EVALUATION OF A POTENTIAL MYCOHERBICIDE FOR CONTROL OF *RANUNCULUS ACRIS*, GIANT BUTTERCUP

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SUMMARY

A fungus, likely to be a *Gnomonia* sp. was isolated from giant buttercup. In the field it caused anthracnose-type lesions on petioles and stems. It grew readily in culture, producing spores for subsequent pathogenicity tests. When inoculated to excised leaves, these were killed within 80 h. Inoculations to excised petiole and flowering stem portions, 80 mm in length, resulted in complete infection in 5 days when inoculated distally, and 7 days when inoculated proximally. Excised crowns and roots were inoculated but no symptoms were produced. At 8 days after inoculation of whole plants with spore suspensions only 30-40% of the plants became infected. Most leaves died but infection of crown tissue did not appear to occur, and plants regrew.

Keywords: *Ranunculus acris, Gnomonia*, mycoherbicide.

INTRODUCTION

The giant buttercup, *Ranunculus acris* L. is an important weed of dairy pastures in Golden Bay and parts of the North Island. It is potentially more serious since Bourdot *et al.* (1990) showed that some populations of this plant have developed resistance to MCPA, following constant use of this herbicide. Cultural and other non-chemical methods of control do need to be investigated. Mycoherbicides, the use of fungal plant pathogens to control weeds, have potential for control of some weeds (Charudattan 1991). During an ecological study of *R. acris*, plants which showed elongate, elliptical lesions, with a dark margin and grey central area, on their petioles and stems were collected (W. Brown, pers. comm.). A fungus, isolated from these lesions, was designated as GBX. A similar fungus was isolated from lesions on creeping buttercup *Ranunculus repens* L, and this was designated as CBY. In preliminary studies both isolates were shown to be pathogenic to *R. acris*. Their potential as mycoherbicides has been investigated in this study.

MATERIALS AND METHODS

Both GBX and CBY were grown on malt extract agar (MEA) at 25°C. Conidia were formed abundantly after 14 days. Spore suspensions for inoculations were prepared by adding 10 ml of 0.01% Tween 20 solution to petri dishes, allowing them to stand for 5 minutes, stirring and leaving for a further 5 minutes. The spore concentration was assessed. Fresh spore suspensions were prepared for every set of experiments, and were never more than 2 h old when used.

All plant material to be inoculated came from *R. acris* plants more than one year old. Excised leaves, petioles, flowering stems, crowns and roots were cut from whole plants. They were surface-sterilised in 5% hypochlorite solution for 5 minutes, and then washed in sterile distilled water for 10 minutes.

Leaves were placed separately in covered petri dishes containing moistened germination pads. Leaves were inoculated by placing 0.5 ml of spore suspension on an area at the base or tip of the leaf damaged by inserting a steel probe five times into a 3 mm^2 area. A similar amount of 0.01% Tween 20 was placed on the healthy control leaves. The leaves were placed in a growth cabinet with a 12 h light-dark regime at 20-25°C. Lesions were recorded by measuring their growth along the midrib.

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Petioles and flowering stems were cut into lengths, and the distal and proximal ends marked. They were cut into 80 mm lengths, with part of each end being removed. The distal or proximal ends were inoculated by dipping them, for 3 seconds, into a spore suspension of GBX or CBY or a 0.01% Tween 20 solution. The excised portions were placed, inoculated end up, in a capped tube with 1.5 ml water in the bottom. They were placed in a growth cabinet with the same conditions as for leaves.

Excised crowns and roots were inoculated by placing a 6 mm diam piece of the 14 day culture directly on to the top of the crown or on to the cut end of an 80 mm root segment. The control treatment was a 14 day old 6 mm diam plug of MEA. The crown or root was placed on a moistened germination pad in a covered petri dish and placed in the growth chamber.

Whole plants of *R. acris*, more than one year old, were inoculated by spraying them with the spore suspension until run-off. The plants were incubated for 72 hours in 100% relative humidity in the growth cabinets, 20/15°C, with 12 h light - 12 h dark. After 72 hours, the RH was reduced to 70-80%. There were ten replicates of each fungal isolate, as well as ten plants treated with a 0.01% Tween 20 solution.

Lesion size, colour and development were recorded every 2 days for 10 days. The germination pads and/or plants were watered as required.

RESULTS

Excised tissue inoculations

In all inoculations, where disease symptoms were present, tissue isolation to MEA confirmed the presence of the original fungal pathogen. Excised crown or root inoculations showed no symptoms of disease. With excised leaves, GBX and CBY produced similar leaf lesions and lesion growth. When inoculated at the leaf tip, the lesions were brown-black, moved down the midrib and within 36 h had reached the base of the leaves. After 36 hours the lesions started advancing into the interveinal areas. Basal inoculation of excised leaves produced the same disease symptoms but it took 5 days for GBX and CBY to move up the mid-vein to the tip of the leaf. This difference in rate of movement was significant ($P = 0.001$).

On excised petioles and flowering stems GBX and CBY lesions were brown to black in colour and were soft and slightly sunken, ie. anthracnose-like. The lesions encircled the excised plant tissue and then moved up or down it at differing rates.

Table 1 shows the growth of lesions on distally-inoculated petioles or flowering stems. GBX grew faster on both types of excised tissue, with significant differences occurring at 60, 80 and 112 hours after inoculation.

TABLE 1: Mean lesion length increase over time in excised tissues, 80 mm in length, inoculated at the distal end.

Time after inoculation (hours)	Petioles LSD 5% level	Petioles GBX (mm)	Petioles CBY (mm)	Flowering stems LSD 5% level	Flowering stems GBX (mm)	Flowering stems CBY (mm)
0	±0.0	0.0	0.0	±0.0	0.0	0.0
36	±0.5	0.2	0.0	±1.0	1.3	0.01
60	±2.0	5.8	0.0	±2.5	4.5	1.0
80	±5.0	45.8	5.9	±5.0	61.1	7.3
112	±12.0	80.5	32.5	±4.0	80.0	44.0
132	±1.0	80.0	80.0	±2.5	80.0	80.0

Table 2 shows the growth of GBX and CBY on proximally-inoculated excised petioles and flowering stems. The acropetal growth of lesions in these tissues was far slower than the basipetal growth (Table 1). With petioles a significant difference in lesion length did not occur until 144 h after inoculation. With flowering stems a major

difference in lesion length, between GBX and CBY, occurred at 100 h. This relative difference was maintained through to the end of the experiment at 172 h.

TABLE 2: Mean lesion length increase over time in excised tissues, 80 mm in length, inoculated at the proximal end.

Petioles				Flowering Stems			
Time after inoculation (hours)	LSD 5% level	GBX (mm)	CBY (mm)	Time after inoculation (hours)	LSD 5% level	GBX (mm)	CBY (mm)
0	±0.0	0.0	0.0	0	±0.0	0.0	0.0
44	±2.0	5.5	1.2	48	±1.5	4.5	2.0
51	±4.5	8.2	3.0	67	±5.0	12.3	6.0
66	±5.0	9.4	5.4	80	±7.5	20.5	8.0
76	±3.0	10.0	10.0	100	±5.0	27.0	12.4
96	±4.0	14.0	13.0	124	±4.5	37.0	17.0
120	±3.0	16.0	17.0	148	±5.0	49.0	24.5
144	±4.0	41.0	28.0	172	±6.5	67.5	38.0
166	±3.5	68.0	39.0				

Whole plant inoculations

All the control treatments were disease-free and healthy. It was difficult to successfully inoculate whole plants, with only four out of 10 being infected with GBX, and three out of 10 with CBY.

At 24 h after inoculation of whole plants lesions up to 14 mm long had developed on leaves and stems. The lesions were chocolate-brown and watery with a chlorotic yellow zone (up to 8 mm) around the lesion. After 48 h infected plants were wilted with epinastic leaves. Lesions were up to 25 mm long, and had started to encircle the petioles. At 96 h, 70 to 80% of the leaves were severely infected, with mainly brown lesions with black edges. The overall effect was to give the plants a blotchy appearance. After 8 days, 70%-80% of all older leaves had died in both the GBX and CBY-infected plants. The plants began to recover at this time, producing new leaves. When infected plants were dissected at 10 days, the crowns and roots were not showing symptoms.

DISCUSSION

Neither of the fungal pathogens produced symptoms of infection when inoculated to excised crown and root tissue. Nor did the crown and roots show symptoms following inoculation of whole plants with spore suspensions, under the conditions in this experiment. Because *R. acris* was able to recover after innundative application of GBX or CBY, this suggests that these may not be of value as mycoherbicides. However, the pathogens may be more active under certain environmental conditions, or may be more severe on plants that have been mechanically or chemically damaged.

The lesions appeared to develop more quickly on whole plants than on excised tissues. Again this may be due to the different environmental conditions used for whole plants and excised tissues.

Only 30 - 40% of whole plants inoculated with GBX and CBY became infected. This may have been due to problems in maintaining the high relative humidity necessary for optimum germination and infection by the conidia. This is a further possible disadvantage to the potential development of GBX or CBY as mycoherbicides. On the other hand both of these fungi produce conidia readily either on agar or in liquid culture, thus providing inoculum for inundative application. Hardwick (1992) reported that conidial germination on agar occurred after 2 h, and by 12 h the germ tubes were 70-90 µm with no appressoria. The conidia were 5-23 x 2-6 µm in size and two-celled.

In excised leaves, petioles and flowering stems the two fungi grew more quickly basipetally than acropetally to the tip of excised tissues. This may be related to the faster transport of fungal toxins and/or conidia basipetally in the phloem. If this is the case then mechanical damage to tissues by mowing or rolling before inundative application could enhance the effectiveness of these fungal pathogens. Leaf clearing and staining of internal fungal structures showed that fungal hyphae could be present in apparently healthy tissue, indicating that lesion development records the rate of disease development and not the progress of the pathogen through the plant.

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