelion, followed by the white clover.

S. minor was virulent on 32 broadleaf weeds, representing many of the common broadleaf weeds in cool season turfgrass environments. Comparison of the efficacy of the fungus on different hosts was not analyzed in this study due to the difference in the time of application within the fall season and the inequality in number of replications between species. High standard deviations of percentage damage were observed among replicate plants in certain species (i.e., *Ambrosia artemisiifolia*, *Malva neglecta*, *Medicago sativa*, *Convolvoulus arvensis*, *Capsella burs-pastoris*, *Conyza canadensis*, *Cirsium arvense* and *Polygonum aviculare*). Several causes could be behind the variability within species response 1) different dew points and soil moisture in the first week of application due to different times of treatment application, 2) the duration that the inoculum remains in direct contact with treated plant, 3) age and biotype variations of the plants and 4) the low number of plants tested for certain species.

Plant survival due to either tolerance after a partial damage or regrowth after a complete damage was variable among weed species ranging from 0% to 100% and this could be the result of the species genotypes and/or the above mentioned factors. Our long-term field results indicated the importance of combining the bioherbicide with proper mowing to control dandelion at a level similar to that of the common widely used herbicide, 2,4-D or improving grass competition by over-seeding (Chapters 6 & 8).

Recorded hosts of *S. minor* include 21 families, 66 genera and 94 plant species, while 19 families are all Dicotyledonae, three plant hosts occurred in the Monocotyledonae (Liliaceae: tulip and asparagus and Muscaceae: banana) (Melzer et al. 1997). Meador & Melouk (2002) mentioned that the host range is as a broad as 222 plant species. Weed species in peanut fields serve as hosts of *S. minor* and aid in maintaining pathogen population in the soil (Meador & Melouk 2002; Hollowell et al 2003). Nine weed species in the present study have been reported as natural hosts of *S. minor* (Melzer et al. 1997; Hollowell et al. 2003) while the other 23, according to our knowledge, are not previously reported as being natural plant hosts of *S. minor*.

Our results indicated that the barley based formulation of *S. minor* is virulent on a wide range of broadleaf weeds with no adverse impact on turfgrass species. From certain fields, sclerotia were seen rarely on the inoculum after the growing mycelia was spread on the weed leaves, however screening of 30 soil samples after one year of treatment application revealed that no sclerotia were available (M.H. Abu-Dieyeh & A. K. Watson, unpublished). Similarly, results from previous experiments showed that sclerotia of *S. minor* do not overwinter in the turfgrass environment and lose their viability within four months (Stewart-Wade et al. 2002a).

The one-weed-one-pathogen strategy has been a major obstacles facing commercialization of bioherbicides (Kennedy & Kremer 1996; Charudattan & Dinoor 2000) which directed researchers to combine a cultural management practice with the bioherbicide to enhance the efficacy and broaden the weed control (Hatcher & Melander 2003). Another approach was the multi pathogen strategy using a mixture of host specific pathogens; each one can control a specific group of weeds (Chandramohan et al. 2002). Recently the use of highly virulent, broad-spectrum bioherbicide has been suggested as an economical and practical alternative after considering the safety release on non-target species (Műller-Scharer et al. 2000; Hallett 2005).

Specifically for turfgrass environment, *S. minor* appears to be a safe biological control agent exerted negative effects on several species of broadleaf weeds through direct infection or indirect ecological effect after creating a new environment that favours the grass growth but not the weeds. The results of this chapter are considered preliminary for further investigations to test the efficacy of *S. minor* on the susceptible weeds using higher number of plant replicates and on different plant biotypes and ages.