

GIANT BUTTERCUP (*RANUNCULUS ACRIS* L.) CONTROL IN DAIRY PASTURE USING A MYCOHERBICIDE BASED ON *SCLEROTINIA SCLEROTIORUM*

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ABSTRACT

Sclerotinia sclerotiorum was applied as a dry kibbled wheat formulation to giant buttercup in trials in permanent dairy pasture in Takaka, Golden Bay. Autumn and late spring broadcast treatment resulted in 30-50% reduction in cover of giant buttercup. Spot treatment of individual plants was unsuccessful in autumn, but resulted in over 50% reduction in cover when conducted in late spring. Reasons for the success and failure of the treatments are discussed together with the future direction of the research and the integration of the technique into a practical giant buttercup management programme for dairy pasture.

INTRODUCTION

Giant buttercup (*Ranunculus acris*) is a tall-growing perennial plant of European origin that is well adapted to persist and spread in pastures under high soil moisture conditions. Introduced into New Zealand around 1910, it has become a widespread and intractable weed in dairy pastures in New Zealand, especially in the Golden Bay region. It is spread by seed carried on hooves of grazing animals or in supplementary feed and thus poses a threat to non-infested dairy pastures throughout New Zealand. Giant buttercup is estimated to cost the Golden Bay dairy industry \$9.5 million per annum in lost production; the national loss is estimated to be \$114 million per annum (G. Bourdôt, unpubl. data).

Giant buttercup is avoided by dairy cows because of its bitter taste, so populations tend to increase under grazing pressure. The ground cover of giant buttercup is highest in November. Measurements made in Takaka show that up to 50% of a paddock may be covered by the weed at this time of year (G. Bourdôt, unpubl. data). It responds positively to fertilizer application (Brown 1993) and has developed resistance to the phenoxy herbicides 24-D, MCPA and MCPB throughout New Zealand where these chemicals have been repeatedly used (Bourdôt et al. 1989; Bourdôt & Hurrell 1988; Bourdôt et al. 1990a; Bourdôt et al. 1990b). Furthermore, the continuing problem with giant buttercup in Golden Bay, despite the widespread use of the newer herbicides, flumetsulam and thifensulfuron, indicate that these products provide no lasting control of the weed.

The plant pathogenic fungus *Sclerotinia sclerotiorum* has been investigated as a mycoherbicide for the control of several pasture weeds, such as California thistle (Bourdôt et al. 1993). A water miscible formulation tested against giant buttercup in dairy pastures gave good knock-down but the plant regenerated from buds on the rhizome or "crown" (Cornwallis et al. 1999). While the rhizome shows some inherent resistance to infection (Green et al. 1994), regrowth after treatment with the water miscible formulation may be due to a lack of persistence of the applied inoculum in the leaf axils on the rhizome and/or phenologically based variation in susceptibility. This paper outlines trials conducted in dairy pastures in Golden Bay to evaluate a dry granule formulation applied by two different methods at two different times in the year.

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METHODS

Fresh isolates of *S. sclerotiorum* were obtained for each of two application times by taking a range of strains from various hosts and assaying them for virulence on excised leaves of *R. acris* using the following method. The fungal isolates were grown on malt extract agar (20% malt; 15% agar) for three days after which 4 mm diameter plugs from the edge of the colonies were placed, mycelium down, on the excised leaves on damp paper towels under humid conditions at ambient laboratory temperatures (15–18°C). The isolates were arranged at random using four replicate leaves and two inoculation points per leaf. After 72 h incubation the largest radius of necrosis from the plug was measured. The isolate that produced the largest mean radius lesion was selected for granule formulation.

The isolate was then grown on fresh plates and after 3 days 100 x 1 mm square plugs of agar were cut from the growing edge of the colonies and placed in 250 ml of malt extract broth (20% malt extract) in 500 ml flasks and stirred at 20°C for 3 days. The resultant broth was then poured over sterile kibbled wheat (double autoclaved 2:1 with water). The inoculated wheat was spread 4–5 cm deep in 40x30x10 cm sterile plastic trays which were then placed in new large plastic bags. Aluminium tubes (5x40 mm) were inserted into the necks of the bags and these were loosely plugged with cotton wool. After 3 days incubation at 20°C, the resultant fungus-infested wheat was placed on paper towels and air-dried at 27°C for 3 days. The wheat was turned after 1 day drying. The granules were then passed through a 4 mm mesh. The granules that did not pass were ground in a kibbling attachment on a Kenwood Food processor and re-passed through the mesh. The fungus-infested wheat was stored in airtight plastic drums at 4°C for 7 to 10 days until applied.

Granule application was compared in autumn and in spring on giant buttercup infested dairy pasture by either single plant application using a hand shaker (Motupipi, Golden Bay, Nelson), or plot application using a hand-propelled granule applicator (East Takaka, Golden Bay, Nelson). The plots used at each application date were in the same paddock but in different locations.

Single plant applications

This application was made to all the individual giant buttercup plants in four randomised 5x5 m plots on the 29 March and 20 November 2000 using a 400 ml shaker jar with a 70 mm diameter perforated lid with 10 x 5 mm diameter holes. Four plots were left untreated. At the March application date, plants were in the vegetative stage of growth, having finished flowering and beginning autumnal growth. At the November application date, plants were in full flower. The soil was dry at the time of the autumn application, and slightly moist at the time of the spring application.

The percentage cover of giant buttercup in each plot at the time of application was estimated by eye to within 5%. The amount of inoculum applied to the individual plants in each plot was measured by volume, and the weight of inoculum applied per square meter of weed cover was then estimated.

Sixteen days after the spring application only, the percentage area of the crowns of the plants that were diseased was estimated on 10 plants along a diagonal transect into each plot. After each application the percentage cover of giant buttercup plants in each plot was estimated 28 or 43 days after treatment (DAT).

Plot applications

At East Takaka, 4x2 m plots were treated with a hand-propelled mechanical granule applicator on the same dates as above. The rates of granule application were 0, 30 and 60 g/m² to each of four replicated, randomised plots. An overall estimation of the percentage cover by giant buttercup of the treated area was made.

Twenty-eight days after the first application in March, a visual estimate of giant buttercup cover was made in each plot. Similar estimates were made 16 and 43 days after the second treatment.

All data were subjected to ANOVA statistical analysis where appropriate.

RESULTS

Single plant applications

Table 1 shows that there was no significant change ($P < 0.05$) in giant buttercup plant cover 28 days after the autumn treatment, whereas there was a change after the spring treatment. Although there was little difference in the initial cover in plots in autumn and spring, the latter application halved the cover from 21% to 11%. Untreated plots in both trials showed no perceptible change in population between the time of application and the assessment time, 28 or 43 days later. Note that the application rate used in the autumn trial was estimated to be less than half of that used in the spring.

Sixteen days after the spring (November) application, the estimated reduction in percentage area of the 10 plants with crowns rotted in plots treated with *S. sclerotiorum* was 35.2%. In the untreated, this was only 7.2% (LSD ($P < 0.05$) = 10.3).

TABLE 1: Change in % cover of giant buttercup in dairy pasture after an autumn and a spring application of *Sclerotinia sclerotiorum* as granules to individual plants using a hand applicator.

Application time	Treatment	Application rate (g/m ² of weed)	Mean percentage cover by giant buttercup		
			Initial cover	Cover 28 DAT ¹ (43 DAT)	Percentage change
March	Nil	0	18.8	18.8	0
	<i>S. sclerotiorum</i>	19.2	21.3	17.5	-3.8
	LSD ($P < 0.05$)		11.2		
November	Nil	0	21.3	(21.3)	0
	<i>S. sclerotiorum</i>	53.0	20	(11.3)	-8.7
	LSD ($P < 0.05$)		7.5		

¹Days after treatment.

Plot applications

Plots inoculated with granules in the autumn had an estimated 40% cover of giant buttercup at the time of application, whereas at the spring application they had 85% cover. The application rate of 30 g/m² gave no significant reduction in giant buttercup cover at either application time, whereas the use of 60 g/m² gave a reduction at both application times but this was not quite significant ($P < 0.05$) in the spring application (Table 2).

DISCUSSION

Previous attempts to control giant buttercup in dairy pastures with a bioherbicide based on *S. sclerotiorum* have not been particularly successful (Cornwallis et al. 1999). Re-growth from the crown has been identified as a major cause of the failure. Recent studies (I. Harvey, unpubl. data) using a formulation based on a slurry preparation applied either topically to the crowns of individual plants or as a broadcast slurry droplet application have also been unsuccessful. These failures were deemed to be due to the inoculum dissipating from the point of application through either drying out and blowing away or being washed away by heavy rain. The granule formulation used in the trial reported here was found to remain at the site of application under both heavy rain and dry conditions. The treatment also remained viable for several weeks, allowing infection to take place when conditions became conducive. Also, when the granules were applied at flowering time, in both application methods used

TABLE 2: The effect of *Sclerotinia sclerotiorum* granules applied at two dates through a mechanical applicator on the population of giant buttercup plants in a dairy pasture.

Application time	Rate of application g/m ²	Mean percentage cover of giant buttercup		
		16 DAT ¹	28 DAT	43 DAT
March	0	-	41	-
	30	-	28	-
	60	-	15	-
	LSD (P<0.05)		17	
November	0	82.2	-	72.5
	30	73.8	-	48.8
	60	57.5	-	38.8
	LSD (P<0.05)	45.4		34.4

¹Days after treatment.

they were observed to lodge at the base of the flowering stem and subsequently rot out this part of the plant. It was common to find that rotting had also progressed into the crown causing complete plant destruction. However, some evidence of regrowth of the giant buttercup plants from the crowns was observed, suggesting that a second clean-up application to individual plants may still be required to control the weed using this method. Further, once the population of giant buttercup has been reduced through the use of a biological control agent, such as one based on *S. sclerotiorum*, then the residual population must be managed to ensure that the weed does not re-infest the pasture. Such practices could include grazing management, the use of alternative pasture species and fertilizer applications to ensure the weed is kept at manageable levels through competition.

This study, and the observations of inoculum persistence and fate, have shown that the successful biological control of giant buttercup (and other susceptible pasture weeds such as thistles) depends on the arrival of the inoculum on the part of the plant that will allow destructive infection to take place. Further, that inoculum must remain viable at that site of inoculation until environmental conditions are conducive for the infection process to either be initiated or re-initiated under changeable microclimatic conditions. *S. sclerotiorum* requires a combination of free water for 48–72 h (depending on the mean daily temperature) and an external source of nutrient (Abawi & Grogan 1979) for infection to take place. These studies suggest that a granular formulation only can satisfy these fastidious requirements. Further, strains of the pathogen must be selected that have high virulence towards the target host. Since this pathogen has been found to be prone to culture mutation (I. Harvey, unpubl. data), the use of fresh selections of isolates appears to be the best method of ensuring acceptable field virulence.

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