

THE BIOLOGICAL CONTROL OF RUSSELL LUPIN IN RIVERBEDS WITH ENDEMIC PLANT PATHOGENS

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ABSTRACT

The control of Russell lupin (*Lupinus polyphyllus*) in wildlife habitats in braided riverbeds in the central South Island is discussed. A survey of plant pathogens as prospective biological control agents for the plant was carried out. Four pathogens, all of them new records for this host in New Zealand, were identified; *Pleiochaeta setosa*, *Colletotrichum gloeosporioides*, *Verticillium albo-atrum* and *Fusarium heterosporum*. Symptoms of a disease very similar to bacterial wilt of lucerne were also found but the suspected causal organism, *Clavibacter michiganensis* subsp. *insidiosus*, has yet to be verified. In the light of experimental infection results, these pathogens were assessed as potential bioherbicides together with *Ascochyta pisi*, *Sclerotinia sclerotiorum* and *S. minor*, already recorded on lupin species in New Zealand.

Keywords: Russell lupin, wildlife habitats, diseases, bioherbicide

INTRODUCTION

The deleterious effects of exotic plants in natural ecosystems such as the braided riverbeds of the Upper Waitaki and Mackenzie Basins of the South Island of New Zealand has been well publicised by the Department of Conservation and outlined by Balneaves and Hughey (1990). The specific damage caused by introduced Russell lupin (*Lupinus polyphyllus* Lindl.) in the Ahuriri River has been the colonisation of braided river beds and loss of the open shingle habitats required by river birds such as the black fronted tern, wrybill and black stilt.

While Russell lupin is not the sole introduced plant species responsible for the loss of river bird habitats, the plant very rapidly colonises this niche. Other plants such as crack willow, gorse and broom which have been in these riverbeds for a much longer period, have not demonstrated the same invasive characteristics. This suggests that Russell lupin has great potential to be an important weed of this habitat, with many riverbeds as yet uncolonised. However, significant populations of the plant occur in the Ahuriri, Tasman, Waimakariri (Balneaves and Hughey, 1990) and Tekapo rivers and small populations are beginning to be found in many others (A.F.J. Warren pers. comm.).

Several options have been utilised for the control of weeds in selected rivers of importance to threatened bird species. These include the use of herbicides such as triclopyr (Grazon) to control Russell lupin. Balneaves and Hughey (1990) discussed these measures and considered biological control as a possible further control strategy. We report here on our search to identify potential pathogens, present within New Zealand, and suitable for use in an inundative biological control programme for Russell lupin. A survey of diseases on Russell lupin was carried out in infested riverbeds, wild stands and seed crops. Suspected causal organisms were isolated, identified and selected ones tested for pathogenicity. Pathogens of other lupin species were also isolated and tested together with some strains of wide host range pathogens previously recorded on lupin species in New Zealand (Pennycook 1989).

METHODS

Disease survey

Stands of Russell lupin were examined for diseased plants in the Ahuriri and Tekapo river beds and in the Mt Cook region in November, 1993, September and November, 1994, and March and May, 1995. Diseased plants were also collected from stands of Russell and blue lupins grown for seed from throughout Canterbury. Further diseased material was received from Department of Conservation field officers.

Microorganisms were isolated and identified from plants exhibiting disease symptoms using a range of techniques that were considered appropriate for the type of disease and the host material that was being processed. Isolates were stored on agar slopes at 4°C.

Pathogenicity testing

In vitro testing of pathogenicity to compare either isolates of a single species, or isolates of different species was carried out on disease-free excised Russell lupin tissue that was collected from plants either grown in the greenhouse or plants from the field. Plant parts were placed on moist paper towels in plastic trays in polythene bags to maintain humidity. The trays were placed under fluorescent lights on the laboratory bench.

Tests for comparative virulence of seven isolates of *Pleiochaeta setosa* (Kirch.) Hughes, two from Russell lupin and five from blue lupin, were carried out on excised leaves. The experiment was performed twice with four replicates per isolate, seven leaves per tray in a block with each leaf being inoculated with an isolate in random order within the tray. Maximum lesion radius of the resulting lesions was measured 96 h after inoculation.

Pathogenicity of a range of different lupin pathogens was also determined *in vitro* on Russell lupin tissue as described above, using leaflets, petioles and stems. The comparative virulence of the pathogens was assessed by the following scoring method: 0 = no symptoms, 1 = lesions 1 - 2 mm from inoculation point, 2 = 2 - 4mm and 3 = greater than 4mm from the inoculation point. The fungi used in this experiment were (from Table 1) *Verticillium albo-atrum* Reinke & Berth., *Ascochyta pisi* Lib., *Sclerotinia sclerotiorum* (Lib.) de Bary and *Sclerotinia minor* Jagger.

Field trials

Field inoculations were carried out in two consecutive years. In year 1, inoculation was in mid-February 1994 with *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. on individual plants grown from seed at Lincoln that were in half flower, half pod set. Inoculum of two isolates of the pathogen, one originating from Lincoln and the other from the Ahuriri river, was produced in a broth containing 2.5% malt extract (Maltex) plus 2% dextrose in 1 litre flasks containing 250 ml of broth and placed on a rotary shaker for 5 days at 20°C. The inoculum concentration was adjusted to 5×10^5 conidia/ml and applied with 0.05% Tween 80 to incipient run-off using a hand-held sprayer. Six randomly selected plants were treated with each strain in each of four blocks of plants. Disease assessments were carried out on pods 12, 25 and 35 days after application using a 0 - 5 disease index scale (0 = no infection, 1 = lesion covering up to approximately 10% of the pod, 2 = 20%, 3 = 40%, 4 = 70% and 5 = 100% of the pods infected).

Year 2 field trial was carried out using both *C. gloeosporioides* (Lincoln strain) and *P. setosa* (strain PS 11) on the regrowth of the plots that had been used the previous season. No account was taken of the previous treatments since there was little disease carry-over to complicate the assessment and the following five treatments were used: 1) Water (control), 2) *C. gloeosporioides* at 3×10^5 conidia/ml, 3) *P. setosa* at 2×10^4 conidia/ml, 4) Mixture of *C. gloeosporioides* and *P. setosa* at 3×10^5 and 2×10^4 conidia/ml respectively, 5) Mixture of *C. gloeosporioides* and *P. setosa* at 1.5×10^5 and 1×10^4 conidia/ml respectively.

Pleiochaeta setosa conidia were produced in bulk using a diphasic liquid (PD broth)/solid (diatomaceous earth) method that was developed during this study, while *C. gloeosporioides* conidia were produced in malt extract plus dextrose broth as above. The treatments were applied three times over the 1994-95 season; pre-flowering, mid-flowering and during maximum pod production, using 20ml of each of the above treatments plus Tween 80 wetting agent per plant. The trial was a randomised block

design, with four blocks of five treatments. Each treatment plot contained four plants. The plants were assessed 4 weeks after the final application for percentage of flowering stems with lesions and percentage stems dead.

RESULTS

Disease survey

Survey results, and a list of organisms assessed for their suitability as biological control agents against Russell lupin are given in Table 1. Several pathogens used in the study were not found on Russell lupin, but were known overseas and in New Zealand (Pennycook 1989) to cause diseases of *Lupinus* spp. There are four new host records for New Zealand on Russell lupin in the list.

TABLE 1: Pathogens located on Russell lupin during the survey plus other lupin pathogens assessed.

Pathogen (and Disease)	Host source(s)	Location	Symptoms
<i>Colletotrichum gloeosporioides</i> (Anthracnose) ¹	Russell lupin (<i>Lupinus polyphyllus</i> Lindl.)	Ahuriri River Lincoln	Twisting of stems and petioles with red-brown lesions (pink spore masses at centre). Similar lesions on pods; stem death
<i>Pleiochaeta setosa</i> (Brown leaf spot) ¹	Russell lupin Blue lupin (<i>Lupinus angustifolius</i> L.)	Ahuriri River Lincoln	Leaf spotting often with dendritic pattern; yellowing and defoliation
<i>Fusarium heterosporum</i> (Bract, crown and root rot) ¹	Russell lupin	Ahuriri River Mt Cook	Red brown rotting of bracts, crowns and roots, plant stunting and death
<i>Verticillium albo-atrum</i> (Wilt) ¹	Russell lupin	Ahuriri River	Wilting, vascular staining and root rotting
<i>Ascochyta pisi</i> (Foot rot)	Blue lupin	Ashburton	Stem lesions and plant collapse ²
<i>Sclerotinia clerotiorum</i> (Watery soft rot)	Californian thistle (<i>Cirsium arvense</i> L.) Strain S9	North Canterbury	Stem lesioning, plant wilting and death ²
<i>Sclerotinia minor</i> (Watery soft rot)	Nodding thistle (<i>Carduus nutans</i> L.) Strain S32	Maniototo	Stem lesions, plant wilting and death ²
<i>Clavibacter</i> sp. (?) (Bacterial wilt)	Russell lupin	Ahuriri River Mt Cook	Witches broom, leaf cupping, plant stunting, root staining and rotting

¹New host record for New Zealand

²Usual symptoms caused by this pathogen; not confirmed on Russell lupin

The wilt pathogen *Verticillium albo-atrum* was not found in the first season survey of Russell lupin in the Ahuriri River, but was in the second.

Symptoms similar to those described for bacterial wilt of lucerne (Close and Mulcock 1972) were commonly seen on Russell lupin both in the Ahuriri River and at Mount Cook Village. Many bacteria were isolated and tested for pathogenicity using root soak and root cutting techniques, but no isolates proved pathogenic. Despite numerous attempts, no isolations of the suspected causal bacterium, *Clavibacter* spp., were made.

Pathogenicity testing

Isolates of *P. setosa* were collected during the survey on diseased Russell lupin, and from stands of blue lupin from the Lincoln area. Results of tests to compare pathogenicity of these isolates are given in Fig. 1. Isolates collected from Russell lupin were generally more virulent on that host than those from blue lupin, but two isolates from blue lupin (PS4 and PS6) gave mean lesion sizes that were not different ($P > 0.05$) to those for isolates from Russell lupin.

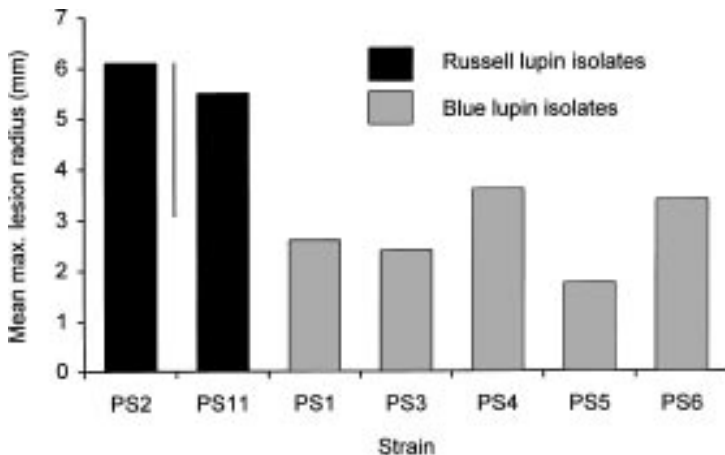


FIGURE 1: Mean lesion radii for seven isolates of *Pleiochaeta setosa* from two host sources, on excised leaves of Russell lupin, 96 h after inoculation. Vertical bar is the LSD ($P < 0.05$).

The proportions of the different types of Russell lupin tissue infected and mean disease scores in the experiment to compare the relative virulence of a range of lupin pathogens are given in Table 2. The data on the percentage of tissue types infected in Table 2 were analysed by chi square test, using 0 = not infected and 1 = infected for each of the eight plant portions per treatment. On leaflets, *A. pisi* was more pathogenic than *S. minor* ($P < 0.01$), with no differences ($P > 0.01$) between other pairs of pathogens. On cut stems, all the pathogens gave 100% infection. On petioles and uncut stems, there were no differences ($P > 0.01$) between all the pathogens. In terms of disease score, *V. albo-atrum*, *S. sclerotiorum* and *A. pisi* were very virulent on cut stems, but not on other tissue types. *S. minor* appeared to be less virulent on cut stems and other tissue types than all the other pathogens tested.

Field trials

When the two isolates of *C. gloeosporioides* were compared directly in the field, the isolate from Lincoln demonstrated higher initial virulence, but 35 days after treatment there was no difference ($P > 0.05$) between the isolates. The Lincoln isolate was therefore selected for inclusion in the second year's experiment because of its apparent faster rate of initial infection (Fig. 2).

TABLE 2: Percentage infection and mean disease score on various tissue types of Russell lupin 5 days after inoculation at 22°C with four pathogens *in vitro*.

Pathogen	Leaflet	Petiole	Stem	Cut stem	Mean
% of tissue portions infected					
<i>A. pisi</i>	75	25	25	100	56
<i>V. albo-atrum</i>	25	25	0	100	38
<i>S. minor</i>	0	0	25	100	31
<i>S. sclerotiorum</i>	37	0	50	100	47
Disease score ¹					
<i>A. pisi</i>	1.1	0.5	0.3	2.8	1.2
<i>V. albo-atrum</i>	0.4	0.3	0.0	3.0	0.8
<i>S. minor</i>	0.0	0.0	0.3	1.8	0.4
<i>S. sclerotiorum</i>	0.8	0.0	0.5	3.0	1.1

¹Disease score - 0 = no symptoms; 1 = lesions 1 - 2 mm from inoculation point; 2 = 2 - 4mm and 3 = greater than 4mm from the inoculation point.

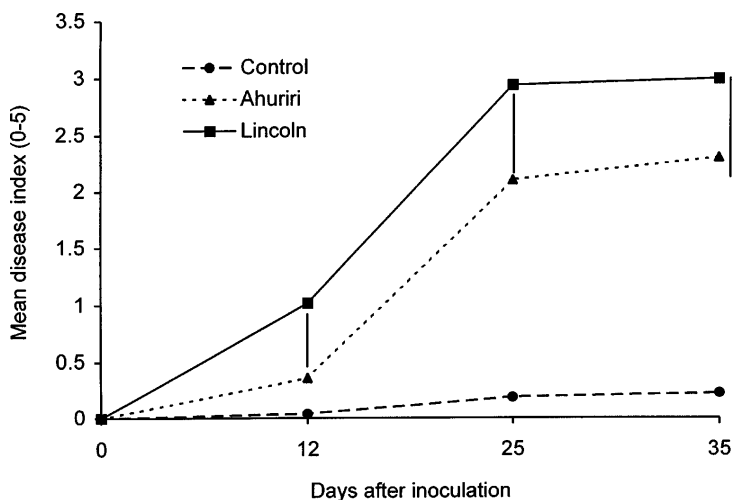


FIGURE 2: Mean disease indices on pods of Russell lupin inoculated with two strains of *Colletotrichum gloeosporioides* (from Ahuriri River and Lincoln) in the field. Vertical bars are the LSD values ($P < 0.05$).

The second year application of both *C. gloeosporioides* and *P. setosa* to the same Russell Lupin stand on three occasions during the season gave results shown in Table 3. In untreated plants, 15% of stems were dead, mostly from natural *C. gloeosporioides* infection. Inoculation with *C. gloeosporioides* gave no increase in the percentage of plants that died, but in combination with *P. setosa*, many additional anthracnose lesions occurred and occasional severe whole plant damage was achieved, that increased the overall disease score. Infection with *P. setosa* was readily initiated by inoculation, but although the pathogen caused rapid initial symptoms, little leaf damage or defoliation occurred. Although *P. setosa* application doubled the percentage

of plants that died compared to the water control, the cause of stem death was always due to anthracnose infection, with *P. setosa* application increasing ($P < 0.01$) the percentage of stems with lesions of *C. gloeosporioides*. The application of *P. setosa* at full strength doubled the impact of *C. gloeosporioides* and at half strength gave an intermediate effect, suggesting that the inoculum rate of *P. setosa* to achieve good infection should not be reduced below 2×10^5 conidia/ml.

TABLE 3: Percentage of Russell lupin stems with lesions and percentage stems dead when treated three times with two pathogens, either alone or in combination, 4 weeks after the final inoculation.

Treatment	% stems with lesions	% stems dead
1. Control	39	15
2. <i>C. gloeosporioides</i>	35	17
3. <i>P. setosa</i>	69	35
4. Full strength mixture	64	33
5. Half strength mixture	53	25
LSD (5%)	19	28
Contrasts		
<i>C. gloeosporioides</i> (Trts 2&4 vs 1&3)	not sig.	not sig.
<i>P. setosa</i> (Trts 3&4 vs 1&2)	$P < 0.01$.	$P < 0.10$.
Interactions	not sig.	not sig.

DISCUSSION

The survey of Russell lupin over 2 years confirmed that there was a range of pathogens that attack the host that have potential as biological control agents. A desirable attribute of a bioherbicide for Russell lupin control in braided riverbeds is its ability to be contained to the area of application, since the plant has considerable aesthetic value in the region. All of the pathogens found in the survey are limited in their distance of spread or are seed-borne, making them suitable for consideration as biological control agents.

Colletotrichum spp. are often preferred as the bases for mycoherbicide development (Templeton 1992). They are normally host specific and readily produce numerous conidia in submerged culture that can be the basis for an applicable formulation. Our studies showed that the species of *Colletotrichum* causing anthracnose on Russell lupin was readily grown in liquid culture and infected stems, petioles and pods in the field.

The success of a biological control agent against Russell lupin will depend not only on the success of the pathogen in killing the foliage, flowering stems and pods, but also on its ability to rot out the crown of the plant so that it will produce no new shoots the following season. Neither *C. gloeosporioides* nor *P. setosa* appeared to be able to penetrate this part of the host. *Colletotrichum gloeosporioides* was capable of causing die-back and apparent plant death in a season, but plants produced new shoots the following season. The addition of *P. setosa* conidia to the inoculum increased the level of anthracnose, but the inclusion of the two pathogens in a formulation is unlikely to be economic. Production of *P. setosa* conidia requires a diphasic system and conditions for sporulation are fastidious (Harvey 1975) meaning that large scale production may be slow and expensive.

Our observations in the field suggest that the pathogen causing bacterial wilt-like symptoms could be eminently suitable as a bioherbicide, since it appeared to cause complete plant death. Bacteria are generally readily produced by fermentation for inclusion in a formulation, and the prospects appeared to be considerable. However, our efforts to isolate and identify the primary cause of the debilitating symptoms were

unsuccessful.

Of the other pathogens tested that were suspected as having possible potential as mycoherbicides against Russell lupin, *A. pisi* showed the most promise. Little is known of the reaction of Russell lupin to this fungus, and further work would be justified to determine the amount of damage that it could inflict, especially to crown material.

Crown and root rot caused by *Fusarium heterosporum* Nees (Table 1) was recognised after the second field experiment as a major cause of plant death in the field plots of Russell lupin at Lincoln, where populations were reduced over two years by the disease. Since crown destruction is a major objective in Russell lupin control as it is with the biological control of giant buttercup (Green *et al.* 1993), further investigation of this pathogen is warranted. It appears from our collections and observations in the field that the pathogen enters plants through bracts, and advances to the crowns, eventually causing complete crown and root rotting.

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REFERENCES

- Balneaves, J.M. and Hughey, K., 1990. The need for control of exotic weeds in braided river beds for conservation of wildlife. *Proc. 9th Aust. Weeds Conf., Adelaide.* 103-108.
- Close, R. and Mulcock, A.P., 1972. Bacterial wilt *Corynebacterium insidiosum* (McCulloch, 1925) Jensen, 1934 of lucerne in New Zealand. *N.Z. J. Agric. Res.* 15: 141-148.
- Harvey, I.C., 1975. Studies on the growth and development of the hyphomycete *Pleiochaeta setosa*, a pathogen of lupins. PhD thesis, Bristol University, 130pp.
- Green, S., Saville, D.J., Bourdôt G.W., Harvey, I.C., Field, R.J. and Close, R.C., 1993. Regeneration sites of *Ranunculus acris* are the targets for *Sclerotinia sclerotiorum*, a potential mycoherbicide. *Proc. 46th N.Z. Plant Prot. Conf.:* 274- 277.
- Pennycook, S. R. 1989. Plant diseases recorded in New Zealand. 1st ed. Vol. 1. Plant Diseases Division, DSIR, Auckland. 276 pp.
- Templeton G. E., 1992. Use of *Colletotrichum* strains as mycoherbicides. Pp 358-380 *In: Colletotrichum: Biology, Pathology and Control.* J.A. Bailey and M.J. Jeger (Eds); CAB International, Wallingford Oxon, U.K.