Chondrostereum purpureum reduces the regeneration of gorse (*Ulex europaeus*) following application to cut stumps

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Summary Twenty four gorse (*Ulex europaeus* L.) bushes each about 1 m tall in a grazed hill pasture in Canterbury, New Zealand, were cut to near ground level in May 2005 before applying three treatments to determine if the stumps could be artificially infected with the fungus *Chondrostereum purpureum* (Pers.) Pouzar. The stumps of eight of the plants were treated with the mycelium of the fungus on agar, a further eight were treated with mycelium formulated as a slurry, and the remaining eight plants were left untreated. One year after treatment, fruiting bodies of the fungus (basidiocarps) were present in large numbers on 15 of the 16 treated stumps. Seventeen months after treatment (November 2006), half of the *C. purpureum*-treated stumps were dead, with an overall reduction in the volume of regenerating shoots of 64% as compared to the untreated bushes. These results confirm that *C. purpureum* has potential as a biological control agent for gorse.

Keywords Scrub-weed control, biological weed control, mycoherbicide.

INTRODUCTION

Gorse (*Ulex europaeus* L.) was recognised as a weed in New Zealand by 1865, when, in Taranaki its planting for fodder and hedging was prohibited (Ivens 1978). It remains a weed of major concern in pastoral agriculture and plantation forestry. Its control, typically a two-stage process of site-preparation to kill existing plants, and follow-up control of seedlings in the new pasture or forest, is facilitated through 70 registered herbicide products, containing one or more of 18 active ingredients; seven effective on seedlings and 11 on mature woody plants (Young 2008). While the effective use of these herbicides, their formulation and their integration with fire, crushing, cutting, root-raking, competition from grasses and grazing, is supported by a considerable body of research (Chavasse and Davenhill 1973a,b, Davenhill 1976, Hamblyn 1951, Hartley *et al.* 1983, Ivens 1978, Matthews 1950, Popay and Field 1996, Preest 1979, Preest and Davenhill 1985, Radcliffe 1990), increasing numbers of pastoral farmers and forest owners in New Zealand are seeking to reduce the use of these chemicals. For example, approximately 700,000 ha of plantation forest area is now certified through the Forest Stewardship Council (FSC 1996). Criterion 6.6 of Principle 6 (Environmental Impact) requires a commitment to the development and adoption of non-chemical methods of pest management and to strive to avoid the use of chemical pesticides. In such systems, integrated pest management is essential, with primary reliance on prevention and biological control methods rather than chemical pesticides. Similarly in pastoral agriculture, increasing numbers of New Zealand farmers are committing to 'organic' production, and hence reduced use of chemical herbicides, through certification schemes such as BioGro (BioGro 2007).

The wood-rotting fungus, *Chondrostereum purpureum* (Pers.) Pouzar, occurs naturally on gorse in New Zealand and is potentially acceptable as an alternative to chemical herbicides for gorse under these certification schemes. Its ubiquitous occurrence in NZ implies a low environmental risk should it be used as a mycoherbicide (Ramsfield 2006). In an experiment in Canada, when applied to the wound surfaces of gorse stems cut near soil level, a local strain of the fungus was slow to act and reduced stump survival by 40% after two years (Prasad 2005). By contrast, in New Zealand when applied (in autumn) to the cut surfaces of decapitated 11–12 mm diameter stems, NZ isolates of the fungus acted rapidly (within 100 days), halving the survival of the distal 100 mm of stem below the cuts from 56 to 29% (Bourdôt *et al.* 2006). Since the depth of penetration of this fungus has been shown to increase from 50 mm from a 10 mm wound, to 250 mm from a 70 mm wound (Spiers and Hopcroft 1988), we hypothesised that the NZ isolates would also kill plants if applied to larger wounds created by cutting the main stems near the ground (Bourdôt *et al.* 2006). Here we report on an experiment that tested this hypothesis.

MATERIALS AND METHODS

The experiment was located on a beef cattle farm at Teddington in Canterbury, New Zealand (43°40.8'S, 172°39.9'E), where 24 separate, but similar-sized gorse plants, were selected in triplets. In May 2005, the foliage and stems of all of these plants were removed 50 mm above the ground with a chain saw. Any lower shoots were also removed, leaving clean stumps with horizontally cut surfaces. The mean (± SD) number of stumps, stump diameter (mm), and stump surface area (cm^2) per plant were 5 ± 3, 38 ± 14, and 62 ± 39 respectively.

The treatments applied to the cut stump surfaces (Table 1) were: (1) control, (2) mycelium on agar + Parafilm® and (3) mycelium formulated as slurry. Eight replicate plants for each treatment were arranged in a randomised-block layout.

An isolate of *C. purpureum* from *Malus domestica* (Borkh.) in New Zealand was inoculated onto malt extract agar (MEA) in Petri dishes. After seven days, the colonies were sub-cultured onto fresh MEA and incubated for a further seven days. For treatment (2) discs of colonised MEA were placed mycelium-side down on the cut stump surfaces and sealed on the stump using Parafilm. For treatment (3) the slurry was prepared by autoclaving a 1.5% malt extract solution in 250 mL lots in 500 mL Erlenmeyer flasks, each flask being inoculated with 20 × 5 mm agar cores of mycelium taken from the 7-day Petri dish cultures. After further incubation in a shaker at 24°C for 14 days, the cultures were chopped in a blender for five seconds to disperse the mycelia, and combined using a magnetic spin-bar stirrer. Canola oil (about 15% v/v) was added until the stirred mixture began to emulsify. A few drops of Tween® were added, and stirring continued for another two hours. Forty mL of this preparation was brushed on to the cut stump surfaces of each plant.

The treatment effects were assessed on three occasions at about six-monthly intervals by measuring the height (h) and width (w) of the regenerating plants and converting these measurements to plant volume (v), by $v = \pi(w/2)^2 \times h$, assuming a cylindrical shape. Plants with no regenerative shoots were deemed dead. The presence of *C. purpureum* basidiocarps was noted.

Table 1. Effects of *Chondrostereum purpureum* applied to cut-stump surfaces of gorse plants 12 and 17 months after treatment.

Treatment	Plants with basidiocarps at 12 months (%)	Plants dead at 17 months (%)	Reduction in re-growth volume at 17 months (%)
Control (1)	0	13	–
Agar (2)	88	63	56
Slurry (3)	100	50	71
LSD (5%)	na	na	56
C *vs* **A** and **S**	*** [A]	ns [A]	***
A *vs* **S**	ns [A]	ns [A]	ns

There were eight replicate plants for each treatment; na not applicable; ns not significant; *** $P<0.001$; [A] using a chi-squared test.

Figure 1. Basidiocarps of *C. purpureum* growing on a gorse stump (40 mm diameter) treated with the fungus in May 2005, Photo – May 2006.

RESULTS AND DISCUSSION

Twelve months after treatment, in May 2006, the stumps of all but one of the 16 gorse plants treated with the *C. purpureum* (seven of the eight agar- and all eight of the slurry-treated plants) bore numerous basidiocarps (sporulating fruiting bodies) (Table 1 and Figure 1). None of the stumps of the 'control' (cut only) plants bore basidiocarps, confirming that the infections were a result of the applied fungus.

Seventeen months after treatment, in October 2006, 13% of the control plants (one of eight) were dead, whereas 63 and 50% of the agar- and slurry-treated plants respectively were dead (Table 1). This mortality, a mean of 57%, combined with fewer and slower-growing regenerative shoots, resulted in a net reduction in volume of the regenerating gorse of 56 and 71% for the agar- and slurry-treated plants respectively (Table 1). This difference was not significant, and the mean effect across 'agar' and 'slurry' was a 64% reduction in regenerating plant volume (Table 1). The effect of the applied *C. purpureum* on the cut gorse stumps was greater than in the Canadian experiment; the mean percent reduction in stump survival attributable to the fungus was 50% $(1 - [100 - (63 + 50)/2]/[100 - 13]) \times 100$ (Table 1), while in the Canadian experiment it was 40% $(1-51/85) \times 100$ (Prasad 2005).

These reductions in the survival of the gorse stumps attributable to the applied *C. purpureum* provide support for the hypothesis that the fungus can kill gorse plants. However the reductions are considerably lower than those of >80% that have been achieved in several other woody species (Bourdôt *et al.* 2006). These greater effects have enabled the commercialisation of *C. purpureum* for stump control in several of these species in North America. The fungus is currently registered as Myco-Tech™ Paste (isolate HQ1, Myco-Forestis Corporation) for inhibiting re-growth of stumps of five tree species east of the Rocky Mountains in the US and Canada, and as Chontrol Paste (isolate PFC 2139, Canadian Forest Service – Pacific Forestry Centre and Mycologic Inc.) for use against two other species.

Whilst this experiment has confirmed that *C. purpureum* has potential as a mycoherbicide for gorse, improvement in its efficacy will be necessary for its commercial application in forestry and pastoral agriculture. Research into formulation, wounding and application methods, time of application, and isolate virulence, could reveal how such improvement might be realised.

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