AN EXPERIMENTAL MYCOHERBICIDE UTILIZING *SCLEROTINIA SCLEROTIORUM* CONTROLS PASTURE POPULATIONS OF *CIRSIIUM ARVENSE* IN CANTERBURY

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SUMMARY

The adventitious shoots of *Cirsium arvense* (Californian thistle) in sheep-grazed pastures in Canterbury were treated in October, November or October and November 1991 with a mycelium-on-wheat formulation of *Sclerotinia sclerotiorum*. Three sites were used. Untreated thistles covered 51.6% of the ground in January 1992, as compared to 28.5%, 21.7% and 16.7% with treatment in November, October or October and November respectively. In December 1992, in the growing season after treatment, the ground area covered by *C. arvense* was again lower on treated plots and the shoots were both fewer and smaller, indicating that the thistle's root system had been debilitated by the *S. sclerotiorum*. The potential of *S. sclerotiorum* as a mycoherbicide for *C. arvense* in New Zealand is discussed.

Keywords: *Cirsium arvense*, Californian thistle, *Sclerotinia sclerotiorum*, mycoherbicide, weed control.

INTRODUCTION

Cirsium arvense L. (Scop.) (Californian thistle) is a perennial weed widely distributed in the temperate agricultural zones of both hemispheres (Holm *et al.* 1977). In New Zealand it is a serious weed in pastures and crops (Cockayne 1917; Bascand and Jowett 1982; Bourdôt and Kelly 1986) where population increase occurs by the production of adventitious shoots from the creeping root system and by the establishment of seedlings. Despite recommendations involving cultivation, herbicides and grazing (Hartley and Butler 1984; Hartley *et al.* 1984; Meeklah and Mitchell 1984), *C. arvense* is rarely adequately controlled. Furthermore, the herbicides commonly used against the weed in pastures (MCPA, 2,4-D, dicamba) remove nitrogen-fixing *Trifolium* species from the treated sward. Three exotic phytophagous insects (*Urophora cardui*, *Lema cyanella*, and *Altica carduorum*) have been released in New Zealand as "classical" biological control agents (Jessep 1989). *L. cyanella* only has established but to date has not controlled the thistle (Jessep 1989).

A mycelial/whole wheat grain preparation of *Sclerotinia sclerotiorum* (Lib.) de Bary, a common fungal pathogen of *C. arvense* and several important crop species (Pennycook 1989), was field-tested as a biological herbicide against the thistle in Montana USA in the early 1980s (Brosten and Sands 1986). Shoot mortalities ranged from 20% to 80% in the season of application and lower shoot densities on treated plots the year following treatment indicated that the root system had also been affected. This early work gave cause for optimism that this pathogen could be used as a mycoherbicide against this weed. The present contribution reports on the effects of applications of an experimental formulation of *S. sclerotiorum* on *C. arvense* in sheep pastures in Canterbury.

METHODS

The experiment was conducted at three sites in *Lolium perenne* L./*Trifolium repens* L. pastures at the AgResearch Experimental farm at Templeton from October

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1991 until September 1992. The pastures were ca. 10 years old and supported extensive and dense populations of *C. arvensis*, usually managed by annual mowing at flowering. This mowing was withheld from the experimental plots. The pastures were flood-irrigated several times during the summer and were rotationally grazed by sheep.

At Site 1 three treatments were allocated randomly to four replicate blocks. The treatments were untreated control, *S. sclerotiorum* applied on 12 October 1991 to the newly emerged spring cohort of adventitious shoots, and *S. sclerotiorum* applied on 12 October followed by retreatment on 15 November when stem elongation and flower bud initiation were occurring in the thistle. At Sites 2 and 3 an additional treatment on 15 November only was included. At these sites there were two untreated plots and two 15 November treatments in each block; the other treatments occurred once only in each block. The *S. sclerotiorum* was broadcast by hand (using jars with perforated lids) onto plots 3 m long and 1.3 m wide, as a dried mycelium-on-kibbled wheat preparation at 50 g/m². Each plot was separated from its neighbour by a 1 m wide untreated buffer. To ensure that the preparation adhered to the thistle foliage, the plots were sprayed immediately before treatment with 200 litres/ha of water through Teejet 8003 hydraulic fan nozzles mounted on a nitrogen-gas-powered boom.

Dry northerly winds prevailed for several days immediately following both application times but frequent showers of rain occurred throughout the following spring and summer months.

The preparation applied to the plots used a wild strain of *S. sclerotiorum* (S9) collected from *C. arvensis* in Canterbury in the summer of 1990. The production and some characteristics of the formulation are described by Bourdôt *et al* (in prep.). When plated on potato/dextrose agar at the time of field application the preparation had 15,000 colony forming units/gram. At the field application rate of 50 g/m², this equates to 77 colony forming units/cm² of treated surface.

Development of the disease was assessed qualitatively at regular intervals after application. On 28 January 1992, 15 and 11 weeks after the October and November applications respectively, the percentage of ground area covered by the thistle was estimated visually by two observers. The data were pooled over the three sites and subjected to analysis of variance with four treatments and 12 blocks; the missing treatment at site 1 was adjusted for using a missing plot technique. Additionally, on 14 December 1992, the cover of thistle and the population density of adventitious shoots were measured at Site 1.

RESULTS

Within 7 days of application, leaf and stem lesions caused by *S. sclerotiorum* were apparent on most of the thistle shoots in the treated plots. Typically lesions developed from the granules of inoculum on the upper surface of lower leaves close to the junction with the stem. These lesions then increased in size and enveloped the stem. Shoot growth slowed markedly, the upper leaves which developed after treatment became chlorotic, and whole shoots soon wilted and died. Regular censuses of the populations (data not given here) revealed that most of the deaths due to the pathogen occurred within 30 days of treatment. Regrowth from axillary buds on stems below the ground occurred only rarely. In all cases when this was observed, the regrowing shoots soon died as the pathogen invaded the new tissue from the dead "parent" tissue. The *T. repens* in the treated swards was largely unaffected, although some small lesions did occur. The grasses showed no symptoms of disease.

The percentage of ground covered by the thistle on 28 January 1992, averaged over the three sites is given in Figure 1. All treatments reduced the ground covered by the thistle ($P < 0.01$). Application in October reduced the area covered by thistle to 42%, in November to 55%, and in October and November to 32% of untreated. The last two of these treatments differed significantly ($P < 0.01$).

The percentage of ground covered by the thistle and the density of shoots on the 14 December 1992 in the growing season after treatment, is given for Site 1 (Fig. 2b).

Figure 1: The effect of timing of applications of the experimental formulation of *Sclerotinia sclerotiorum* in 1991 on the ground cover of *Cirsium arvense* in January 1992. The data given are the means over the three sites. Vertical lines are LSD($P<0.05$) values. The lefthand LSD is for comparing the October treatment with the double October/November treatment. The righthand LSD is for comparing the November treatment with untreated. The middle LSD is for all other comparisons.

The cover of thistle in the previous year (treatment year) is given for comparison (Fig.2a). The effects on thistle ground cover in the year of treatment at Site 1 mimic the effects averaged over the three sites (Fig.2a cf. Fig.1). The October and October plus November treatments reduced the thistle cover to 39% and 24% of untreated respectively by 28 January ($P<0.01$); the difference between the two treatments was not statistically significant (Fig. 2 a).

The treatment effects were evident again in the year after treatment (Fig.2b). On 14 December, 14 and 13 months respectively after the October and November 1991 treatments, the ground cover of the 1992 population of thistle shoots on the treated plots was only 19% of untreated (Fig.2b). In addition the densities of the shoot populations in December 1992 on the plots treated in October and October plus November 1991, were 48% and 39% of untreated respectively. Since the proportional reduction was less for shoot density than it was for percentage ground cover, the shoots apparently had less leaf area on the treated plots than on the untreated in the year after treatment. These shoots were less than half as tall as those on the untreated plots and flowered later and less profusely.

DISCUSSION

In this experiment the fungal pathogen *S. sclerotiorum* applied to *C. arvense* foliage as a mycelium-on-kibbled wheat formulation was effective in reducing the pasture cover of the thistle in the year of application. It killed a high proportion of the shoots present at the time of application. The effect of the pathogen was evident again in summer of the year after treatment when the treated shoot population consisted of fewer shoots. This implies that the regenerative capacity of the root system had been

Figure 2: The effect of spring applications of the experimental formulation of *Sclerotinia sclerotiorum* in 1991 on *Cirsium arvense* at Site 1; a) % ground cover of *C. arvense* shoots on 28 January 1992 and b) % ground cover and density of shoots on 14 December 1992; the year after treatment. The vertical lines to the left of the lefthand bars in a) and b) are the LSD(P<0.05) values for comparing % ground covers between treatments; the line to the right of the lefthand hatched bar in b) is the LSD(P<0.05) for comparing shoot densities between treatments.

reduced by the pathogen. Bourdôt *et al* (in prep.) have shown this to be due to ca. 70% reduction in the biomass of roots by the autumn following treatment.

Several explanations seem possible for the smaller size and developmental delay of shoots on the treated plots in the year after treatment. While root death provides an explanation for the lower density of shoots in the season after treatment, it's difficult to see how root death *per se* could result in smaller or developmentally delayed shoots in the year after treatment. But if the reduction in root biomass in the year of treatment had occurred predominantly in the upper soil layer, then in the next year a higher

proportion of shoots would arise from deeper depths than is usual. These might be expected to be later emerging than shoots from more shallow depths, and therefore smaller and slower developing. Another possible explanation is that the pathogen caused disease in the thistle in the year after treatment; infection could have occurred from overwintering mycelium in the roots, from myceliogenic germination of sclerotia formed in the treatment year, or from ascospores produced following carpogenic germination of the soil-borne sclerotia. There was evidence of disease "carry-over" in the thistle since sclerotia were found in 3% of the senescing stems present on the treated plots in May 1993 (Harvey *et al.* 1993). The precise reason for reduced shoot vigour the year after treatment, will remain obscure until more definitive studies, which are currently underway, are completed.

The results from this first experimental use of a fungus as a biological herbicide in New Zealand, show that *S. sclerotiorum* has potential for controlling *C. arvense* in pastures. The lack of any substantial effect on pasture grasses and clovers contrasts with the debilitation of clovers which follows the use of other pasture herbicides such as the phenoxy acetic acids (Hurrell and Bourdôt 1993). Its wide host range, which includes many weeds in addition to *C. arvense* (Pennycook 1989), maybe a "two-edged-sword" for whilst this widens its market potential as a bio-herbicide, it carries with it the risk of disease incitement in adjacent or subsequent susceptible crops. This risk may be averted by spatial and temporal containment of the pathogen in the inoculated field by employing auxotrophic or nonsclerotial mutants (Miller *et al.* 1989a,b; Sands *et al.* 1990) or heterokaryons (Forde *et al.* 1992) rather than wildtypes. However the risk of using a wildtype *S. sclerotiorum* is unknown, except in a qualitative sense. Research is therefore required to evaluate this risk. It does not necessarily follow that a mycoherbicide based on a fungus with a broad host range presents an unacceptable risk to susceptible crops. The risk analysis conducted by de Jong *et al.* (1990) showed that the widely occurring fungus *Chondrostereum purpureum*, which causes silverleaf disease, could be safely used as a mycoherbicide against the weedy tree *Prunus serotina* in pine forests in the Netherlands without increasing the risk of the disease in adjacent fruit growing areas.

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