**REGENERATION SITES OF *RANUNCULUS ACRIS* ARE THE
TARGET FOR *SCLEROTINIA SCLEROTIORUM*,
A POTENTIAL MYCOHERBICIDE**

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SUMMARY

Plants of the perennial weed, giant buttercup (*Ranunculus acris* L.), were inoculated with an isolate of *Sclerotinia sclerotiorum* and the influence of the following treatments were determined; i) plant size, ii) wounding at inoculation site and iii) position of inoculum placement relative to the plant's regeneration reserves in the crown. Disease level and plant mortality 21 days after inoculation were measured. Small plants were most susceptible and large plants least susceptible to the pathogen. Wounding the crown had no effect. Disease levels and mortality were higher in plants inoculated near the apex of the crown than near its base. It is concluded that application of inoculum to ensure initial and rapid infection of the crown apex is important in determining the efficacy of *S. sclerotiorum* as a mycoherbicide for control of giant buttercup.

Keywords: giant buttercup, *Sclerotinia sclerotiorum*, mycoherbicide

INTRODUCTION

Giant buttercup (*Ranunculus acris* L.) is a serious weed in a number of dairying regions in New Zealand. Cattle avoid eating it so that it invades pastures at the expense of grasses and clovers, and reduces productivity. Tolerance to the chemical herbicides MCPA and MCPB (Bourdôt and Hurrell 1988) has prompted the search for alternative methods of control (Bourdôt and Harvey 1992). Over the past 20 years there has been increasing interest in the use of fungi as biological control agents of weeds using the mycoherbicide approach. Mycoherbicides can be defined as plant pathogenic fungi developed and used inundatively to control weeds in the way chemical herbicides are used (Charudattan 1991). Pathogenicity of *Sclerotinia sclerotiorum* to a large number of important crop plants has previously deterred its use in biological control systems. However, host range, and the ability to spread to non-target plants may be reduced by the use of new strains selected using recently developed biotechnological techniques. This will improve the potential of *Sclerotinia* spp. in biological weed control (Miller *et al.* 1989).

Previous experiments in which giant buttercup plants have been inoculated with *S. sclerotiorum* have shown that it has a large capacity for regrowth post-infection, with regeneration occurring from the apical region and also from axillary buds. All of these regeneration sites are situated in the crown at the base of the plant. Observations from these studies show that recovery after infection is dependent on the presence of some remaining healthy crown tissue, and will occur even after extensive necrosis of the roots and shoots. This paper describes an experiment to determine how plant size, wounding at the site of inoculation, and site of inoculation relative to the apical region of the crown, affect disease progression and plant mortality in giant buttercup plants inoculated with an isolate of *S. sclerotiorum*.

METHODS

The inoculum used in the experiment was air-dried kibbled wheat infested with isolate S13 of *S. sclerotiorum*, originally isolated from squash (Harvey, pers. comm). Three sizes of plants, small, medium and large, were used, with 40 plants in each size category. All plants were grown in a glasshouse prior to the experiment. The small plants were 3 months old and grown in 70 mm diameter pots which restricted growth. They had small crowns. Medium plants were 3 months old but grown in 125 mm diameter pots which allowed development of a greater amount of foliage and crown tissue. Large plants were 6 months old, grown in 125 mm diameter pots and had a dense rosette of leaves around a large crown which often protruded several cm above the soil level. All inoculations were carried out by placing a single grain of inoculum (2-3 mm in length) adjacent to the crown at the petiole base of each of three leaves per plant. Ten plants from each size category were inoculated either on the upper or lower leaves, with and without wounding of the inoculation site. The plants were inoculated either at the petiole base of the three youngest leaves, around the apical region of the crown, or at the petiole base of the three oldest leaves, at the base of the crown. The wounding was carried out by making a small incision into the crown (approximately 2 mm deep, 0.5 mm wide and 3 mm long) at the petiole base using a sharp scalpel blade. Inoculum was placed adjacent to this wound.

The experimental design was a split plot incorporating plant size (small, medium, large) as the main plot treatment factor, and wounding (plus, minus) and inoculation position (lower, upper leaves) as the sub-plot treatment factors. Main plots were arranged in a randomised block design, with ten replicates.

Plants were misted with water prior to and immediately after inoculation. All plants were incubated for the duration of the experiment in a glasshouse, under a misting unit which maintained almost constant leaf wetness using an electronic "wet leaf" sensor device connected to the misting supply. The glasshouse had supplementary lighting for a 12 h daylength and the temperature was maintained between 17 and 22°C. The experiment was carried out in September, before the onset of flowering.

Disease assessment

Disease progression on each plant was assessed daily for the first 7 days of the experiment, and then at 9, 12, 14 and 21 days after inoculation (DAI) using the 0-7 scale disease index described below.

- 0 = No disease
- 1 = Brown, water-soaked lesion on petiole base immediately surrounding site of inoculation.
- 2 = Lesion completely encircling infected petiole bases. Infected petiole losing rigidity.
- 3 = As in 2 plus some necrosis of leaf laminae.
- 4 = Necrosis and total loss of rigidity of all petioles. Necrosis on less than 50% of leaf laminae.
- 5 = As in 4 plus necrosis of over 75% of leaf laminae.
- 6 = Entire plant collapsed and withered. All tissue completely or almost completely necrotic/chlorotic. Crown potentially viable.
- 7 = Plant dead (crown completely rotten).

At 21 DAI all plants were harvested and the percentage area of infected crown tissue was determined by visual examination after slicing the crowns in half. The fresh weight of each plant was recorded and plants were then dried in an oven at 70°C for 4 days. Oven dry weights were recorded and percentage dry matter (DM%) calculated. The mean disease rating, mean percentage of crown which had rotted and mean DM% were analysed using analysis of variance. The percentage mortality 21 days after inoculation was analysed using a χ^2 test.

RESULTS

All inoculated plants developed symptoms of disease typical of those caused by *S. sclerotiorum* (Purdy 1979). In the most effective treatments, plants died within 21

DAI. In the least effective treatments, plants were able to recover from disease by producing new shoots from the apical region of the crown and from axillary buds associated with infected leaves. The disease ratings at 21 DAI only were used in the analysis, as this gave the best indication of extent of infection. The main effects of plant size, wounding at inoculation site and position of inoculation on mean disease rating, percentage of crown which had rotted, DM% and percentage mortality of giant buttercup plants 21 days after inoculation with isolate S13 of *S. sclerotiorum* are presented in Table 1.

TABLE 1: Main effects of a) plant size, b) wounding at the site of inoculation, and c) inoculation position in *Ranunculus acris* on disease rating, percentage of crown rotted, DM% and percentage mortality 21 days after inoculation with isolate S13 of *Sclerotinia sclerotiorum*.

	Disease rating	% of crown rotted	DM %	% mortality
a) Plant size				
Small	5.0	56.7	39.9	42
Medium	4.5	42.4	34.4	30
Large	4.4	33.1	29.5	17
LSD (P<0.05)	0.4	19.3	5.2	-
Significance	*	*	*	*
b) Wounding				
- wound	4.5	38.6	33.8	23
+ wound	4.8	49.6	35.5	36
LSD (P<0.05)	0.3	11.4	3.1	-
Significance	NS	NS	NS	NS
c) Position of inoculation				
Upper leaves	5.7	70.2	40.8	55
Lower leaves	3.6	18.0	28.4	5
LSD (P<0.05)	0.3	11.4	3.1	-
Significance	**	**	**	**

In this and Table 2: * = significant at P<0.05; ** = significant at P<0.01; NS = not significant.

TABLE 2: Interaction between plant size and position of inoculation in *Ranunculus acris* on disease rating, percentage of crown rotted, DM% and percentage mortality 21 days after inoculation with isolate S13 of *Sclerotinia sclerotiorum*.

	Disease rating	% of crown	DM %	% mortality
Upper leaves inoculated				
Small plants	6.0	85.5	48.0	75
Medium plants	5.9	77.5	44.6	60
Large plants	5.4	47.5	29.9	30
Lower leaves inoculated				
Small plants	4.2	28.0	31.8	10
Medium plants	3.2	7.3	24.3	0
Large plants	3.5	18.7	29.2	5
Significance of difference in linear trends	NS	*	**	NS

Large plants had significantly lower disease ratings, percentage crown rot, DM % and percentage mortality than small plants (Table 1a). Although no significant differences occurred between medium and large plants in any of the variables there was a significant linear decline with increasing plant size. Wounding at the site of inoculation resulted in apparently higher disease ratings, crown rot, DM% and plant mortality than non-wounding, but these effects were not significant (Table 1b). Positioning the inoculum at the base of the upper leaves resulted in significantly ($P < 0.01$) greater values for all four variables than positioning it at the base of the lower leaves (Table 1c). The only significant interactions were those between plant size and position of inoculation (Table 2). Differences between small and large plants were accentuated when upper leaves were inoculated, as might be expected from the greater efficacy of this treatment (Table 2).

DISCUSSION

Disease level and plant mortality 21 DAI decreased as plant size (and therefore crown size) increased (Table 1). The pathogen appeared to invade the small crowns more rapidly and completely. Larger plants have substantial crown tissue and therefore present a much greater area which must be completely infected before the plant will die. There was no evidence that wounding affected pathogenicity (Table 1b). This suggests that the outer surface of the crown is unlikely to act as a physical barrier to penetration by the pathogen and that the slow rate of disease progression in crowns is caused by some other factor, possibly within the crown tissue itself. The position of inoculum placement on the host had the largest effect on the level of infection 21 DAI. Inoculation of the upper leaves resulted in significantly greater disease development, DM%, percentage of crown which had rotted and percentage mortality than inoculation of the lower leaves (Tables 1c and 2). This was possibly because the pathogen was able to invade the region of most active tissue production at the crown apex first before invading the rest of the plant, thus slowing down and greatly reducing the plant's capacity for regrowth.

Therefore the crown of the giant buttercup is considered to be the most important target site for a *Sclerotinia* mycoherbicide. Templeton and Heiny (1990) suggested that perennial plants which have protected reserve tissue are more likely to regrow away from infection, and that mycoherbicides may be less effective on them than annual weeds. This experiment has shown that *S. sclerotiorum* has sufficient virulence to completely infect plants of the giant buttercup, but inoculum must be targeted to facilitate rapid infection of the crown apex for high rates of plant mortality to occur.

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