SCLEROTINIA SCLEROTIORUM SHOWS POTENTIAL FOR CONTROLLING WATER LETTUCE, ALLIGATOR WEED AND WANDERING JEW

N.W. WAIPARA[1], G.W. BOURDÔT[2] and G.A. HURRELL[2]

[1]*Landcare Research, 231 Morrin Road, P Bag 92170, Auckland, New Zealand*
[2]*AgResearch Ltd, Gerald Street, P O Box 60, Lincoln, New Zealand*

Corresponding author: WaiparaN@landcareresearch.co.nz

ABSTRACT

The responses of six aquatic environmental weeds (water hyacinth (*Eichhornia crassipes*), alligator weed (*Alternanthera philoxeroides*), water lettuce (*Pistia stratiotes*), ferny azolla (*Azolla pinnata*), parrot's feather (*Myriophyllum aquaticum*) and bladderwort (*Utricularia giba*) and a terrestrial weed (wandering Jew (*Tradescantia fluminensis*)) to *Sclerotinia sclerotiorum* were evaluated. The fungus was applied as a mycelium-on-barley formulation to individual container-grown plants. Visual scores of lesion development revealed that a watery soft-rot disease, caused by the pathogen, developed in the treated water lettuce, alligator weed and wandering Jew plants. In water lettuce, the pathogen resulted in 100% mortality of treated plants 54 days after application. Shoot necrosis was 4% (control) and 24% (treated) for wandering Jew and 9% (control) and 17% (treated) for alligator weed, at 35 and 52 days after inoculation respectively. These results indicate that *S. sclerotiorum* has potential as a mycoherbicide for controlling water lettuce and possibly also alligator weed and wandering Jew.
Keywords: environmental weeds, aquatic weeds, terrestrial weeds, biocontrol, biological herbicide, mycoherbicide.

INTRODUCTION

New Zealand's aquatic and riparian ecosystems are under increasing threat from a growing list of aquatic weeds that have naturalised but not yet reached their potential geographic distributions. Their control can be difficult and expensive requiring labour-intensive removal or high rates and frequent applications of herbicides. Inundative and classical biological control approaches using plant pathogens have both been proposed to mitigate the impacts of such weeds in New Zealand (Standish 2001; Winks & Fowler 2001; Ward & Gianotti 2002).

Sclerotinia sclerotiorum (Lib.) de Bary, a naturally occurring pathogen of many weeds (Pennycook 1989), has been shown to have potential as a mycoherbicide for controlling Californian thistle (*Cirsium arvense*) (Hurrell et al. 2001; Bourdôt et al 2006), giant buttercup (*Ranaunculus acris*) (Harvey & Bourdôt 2001; Verkaaik et al. 2004) and a number of other pastoral weed species (Waipara et al. 1993). A new host record for *S. sclerotiorum* was recently observed for wandering Jew (*Tradescantia fluminensis*) in New Zealand (N.W. Waipara, unpubl. data). Evaluations of *S. sclerotiorum* for biocontrol of water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) have been conducted previously and showed that the fungus was pathogenic towards both species (M. de Jong, pers. comm.). These previous observations on *S. sclerotiorum*, along with its wide host range, and need for free water (Harvey et al. 1994), suggested that many weeds of damp or watery environments may be controllable with this pathogen. To test this hypothesis, experiments were undertaken in 2005 to assess its efficacy against six aquatic weed species: water hyacinth (*E. crassipes*), alligator weed (*Alternanthera philoxeroides*), water

lettuce (*P. stratiotes*), ferny azolla (*Azolla pinnata*), parrot's feather (*Myriophyllum aquaticum*) and bladderwort (*Utricularia giba*) and one riparian and terrestrial weed, wandering Jew (*Tradescantia fluminensis*). These experiments are described in this paper.

MATERIALS AND METHODS

Formulation of *S. sclerotiorum*

A mycelium-on-barley formulation of *S. sclerotiorum*, MB1, was produced from an isolate of *S. sclerotiorum,* S36, which had originated from *Cirsium arvense* at Tai Tapu, Canterbury, and had been stored as dried sclerotia at 4°C. Fifty 5 mm diameter cores from 4-day-old cultures on potato dextrose agar (Merck 1.10130, 39 g/litre) were used to inoculate sterile potato dextrose broth solutions (Difco 254920, 27 g/litre). The broths were incubated for 4 days at 25ºC in shaken flasks, then homogenised in a blender at low speed for 2 min and poured over moist autoclaved spent barley (from a brewery). The inoculated barley was incubated at 25ºC for 4 days then dried at 28ºC for 3 days and ground with a Kenwood coffee grinder. The final experimental product (MB1) was composed, after sieving, of ca 1100 particles per gram of infested barley ranging in size from 1.0 to 3.0 mm of which 88% were viable colony forming units. The viability of the MB1 was checked at each time of inoculation by sprinkling a sub sample of the particles onto malt extract agar in Petri dishes and counting the colonies after incubation at 22ºC for 7 days.

Experiment 1

Five host species (water hyacinth, alligator weed, water lettuce, ferny azolla and parrot's feather), all declared as National Surveillance Pests under the Biosecurity Act 1993, were propagated, in 25 cm-diameter plastic containers (one plant/container), at the NIWA Quarantine Facility at the Ruakura Research Centre, Hamilton. A 5 cm layer of top soil was placed at the bottom of each container and then water was poured to approximately 5 cm from the top of each container to simulate an aquatic habitat. In November 2004, previously grown plants were transplanted from propagation tanks into each container where they were left to establish for a further 3 months before inoculation. Both alligator weed and parrot's feather plants were rooted into the soil layer, while the remaining species were floated on the water surface of each container. Water levels were maintained at approximately 15 cm depth for the duration of the trial. In March 2005, alligator weed and parrot's feather plants both comprised submerged and emergent stems that filled the entire area of each container. Water hyacinth and water lettuce plants comprised up to 30% of the water surface area, while ferny azolla had formed floating mats over the entire water surface in each container. At day 0, the plants were inoculated with the described formulation of *S. sclerotiorum*. The treated plants were dosed at an estimated rate of 100 kg/ha or 0.5 g MB1/pot. The MB1 particles were sprinkled through a 3 mm sieve placed on top of a settling tower, 0.6 m above the container surface to ensure the particles were dispersed evenly over the treated plants. Control plants were uninoculated. The sixth host, bladderwort, was propagated as a floating monoculture across the entire surface of a plastic trough that was 1.25 m diameter, 0.5 m deep, and the water level maintained at a depth of 0.4 m. Within this population, five plots of 10 cm^2 were inoculated at the same dose described above, with another five used as untreated controls. All plants (groups of shoots in the case of bladderwort) were assessed on four occasions (days 10, 20, 27 and 54) after inoculation. Both treatments (inoculated and control) for each host were replicated five times, apart from water lettuce where three plants were inoculated and two control plants were untreated. The lower replication for water lettuce was necessary due to the limited number of containment plants available at the time of inoculation.

Experiment 2

A second pot trial was undertaken at the Landcare Research glasshouse facility at Tamaki, Auckland, in which alligator weed was transplanted and propagated as an aquatic plant using the same container method described above. Additionally alligator weed was propagated for the same growth period as a "terrestrial plant" in potting mix in 20 cm diameter pots. All plants were maintained at constant room temperature of 22ºC. At

day 0 plants were inoculated using the method described above. Alligator weed was assessed on days 10, 20, 30 and 52. Both treatments (inoculated and control) were replicated five times for each propagation method (aquatic and terrestrial).

Experiment 3

A third pot trial was undertaken at the Landcare Research glasshouse facility at Tamaki, Auckland, in which wandering Jew was propagated from 20 cm excised stems (1 stem per pot) for 8 weeks using the same pot method as for "terrestrial" alligator weed described above. At day 0 plants were inoculated using the method described above. Plants were assessed on days 7, 14, 22 and 35. Both treatments (inoculated and control) were replicated 15 times.

Disease assessment

Disease progression on each plant in each of the experiments was assessed visually and scores of 0-5 were assigned according to a system developed earlier (Table 1) (Green et al. 1993; Waipara et al. 1993)

TABLE 1: Disease scoring system used to quantify the disease caused by the *Sclerotinia sclerotiorum* in the plants in Experiments 1-3.

Score	Description of disease	% shoot area exhibiting disease
0	None – no symptoms	0
1	Very low – small superficial lesions	1-10
2	Low – small discreet lesions	11-25
3	Medium – large systemic lesions	26-75
4	High – significant plant necrosis	78-99
5	Plant dead	100

To confirm that the applied *S. sclerotiorum* caused the disease measured in the plants (completing Koch's postulates), a re-isolation method outlined previously (Waipara et al. 2005) was used in which samples of diseased plant tissue from each host exhibiting necrosis were surface sterilised and placed onto Potato Dextrose Agar (Difco) amended with antibiotic.

Experimental layout and data analysis

For all three experiments, inoculated and untreated plants were allocated at random to species and treatments. The requirement to avoid cross contamination between the inoculated and control treatments within a very limited containment space precluded the randomisation of the species and treatments within replicates.

The scores for the treated and control plants were analysed separately for each species in each of the three experiments by calculating their 95% confidence intervals. These intervals were used to determine if there was a treatment effect. The means of these scores were converted back to % necrosis.

RESULTS AND DISCUSSION

Sclerotinia sclerotiorum was observed to initiate infection across all inoculated targets apart from bladderwort, which showed no visible symptoms of infection over the assessment period. However, only in water lettuce, alligator weed and wandering Jew was there statistical evidence for an effect of the pathogen in any of the three experiments (Table 2). On water lettuce, the pathogen resulted in an increase from 0 to 100% necrosis (100% mortality) between control and treated plants at 54 days after treatment. For aquatic and terrestrial alligator weed in Experiment 2, the corresponding differences were 3 to 15% necrosis and 5 to17% necrosis respectively, at 52 days after treatment. In contrast, no treatment difference was observed for the aquatic alligator weed in Experiment 1. For wandering Jew, the treatment difference was 4 to 24% necrosis between control and treated plants at 35 days after application. There was no plant mortality of either

alligator weed or wandering Jew. These results indicate that *S. sclerotiorum* has potential as a mycoherbicide for controlling water lettuce in particular, and possibly also alligator weed and wandering Jew.

Although infection was confirmed on ferny azolla, parrot's feather and water hyacinth, subsequent disease progression was slow and restricted allowing these host plants to resist systemic infection (Table 2). Therefore, these species were not considered to be suitable targets for a *S. sclerotiorum*-based mycoherbicide. However, preliminary tests in Europe have demonstrated high levels of disease in both alligator weed and water hyacinth plants treated with *S. sclerotiorum* (M. de Jong, unpubl. data) indicating that further work with either alternative strains of *S. sclerotiorum* or formulation technologies could be warranted.

TABLE 2: Mean disease score (±95% CI) and % plant necrotic (estimated from disease scores) for seven weeds treated with *Sclerotinia sclerotiorum* or left untreated (control). Values are the means at the final assessment.

	Mean disease score (±95%CI)			% plant necrotic	
	Control	Treated	P-value[1]	Control	Treated
Experiment 1					
Alligator weed	2.0 (±0.0)	2.0 (±0.0)	ns	17.5	17.5
Ferny azolla	0.2 (±0.6)	1.0 (±2.8)	ns	1.1	5.5
Parrots feather	0.6 (±0.7)	1.4 (±0.7)	ns	3.3	10.3
Water hyacinth	0.6 (±0.7)	0.8 (±1.0)	ns	3.3	4.4
Bladderwort	0.4 (±0.7)	0.4 (±0.7)	ns	2.2	2.2
Water lettuce	0.0 (±0.0)	5.0 (±0.0)	P<0.001	0.0	100.0
Experiment 2					
Alligator weed (Aq)[2]	0.6 (±0.7)	1.8 (±0.6)	P<0.01	3.3	15.1
Alligator weed (Te)[2]	1.0 (±0.0)	2.0 (±0.0)	P<0.001	5.5	17.5
Experiment 3					
Wandering Jew	0.7 (±0.3)	2.2 (±0.5)	P<0.001	3.9	24.0

[1]Significance of difference between control and treated plants, ns=not significant.
[2]Aquatic (Aq) and terrestrial (Te) forms of alligator weed.

Re-isolation of *S. sclerotiorum* from necrotic lesions confirmed this fungus as the causative agent of disease symptoms for all species except bladderwort. In addition, a previously described pathogen of alligator weed, *Nimbya alternantherae*, was isolated from reddish brown lesions on alligator weed in both Experiments 1 and 2 across both control and *Sclerotinia* treatments. The presence of *Nimbya* leaf symptoms on alligator weed contributed to the disease score and necrosis observed on the control plants (Table 2). Infection by this naturally occurring pathogen could have influenced the efficacy of *S. sclerotiorum,* particularly in Experiment 1 where no significant treatment effect was observed. This fungus has been isolated in Australia (Gilbert et al. 2005), and is currently being developed as a mycoherbicide for alligator weed. Two other related *Alternaria* species were also recovered from several black lesions on inoculated water hyacinth leaves (*A. alternata* and a second species yet to be confirmed as *A. eichhorniae*) and a powdery mildew, *Microsphaera alphitoides,* was identified on some of the parrot's feather plants. Despite the autonomous appearance of these primary leaf pathogens with *S. sclerotiorum,* a more virulent and synergistic disease complex was not observed.

The previously recorded host range for *S. sclerotiorum* is very broad and includes economic crop species (Pennycook 1989) and further work would be required to determine the relative risk for this pathogen to damage non target species in aquatic and riparian environments.

In summary, this study has demonstrated that there is potential to broaden the range of targets for inundative biocontrol using a *S. sclerotiorum* mycoherbicide product

to include several important environmental weeds, water lettuce, alligator weed and wandering Jew.

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