A review of recent research on the microbial control of Californian thistle and other pasture weeds using the fungus *Sclerotinia sclerotiorum* as a biological herbicide

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Abstract

In this paper we review the research findings published to date on the development of a plant pathogenic fungus, *Sclerotinia sclerotiorum,* as a mycoherbicide for Californian thistle, *Cirsium arvense,* and other weeds of pastures. A strain of the fungus isolated from Californian thistle was virulent also on Scotch thistle *(Cirsium vulgare)*, nodding thistle *(Carduus nutans)* and ragwort *(Senecio jacobaea)* when applied as a mycelium-on-wheat formulation to the foliage of these weeds under glasshouse conditions. In field trials in which this formulation of the mycoherbicide was applied to the new spring foliage of Californian thistle in Canterbury sheep pastures, the thistle was controlled, to a high level both in the season of application and during the following growing season. Debilitation of root systems of the thistle occurs through reductions in photosynthetic capacity through death of treated shoots, and by invasion and rotting of roots by the pathogen. The strain of the fungus used in the field trials did not infect either grasses or clovers. The results of research to date indicate that *S. sclerotiorum* has considerable potential as a mycoherbicide for use in pastures against Californian thistle and some other species of weeds

Keywords: biological weed control, *Carduus nutans, Cirsium arvense, Cirsium vulgare*, mycoherbicide, *Sclerotinia sclerotiorum, Senecio jacobaea*

Introduction

Many weeds impede pastoral farming in New Zealand and one of the most important is Californian thistle, *Cirsium arvense,* a perennial widely distributed in the temperate agricultural zones of both hemispheres (Holm et al. 1977). In New Zealand it occurs in pastures and crops (Cockayne 1917; Bascand & Jowett 1982; Bourdôt & Kelly 1986) where population increase occurs by adventitious shoots from the creeping root system and by the establishment of seedlings on open land. Despite recommendations involving cultivation, herbicides and grazing (Hartley & Butler 1984; Hartley et al. 1984; Meeklah & Mitchell 1984). Californian thistle is rarely adequately controlled. Furthermore, the herbicides commonly used against the weed in pastures (MCPA, 2,4-D, dicamba) remove nitrogen-fixing clovers from the treated sward, reducing pasture production. As a consequence of the tenacity of this weed, the 'classical' biological control approach has been attempted. To this end three exotic phytophagous insects *(Urophora cardui, Lema cyanella, and Altica carduorum)* have been released in New Zealand over the last 19 years (Jessep 1989). Unfortunately *L. cyanella* only has established but to date has not controlled the thistle (Jessep 1989). An alternative biological approach was first investigated about 10 years ago in the USA when a soil-applied mycelial/whole wheat grain preparation of *Sclerotinia sclerotiorum* (Lib.) de Bary, a common fungal pathogen of Californian thistle and several important crop species (Pennycook 1989), was field-tested as a mycoherbicide against the thistle in Montana (Brosten & Sands 1986). However, because of the variable results in the field, and the very high application rates (500 to 1000 kg/ha) as a consequence of using whole-seeds-as-the food source/carrier for the fungus, commercial interest in the USA declined and a mycoherbicide based on S. *sclerotiorum* was never developed..

Research began in New Zealand on *S. sclerotiorum* and its use as a mycoherbicide in 1989 following the success of greenhouse trials on Californian thistle using a foliage applied mycelium-on-kibbled wheat formulation. In this paper we summarise the research published to date and discuss the potential and limitations of this broad-host-range pathogen for controlling Californian thistle and several other herbaceous pasture weeds.

Experiments on the pathogenicity of S. sclerotiorum on thistles and other pasture weeds

Waipara et al. (1993) reported the results of two experiments in which an isolate of *S. sclerotiorum* (S9) from a Californian thistle plant in Canterbury, was

tested for virulence on several weed species. Inoculum containing about 15 000 colony forming units per gram was prepared by growing the pathogen on kibbled wheat and milling to a tine granule. This was applied to pre-misted plants in a glasshouse at an equivalent rate of 50 g/m². In the first experiment 12 plants (one per pot) of each of the following weeds were inoculated: Californian thistle, nodding thistle (Carduus nutans), winged thistle (Carduus tenuiflorus), Scotch thistle (Cirsium vulgare), and broad-leaved dock (Rumex obtusifolius). In the second experiment, eight plants each of Californian thistle, hawksbeard (Crepis capillaris), catsear (Hypochaeris radicata), dandelion (Taraxacum officinale) and ragwort (Senecio jacobaea) were inoculated. Mortality was assessed 12 and 18 days after inoculation in Experiments 1 and 2 respectively. The S. sclerotiorum infected all species, but some were more affected than others with symptoms ranging from superficial lesions on leaf laminae to general necrosis and death of whole plants. Californian, Scotch and nodding thistles and ragwort had high levels of mortality (Table 1). However, there was no mortality in broadleaved dock, hawksbeard, dandelion or catsear although the pathogen caused variable levels of leaf necrosis in these species.

Table 1 Plant mortality in nine species of weeds following inundative foliage inoculation with a mycelium-on-wheat formulation of *Sclerotinia sclerotiorum* under glasshouse conditions. Mortalities were determined 12 and 18 days after inoculation in Experiments 1 and 2 respectively. (Adapted from Waipara *et al.* 1993).

	Mortality (%)
Experiment 1	
Californian thistle (*Cirsium arvense*)	100
Scotch thistle (Cirsium *vulgare*)	75
nodding thistle (*Carduus nutans*)	58
winged thistle (*Carduus tenuiflorus*)	8
broad leaved dock (*Rumex obtusifolius*)	0
Experiment 2	
Californian thistle	100
Ragwort (Senecio jacobaea)	50
Hawksbeard (*Crepis capillaris*)	0
Dandelion (Taraxacurn *officinale*)	0
Catsear (Hypochaeris *radicata*)	0

## Evaluation of S. *sclerotiorum* for Californian thistle control in pastures

An experiment was conducted by Bourdôt *et al.* (1993) at three sites in *Lolium perenne/Trifolium repens* pastures at Templeton from October 1991 until September 1992. They were 10 years old and supported extensive populations of C. *arvense*. They were flood-irrigated several times during the summer and were rotationally grazed by sheep.

At site 1 three treatments were replicated four times; (1) untreated control, ('2) S. sclerotiorum applied on 12 October 1991 to the newly emerged spring cohort of adventitious shoots, (3) 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 a fourth treatment on 15 November only was included. The treatments used the formulation described above which was broadcast by hand at 50 g/m² onto small plots. Other experimental details are described by Bourdôt *et al.* (1992).

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 adhering to 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 and the upper leaves developed after treatment became chlorotic and whole shoots soon wilted and died. Most deaths due to the pathogen occurred within 30 days of treatment. Regrowth from axillary buds on stems below the ground rarely occurred; any regrowing shoots soon died as the pathogen invaded the new tissue from the dead parent tissue.

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. (From Bourdôt *et al.* 1993).
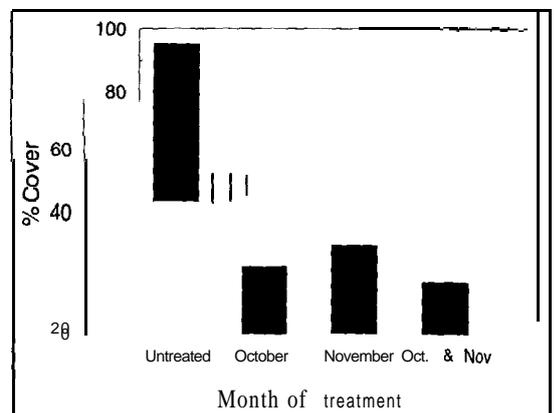
Averaged over the three sites, all treatments significantly reduced the ground area covered by the thistle when measured on 28 January 1992 in the season of treatment but there were no significant differences between the treatments (Figure 1). Application in October reduced the area covered by thistle to 42%, in November to 55%, and in October and November to 32% of untreated.

The percentage of ground area 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 in Figure 2. The effects on thistle ground cover in the year of treatment at Site 1 mimic the effects averaged over the three sites (Figure 2a cf. Figure 1). 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 (Figure 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.

Selectivity of *S. sclerotiorum* in grass/clover pasture

Hurrell & Bourdôt (1993) conducted an experiment on a Templeton silt loam in Canterbury to compare the effects of S. *sclerotiorum* and MCPA (the usual treatment for this weed) on pasture grasses and clovers. The pasture was 12 years old and contained perennial ryegrass (cv. Ellett) and white clover (cv. Grasslands Huia); Californian thistle was absent. Two treatments were applied on 2 November 1992; (1) MCPA at 1.5 kg/ha (IWD MCPA, 375 g/litre) and (2) the mycelium-on-wheat preparation of *S. sclerotiorum* described above at 500 kg/ha. An untreated control was also included. Other experimental details are given in Bourdôt & Hurrell (1993).

The dry matter yields of white clover and ryegrass on four harvest occasions are given in Figure 3. The yield of white clover was greatly reduced by MCPA at each time, The percentage reductions were 85%, 90%, 74% and 71% at 23, 65, 118 and 168 days after treatment (DAT) respectively. There was no evidence that *S. sclerotiorum* reduced the clover yields. Neither MCPA nor *S. sclerotiorum* reduced ryegrass yields.

This experiment demonstrated the severe effects

Figure 2 The effect of spring applications of the experimental formulation of *Sclerotinia sclerotiorum* in 1991 on *Cirsium* awense 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. (From Bourdôt et al. 1993).
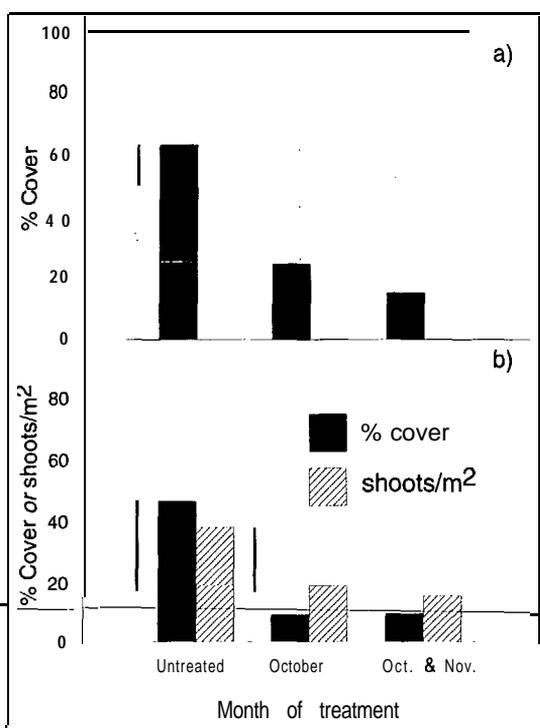
of MCPA on clovers that have been documented by others (Thompson 1956; Maclean 1957; Meeklah 1958; Meeklah & Mitchell 1984; Honore *et al.* 1980) but provided no evidence that the *S. sclerotiorum* affected-white-clover production. The effect-of MCPA on the clover lasted almost the entire growing season. Previous research has attempted to determine the impact of this on livestock production. Hartley & Thomson (1982) reported that repeat applications of MCPA at 1 kg/ha, reducing clover cover by 37%. caused an 18% reduction in liveweight gain in young dairy cattle. Lewis (1956) showed that a single application of MCPA at 1.1 kg/ha gave approximately 75% reduction in clover productivity resulting in a 15% reduction in liveweight gain in pre-weaned lambs during the month immediately following treatment.

Figure 3 The effect of *Sclerotinia sclerotiorum* and MCPA on the productivity of ryegrass and clover on four occasions after treatment. Yields given as back-transformed means of the logarithms ($\log_{10}$) of the data. Values above each group of three treatments are the Least Significant Ratios (P<0.05). Treatment means are significantly different if the ratio between them is greater than the LSR (e.g. for the first occasion, one clover dry matter mean differs significantly from another mean if it is 1.8 times greater). (From Hurrell & Bourdôt 1993).
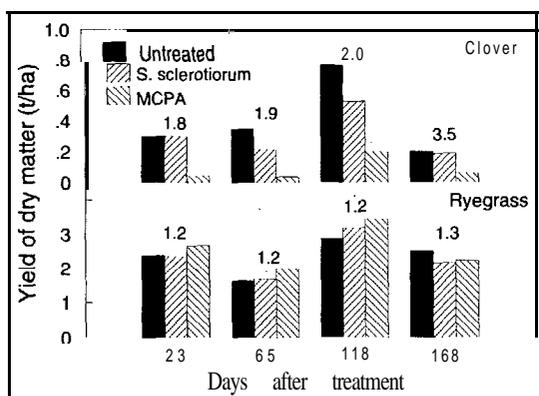
Discussion

The term mycoherbicide is applied to fungal plant pathogens when used inundatively to control a weed population. The chose? pathogens are often already present in the country or region but affect the target weed only spasmodically as a result of natural constraints to epidemic development. This approach to biological weed control stands in contrast to the more conventional "classical" biological control where the pathogen (or other agent e.g. an insect) is imported from another country or region and is released, after thorough testing, to build up population levels that eventually may suppress populations of the target weed.

The pathogen we have chosen as a mycoherbicide for pasture weed control in New Zealand is *S. sclerotiorum.* Its advantages are the ease with which its mycelial phase can be cultured en masse and its naturally high pathogenicity towards a variety of pasture weeds. It appears from initial virulence tests *with S. sclerotiorum* (strain S9) that in addition to Californian thistle, from which this strain was obtained, Scotch thistle, nodding thistle and ragwort are also excellent candidates for a mycoherbicide using this pathogen (Table 1). The last two species were hitherto unknown as hosts of S. *sclerotiorum*

(Pennycook 1989; Farr *et al.* 1989). Although there was no mortality in broadleaved dock, hawksbeard, dandelion or catsear, and only 8% mortality in winged thistle, all plants of these species were infected by the pathogen. Neither catsear nor hawksbeard have been recorded as hosts to *S. sclerotiorum* (Pennycook 1989; Farr *et* al. 1989). Other strains may be more effective on these weeds than strain S9 used in the experiments. This contention is supported by recent research by Riddle *et al.* (1991) showing that the mortality rates of dandelion in turfgrass after application of different strains of *S.* sclerotiorum, was positively-correlated with the *in vitro* virulence of the strains; mortality in the field was as high as 81% with the most virulent strain.

To date only Californian thistle has been the subject of field evaluations and the results have been most encouraging (Figures 1 and 2). When applied as a mycelium-on-kibbled wheat formulation to the foliage of the thistle in spring, *S. sclerotiorum* (S9) was effective in reducing the pasture cover of the thistle in the year of application. It achieved this by killing 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 reduced by the pathogen. This effect on the roots was probably due in part to the reduction in photosynthetic capacity of the thistle stands which would have resulted in reduced ability to support current roots and reduced ability to produce new roots. In addition the pathogen invades the roots down to a depth of at least 300 mm (Bourdôt *et al.* 1994) causing them to rot.

The shoots emerging on treated plots the year after treatment were developmentally delayed and several explanations seem possible for this. While root death provides an explanation for the lower density of shoots in the season after treatment, it is difficult to see how root death *per* se could result in smaller or develop-mentally delayed shoots in the year after treatment. A possible explanation is that the pathogen persisted in root tissue over winter, and weakened the spring shoots by continuing to destroy feeding roots in the year after treatment. New infections may also have occurred from overwintering sclerotia, and weakened the developing shoots. There was however no evidence of shoot mortality due to *S. sclerotiorum* on the treated plots the year after treatment. The reason for reduced shoot vigour the year after treatment, will remain obscure until more definitive studies, which are currently underway, are completed.

The mycelium-on-wheat formulation used in this research was applied in the field at 500 kg/ha. Application rates as high as this are obviously neither feasible nor would they be commercially viable broadcast over large areas of pasture. A food source must be applied along with the fungal mycelium in order to allow a period of saprophytic growth to facilitate eventual pathogenesis. The research to date has used kibbled wheat but AgResearch has recently entered into a 3-year research partnership with ICI Crop Care to develop a formulation that has both the necessary biological characteristics and commercial viability.

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 Californian thistle and other weeds in pastures. The lack of any substantial effect on pasture grasses and clovers contrasts with the debilitation of clovers which follows the use of MCPA (Figure 3). Its wide host range 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; 1989b; Sands et al. 1990) or heterokaryons (Forde et al. 1992) rather than wildtypes. However the risk of using a wildtype *S. sclerotiorum* is unknown, expect 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 in fruit trees, 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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