The potential of the fungus *Sclerotinia sclerotiorum* as a biological herbicide for controlling thistles in pasture

G.W. Bourdôt and **I.C. Harvey**, AgResearch, PO Box 60, Lincoln, New Zealand.

Summary

Research is reviewed on the development of *Sclerotinia sclerotiorum*, isolated form Californian thistle (*Cirsium arvense*) as a biological herbicide for controlling thistles in pastures. It has been shown, in glasshouse tests, to be virulent against two other thistles (*C. vulgare* and *Carduus nutans*), but not against *Carduus tenuiflorus*. In field trials against *C. arvense* it causes high mortality of vegetative shoots, thus reducing the cover of the thistle foliage. Only 8% of treated shoots survived to flowering compared to 28% on untreated plots. Root biomass is also reduced, resulting in lower shoot growth in the season following treatment. The fungus did not infect the key pasture species *Lolium perenne* or *Trifolium repens*. The potential for the development of *S. sclerotiorum* as a mycoherbicide against *Cirsium arvense, C. vulgare* and *Carduus nutans* is discussed.

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 and Jowett 1982, Bourdôt and Kelly 1986) where population increase occurs by recruitment of adventitious shoots from the creeping root system, and by the establishment of seedlings on open land. Despite recommendations involving cultivation, herbicides and grazing (Hartley and Butler 1984, Hartley *et al.* 1984, Meeklah and Mitchell 1984), Californian thistle is rarely adequately controlled. Furthermore, the herbicides commonly used against the weed in pastures (MCPA, 2,4-D and 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 21 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 12 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, many other weeds, and several important crop species (Pennycook 1989), was field-tested as a mycoherbicide against the thistle in Montana (Brosten and Sands 1986). However, because of the variable results in the field, and the very high application rates 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 not developed.

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

Experiments on the pathogenicity of *S. sclerotiorum* on thistles

Waipara *et al.* (1993) reported the results of an experiment in which an isolate of *S. sclerotiorum* (S9) from a Californian thistle plant in Canterbury, was tested for virulence on four thistle species. Inoculum containing about 15 000 colony forming units per gram was prepared by growing the pathogen on kibbled wheat and milling to a fine granule. This was applied to premisted plants in a glasshouse at an equivalent rate of 50 g m^{-2}. Twelve plants (one per pot) of each of the following weeds were inoculated: Californian thistle, nodding thistle (*Carduus nutans*), winged thistle (*Carduus tenuiflorus*) and Scotch thistle (*Cirsium vulgare*). Mortality was assessed 12 days after inoculation. 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 all had high levels of mortality whereas mortality was low in winged thistle (Table 1).

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 in Canterbury from October 1991 until September 1992. The pastures were 10 years old and supported extensive populations of *C. arvense*. The pastures were flood-irrigated several times during the summer and were rotationally grazed by sheep.

At site 1 three treatments were replicated four times: (i) untreated control, (ii) *S. sclerotiorum* applied on 12 October 1991 to the newly emerged spring cohort of adventitious shoots, (iii) *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 m^{-2} onto small plots. Other experimental details are described by Bourdôt *et al.* (1992).

Within seven 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 leaves which developed after treatment became chlorotic and whole shoots soon wilted and died.

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. Root dry weights measured at the end of the growing season in late autumn revealed that roots had been affected to similar extent as the aerial shoots (Bourdôt *et al.* 1996).

The percentage of ground area covered by the thistle and the density of shoots on 14 December 1992 in the growing season

Table 1. Mortality in four thistle species following inundative foliage inoculation with a mycelium-on-wheat formulation of *Sclerotinia sclerotiorum* under glasshouse conditions. Plant mortalities were determined 12 days after inoculation. (Adapted from Waipara *et al.* 1993).

	Mortality (%)
Californian thistle (*Cirsium arvense*)	100
Scotch thistle (*Cirsium vulgare*)	75
Nodding thistle (*Carduus nutans*)	58
Winged thistle (*Carduus tenuiflorus*)	8

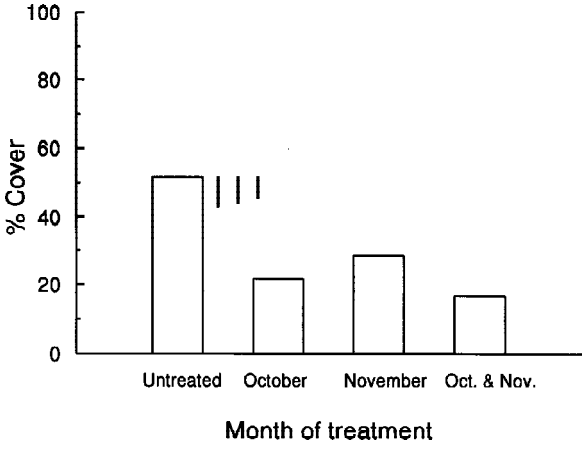

Figure 1. The effect of timing of applications of the experimental formulation of *S. sclerotiorum* in 1991 on the ground cover of *C. arvense* in January 1992. The data given are the means over the three sites. Vertical lines are LSD (P<0.05) values. The lefthand LSD is for comparing the October treatment 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).

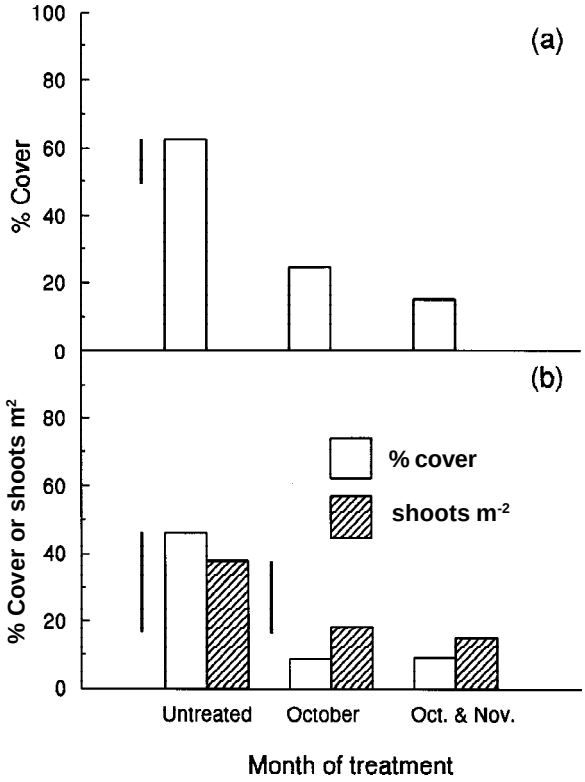

Figure 2. The effect of spring applications of the experimental formulation of *S. sclerotiorum* in 1991 on *C. arvense* at Site 1; (a) % ground cover of *C. arvense* shoots on 28 January 1992 and (b) % ground cover and density of shoots on 14 December 1992; the year after treatment. The vertical lines 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. (From Bourdôt *et al.* 1993).

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 also less than half as tall as those on the untreated plots and flowered later and less profusely.

Demographic analysis of the treated and untreated populations was conducted by mapping all shoots in permanent sample areas with the plots. Three life history stages were recognised; vegetative shoots, shoots with flower buds and flowering / seeding shoots. This analysis revealed that the vegetative shoot stage to budding shoot stage was the most affected transition; only 13% of shoots surviving to become budders on treated plots, compared to 32% on untreated plots (Figure 3). A greater mortality also occurred at the flower bud stage on treated plots. The net effect of the treatment was that only 8% of adventitous shoots emerging during the season survived to flowering on treated plots compared to 28% on untreated plots. Most deaths due to the pathogen occurred within 35 days of treatment and all were restricted to shoots present at the time of application. 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.

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

Hurrell and 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. Huia); Californian thistle was absent. Two treatments were applied on 2 November 1992: (i) MCPA at 1.5 kg ha^{-1} (IWD MCPA, 375 g L^{-1}) and (ii) the mycelium-on-wheat preparation of *S. sclerotiorum* described above at 500 kg ha^{-1}. An untreated control was also included. Other experimental details are given in Hurrell and Bourdôt (1993).

Neither MCPA nor *S. sclerotiorum* reduced ryegrass yields. The dry matter yields of white clover and ryegrass on four harvest occasions are given in Figure 4. The yield of white clover was greatly reduced by MCPA at each time (as has been documented by others (Thompson 1956, Maclean 1957, Meeklah 1958, Meeklah and Mitchell 1984, Honore *et al.* 1980)). The percentage reductions were 85, 90, 74 and 71% at 23, 65, 118 and 168 days after treatment (DAT) respectively. By comparison there was no significant effect of *S. sclerotiorum* on clover yields. The effect of MCPA on the clover lasted almost the entire growing season.

Discussion

The term mycoherbicide is applied to fungal plant pathogens when used inundatively to control a weed population. The chosen 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 chosen as a candidate mycoherbicide for thistle 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 several important thistle species. It appears from initial virulence tests with *S. sclerotiorum* (strain S9) that in addition to Californian thistle, from which this strain was obtained, both Scotch thistle and nodding thistle are also excellent targets (Table 1). Nodding thistle was hitherto unknown as a host of *S. sclerotiorum* (Pennycook 1989, Farr *et al.* 1989). To date only Californian thistle has been the subject of field evaluations and the results have been most

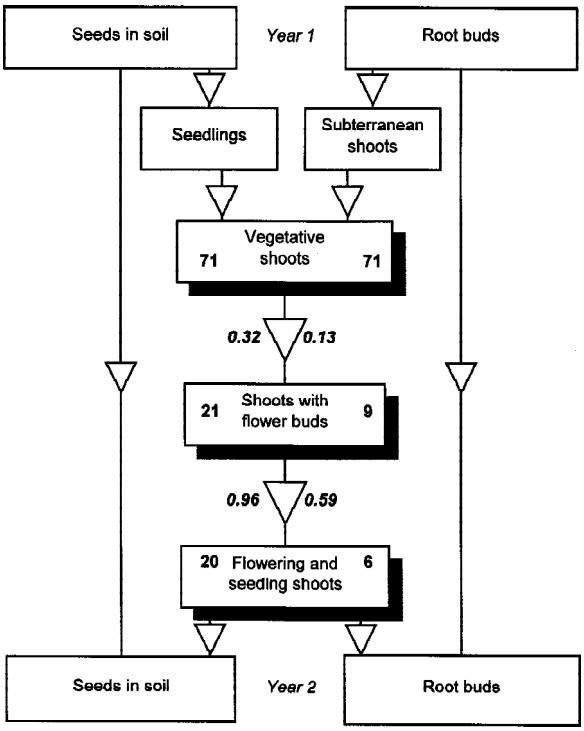

Figure 3. Life table for *Cirsium arvense* showing the impact of *Sclerotinia sclerotiorum* on transitions between two life history stages. Numbers on the right apply to a *C. arvense* treated with *S. sclerotiorum* and numbers on the left are for untreated *C. arvense*. Numbers in boxes are the number of shoots in each stage (shoots m^{-2}) and numbers beside the triangles are the probabilities assigned to the transitions between stages. (Modified from Bourdôt *et al.* 1996).

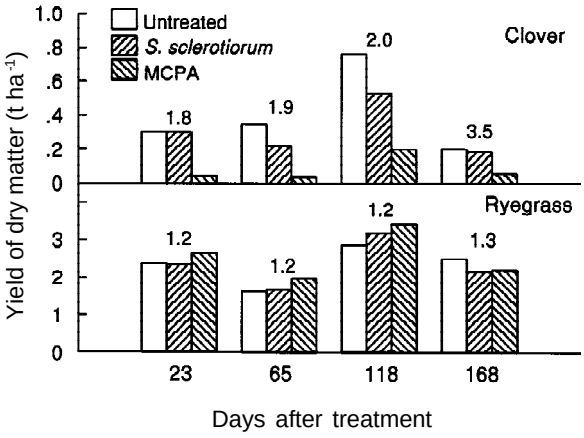

Figure 4. The effect of *Sclerotinia sclerotiorum* and MCPA on the productivity of ryegrass and clover on four occasions after treatment. Yields given as backtransformed means of the logarithms (log_{10}) of the data. Values above each group of three treatments are the Least Significant Ratios (P<0.05). 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 and Bourdôt 1993).

encouraging (Figures 1, 2 and 3). When applied as a mycelium-on-kibbled wheat formulation to the foliage of the thistle in spring, *S. sclerotiorum* (S9) was effective in reducing thistle cover 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 was a direct consequence of a reduction in the size of the root system. 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. Additionally the pathogen invaded and killed roots down to a depth of at least 300 mm on treated plots (unpublished).

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 developmentally 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 over wintering sclerotia, weakening 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.

The mycelium-on-wheat formulation was applied in the field at 500 kg ha^{-1} but this rate would not be commercially viable broadcast over large areas of pasture. However a food source must be applied along with the fungal mycelium in order to allow a period of saprophytic growth to facilitate eventual pathogenesis. Other food source / carriers are currently under investigation in a joint project between AgResearch and Crop Care Holdings Ltd., New Zealand.

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 and other thistles 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 4). However, the pathogen's wide host range is a 'two-edged-sword' for whilst this widens its market potential as a bio-herbicide, it carries with it a possible increased risk of disease in adjacent or subsequent susceptible crops.

Two different approaches to this problem seem possible; risk avoidance and risk acceptance / management. Genetic modification to render the pathogen host-specific or incapable of spreading as ascospores from the treated thistles or surviving as sclerotia to infect later sown crops, is one approach (Sands and Miller 1993). This approach offers many scenarios of which auxotrophic and sclerotiumless mutants are two examples developed to date (Miller *et al.* 1989a,b, Sands *et al.* 1990). Because the chemical dependency of auxotrophs may be satisfied by naturally occurring nutrients on the leaf surfaces of some susceptible plant species (Sands *et al.* 1990), auxotrophs may not be completely safe. Furthermore, auxotrophs and sclerotiumless mutants tested to date have been less pathogenic than wildtypes (Sands and Miller personal communication) although Ford *et al.* (1992) showed that heterokaryons, produced by combining auxotrophic strains, are as pathogenic as wildtypes, and likely to segregate into 'safe' auxotrophs at ascospore formation (Ford personal communication). An alternative to using 'safe' mutants, is to use wildtypes in a risk acceptance / risk management approach. To this end, a risk analysis is being conducted in New Zealand in which the expected additional infection from a mycoherbicide source of *S. sclerotiorum* is being compared to the existing natural infection using techniques employed by de Jong *et al.* (1990). This approach accepts that there is a risk and attempts to quantify it so that the biocontrol agent may be used and managed to satisfy an acceptable level of relative risk.

References

Bascand, L.D. and Jowett, G.H. (1982). Scrubweed cover of South Island agricultural and pastoral land. 2. Plant distribution and managerial problem status. *NZ Journal of Experimental Agriculture* 10, 455-92.

Bourdôt, G.W., Harvey, I.C., Hurrell, G.A.

and Alexander, R.T. (1993). An experimental mycoherbicide utilizing *Sclerotinia sclerotiorum* controls pasture populations of Cirsium arvense in Canterbury. Proceedings 46th NZ Plant Protection Conference, pp. 251-6.

Bourdôt, G.W., Harvey, I.C., Hurrell, G.A. and Saville, D.J. (1996). Demographic and biomass production consequences of inundative treatment of *Cirsium arvense* with *Sclerotinia sclerotiorum*. *Biocontrol Science and Technology* 5, 11-25.

Bourdôt, G.W. and Kelly, D. (1986). Density and cover estimates of some non-palatable herbaceous pasture weeds. Proceedings 39th NZ Weed and Pest Control Conference, pp. 183-6.

Brosten, B. and Sands, D. (1986). Field trials of *Sclerotinia sclerotiorum* to control Canada thistle (*Cirsium arvense*). *Weed Science* 34, 377-80.

Cockayne, A.H. (1917). Noxious weeds in New Zealand. *Journal of Agriculture* 14, 339-44.

de Jong, M.D., Scheepens, P.C. and Zadocks, J.C. (1990). Risk analysis for biological control: A Dutch case study in biocontrol of *Prunus serotina* by the fungus *Chondrostereum purpureum*. *The Plant Disease* 74, 189-94.

Farr, D.F., Bills, G.F., Chamuris, G.P. and Rossman, A.Y. (1989). 'Fungi on plants and plant products in the United States'. (APS Press-American Phytopathological Society, Minnesota, USA).

Ford, E.J., Casquilho, H., Miller, R.V., and Sands, D.C. (1992). First report of heterokaryon formation by *Sclerotinia sclerotiorum*. Abstract (A181) of the joint meeting of The American Phytopathological Society and the Mycological Society of America, Portland, 1992.

Hartley, M.J., and Butler, J.H.B. (1984). Californian thistle-weed of pasture and crops-biology and control. *Aglink FPP* 651. (MAF Information Services, Wellington).

Hartley, M.J., Lyttle, L.A. and Popay, A.I. (1984). Control of Californian thistle by grazing management. Proceedings 37th New Zealand Weed and Pest Control Conference, pp. 24-7.

Hartley, M.J. and Thomson, N.A. (1982). Effect and control of Californian thistle in dairy pasture. Proceedings NZ Grasslands Association 43, 104-7.

Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. (1977). *Cirsium arvense* (L.) Scop. *In* 'The world's worst weeds-distribution and biology', p. 217. (East-West Centre, University of Hawaii).

Honore, E.N., Rahman, A. and Dyson, C.B. (1980). Effects of winter application of phenoxy herbicides on white clover and pasture production in lower Northland. Proceedings 33rd NZ Weed and Pest Control Conference, pp. 55-8.

Hurrell, G.A. and Bourdôt, G.W. (1993). Pasture grasses and clovers unaffected by an inundative application of *Sclerotinia sclerotiorum*. Proceedings 46th NZ Plant Protection Conference, pp. 257-60.

Jessep, C.T. (1989). *Cirsium arvense* (L.) Scopoli, Californian thistle (Asteraceae). *In* 'A review of biological control invertebrate pests and weeds in New Zealand 1974–1987', eds. P.J. Cameron *et al.*, p. 343. (CAB Technical Communication No. 10).

Maclean, S.M. (1957). Effect of time of spraying on pasture production. Proceedings 10th NZ Weed Control Conference, pp. 34-43.

Meeklah, F.A. (1958). Comparison of the substituted phenoxy-acetics and the phenoxy-butyrics on a white clover-perennial ryegrass. Proceedings 11th NZ Weed Control Conference, pp. 61-7.

Meeklah, F.A. and Mitchell, R.B. (1984). Evaluation of herbicides for control of Californian thistle. Proceedings 37th New Zealand Weed and Pest Control Conference, pp. 20-3.

Miller, R.V., Ford, E.J. and Sands, D.C. (1989a). A nonsclerotial mutant of *Sclerotinia sclerotiorum*. *Canadian Journal of Microbiology* 35, 517-20.

Miller, R.V., Ford, E.J., Zidack, N.J. and Sands, D.C. (1989b). A Pyrimidine auxotroph of *Sclerotinia sclerotiorum* for use in biological weed control. *Journal of General Microbiology* 135, 2085-91.

Pennycook, S.R. (1989). 'Plant diseases recorded in New Zealand'. (DSIR, Auckland).

Sands, D.C. and Miller, R.V. (1993). Evolving strategies for biological control of weeds with plant pathogens. *Pesticide Science* 37, 399-403.

Sands, D.C., Miller, R.V. and Ford, E.J. (1990). Biotechnological approaches to control of weeds with pathogens. *In* 'Microbes and microbial products as herbicides', ed. R.E. Hoagland. (American Chemical Society, Washington DC).

Thompson, F.B. (1956). Clover tolerance. Proceedings 9th NZ Weed Control Conference, pp. 89-93.

Waipara, N.W., Harvey, I.C., and Bourdôt, G.W. (1993). Pathogenicity of *Sclerotinia sclerotiorum* on common thistle species and other pasture weeds. Proceedings 46th NZ Plant Protection Conference, pp. 261-4.