WOUNDING OF *CIRSIUM ARVENSE* ENHANCES THE EFFICACY OF *SCLEROTINIA SCLEROTIORUM* AS A MYCOHERBICIDE

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ABSTRACT

Two experiments were conducted in which mycelial fragments of the plant-pathogenic fungus *Sclerotinia sclerotiorum* were applied with an organic food source to *Cirsium arvense* shoots with and without prior wounding. In the first experiment, more shoots developed disease when the fungus was applied to crush wounds on stems (100% of stems diseased) than when applied to the wound of decapitated stems (38%) or to the uppermost leaf axil of decapitated stems (13%). In the second experiment, reduction in the autumnal shoot dry mass of *C. arvense* due to the mycoherbicide (broadcast in early December at 60 kg/ha) was greater when the shoots were wounded with a spade before applying the mycoherbicide (49% reduction) than when not wounded (26%). These results imply a greater susceptibility of wounded *C. arvense* tissues to *S. sclerotiorum* that could be exploited to enhance the efficacy of this fungus as a mycoherbicide.

Keywords: fungi, pathogen, biocontrol, weed, Californian thistle.

INTRODUCTION

Cirsium arvense L. Scop. (Californian thistle) is a serious weed of pastures in New Zealand (Bascand & Jowett 1982) and its tenacity in the face of conventional control methods, particularly in inaccessible hill land, has prompted research into biological control. Phytophagous insects as classical biological control agents for *C. arvense* (Fowler 1999) have not yet proven effective in New Zealand. Plant pathogenic fungi have also been considered as classical biocontrol agents for *C. arvense* (Cunningham 1927; Fowler 1999; Johnston 1990) but to date there have been no successes.

Sclerotinia sclerotiorum (Lib.) de Bary, a naturally occurring pathogen of many weeds (Pennycook 1989), has been shown to have potential as a mycoherbicide for controlling *C. arvense* (Brosten & Sands 1986; Waipara et al. 1993). Considered by some authors to be too risky on account of its wide host range (Cunningham 1927; Johnston 1990), recent research in New Zealand has shown that the risk of additional disease in susceptible crops is low with appropriate management of the treated pasture and that a safety zone around treated pastures would not be necessary (de Jong et al. 2002). Field evaluations of *S. sclerotiorum* against *C. arvense* were first conducted in the USA using a mycelium-on-canola seed formulation (Brosten & Sands 1986), while more recently in New Zealand field tests using granule and water-miscible formulations of mycelium have been conducted (Bourdôt et al. 1993; Bourdôt et al. 1995; Hurrell et al. 2001).

The efficacy of these formulations of *S. sclerotiorum* has been highly variable. For example, Brosten & Sands (1986) obtained kills of *C. arvense* shoot populations ranging from 20 to 80% in different applications of their canola-based preparation. Similarly variable results have also been obtained in field trials in New Zealand. For example, in an experiment in which small fragments of wheat grain (< 3.0 mm diameter) colonised by *S. sclerotiorum* mycelium were applied to *C. arvense* shoots in October and November (spring) over three successive years in three regions, significant reductions

in the ground area covered by the thistle occurred in 67% (12 out of 18) of the applications, and these reductions varied from 19 to 62% (Hurrell et al. 2001).

This variability in field performance may in part be due to a failure of a proportion of the applied inoculum particles to infect the host tissue. Variation in the nutritional environment on the plant surface is an unlikely explanation. Although this fungus requires an exogenous food source to support its saprophytic stage, during which hyphae produce the 'appressoria' that enable penetration of the host cuticle (Purdy 1958), growth-supporting nutrients are supplied in the formulations. A more probable explanation is inadequate hydration at inoculum reception sites on treated plants. Previous studies have shown that creating wounds on susceptible cabbage plants that result in cell content exudation increases the probability of ascosporic infection by *S. sclerotiorum* (Dillard & Cobb 1995; Porter & Powell 1978). We hypothesised that the probability of myceliogenic infection caused by the mycelium-based mycoherbicide formulations of this fungus, and therefore the severity of the disease and its impact on the target weed populations, would also be greater with wounding. The objective of this study was to test this hypothesis.

MATERIALS AND METHODS

Experiment 1

In January 1996 thirty-two separate flowering shoots of *C. arvense* were identified in a grazed pasture at Lincoln (172° 28'E, 43° 38'N), Canterbury. Four treatments replicated eight times were applied to the individual shoots. On 18 January all shoots were decapitated (removing ca one third of the shoot) with a sharp scalpel immediately before applying the treatments to the remainder of each stem: (1) stem crushed with pliers and *S. sclerotiorum* applied into the crushed tissue, (2) *S. sclerotiorum* applied to the cut surface of the decapitated stem, (3) *S. sclerotiorum* applied in the axil of the uppermost leaf and (4) not treated. The *S. sclerotiorum* isolate used, S36, was from a single sclerotium taken from a *C. arvense* stem at Tai Tapu, Canterbury in 1995, and stored at 4°C. The *S. sclerotiorum* applied to the *C. arvense* was formulated by Crop Care Holdings NZ Ltd. as a water-miscible powder comprising mycelial fragments and an organic food source. This powder was mixed with water (15 g/100 ml water) to create a viscous slurry before application to the *C. arvense* shoots with a syringe. Sufficient of the formulated *S. sclerotiorum* was applied to completely occupy the wound sites and leaf axils, with up to twice as much of the slurry being applied to treatment (1) than to treatments (2) and (3).

The treatment effects were assessed on one occasion, six weeks after treatment (1 March 1996). The length of any *S. sclerotiorum* lesion originating at the application site was measured, as was the length of the stem to enable an estimate of the proportion of stem diseased. These data were subjected to an analysis of variance.

Experiment 2

An experiment was conducted in the summer of 1997-98 at Templeton, Canterbury (172° 27'E, 43°C 34'N), in a flood irrigated, sheep-grazed, ryegrass (*Lolium perenne*)-clover (*Trifolium repens*) pasture. A uniform population of *C. arvense* was present at an estimated density of 30 shoots/m^2. Eleven treatments were arranged in plots measuring 6 m x 1.5 m. Four treatments relevant to the current discussion (a 2 x 2 factorial structure) were: (a) no wounding or *S. sclerotiorum*, control, (b) no wounding plus *S. sclerotiorum,* (c) wounding without *S. sclerotiorum* and (d) wounding plus *S. sclerotiorum*. Treatments (a), (c) and (d) were replicated five times and treatment (b) was replicated 10 times in a randomised block arrangement.

The treatments were applied to the *C. arvense* on 8 December 1997 when both vegetative and reproductive shoots (with flower buds) averaging 30 cm in height were present in the plots. Wounding was achieved by hitting every shoot in the plot with a garden spade, creating contusions on the stem and foliage. This procedure was carried out immediately prior to inoculation. Isolate S36 of *S. sclerotiorum* prepared as a slurry (as in experiment 1), was applied under low-pressure (10 kPa) through a 1.5 m-wide

boom equipped with "dripper" nozzles spaced 75 mm apart and calibrated to apply 120 litres/ha. The application rate of 60 kg/ha of formulated *S. sclerotiorum* was achieved by passing the boom over each plot four times giving a droplet density of 1400 droplets/m^2 at an average droplet size of 34 μl.

The effects of the treatments were measured by harvesting the *C. arvense* shoots from two randomly located 0.25 m^2 quadrats per plot on 27 April (autumn) 1998. The resulting biomass was oven dried at 80°C for 24 hours. The dry mass data was transformed to logarithms and subjected to an analysis of variance.

RESULTS AND DISCUSSION

Experiment 1

During the 43-day period of this experiment, rainfall totalling 34 mm occurred on 14 of the days (Table 1). In addition there was potential for dew (when the saturated vapour pressure, at the minimum temperature > vapour pressure + 0.02) on nine of the rainless days giving a total of 23 days on which free moisture was present. Thus free water occurred on about 50% of the days. Mean daily air temperature was within the optimum range of 15–25°C for mycelial growth of *S. sclerotiorum* (Abawi & Grogan 1979) on all but one day during the period.

TABLE 1: Meteorological conditions prevailing during Experiment 1. The number of dew days/month was calculated as the number of days when the vapour pressure exceeded the saturated vapour pressure at the minimum temperature.

	18-31 January 1996	All February 1996
Total rainfall (mm)	1.4	32.3
Mean of maximum temperatures (°C)	21.5	22.2
Mean of minimum temperatures (°C)	12.4	11.6
Rain days (a)	2	12
Dew days	7	7
Rainless dew days (b)	5	4
Wet days (a + b)	7	16

All eight stems that were crushed and had *S. sclerotiorum* applied into the wound became infected, developing large lesions covering on average 69% of the stem length by March, six weeks after treatment (Table 2). Two of the shoots treated in this way had died back completely to ground level. By contrast, only three shoots inoculated onto the horizontal cut made to decapitate the stem, and one shoot where the *S. sclerotiorum* was applied in the leaf axil became infected, developing much smaller lesions. These data provide evidence that *S. sclerotiorum* is more able to infect severely wounded stem tissue in *C. arvense* than intact tissue. This conclusion was made under the assumption that the differences in application dose of the *S. sclerotiorum* between the sites of application did not influence the level of infection obtained. Observations revealed that the *C. arvense* shoots exuded sap after wounding, a response more pronounced on crushed than cut shoots. While this experiment cannot provide evidence of the mechanism of the disease facilitation in the "crushed stem" treatment, it seems likely that the sap provided a highly suitable moisture and/or nutritional environment for the fungus. Additionally, the many ruptures of the cuticle would have allowed direct access by the fungus to the subcuticular tissues within which it is able to grow pathogenically (Lumsden & Dow 1973). In histological studies of *S. sclerotiorum* infection in *Ranunculus acris,* foliage wounded prior to treatment also exhibited higher disease levels than non-wounded foliage (Green et al. 1998; Green et al. 1993).

TABLE 2: Responses of decapitated *Cirsium arvense* stems to applications of *Sclerotinia sclerotiorum* in January 1996 as measured in March 1996 (Experiment 1).

S. sclerotiorum applied to:	No. infected shoots[1]	No. dead shoots[1]	Lesion length (mm)	Percentage of stem diseased
Crushed region of decapitated stem	8	2	27.0	69.0
Wounded surface of decapitated stem	3	0	1.4	3.0
Top leaf axil of decapitated stem	1	0	0	0.3
Control – decapitated stem not treated	1	0	0	0.1

[1]Number of shoots dead or infected out of a total of eight shoots.

Experiment 2

The meteorological conditions that prevailed at the site of Experiment 2 over the period December 1997 to April 1998 are summarised in Table 3. Conditions when free moisture was present, either as dew or rain, occurred infrequently during this experiment. Rainfall during the 4 week period after treatment (8 December until 8 January) was 67% of the long-term average rainfall for this period. During the months of January to April only 34% of the average rainfall for the period was recorded. Free water was predicted to have occurred on foliage (wet-days) on only 11 and 10 days in the first two months respectively of this experiment. The air temperatures during the period were within the optimal range for mycelial growth of *S. sclerotiorum* to occur.

TABLE 3: Meteorological conditions prevailing during Experiment 2.

	December 1997	January 1998	February 1998	March 1998	April 1998
Total rainfall (mm)	41	17	14	31	9
Mean maximum temperature (°C)	22.1	24.5	25.9	23.0	19.1
Mean minimum temperature (°C)	10.2	12.0	13.7	11.2	7.6
Rain days (a)	6	4	4	11	5
Dew days	6	9	9	10	2
Rainless dew days (b)	5	6	5	4	1
Wet days (a + b)	11	10	9	15	6

Sclerotinia sclerotiorum reduced the yield of shoots by 26% (from 188 to 139 g/m^2) in the nonwounded population, but by 49% (from 92 to 47 g/m^2) in the wounded population (Table 4). This interaction was statistically significant ($P<0.10$), providing some support for the hypothesis that severity of the disease caused by *S. sclerotiorum* is enhanced when the fungus is applied to freshly wounded leaf and stem tissue in *C. arvense*. The overall effect of wounding plus *S. sclerotiorum* was a 75% reduction in the autumnal shoot biomass.

The reductions in *C. arvense* biomass due to *S. sclerotiorum*, despite the intermittent occurrence of free external moisture (wet days) (Table 3), indicate that another source of moisture may have been supporting the infections. A probable explanation is that the sap exuding from the wounds made by the spade was trapped along with transpired water in the wounded tissue beneath droplets of the *S. sclerotiorum* formulation that tends to form a crust after application (Hurrell et al. 2001). Because the wounded *C. arvense* shoots were also flattened, a greater surface area was exposed to the *S. sclerotiorum* formulation and hence a greater dose would have been received per shoot. This greater interception of the

S. sclerotiorum in the wounded shoots may also have contributed to the greater effect of the pathogen when applied to wounded tissue.

TABLE 4: Response of the autumnal dry mass of *Cirsium arvense* (g/m²) to *Sclerotinia sclerotiorum* and wounding treatments applied in December 1997 (Experiment 2). Values shown are the back-transformed means of the logarithms of the raw data.

Treatment	Not wounded	Wounded
No *S. sclerotiorum*	188	92
S. sclerotiorum	139	47
LSR (P<0.05)[1]	1.36	
Significance of the wounding x *S. sclerotiorum* interaction	P<0.10	

[1]LSR is the least significant ratio. Two means differ significantly if their ratio exceeds the LSR.

Overall, the two experiments support the hypothesis that wounding enhances the ability of *S. sclerotiorum* to cause disease in *C. arvense* plants. Therefore, mechanical wounding of the thistle prior to application of mycoherbicide preparations of the fungus may be a worthwhile field practice.

ACKNOWLEDGEMENTS

The authors acknowledge Christine Galbraith for assistance with the field study, and Dr Ian Harvey (Plantwise) and John Lloyd (Crop Care Holdings NZ Ltd) for assistance with formulation development.

REFERENCES

Abawi, G.S.; Grogan, R.G. 1979: Epidemiology of diseases caused by *Sclerotinia* species. *Phytopath. 69*: 899-903.

Bascand, L.D.; Jowett, G.H. 1982: Scrubweed cover of South Island agricultural and pastoral land 2. Plant distribution and managerial problem status. *N.Z. J. Exp. Agric. 10*: 455-492.

Bourdôt, G.W.; Harvey, I.C.; Hurrell, G.A.; Alexander, R.T. 1993: An experimental mycoherbicide utilizing *Sclerotinia sclerotiorum* controls pasture populations of *Cirsium arvense* in Canterbury. *Proc. 46th N.Z. Plant Prot. Conf. 46:* 251-256.

Bourdôt, G.W.; Harvey, I.C.; Hurrell, G.A.; Saville, D.J. 1995: Demographic and biomass production consequences of inundative treatment of *Cirsium arvense* with *Sclerotinia sclerotiorum*. *Bioc. Sci. and Tech. 5*: 11-25.

Brosten, B.S.; Sands, D.C. 1986: Field trials of *Sclerotinia sclerotiorum* to control Canada Thistle *(Cirsium arvense). Weed Sci. 34*: 377-380.

Cunningham, G.H. 1927: Natural control of weeds and insects. *N.Z. J. Agric. 20*: 244-251.

de Jong, M.D.; Bourdot, G.W.; Hurrell, G.A.; Saville, D.J.; Erbrink, H.; Zadoks, J.C. 2002: Risk analysis for biological control - simulating dispersal of *Sclerotinia sclerotiorum* (Lib.) de Bary ascospores from a pasture after biological control of *Cirsium arvense* (L.) Scop. *Aerobiologia 18*: 211-222.

Dillard, H.R.; Cobb, A.C. 1995: Relationship between leaf injury and colonization of cabbage by *Sclerotinia sclerotiorum*. *Crop Prot. 14*: 677-682.

Fowler, S.V. 1999: Interim reports on worldwide progress with the biological control of californian thistle, *Cirsium arvense*. Landcare Research Contract Report: LC 9900/23. Landcare Research, Lincoln, New Zealand. 18 p.

Green, S.; Gaunt, R.E.; Harvey, I.C.; Bourdôt, G.W. 1998: Histopathology of *Ranunculus acris* infected by a mycoherbicide, *Sclerotinia sclerotiorum*. *Aust. Plant Path. 27*: 73-79.

Green, S.; Saville, D.J.; Bourdôt, G.W.; Harvey, I.C.; Field, R.J.; Close, R.C. 1993: Regeneration sites of *Ranunculus acris* are the target for *Sclerotinia sclerotiorum*, a potential mycoherbicide. *Proc. 46th N. Z. Plant Prot. Conf. 46:* 274-277.

Hurrell, G.A.; Bourdôt, G.W.; Saville, D. 2001: Effect of application time on the efficacy of *Sclerotinia sclerotiorum* as a mycoherbicide for *Cirsium arvense* control in pasture. *Biocontrol, Sci. Technol. 11*: 317-330.

Johnston, P.R. 1990: Potential of fungi for the biological control of some New Zealand weeds. *N.Z. J. Agric. Res. 33*: 1-14.

Lumsden, R.D.; Dow, R.L. 1973: Histopathology of *Sclerotinia sclerotiorum* infection in bean. *Phytopath. 63*: 708-715.

Pennycook, S.R. 1989: Plant diseases recorded in New Zealand. DSIR, Auckland. 502 p.

Porter, D.M.; Powell, N.L. 1978: Sclerotinia blight development in peanut vines injured by tractor tires. *Peanut Sci. 5*: 87-90.

Purdy, L.H. 1958: Some factors affecting penetration and infection by *Sclerotinia sclerotiorum*. *Phytopath. 48*: 605-609.

Waipara, N.W.; Harvey, I.C.; Bourdôt, G.W. 1993: Pathogenicity of *Sclerotinia sclerotiorum* on common thistle species and other pasture weeds. *Proc. N.Z. Plant Prot. Conf. 46:* 261-264.