

## EVALUATION OF *SCLEROTINIA SCLEROTIUM* FOR GIANT BUTTERCUP CONTROL IN DAIRY PASTURES

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### ABSTRACT

An experiment was established in November 2002 on each of two dairy farms in Golden Bay (East Takaka and Pupu Valley) to evaluate a mycelium-on-wheat formulation of the fungus *Sclerotinia sclerotiorum* as a mycoherbicide against giant buttercup (*Ranunculus acris*). Granules were applied to individual giant buttercup plants or manually broadcast onto infested pasture at 500 kg/ha. The mortality of the giant buttercup plants reached 63% at East Takaka compared with 13% at Pupu Valley. Survivors were stunted so that the mean size of a treated plant was 27% and 56% of that of untreated plants 100 days after treatment at the East Takaka and Pupu Valley sites respectively. These effects remained evident 12 months after application. A second experiment confirmed these results. **Keywords:** dairy pasture, biocontrol, weed, *Ranunculus acris*, biological herbicide.

### INTRODUCTION

Giant buttercup (*Ranunculus acris*) is a tenacious and economically significant weed in New Zealand's major dairying regions. In the 2001-02 milking season it was estimated to have caused a loss in milk solids revenue of \$156m nationally (Bourdôt et al. 2003). Herbicides have increasingly failed to give satisfactory control, in part due to the build up of resistance to the commonly used phenoxy herbicides MCPA and MCPB (Bourdôt & Hurrell 1991; Bourdôt et al. 1990). These difficulties and the global trends towards organic or chemical-free production are encouraging dairy farmers to seek biological alternatives to chemical herbicides.

One potential alternative is a mycoherbicide based on the plant pathogenic fungus *Sclerotinia sclerotiorum*. In dairy pastures, a 57% reduction in giant buttercup biomass was measured 19 weeks after application of a slurry formulation of this pathogen in early December (Cornwallis et al. 1999). Similarly, 47% reduction in ground cover of the weed was measured when the fungus, formulated as a dry, kibbled wheat granule, was applied in November to individual plants in a dairy pasture at 530 kg/ha (Harvey & Bourdôt 2001). Anecdotal evidence from plots treated by Harvey and Bourdôt (2001) suggested that the effects on the giant buttercup population remained evident for two years after the application (G.A Milne, pers. comm.). In addition, *S. sclerotiorum* applied to dairy pasture has no effect on perennial ryegrass (*Lolium perenne*) or white clover (*Trifolium repens*) (Hurrell & Bourdôt 1993) and does not result in an increase in disease risk in adjacent susceptible crops (de Jong et al. 2002).

The current work involved two experiments to determine the effects of a targeted and broadcast November application of *S. sclerotiorum* on giant buttercup populations. The rate of appearance and persistence of these effects were monitored for twelve months following application.

## MATERIALS AND METHODS

### Formulation of *S. sclerotiorum*

A mycelium-on-wheat formulation of *S. sclerotiorum*, WH1, was produced from an isolate of *S. sclerotiorum*, S36, which had originated from Tai Tapu, Canterbury, and was stored as dried sclerotia at 4°C. Fifty 5 mm diameter cores from four-day-old cultures made from stored sclerotia on potato dextrose agar (Merck 1.10130, 39 g/litre) were used to inoculate sterile potato dextrose broth solutions (Difco 254920, 27 g/litre). The broths were incubated for four days at 25°C in shaken flasks, then homogenised in a blender at low speed for two minutes and poured over moist autoclaved kibbled wheat. Inoculated wheat was incubated at 25°C for four days then dried at 28°C for three days and ground with a Kenwood coffee grinder. The final product (WH1) yielded particles with 54%, 44% and 2% in diameter classes of 1-2, 2-3 and 3-4 mm respectively.

### Experiment 1

This experiment was established on giant buttercup-infested dairy pasture at each of two sites in Golden Bay, Pupu Valley (Pupu) and East Takaka (Takaka). There were four treatments: (1) WH1 (targeted application), (2) WH1 (broadcast application), (3) Nil (no material applied) and (4) Dead WH1 (targeted application of heat-killed WH1). Each was applied to a group of individually-marked flowering giant buttercup plants, which constituted a plot. There were five individual plants in each plot for the WH1 targeted, Dead and Nil treatments, and four for the WH1 broadcast treatment. The statistical design was a randomised complete block with six replicates.

The 'targeted' applications of WH1 and dead WH1 were made by applying 2 g of formulation to the basal leaf axils of each of the five plants per plot. The 'broadcast' applications were applied manually at a rate of 50 g/m<sup>2</sup> over 2x2 m areas of pasture that included the individually marked plants. The treatments were applied on 11 and 12 November 2002 at Pupu and Takaka respectively.

The treatment effects were assessed 0, 15, 35, 69, 98, 134, 197 and 358 days after treatment (DAT) at Takaka and 0, 14, 35, 69, 98, 133, 196 and 357 DAT at Pupu. On each occasion the diameter of each plant was measured in two directions at right angles to each other and an assessment of the extent of disease (including mortality) was recorded.

### Experiment 2

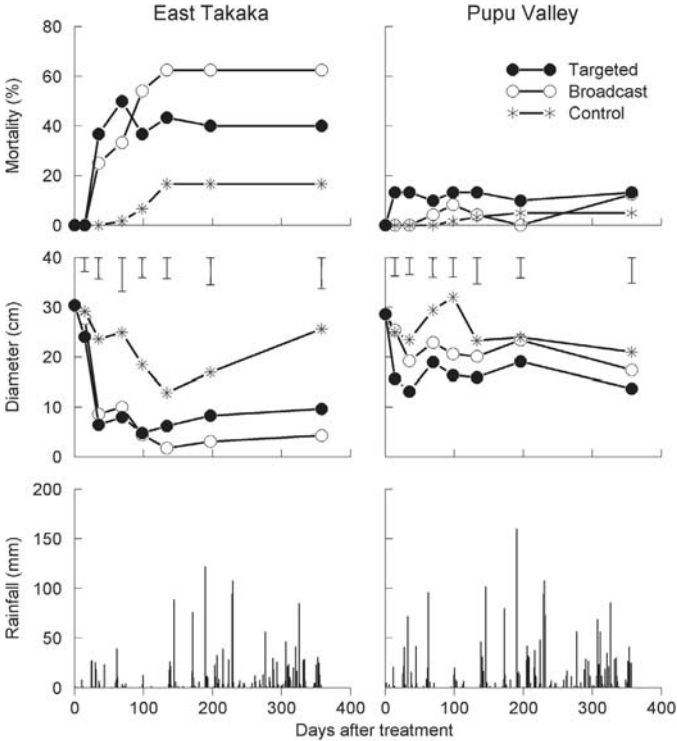
This experiment was established at the same time and at the same two sites as Experiment 1. Plots measuring 2x2 m were located in areas of the paddock with a high density of giant buttercup. At each location there were two treated plots (one treated by the farmer and the other by an AgResearch technician) and one control (Nil) plot in randomised blocks replicated four times. Treated plots received 50 g/m<sup>2</sup> of WH1 broadcast evenly over the plot as in Experiment 1, while the control plots had no material applied. Within each plot four individual flowering plants were marked with wooden pegs and plant diameters and disease scores were recorded on these in the same way and at the same intervals as for Experiment 1. Additionally, the percentage of ground covered by giant buttercup in each plot was estimated visually at each sampling time (except for 14 and 15 DAT).

### Climate data and statistical analysis

Temperature was recorded hourly at both sites using "Tinytag" (Gemini Dataloggers UK Ltd, Chichester, West Sussex, UK) data loggers. Rainfall was recorded hourly at Takaka using a tipping bucket attached to a "Tinytag" data logger. At the Pupu site, daily rainfall records were supplied by the farmer until July 2003, after which rainfall was recorded hourly with a "Tinytag" logger. Plant diameter data from both experiments were statistically analysed by analysis of variance (GenStat Version 7.1), using the plant size at the time of application (0 DAT) as a covariate. A chi-square test was used for the analysis of plant mortality.

### RESULTS AND DISCUSSION

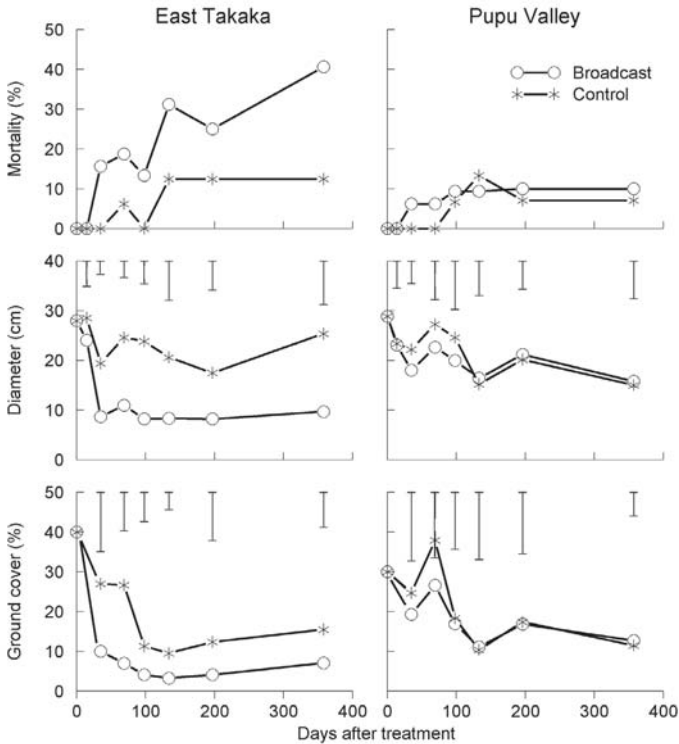
The effects of *S. sclerotiorum* on mean plant mortality, diameter and ground cover from Experiments 1 and 2 are given in Figures 1 & 2 respectively. In Experiment 1 there was no significant difference ( $P>0.05$ ) between the 'dead' and the 'nil' treatments for either mortality or plant diameter at any time so these two treatment means were averaged and are referred to as the 'control' (Fig. 1). In Experiment 2 there was no significant difference between the farmer and technician-applied treatments so these two treatment means were averaged (Fig. 2). This averaging was carried out to increase the accuracy of subsequent comparisons.



**FIGURE 1:** Mortality (%) and mean diameter (cm) of giant buttercup plants in dairy pasture treated with *S. sclerotiorum* (WH1) either targeted on the lower leaf axils of individual plants or broadcast onto infested pasture in Experiment 1. Vertical bars are LSDs ( $P<0.05$ ) for comparing between targeted and broadcast; for comparison of a treatment with control, multiply this LSD by 0.866. Daily rainfall (mm) is also presented.

At Takaka the *S. sclerotiorum* treatments in both experiments increased the mortality and reduced the mean size of the giant buttercup individuals (Figs 1 & 2). For both experiments the effects on plant size were significant from one month after application and continued until the next spring. In Experiment 1, the effect on mortality was highly significant ( $P<0.001$ ) for the broadcast treatment throughout the experiment and significant to a varying degree through time ( $P<0.05$  to  $P<0.001$ ) for the targeted

application (Fig. 1). In Experiment 2 mortality differences, although large, were not statistically significant (Fig. 2). This persistent weed control effect supports the anecdotal evidence from the earlier experiment by Harvey and Bourdôt (2001) where treated plots were observed to have less giant buttercup than the untreated plots two years after *S. sclerotiorum* application.



**FIGURE 2:** Mortality (%), mean diameter (cm) and ground cover (%) of giant buttercup in dairy pasture after application of *S. sclerotiorum* (WH1) as a broadcast treatment to infested pasture in Experiment 2. Vertical bars are LSDs ( $P < 0.05$ ) for comparing between treated and control.

The reductions in mean plant size caused by the treatments at Takaka are not completely explained by the increased plant mortality (Figs 1 & 2). For example, at the final assessment 358 DAT (Fig. 1), survival in the treated plots was 72% of the survival on the control plots for the targeted application and 45% for the broadcast treatment. Dead plants were recorded as having zero diameter, so if there was no stunting, there would be a similar effect on mean diameter. By comparison, the observed mean diameters of the treated plants were 38% and 17% of that of the control plants for the targeted and broadcast treatments respectively. These greater proportional reductions in mean plant size than the proportional reductions in survival reveal that the giant buttercup plants surviving the targeted and broadcast treatments were stunted compared to the control plants, apparently suffering sub-lethal growth-inhibiting effects of the disease caused by *S. sclerotiorum*.

The treatments had much less effect on the mortality and size of the giant buttercup plants at Pupu than at Takaka in Experiment 1 (Fig. 1), and no effect at this site in Experiment 2 (Fig. 2). It seems unlikely that this was due to a genetically based resistance in the Pupu plants since mortalities at this site were evident earlier than at the Takaka site, indicating the plants were equally, if not more, susceptible at the Pupu site. Similarly, climatic events do not provide an explanation. Both sites had similar air temperatures that were within the range required for the fungus to develop (Abawi & Grogan 1975) and rainfall appeared to be adequate early in the experimental period at both sites for the infection process to occur (Fig. 1). Nevertheless, there is a possibility that a brief drought at Takaka later in the experimental period may have inhibited regrowth in diseased plants and brought about the higher level of mortality amongst the control plants at this site. A more probable explanation for the smaller effects on plant mortality and size at the Pupu site is that the progress of the disease at this site was prematurely curtailed by some catastrophic environmental change. The experimental paddock at Pupu received a combined fertiliser application of potash, reactive phosphate rock and elemental sulphur 2-3 weeks after treatment application (G. Ball, pers. comm.) coinciding with the cessation of the treatment effects on mortality and plant size at the Pupu site (Fig. 1). It is possible that these chemicals prevented the disease from developing further since it is known from detached leaf studies that some fertilizers can substantially interfere with the growth of this pathogen (Pottinger et al. 2004).

The earlier initial occurrence of plant mortality at Pupu than at Takaka can be explained by the timing of rainfall in relation to the treatment applications. Rain was falling at the Pupu site during application of the *Sclerotinia* treatments whereas conditions were dry and windy at Takaka during application and no rain events were recorded until four days later. The presence of free moisture during treatment applications at Pupu would have allowed the infection to begin immediately whereas at Takaka the onset of infection would in all probability have not occurred for several days until free moisture was present.

A complication in the interpretation of the data presented here is that the mortalities and plant sizes may be underestimates of the true values. This applies particularly to the Pupu site where a higher density of giant buttercup plants made it difficult to discriminate between the marked experimental plants and vegetative extensions of their close neighbours. The untreated neighbours tended to invade, by rhizome extension, the spaces left by dead experimental plants, causing dead plants to be recorded as living. The extent to which this problem contributed to the lower mortalities and plant sizes at the Pupu site is unknown. Despite this difficulty, these experiments have confirmed that *S. sclerotiorum* applied to giant buttercup plants in a dairy pasture can not only kill >60% of the plants in the population, but can also leave survivors with sub-lethal symptoms of stunted development that persist for at least 12 months from the time of treatment. Further experiments are planned to determine the relative importance of pasture management and climate on the efficacy of *S. sclerotiorum* as a mycoherbicide.

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