RESISTANCE OF *RANUNCULUS ACRIS* CROWN TISSUE ENABLES SURVIVAL AFTER INFECTION BY *SCLEROTINIA SCLEROTIORUM*

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SUMMARY

Sclerotinia sclerotiorum was inoculated onto plants of the giant buttercup (*Ranunculus acris* L.) with and without pre-treatment with chlorsulfuron to inhibit regenerative growth. This tested the hypothesis that *S. sclerotiorum* is unable to kill *R. acris* because of its very high regenerative capacity. Plants pre-treated with chlorsulfuron had fewer regenerative shoots and higher levels of leaf and crown infection 28 days after inoculation than untreated, but complete invasion of crown tissue did not occur. All plants retained some crown tissue with viable axillary buds. It is concluded that resistance of crown tissue to decay by *S. sclerotiorum* enables survival of regenerative buds, and that this is a more important factor limiting the effect of *S. sclerotiorum* on *R. acris* than the rapid recovery rate of this weed.

Keywords: *Sclerotinia sclerotiorum*, *Ranunculus acris*, chlorsulfuron, mycoherbicide

INTRODUCTION

Giant buttercup (*Ranunculus acris* L.) is a persistent, perennial weed which invades pastures in dairying regions of New Zealand. Resistance to the herbicides MCPA and MCPB (Bourdôt and Hurrell 1990) has prompted research into using the pathogenic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary as a mycoherbicide for the biological control of this weed. Mycoherbicides are plant pathogenic fungi used inundatively to control weeds in the way chemical herbicides are used (Charudattan 1991). *S. sclerotiorum* is pathogenic to many important crop plants but its ability to sporulate and cause unwanted disease is poorly understood at present. The use of auxotrophic strains (Miller *et al.* 1989) may reduce the potential hazards of using *S. sclerotiorum* as mycoherbicides in biological weed control.

Regeneration sites on the crown of *R. acris* have been identified as the target for an *S. sclerotiorum* myocherbicide (Green *et al.* 1993). However, further experiments have shown that plants inoculated at regeneration sites still exhibit a high rate of regeneration from the crown following the rapid leaf death caused by the pathogen (Green, unpublished data). This may occur because *R. acris* can produce regenerative tissues faster than the rate at which the pathogen can invade the plant. The experiment reported here tested this by inoculating plants with and without suppression of regenerative growth and comparing their susceptibility to *S. sclerotiorum*. Preliminary tests showed that the herbicide chlorsulfuron could inhibit the growing shoots of *R. acris* plants when applied at 0.75 g/ha (1/20th of the recommended field rate). Chlorsulfuron at this concentration had no effect on the *in vitro* colony growth of *S. sclerotiorum* when added to an agar medium (Green, unpublished data). Therefore chlorsulfuron was used as an inhibitor of regeneration for the purpose of this experiment.

METHODS

The inoculum used in the experiment was air-dried, ground kibbled wheat infested with isolate S13 of *S. sclerotiorum*, originally isolated from squash (Harvey, pers.

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comm). Fifty plants of *R. acris* were grown from seed in a glasshouse for 4 months prior to the experiment. In August, 25 plants were treated with a single dose of chlorsulfuron (Glean) at 0.75 g/ha + 0.5 ml/litre of Tween 80 surfactant, and the remaining plants treated with water + surfactant. The treatments were applied with a nitrogen-gas-powered moving belt sprayer delivering 60 litres/ha at 210 kPa through a single 8002E flat-fan Teejet hydraulic nozzle mounted 50 cm above the plants. The plants were passed under the stationary nozzle in single file at 1.5 m/sec. The plants were transferred to a glasshouse and the length of the youngest shoot measured daily for the first 10 days after spraying (DAS) to determine shoot growth per day. For analysis, this was summed to give the total growth during the 10 day period. At 10 DAS inoculations were carried out by placing inoculum (0.5 g/plant) around the base of petioles adjacent to the crown. Of the 25 plants within each treatment, 20 were inoculated with *S. sclerotiorum*, and five inoculated with sterile inoculum as control treatments. Plants were misted with water prior to and immediately after inoculation and incubated for 28 days in a glasshouse under a misting unit which maintained almost constant leaf wetness using an electronic "wet leaf" sensor device. The glasshouse had supplementary lighting to give a 12 h daylength and the temperature was maintained between 17 and 22°C. The experiment was carried out in August/ September before the onset of flowering.

The experimental design was a randomised block with five replicates.

Disease assessment

Disease progression was assessed daily for the first 7 days after inoculation (DAI), and then at 11, 14, 19, 21, 25 and 28 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, youngest shoot collapsed.
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).

The disease index was amended for regenerating plants. This is described below.

5 = Original leaf tissue completely necrotic, regenerative shoots just appearing.
4 = Regenerative shoots form 25% of all plant tissue.
3 = Regenerative shoots form 25-50% of all plant tissue.
2 = Regenerative shoots form 50-75% of all plant tissue.
1 = Regenerative shoots form 75% + of all plant tissue.

At 28 DAI, the number of regenerative shoots were recorded and the number of infected leaves on each plant were counted and calculated as a percentage of the total number of leaves per plant. The area of infected crown tissue as a percentage of total crown tissue was determined by visual examination after slicing the crowns in half. All data were analysed using analysis of variance.

RESULTS

Chlorsulfuron significantly ($P<0.01$) reduced the growth of the youngest shoot during the first 10 DAS (Table 1). All young tissues on chlorsulfuron treated plants had developed symptoms of stunting and chlorosis by 10 DAS. By 38 DAS, growth of the youngest shoots had almost ceased in control plants pre-treated with chlorsulfuron, but these plants were not killed and eventually recovered.

TABLE 1: Effect of chlorsulfuron on the growth of the youngest shoot of *Ranunculus acris* during the first 10 days after spraying (DAS).

	Shoot growth (mm)
+ Chlorsulfuron	27.6
- Chlorsulfuron	49.3
LSD (P<0.01)	19.5

TABLE 2: Effect of chlorsulfuron pre-treatment on disease rating up to 28 days after inoculation, and on the percentage of infected leaves, number of regenerative shoots, and percentage of crown rotted in *Ranunculus acris* 28 days after inoculation with isolate S13 of *S. sclerotiorum*.

	Disease rating	% infected leaves	No. of regenerative shoots[a]	% of crown rotted
Chlorsulfuron + *S. sclerotiorum*	4.1	96.6	0.9	74.5
Water + *S. sclerotiorum*	3.7	67.1	11.9	27.2
Chlorsulfuron - *S. sclerotiorum*	0	0	-	0
Water - *S. sclerotiorum*	0	0	-	0
LSD (P<0.01)[1]	0.3	9.5	4.2	17.1
LSD (P<0.01)[2]	0.5	15.1	-	27.0

[a] No data for regenerative shoots in the control treatments.
[1] LSD for comparison between inoculated treatments.
[2] LSD for comparison between inoculated and control treatments.

Plants inoculated with *S. sclerotiorum* developed symptoms of disease typical of this pathogen (Purdy 1979). Inoculated plants without chlorsulfuron pre-treatment began to produce regenerative shoots from the apex, or from axillary buds if the apex had rotted, by 11 DAI. At 28 DAI, 19 out of the 20 plants in this treatment had begun to regenerate. Only five out of the 20 inoculated plants pre-treated with chlorsulfuron had begun to regenerate 28 DAI, with regenerative shoots appearing at 25 DAI. Inoculated plants pre-treated with chlorsulfuron had significantly (P<0.01) higher disease ratings, percentage infected leaves and percentage crown rot, and a significantly lower (P<0.01) number of regenerative shoots 28 days after inoculation with *S. sclerotiorum* than plants not treated with chlorsulfuron (Table 2). Plant mortality in all treatments was zero. No plants had crowns that were completely rotted on dissection 28 DAI. All retained some crown tissue with at least one or more viable axillary buds.

DISCUSSION

Chlorsulfuron applied at 1 g/ha proved to be an effective regeneration inhibitor for this experiment as it significantly reduced the growth of young shoots (Table 1) but did not kill plants. Plants pre-treated with chlorsulfuron were more susceptible to *S. sclerotiorum* than non-treated plants, having greater levels of leaf and crown infection and significantly fewer regenerative shoots 28 DAI. In addition, the interval between inoculation and appearance of the first regenerative shoots was greater in chlorsulfuron treated plants. Despite these effects, *S. sclerotiorum* was unable to completely invade crown tissue and kill plants which had been pre-treated with chlorsulfuron. All plants pre-treated with chlorsulfuron had some undegraded crown tissue 28 DAI with one or more viable axillary buds which indicated that recovery was possible, even after death of all leaf tissues. If crown degradation by *S. sclerotiorum* was as rapid as the observed

rate of leaf degradation by this pathogen, the regeneration sites would be rapidly infected and mortality in *R. acris* would be higher. These results suggest that crown tissue of *R. acris* has greater resistance to degradation by *S. sclerotiorum* than leaf tissues. This causes crown decay either to cease, or to continue at a rate slower than the regenerative rate of the crown buds, enabling them to survive and produce new shoots after target inoculation. Observations have shown that a single surviving axillary bud on remaining crown tissue can eventually develop into an individual plant with its own crown, shoot and root system (Green, unpublished data). Crown tissue is densely packed with starch reserves (Harper 1957) which helps to facilitate the production of regenerative tissues. Therefore as long as some crown tissue remains intact, it is likely that the plant will survive and regenerate. It is concluded that the resistance of crown tissue to decay by *S. sclerotiorum* is more important than the rapid regenerative rate of *R. acris* as a factor limiting the success of *S. sclerotiorum* as a possible mycoherbicide for this perennial weed.

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