Comparative dose-responses of five pasture weeds to a *Sclerotinia sclerotiorum* mycoherbicide

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Abstract A gel preparation of mycelia of the plant pathogen *Sclerotinia sclerotiorum* was applied to the pasture weeds *Carduus nutans* L., *Carduus tenuiflorus* L., *Cirsium arvense* (L.) Scop., *Jacobaea vulgaris* L. and *Ranunculus acris* L. Doses ranging from 0 to 1,000 µl gel/plant were applied as 10 or 50 µl droplets, one per leaf axil. Statistically significant responses to dose were evident in *C. arvense*, *J. vulgaris* and *R. acris*, leading to 90, 74 and 100% reductions in living tissue respectively at 200 µl/plant compared to the control 29 days after treatment. For *C. nutans* and *C. tenuiflorus*, where even low doses gave good control, the reductions (averaged over all doses) were 98 and 88% respectively indicating that these two annual thistles were exceptionally susceptible to this *S. sclerotiorum* formulation. For *C. nutans*, 1 litre of the gel would, if precision applied, be sufficient to control at least 20,000 plants, suggesting broad-acre use may be commercially viable.

Keywords *Carduus nutans* L., *Carduus tenuiflorus* L., *Cirsium arvense* (L.) Scop., *Ranunculus acris* L., *Jacobaea vulgaris* L.

INTRODUCTION

The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, formulated as mycelia on a solid food-source substrate has, in small-scale experiments with the pasture weeds *Cirsium arvense* (Bourdôt et al. 2006a) and *Ranunculus acris* (Verkaaik et al. 2004; Bourdôt et al. 2007b), revealed its potential as a mycoherbicide. Other experiments and models demonstrate that despite its wide host range, it could be safely deployed for weed control in permanent pastures without risk to neighbouring susceptible crops (Bourdôt et al. 2006b). However, solid substrate formulations of *S. sclerotiorum* may not be commercially viable for use in pastures due to the high costs associated with the purchase and sterilisation of the substrate (e.g. kibbled wheat, spent brewers

barley) and with the storage, transport, handling and field application of large volumes of material. Application rates of 30–50 g/m² (300–500 kg/ha) have commonly been required in field trials with *S. sclerotiorum* (Verkaaik et al. 2004; Bourdôt et al. 2006a). In contrast, for *Sclerotinia minor* a solid state formulation of this fungus applied at 60 g/m² to control *Taraxacum officinale* may be commercially viable for use in high-value fine turf swards (Abu-Dieyeh & Watson 2006, 2007).

As a first step toward determining the commercial potential of an alternative, liquid formulation of *S. sclerotiorum* as a mycoherbicide for use in pastures, this study compared the dose-responses of five pasture weeds to a novel formulation of the fungus

prepared by liquid fermentation and applied as a gel. The main purpose was to determine the minimum dose required for good control as a basis for considering the likely commercial potential of such a formulation. The five weed species were *C. arvensis* and *R. acris* along with the three additional species *Carduus nutans* L., *Carduus tenuiflorus* L. and *Jacobaea vulgaris* L. These five species were considered to be good targets for a mycoherbicide. *Ranunculus acris* is a good target because its control in dairy pastures using chemical herbicides has been seriously compromised throughout New Zealand by evolved herbicide resistance (Bourdôt et al. 1990). The other four species represent weeds from the family *Asteraceae*. This family provides 24% of New Zealand's pasture weeds (Bourdôt et al. 2007a), none of which can be controlled with currently available synthetic herbicides without significant damage to clovers (Young 2010). While classical biocontrol of *C. arvensis* and *J. vulgaris* has been attempted in New Zealand, it has been unsuccessful in *C. arvensis* (Cripps et al. 2011) and only locally successful in *J. vulgaris* (Landcare Research 2007), leaving an unfulfilled need, and commercial opportunity, for the selective biological control of these two weeds in New Zealand's clover-based pastures.

METHODS

The isolate of *S. sclerotiorum* used was S36 originating from Tai Tapu, Canterbury, stored as dried sclerotia at 4°C. Sclerotia halves (from stored sclerotia) were plated onto malt extract agar (20 g Malt Extract in 15 g/litre BD Bacto agar) on 19 January 2006 to create starter cultures. On 23 January, 5 × 150 ml Malt Extract + yeast extract cultures were prepared and inoculated with 15 × 5 mm cores from the 4-day-old starter cultures and incubated at 24°C for 72 h. A gel biopolymer was added to the ferment at the rate of 25 g/litre on 26 January 2006 and this "gel" formulation of the mycoherbicide (NF843) was then refrigerated overnight.

On 27 January 2006, between 9 am and 3 pm, nine treatments (different doses of the *S. sclerotiorum* gel) were applied to five different weeds. The seeds

of these weeds had been collected from plants of *C. nutans* and *C. arvensis* at Lincoln, from *C. tenuiflorus* on the Port Hills near Christchurch, from *J. vulgaris* in North Canterbury and from *R. acris* in Golden Bay and used to establish a single plant in potting mix in each of 239 500 ml plastic pots. Eight doses (50, 100, 150, 200, 250, 500, 750 and 1,000 µl/plant) were created by applying either 5, 10, 15 or 20 droplets of the *S. sclerotiorum* preparation per plant either as a 10 or 50 µl droplet, one per leaf axil, using a micropipette. Where fewer axils were present than the number of droplets required, the extra drops were applied to the leaves and petioles. The control treatment consisted of 10 × 50 µl droplets per plant of 'dead' mycelium ferment that had been killed by microwaving.

For each species, the treated plants were arranged in a randomised complete block layout in the AgResearch shade-house at Lincoln with the number of blocks (replicates) varying with species. There was a total of 239 pots (8 treatments × 4 replicates + 9 controls for *C. tenuiflorus*; 8 × 2 + 8 for *C. nutans*; 8 × 8 + 16 for *C. arvensis*; 8 × 4 + 12 for *J. vulgaris*; 8 × 5 + 10 for *R. acris*). Sprinklers were set to irrigate for 10 min on the first night after treatment, then 1 h/day each day thereafter. The size of each plant relative to its respective replicate control plants was visually assessed on 24 and 25 February 2006, at 28–29 days after treatment by two independent observers. The results were then averaged across observers and re-expressed as % reduction in area of green tissue per plant. These data were analysed using an analysis of variance that included two orthogonal contrasts, corresponding to the linear trends within each of the two droplet sizes (10 or 50 µl).

RESULTS

Carduus nutans and *C. tenuiflorus* were highly susceptible to the gel formulation of the fungus at the lowest dose applied (50 µl or 0.05 ml per plant) (Figure 1). Averaging across all eight doses, the two species were reduced by 98 and 88% respectively relative to the controls.

Figure 1 Responses of five pasture weeds to dose of applied *Sclerotinia sclerotiorum*. Points are the means over n replicate plants, where $n=4, 2, 8, 4$ and 5 for *Carduus tenuiflorus*, *C. nutans*, *Cirsium arvense*, *Jacobaea vulgaris* and *Ranunculus acris* respectively. Curves are linear regressions fitted to the 10 µl droplets/plant series (first four points) and to the 50 µl droplets/plant series (other four points). Vertical bars are the LSD (5%) values for comparison between any means (except in the case of *C. nutans* where statistical analysis was not appropriate since most data values were 100%).

In contrast, for *Cirsium arvense*, *Jacobaea vulgaris* and *Ranunculus acris*, pronounced dose-responses were evident (Figure 1). For the small (10 µl) droplet size, there was a highly significant upward trend ($P<0.01$) in the % reduction in green tissue area for all three species. For the large (50 µl) droplet size, there was a significant upward trend ($P<0.01$) in the % reduction in green tissue area only in the case of *J. vulgaris*.

An additional feature of these data are the steps down in the responses of *C. arvense*, *J. vulgaris* and *R. acris* at 250 µl/plant, the point at which the increase in dose (from 200 to 250 µl/plant) was achieved by increasing the size of droplets from 10 to 50 µl/plant and reducing the droplet number from 20 to 5/plant (Figure 1). This effect was statistically significant for *J. vulgaris*.

DISCUSSION

The main purpose of this experiment was to determine the minimum dose required of a liquid formulation of the mycelia of the fungus *Sclerotinia sclerotiorum* for the control of five important pasture weeds as a basis for considering the commercial potential of such a formulation.

The annual thistles, *Carduus tenuiflorus* and *C. nutans*, were highly susceptible, and although not directly reported here, a high level of mortality was evident in both species; the plants that were assessed as having 100% reduction in green tissue relative to the control plants 29 days after treatment (Figure 1) had rotted away completely and there was evidently no prospect of re-growth. The dose-responses for these two thistle species are, however, poorly determined; for *C. nutans*, the minimum dose required for good control is apparently less than the lowest dose tested in the experiment (50 µl/plant). Nevertheless, it is apparent from these data that 1 litre of this preparation would be sufficient to control at least 20,000 plants of *C. nutans* (Figure 2), assuming precision application as in the experiment. For broadcast application the amount applied per plant controlled would be higher than in the experiment (to account for non-target deposition), but may

Figure 2 The theoretical number of plants that could be controlled to the extent shown in Figure 1 per 1 litre of the liquid formulation of *Sclerotinia sclerotiorum*, calculated as $10^6/(\mu\text{l}/\text{plant})$.

be low enough for the mycelia of *S. sclerotiorum* formulated as a gel to be a commercially acceptable treatment for *C. nutans* in pasture.

For *C. tenuiflorus*, the minimum dose required for good control is probably higher than that for *C. nutans* (Figure 1). For the other three weeds, application rates higher again than that required for *C. tenuiflorus* would apparently be required. In the case of *Ranunculus acris* and *Cirsium arvense*, which were reduced by approximately 90% at doses between 150 and 200 $\mu\text{l}/\text{plant}$, 1 litre of the gel would apparently be sufficient, if precision applied, to achieve 90% control of 5,000–6,667 plants (Figure 2).

The differences in dose-responses between the species (Figure 1) suggests that they vary inherently in susceptibility to *S. sclerotiorum*; the differences are not explicable in terms of differences in the adhesion of the gel since it was retained equally well by all five species.

The steps down in the dose-responses (particularly evident in *J. vulgaris*) when the dose of the mycelia-containing gel was increased from 200 to 250 $\mu\text{l}/\text{plant}$ by applying fewer larger droplets of the gel, reveals that a high number of infection points per plant is crucially

important to achieving high levels of disease, particularly in the less susceptible species. It implies that these less-susceptible weed species may be better controlled by applying many small mycelia-containing droplets rather than a few large droplets. It also implies that droplets smaller than 10 μl , the smallest applied in this experiment, may enable even lower effective dose rates to be achieved. This could be tested in further experiments. To optimise droplet size, such experiments would need to explain how the success of a droplet (probability of it causing an infection) is affected by its size, the mass of mycelia and post-fermentation residual food source it contains and the density at which it is applied to the plant surface.

The optimisation of the size of a droplet containing mycelial fragments of a plant-pathogenic fungus is a field of research crucial to the application of fungal mycelia in weed biological control that has not to date been addressed in the scientific literature. As a result, there are no empirical data or existing theory against which to evaluate the results of the current experiment. This is evident in a recent review of published research into the nature of the relationship between plant disease and inoculum density (Bourdôt & Saville 2010). Here, surprisingly, only six studies were found that had quantified the plant disease severity response to inoculum density and all of these had used spores rather than mycelia as the inoculum.

Despite the lack of published comparative data, this novel experiment has revealed that *Carduus tenuiflorus* and *C. nutans* are both highly susceptible to low doses of a gel preparation of the mycelia of *Sclerotinia sclerotiorum*. This potentially enables field application rates (of a mycoherbicide utilising this fungus) for these and other weed species to be much lower than the 30–50 g/m^2 found necessary for controlling *Ranunculus acris* and *Cirsium arvense* in pastures using solid state formulations (Verkaaik et al. 2004; Bourdôt et al. 2006a).

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