Abiotic factors affecting the pathogenicity of a Sclerotinia sclerotiorum-based mycoherbicide

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Summary  A mycoherbicide based on Sclerotinia sclerotiorum (Lib.) de Bary has shown promise for control of Ranunculus acris subsp. acris L. (giant buttercup) in New Zealand dairy pastures. The ability of S. sclerotiorum to infect giant buttercup plants treated with urea and/or lime at various application times was, therefore, assessed. Urea applied at the recommended field rate of 50 kg ha⁻¹ did not reduce the pathogenicity of S. sclerotiorum compared with the control in two experiments. By contrast, lime at the recommended field rate of 10 t ha⁻¹ significantly reduced the pathogenicity (P < 0.05) of S. sclerotiorum on giant buttercup plants compared with the control in the first experiment, but this effect was not repeated in the second experiment.

When S. sclerotiorum-inoculated plants were exposed to varying moisture combinations (6, 12 and 24 hours), there was a significant reduction (P < 0.05) in pathogenicity of the fungus in the dry only treatment. There was no significant difference between the other treatments.

Results indicate that farm fertiliser management practices and moisture levels are likely to be important variables affecting the on-farm efficacy of S. sclerotiorum used as a mycoherbicide for controlling giant buttercup.

Keywords  Sclerotinia sclerotiorum, Ranunculus acris, mycoherbicide, pathogenicity, urea, lime.

INTRODUCTION

Giant buttercup (Ranunculus acris L.) is a serious weed in New Zealand dairy pastures. A national milk solids revenue loss of $156 million in the 2001–02 season has been attributed to this weed species (Bourdôt et al. 2003). Plant populations increase under grazing pressure as giant buttercup is avoided by dairy cows because of its bitter taste. Giant buttercup is difficult to control because of resistance developed to the phenoxy-herbicides 2,4-D, MCPA and MCPPB throughout New Zealand where these chemicals have been repeatedly used (Bourdôt et al. 1989, Powles and Holtum 1994). In addition, cultural practices are considered impractical and/or uneconomic by farmers.

Since its introduction by scientists at AgResearch, Lincoln, Canterbury, a Sclerotinia sclerotiorum-based mycoherbicide has shown great potential for the control of important weed species in New Zealand pastures, including Californian thistle (Cirsium arvense L. (Scop.)) (Bourdôt et al. 1993) and giant buttercup in dairy pastures (Cornwallis et al. 1999). Nitrogen fertilisers and lime are used extensively in New Zealand pastures, and farmers would like to be able to apply S. sclerotiorum along with their fertiliser (Bourdôt pers. comm. 2004). Limited research has been conducted on the effect of specific farm management practices and environmental conditions on the pathogenicity of S. sclerotiorum. The objectives of this research, therefore, were to investigate: (1) the effect of application timing of urea and/or lime on the pathogenicity of S. sclerotiorum, and (2) the effect of different moisture conditions on the pathogenicity of S. sclerotiorum. From this research, practical recommendations can be developed for dairy farmers that will enhance the efficacy of the mycoherbicide and allow for consistent control of giant buttercup in infested pastures. A review of this research is presented.

MATERIALS AND METHODS

Experiment 1. Effect of timing of urea and/or lime application on pathogenicity  Experiments took place in a shadehouse located at the Lincoln University Nursery, Canterbury, New Zealand. Four-month-old plants were grown from seeds collected from Salt Water Creek, Canterbury and maintained under controlled glasshouse conditions.

One millilitre (mL) of two-day-old mycelial infested malt extract agar (MEA) paste was inoculated onto the crown tissue of each plant using a 20 mL syringe (Terumo Europe, Leuven, Belgium). Prior to inoculation, plant tissue was treated with urea and/or lime as outlined in Table 1. Urea was applied to run-off at the recommended field rate of 50 kg ha⁻¹ and lime was applied to run-off at the recommended field rate of 10 t ha⁻¹. Inoculated plants treated with water only were positive controls and uninoculated plants were negative controls. To ensure adequate plant hydration for the duration of the experiment, all plants were positioned in individual trays and bottom-watered as required. In the first experiment, the average temperature ranged...
between 8.8 and 29.3°C, while in the repeat of this experiment, the average temperature ranged between 15.5 and 30.1°C.

The experimental design was a completely randomised block design replicated four times. Disease was measured using a disease severity index ranging between 0–7, with 0 indicating no disease and 7 indicating complete plant death. Disease development was assessed every two days for a total of 28 days. Results of disease severity index measurements at each assessment time were analysed by ANOVA using the Genstat computer programme.

**Experiment 2. Effect of different moisture combinations on pathogenicity**  Plants were inoculated as described in Experiment 1. Prior to inoculation, plants were sprayed with water to run-off and then placed on the shadehouse ground under open metal frames. To provide moist conditions that allowed for free moisture for disease development, the frames were then covered with transparent plastic and, based on local weather forecasts, opposite ends of the plastic were raised approximately 8 cm from the ground to limit excessively high temperatures or humidity levels within the covered environment. All plants, except the dry only treatment, were initially covered for 6, 12 or 24 h (Table 2), after which they were either returned to the covered environment (Treatments 1, 2, 3) or left exposed (Treatments 4, 5, 6) for the remainder of the experiment. For the exposed conditions, plants were positioned adjacent to the moist treatment area on the shadehouse ground and exposed to the open-air environment. Inoculated plants held under the covered environment (Treatment 7) or in the uncovered environment (Treatment 8) for the duration of the experiment were positive controls while uninoculated plants were negative controls. Plants were bottom-watered as described in Experiment 1. The experimental design and disease assessment was as described in Experiment 1.

**RESULTS**

**Experiment 1. Effect of timing of urea and/or lime application on pathogenicity**  In the first experiment, lime applied either on Day 0 or 24 h prior to inoculation reduced the pathogenicity of *S. sclerotiorum* compared with the positive control. By contrast, urea did not reduce the pathogenicity of *S. sclerotiorum* compared with the inoculated control (Figure 1). In the repeat experiment, the pathogenicity of *S. sclerotiorum* on lime treated plants did not differ significantly from the inoculated control plants. Urea also did not reduce the pathogenicity of *S. sclerotiorum* compared with the positive control (Figure 2).
Experiment 2. Effect of different moisture combinations on pathogenicity

A significant difference (P < 0.05) was observed between the dry only treatment and all other moisture treatments at 2, 8, 10, 12 and 14 DAI. There was no significant difference (P > 0.05) in the pathogenicity of S. sclerotiorum between all other treatments at any assessment time (Figure 3).

DISCUSSION

The effect of farm management practices, specifically the timing of urea and/or lime application, on the pathogenicity of S. sclerotiorum on whole giant buttercup plants was studied. Overall, urea did not reduce the pathogenicity of S. sclerotiorum. Lime reduced the pathogenicity of the fungal isolate in the first experiment but had little effect on pathogenicity in the repeat experiment. As no other treatment differed between the two experiments except the lime treatment, a physicochemical alteration of the lime may have occurred.

It is postulated that the colder minimum temperatures within the shadehouse environment in the first experiment resulted in the presence of free moisture within the inoculated crown tissue. This may have allowed for dissociation of the lime (Ca₂CO₃), thereby resulting in very high pH levels. S. sclerotiorum requires a pH of 5 for optimal growth and host invasion (Abawi and Grogan 1979, Rollins and Dickman 2001). Under alkaline conditions, important fungal processes may have been inhibited, resulting in reduced pathogenicity of S. sclerotiorum. Molecular and biochemical studies will be conducted to further investigate the effect of urea and lime on the infection process of S. sclerotiorum.

Irrigation schedules are an important dairy farm management practice. As moisture is one of the main driving factors for disease development in S. sclerotiorum, it was important to determine the effect of drying periods after inoculation on disease development. Pathogenicity was reduced when S. sclerotiorum was exposed to dry only conditions, while the pathogen was able to infect the host with a minimum of 6 h moisture, regardless of a dry period after inoculation. It has been well established that S. sclerotiorum requires 48–72 h of free moisture for successful penetration and disease development in the host plants studied (Abawi and Grogan 1979). The agar-infested mycelial inoculum may have provided adequate moisture levels during the first few days of the experiment, allowing for maximum disease development in all moisture treatments. Future research investigating the effect of different moisture combinations on a wheat grain-based mycoherbicide is planned.

Overall, farm management practices and environmental conditions were important factors influencing the ability of S. sclerotiorum to infect giant buttercup plants. Practical recommendations to dairy farmers to control this significant weed species will be developed as more information is gained regarding the effect of these variables on S. sclerotiorum.

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REFERENCES


