Californian thistle and its variable response to the biological control agent, *Sclerotinia sclerotiorum*

B. Smith¹, S.G. Casonato¹, A. Noble² and G. Bourdôt²

¹Lincoln University, Ecology Department, PO Box 85084, Lincoln 7647

²AgResearch Lincoln, Private Bag 4749, Christchurch 8140

Corresponding author: seona.casonato@lincoln.ac.nz

Abstract Californian thistle (*Cirsium arvense*) is a problematic weed, particularly in permanent pastures. The fungus, *Sclerotinia sclerotiorum*, has potential as a bioherbicide to control this weed, but its variable efficacy in historical field trials suggest that there are differences in susceptibility to *S. sclerotiorum* within the species. To test this hypothesis, the responses of 32 New Zealand provenances of *C. arvense* to a foliage-applied mycelium-on-barley preparation of *S. sclerotiorum* were compared under common conditions. Significant differences between provenances were found, supporting the hypothesis that there is variation within *C. arvense* in New Zealand in its susceptibility to *S. sclerotiorum*. Further work will examine differences in the efficacy of fungal isolates against different *C. arvense* provenances.

Keywords weed, biocontrol, *Cirsium arvense*, fungus, susceptibility

INTRODUCTION

Californian thistle (*Cirsium arvense* (L.) Scop.) is a well-known agricultural weed in pastures. It is a perennial herb indigenous to Eurasia, now present throughout temperate regions of the world (Cripps et al. 2011). This economically significant weed is constantly ranked as highly troublesome (Bourdôt et al. 2014), mainly due to its propensity to regrow from an extensive creeping root system. This root system can spread up to two metres during a growing season (spring to autumn) and it persists over winter, forming adventitious buds which form aerial new shoots in the next growing season (Bourdôt et al. 2000). *C. arvense* has many negative effects: wool contamination; degradation of the quality of hay, grain and winter feed; reduction in pasture quality and loss of clover by herbicide control (Bourdôt et al.

2014). There have been many attempts at classical biological control to overcome these detrimental qualities of *C. arvense* (Cripps et al. 2011).

The current research examined the potential of the fungus *Sclerotinia sclerotiorum* ((Lib.) de Bary) as a biological herbicide. It readily infects *C. arvense* causing reduced vigour and death of plants in the field when applied inundatively as a bioherbicide. However, there has been considerable variation in its efficacy (Hurrell et al. 2001). A plausible explanation for this variability is the genetic variation between populations of *C. arvense* in their susceptibility to the fungus. The research presented here tested this hypothesis by applying the fungus under uniform conditions to provenances of *C. arvense* collected from throughout New Zealand.

Table 1 Geographic region of origin in New Zealand of the 32 provenances of *Cirsium arvense*.

Provenance ID number	Island	Geographic region
5	North	Central
3, 6, 8, 10, 11, 12, 13	North	East
2, 7, 9	North	South-West
20, 21, 25, 28, 47	South	East
33, 36, 37	South	Inland
1, 19, 22, 23	South	North
15, 16, 18, 40, 41	South	South
14	South	South-West
24, 27, 31	South	West

METHODS

C. arvense plants from 32 different provenances were established from root fragments obtained from individual plants sourced from around New Zealand. Each provenance consisted of a root sample taken from a single plant in the field which was planted and later propagated clonally (by splitting up the new roots) to obtain the replicates for the experiment. Of the 32 provenances, 11 were from the North Island and 21 from the South Island (Table 1). The provenances were grouped according to geographic region of origin: Central, East and South-West in the North Island; East, Inland, North, South, South-West and West in the South Island. The root fragments were planted into 2L pots containing a standard potting mix on the 25 and 27 August 2015. The pots were placed in a shade house and received regular overhead irrigation.

For each provenance there were 10 replicate plants (clones) ranked from smallest to largest in

their *C. arvense* biomass, with the sixth and seventh ranked plants used as controls. The experiment was set up as a randomized block design, with each block (eight in total) containing all provenances (32) of the same ranking and randomly chosen control plants (8) (Figure 1). Each block in total contained 40 plants, with 320 plants used for the entire trial.

C. arvense plants were each inoculated on 27 November 2015 with 5g of a dry mycelium-on-brewer's waste barley formulation of *S. sclerotiorum* by sprinkling the material onto the foliage of the target plant. Both treated and control plants were sprayed with water until runoff immediately before inoculation enabling the bioherbicide preparation to adhere to the foliage. Each plant, including the controls, was enclosed in a plastic bag for 2 days after treatment. Supplemental overhead watering continued throughout the course of the trial with plants receiving 15 min of water every 90 min.

Destructive measurements of plant biomass were made 3 weeks after treatment application.

40-C	24	2	40	6	15	31	36
8	22-C	18-C	11	1	33	19	21
41	47	25	12-C	11-C	3	12	9
16	37	28	23	27	10-C	16-C	18
22	10	7	13	5	14	20	3-C

Figure 1 Representation of the layout of one of the eight blocks of *Sclerotinia sclerotiorum*-treated (non-shaded cells) and non-treated control pots (C, shaded cells), with 32 provenances (one replicate of each per block) treated and eight control pots. Provenances for treated and control pots were positioned randomly within the block.

All aerial shoots in each pot were severed at ground level, placed in a forced-air drying oven at 80°C for 48 h and then weighed. The roots in each plot were washed to remove excess soil before being dried in the oven for 48 h then weighed.

Statistical analyses

The data were analysed in Genstat using an unbalanced ANOVA. The fixed effects being: Treatment (*S. sclerotiorum* or control), Date, Island (North or South) and Provenance nested within the geographic region relating to each Island. Random effects were Blocks. Residuals were checked for normality.

RESULTS

The root and shoot mean dry weights differed significantly ($P < 0.001$) between provenances (Figure 1). The shoots dry weight for the *Sclerotinia*-treated plants ranged from a mean of nearly 10 g (Provenance 36) to 33 g (Provenance 27) while the mean root dry weights ranged from as little as 2 g (Provenance 10) to 9.8 g (Provenance 8) (Figure 2).

There was a significant ($P = 0.007$) difference between that North and South Island provenances in the shoot dry weight of treated plants, with plants from the South Island having greater mean dry weight (North Island 16.3g, South Island 19g). Within the South Island, there were significant differences between the five geographical locations

Figure 2 The aerial shoot and root dry weights of *Cirsium arvense* (g per pot) for the 32 provenances, either treated with a bioherbicide preparation of *Sclerotinia sclerotiorum* or left untreated (Control). There were 8 treated and two control plants/provenance. Note the scale differs for shoot and root weights

of the *C. arvense* provenances; West, South, Inland, North and East of the South Island. The greatest difference was between the West and the East of the South Island with the mean dry shoot weight of provenances from the East being greater (22.3g) than the mean dry shoot weight of the provenances from the West of the South Island (14.3g).

There were no significant ($P>0.05$) differences in dry root mass between the North and South Island and no differences between the geographic regions of the *C. arvense* provenances ($P>0.05$). There were no significant ($P>0.05$) differences in the dry root mass between the North and South Island for the provenances tested.

DISCUSSION

This study has highlighted differences in the tested *C. arvense* provenances in their susceptibility to the plant fungal pathogen *S. sclerotiorum* when formulated as a mycelium-on-barley preparation. There was no difference between the North Island and South Island provenances of *C. arvense*, with differences in susceptibility related only to the individual provenances and the geographical region of the provenances. The results indicate that when Californian thistle plants are susceptible to *S. sclerotiorum*, this bioherbicide is effective in reducing the vigour of this weed.

This study improves our understanding of the interaction between *S. sclerotiorum* and *C. arvense*. It supports the anecdotal evidence that there are differences in the field, at a local level, in the susceptibility of *C. arvense* to the fungus when applied as a bioherbicide. Research carried out in Montana (USA) showed a variation of between 20-80% in shoot death, along with a weakened root system, in response to the bioherbicide (Bourdôt et al. 1995). They found that some shoots seemed to be resistant to *S. sclerotiorum*, which could be due to genetic differences between provenances. Houlistan & Goeke (2015) demonstrated genetic differences of *C. arvense* in these same 32 provenances, and phylogenetic placement of these provenances within specific clades may be related to susceptibility differences observed in this work.

Whilst these results do indicate large differences between provenances in their response to *S. sclerotiorum*, these differences might not be due to regional differences. In this work, each provenance represents a single plant and variability in the response to the bioherbicide may occur at a micro-geographic scale, such as between farms or even paddocks. The experiment would need to be repeated using plants from relatively small areas. If variation on this smaller scale occurs, it could be problematic for the development of bioherbicides as it could indicate the potential for a single Californian thistle population in a small area to evolve resistance to a *S. sclerotiorum* bioherbicide.

Knowing there are differences in the susceptibility of Californian thistle populations in New Zealand to *S. sclerotiorum* applied as a bioherbicide highlights the need for more research to enhance the application and subsequent effectiveness of the fungus. Such practices as wounding the plant prior to bioherbicide application has shown to increase the susceptibility of *C. arvense* (Bourdôt et al. 2004). The increase in susceptibility from wounding could negate the different susceptibility levels between the different provenances found in this experiment.

The results open up new possible research opportunities to continue the development of *S. sclerotiorum* as a bioherbicide. Investigation could focus on the virulence of different *S. sclerotiorum* isolates, the related susceptibility of *C. arvense* plants, and different bioherbicide formulations.

REFERENCES

- Bourdôt GW, Harvey IC, Hurrell GA, Saville DJ 1995. Demographic and Biomass Production Consequences of Inundative Treatment of *Cirsium arvense* with *Sclerotinia sclerotiorum*. *Biocontrol Science and Technology* 5: 11-26. DOI: 10.1080/09583159550039981
- Bourdôt GW, Hurrell GA, Saville DJ 2004. Wounding of *Cirsium arvense* enhances the efficacy of *Sclerotinia sclerotiorum* as a mycoherbicide. *New Zealand Plant Protection* 57: 292-297
- Bourdôt GW, Hurrell GA, Saville DJ 2014. Frequency of occurrence and ground cover

- of *Cirsium arvense* on pastoral farms in New Zealand: a farmer opinion survey. New Zealand Journal of Agricultural Research 57: 1-13. DOI:10.1080/00288233.2013.859626
- Bourdôt GW, Leathwick DM, Hurrell GA 2000. Longevity of Californian thistle roots. New Zealand Plant Protection 53: 258-261
- Cripps MG, Gassmann A, Fowler SV, Bourdôt GW, McClay AS, Edwards GR 2011. Classical Biological Control of *Cirsium arvense*: Lessons from the Past. Biological Control 57: 165-174. DOI: 10.1016/j.biocontrol.2011.03.011
- Houliston GJ, Goeke DF 2015. *Cirsium* genetics in New Zealand: Initial report for AgResearch Undermining Weeds Subcontract. AgResearch.
- Hurrell GA, Bourdôt GW, Saville D 2001. Effect of application time on the efficacy of *Sclerotinia sclerotiorum* as a mycoherbicide for *Cirsium arvense* control in pasture. Biocontrol Science and Technology 11: 317-330.