Waterford Institute *of* Technology

INSTITIÚID TEICNEOLAÍOCHTA PHORT LÁIRGE

Achieving effective Rhododendron control by investigating novel methods of forest vegetation management

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Submitted in accordance with the requirements for the degree in Doctor of Philosophy

Waterford Institute of Technology

School of Science

2014

Abstract

In Ireland one of the most serious invasive alien species which poses a threat to local biodiversity, particularly to our native woodlands, is *Rhododendron ponticum* L.. Rhododendron was first introduced to Ireland during the 19th century as an ornamental garden plant and has since become an established invasive species throughout Ireland. Rhododendron has also, in recent decades, become a significant management issue in plantation forests throughout Ireland. This study sets out to improve our understanding of the auto-ecology and invasion dynamics of rhododendron in Irish forests and to investigate control options to inform future rhododendron management plans. The study was divided into three broad areas.

The first study sought to investigate the efficacy of a recently discovered Irish isolate of the fungal pathogen *Chondrostereum purpureum* as an inhibitor of rhododendron and *Betula pendula* (birch) sprouting in Ireland. The treated stumps were monitored for fungal colonisation and adventitious sprouting for the ensuing 18 months. The results demonstrated that a combination of mechanical cutting and the subsequent application of *C. purpureum* is not an effective method of vegetation management for either rhododendron or birch.

As a successful primary invader, rhododendron is often found in areas when recent land management activities have taken place. Disturbed substrate coverage and the absence of predators provide rhododendron with optimum regeneration conditions. Many land management practices (particularly in a forestry situation) expose soil. To assess how the disturbance of vegetation relates to successful rhododendron establishment the degree to which the depth of substrate affects the germination of rhododendron seeds was tested. This second study

demonstrated that even small decreases in forest litter depth, sufficient to expose bare substrate, facilitates rhododendron seedling establishment.

The third study set out to investigate whether the prevention of grazing of native scrub species in woodland sites could be used to prevent the spread of rhododendron. The findings to date suggest that fencing does increase the survival of native scrub species birch and *Ilex aquifolium* (holly). Also of note, in some of the plots, the holly and holly/ birch mix have successfully suppressed the re-growth of rhododendron.

In conclusion, rhododendron has become a huge problem in plantation forest habitats and in order to control it effectively and economically an integrated pest management strategy will have to be employed utilising a mixture of novel bio-controls, innovative management strategies exploiting weaknesses such as seed longevity, viability and litter depth, and ensuring that other plant species can compete free of grazing pressures.

Acknowledgements

I am very grateful to my supervisor Dr. Nick McCarthy, whose help and guidance has been invaluable through these years. Also special thanks to Dr. Sandra Irwin and Prof. John O'Halloran for their suggestions, corrections and ideas for all the manuscripts in this thesis.

I would like to thank the Head of the Science department at Waterford Institute of Technology Peter McLoughlin for providing the working facilities within the department. Also special thanks to Dr. Milo Ó Rathaille for his invaluable help with the statistical analysis.

I would like to thank Dr. Marion K. Seier, senior plant pathologist at the Commonwealth Agricultural Bureaux International (CABI) in the UK, who helped locate and isolate the Irish strain of *C. purpureum*. I am grateful to Dr. Aoife Dillon (Coillte) and the Lismore forest estate manager Mr Adrian Booth, both of whom provide the field sites for these experiments along with help and advice on establishing them.

An Advisory Group was established as part of the PLANFORBIO research programme and met three times during this project. I am grateful to Dr. Daniel Kelly, Ms Sue Iremonger, Dr. John Cross, Mr Noel Foley, Mr Keith Kirby, Mr Alistair Pfeifer, Mr Tor-Bjorn Larsson and Mr Allan Watt for their advice and support on this project.

Finally I wish to thank my family and friends; without their support this chapter in my life would not have been possible.

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1. Introduction

1.1 Invasive Alien Species

The introduction of non-native species into new habitats, compounded by favourable environmental conditions and a lack of associated natural enemies, sometimes permits these species to become invasive. Successful invasions by exotic or alien species can have severe impacts on the biodiversity and integrity of the invaded habitats as well as on local economies (Mack et al. 2000, Manchester and Bullock 2000, Mooney and Cleland 2001, Gherardi and Angiolini 2004, Charles and Dukes 2007, Theoharides and Dukes 2007, Barratt et al. 2010). As human population levels continue to increase and resources become more and more stretched, the impact of invasive species is likely to become more problematic in tandem with the associated increase in habitat modification and climate change (World Resources Institute 2005, Pejchar and Mooney 2009).

Referred to as Invasive Alien Species (IAS) (Shine et al. 2010), these species exert specific negative pressures on native flora and fauna including predation, competition, introduction of pathogens and parasites and dilution of native gene pools. These burdens can result in the alteration of native habitats and food webs putting pressure on native biota. To be considered successfully established an IAS must colonise a site by creating self-sustaining, expanding populations. All of which depends on their encountering compatible environmental conditions and biotic processes in the local ecosystem (Theoharides and Dukes 2007). These IAS's must prevail over a lack of genetic variability, the Allee effect residing with well established native species and biotic and abiotic stochasticity (Sakai et al. 2001).

Few IAS are successful, with just 10% of exotic species developing into naturalised populations (Williamson and Fitter 1996). Successful management of invasive species relies on co-operation between scientific researchers, the commercial sector and policy makers (Stokes et al. 2006). In Ireland one of the most serious invasive alien species posing a threat to biodiversity is rhododendron (*Rhododendron ponticum* L.) (Parrott 2013).

1.2 Rhododendron

Rhododendron was first introduced into Ireland in the 19th century (Cross 1981) as an ornamental garden plant. Its suitability as game cover saw the species introduced into many woodland habitats and it has since become an established invasive species throughout Ireland and the UK (Cross 1981, Kelly 1981, Gritten 1995, Dehnen-Schmutz et al. 2004). Rhododendron is native to an area south of the Black Sea (the Caucasus, northern Turkey and the southeast corner of Bulgaria). Rhododendron was first introduced into Britain in 1763 from southwest Spain (Coats 1963). Subsequent introductions to the UK have not been well documented and are believed to have originated from the Black Sea (Cross 1975, Stace 1991). Morphological studies of naturalised rhododendron suggest that it has been subject to introgressive hybridisation with other introduced rhododendron species since it first arrived in Ireland and the UK (Milne and Abbott 2000).

The current understanding is that three American species of rhododendron; *R. catawbiense* Michx., *R. maximum* L., and *R. macrophyllum* G. have hybridized with rhododendron (Davidian 1992, Stace 1997, Cullen 2011). These four species, referred to as the ‘ponticum group’ are responsible for the invasive plants found today across Ireland and Britain (Cullen 2011). They have hybridised and back-crossed both naturally and by design (it is thought that the genetic input from *Rhododendron catawbiense* has increased its cold tolerance (Milne and Abbott 2000)). The result is a naturalised rhododendron better equipped to flourish in its new environment. This hybrid is stable and a successful invasive which acts much like a distinct species found only in Ireland and Britain (Erfmeier and Bruelheide 2005, Cullen 2011). As such a new name has been proposed to describe the hybrid – *Rhododendron x superponticum* Cullen.

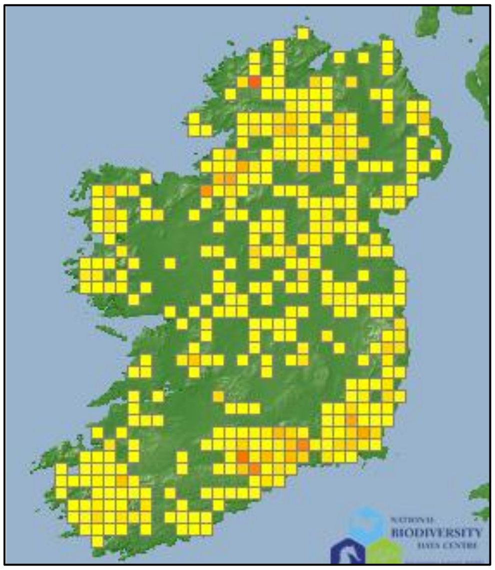

Plate 1.
Distribution of rhododendron across Ireland. Recorded sightings are graded from yellow (1/km2) to red (30/km2). (Biodiversity Data Centre, accessed Feb 2014)

Rhododendron is an extremely successful invasive species in Ireland (Maguire et al. 2008) where it has become established in several Annex 1 habitats listed under the EU Habitats Directive, including old oak woodland (this woodland is classified as WN1 by The Heritage Council (Fossitt 2000))(Plate 1). Rhododendron is now one of the greatest conservation problems for protected oak woodlands in Ireland (Kelly 2007). Where rhododendron becomes invasive it poses a serious threat to the biodiversity of native flora and fauna as it has important competitive advantages over native understory species (Cross 1975, Manchester and Bullock 2000, Peterken 2001, Dehnen-Schmutz and Williamson 2006, Parrott 2013).

Many morphological and ecological characteristics allow rhododendron the advantage in non-native habitats and facilitate its invasiveness, for example better growth rates and seedling recruitment compared with competing native species (Erfmeier and Bruelheide 2004). The dark undergrowth and toxic leaf litter can combine to produce a sterile ground layer which prevents natural regeneration (Edwards 2006) and has negative impacts on the biodiversity potential of the invaded site (Cross 1975). The reduction in native flora species has a knock-on effect on the abundance of native fauna that rely on native plants for resources such as food and shelter (Cross 1975).

1.3 Rhododendron as an environmental threat.

Where conditions favour its regeneration rhododendron can spread prolifically into neighbouring areas, particularly where little or no land management operations are carried out. Once rhododendron has become established at a site, its allelopathic effect and shading of the ground layer with its large waxy leaves smothers the regeneration of local trees and other plants at the site (Cross 1982, Rotherham and Read 1988). Rhododendron also invades commercial plantation forests in Ireland where it causes problems of accessibility and interferes with forest management operations where the dense understory presents financial and logistical challenges (Dehnen-Schmutz et al. 2004). Rhododendron infestation increases the cost of all silvicultural operations and the cost of reclaiming infested land threatens the profitability of commercial forest plantations (Robinson 1980). Rhododendron also acts as a reservoir for the plant pathogen *Phytophthora ramorum* which causes Sudden Oak Death (Maguire et al. 2008).

Forest cover in Ireland today is 10.5%, well below the EU average of 30% (Forest Service 2013). Afforestation in Ireland got underway at the beginning of the 20th century and remained low (compared to other European counties) in Ireland until the end of the 1980s when an improved grant system was introduced. The new system focused on encouraging private land owners to invest in forestry with financial incentives, including incentives to facilitate biodiversity conservation in plantation forests. Plantation forests in Ireland are required to set aside at least 15% of their land cover for biodiversity enhancement, which essentially means that these designated areas must remain free from major management operations such as vegetation clearing, clearfell etc.

Should rhododendron establish itself in these areas, as well as significantly reducing the biodiversity viability of the immediate area, the effect of the rhododendron spreading throughout the plantation undermines the philosophy of many regenerative schemes including the Native Woodland Scheme. Under the Native Woodland Scheme forest owners are supported, through the awarding of grants, to plant and maintain stands of native Irish trees, including oak (*Quercus* spp.) ash (*Fraxinus excelsior)*, Scots pine (*Pinus sylvestris)*, holly (*Ilex aquifolium)* etc. However, some of the acidic soils favoured by these species are ideal for rhododendron.

A study conducted by Harris et al (2011) into the invasion potential of the species in different habitats found that the highest invasion speeds were found in conifer habitats and the highest invasion densities were found in open habitats, however deciduous habitats displayed " intermediate invasion potential that is most vulnerable to relative rapid and dense invasion". Should it establish itself as an understory in these stands, it invariable prevents the natural regeneration of native trees and suppresses other ground flora (Plate 2).

Plate 2.
A typical example of rhododendron encroaching through a mixed species plantation forest.

A study conducted by Baker (1974) identified the attributes of an ‘ideal weed’, including fast vegetative growth to flowering, production of large quantities of seed, vegetative propagation and non-specialised pollination systems and germination requirements. While these characteristics describe the ecophysiology of rhododendron they are not the only reasons for its successful establishment in Ireland. In its native habitat (The Black Sea), rhododendron occurs in small relict populations and is not considered invasive (Mejías et al. 2002). In Turkey and Georgia it is an integral part of the forest flora and its abundance is limited by drought and by vigorous competition. Where a forested area is clear-felled rhododendron becomes aggressive, quickly colonising the disturbed site much in the same manner as in Ireland.

The continued requirement for rhododendron control in Ireland’s remaining native woodlands means that the demand for rhododendron control remains an important management consideration across the Irish forest estates and non-native woodland. Despite the clear need for effective rhododendron control strategies, no definitive intervention has been established to date and there is a lack of clear scientific evidence for some commonly implemented management and control strategies (Tyler et al. 2006).

1.4 Rhododendron control

Effective rhododendron control can greatly increase the economic value and prevent negative ecological impacts on invaded land. Current best practice for rhododendron control combines three strategies: initial removal, control of stems and root and follow up control (Barron 2008, Higgins 2008, Maguire et al. 2008). A number of options are currently available for the control of stems and root following initial removal including digging out of stumps; stump treatment with herbicide; stem injection and spraying of re-growth (Higgins 2008). The most common method of rhododendron control is a combination of manual cutting and herbicide application (Dehnen-Schmutz et al. 2004, Edwards 2006, Maguire et al. 2008). These control strategies are long-term, incur recurring costs and do not offer guaranteed effectiveness (Dehnen-Schmutz and Williamson 2006, Tyler et al. 2006, Baars 2011). The cost of management activities can be prohibitive (€200 - €10000 per hectare depending on site accessibility, control method, density of bushes) (Dehnen-Schmutz et al. 2004, Willoughby et al. 2004, Edwards 2006, Maguire et al. 2008, Parrott 2013).

Herbicide use, while efficient and cost effective, has caused increasing problems including public concern over contamination of water sources and harmful effects to non-target flora and fauna (Jobidon 1991, Gherardi and Angiolini 2004). Negative effects can also arise through over application and soil persistence of some herbicides (Gianelli et al 2014, Malone et al 2012). Herbicide resistance has also been noted in many weed species (Heap 2009). There is particular concern over the use of chemicals in environmentally sensitive sites as many herbicides, including glyphosate, can accumulate in soil and underground water (Wilson 1969, Aparicio et al 2013, Bailey 2014).

1.5 Bio-herbicide control

For many years glyphosate has been the mainstay of vegetation management practices in Ireland (Mc Carthy 2005, Barron 2008). Its use has become so prevalent that it is being used from invasive weed control programs, e.g. rhododendron eradication, to control of re-growth in thinned broadleaved stands. However concerns with overuse, and documented effects to the residual crop such as ‘flashback’, coupled with certification process bodies such as the Forest Stewardship Council (FSC) demanding a reduction in pesticide use and also a move towards Continuous Cover Forestry and Close to Nature Forestry silvicultural programs has prompted foresters to start looking at viable alternatives (World Resources Institute 2005, Heap 2009, Mc Carthy et al. 2011). A relatively new innovation in vegetation control generally is to use, in tandem with mechanical treatments, biological vegetation control (Julien and Griffiths 1998, Green 2003).

Biological control is defined as the suppression of a population through the action of predators, parasites, or disease organisms (Baker 1987). It is a progressive and environmentally friendly method of keeping invasive species in check while also satisfying the general trend towards phasing out chemical use in forest management (Templeton et al. 1979, Bailey 2014). Plant pathogens can be targeted towards specific weed species while not affecting neighbouring non-target species (Wilson 1969). Bio-herbicides based on living microbes and their bioactive compounds have been researched and promoted as replacements for synthetic pesticides for many years, but lack of efficacy, inconsistent field performance and high costs have meant that there has not been a significant uptake of these products (Glare et al. 2012b). Rhododendron has been identified as a suitable target weed for control using the bio-herbicide approach (Green 2003). The most appropriate biological control method for rhododendron is an inundative bio-control strategy (the release of an overwhelming number of a mass produced biological control agent in expectation of a rapid reduction of the target pest population), which is based on the use of indigenous control agents (Gherardi and Angiolini 2004). A suitable indigenous pathogen is mass produced and then applied to the target vegetation in a manner similar to chemical control agents with the goal of repressing or eliminating the target species (Templeton et al. 1979). Indigenous control agents are used to reduce the potential of the agent to persist in the treatment area at unnatural or endemic levels or to migrate from the treatment area. Pre-existing biotic and abiotic controls will prevent the introduced agents from spreading beyond the target area. For example, species of flora surrounding the treated area not susceptible to the pathogen or a change in land use type which may physically prevent the pathogen from spreading (Wall et al. 1992).

Several bio-control strategies have recently been developed including a box aerial-release system for the mass release of the gall forming wasp, *Tetramesa romana*, a biological control agent of the giant reed, *Arundo donax* which is an invasive weed of waterways and riparian areas in the south-western U.S. and Mexico (Goolsby and Moran 2009). Another control strategy involves entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis downes*) which can kill larvae of the pine weevil, *Hylobius abietis* which are a major pest in reforestation sites in Europe. This method of weevil control has been used at an operational level in Ireland and Britain (Williams et al. 2013).

The procedure for developing a bio-control agent is specific to each individual control agent and target. It can be simplified into three distinct stages: discovery, development and deployment (Watson and Wall 1995). During the discovery stage the problem weed is identified and diseased specimens are located. The offending pathogen is then isolated from the weed and identified. Pure cultures of the pathogen are then tested, in laboratory conditions, on healthy weeds. The disease process is monitored and once symptoms manifest themselves, the pathogen is re-isolated from the diseased tissue and identified. Once the re-isolated pathogen is confirmed as the same pathogen that the weed was infected with the process can continue to the second stage. In the development stage tests are undertaken in order to assess the efficacy of the pathogen as a bio-control agent at landscape level including identifying the host range followed by optimal inoculation production and monitoring disease development. During the final stage various strategies are developed that will facilitate the bio-control route to commercial market, included mass production, marketing and regulation (Watson and Wall 1995).

The keys to a successful inundative bio-control programme rests on three criteria; 1: effectively producing durable and abundant inoculums, 2: genetic stability compatibility with host target and 3: efficacy of bio-control in functioning under variable environmental conditions. Should potential bio-control fail to meet any one of these criteria then an alternate strategy will be necessary (Watson and Wall 1995, Julien and Griffiths 1998). One fungal pathogen with the potential to repress or eliminate broadleaf trees species and act as a bio-control agent is *Chondrostereum purpureum* (syn. *Stereum purpureum* (Pers.:Fr)) (Pouzar 1959).

1.5.1 Chondrostereum purpureum

1.5.1.1 Biology

C. purpureum is a basidiomycete fungus distributed in temperate regions of both the Northern and Southern hemispheres. The basidiocarp (fruiting body) of *C. purpureum* is resupinate to reflexed, monomitic, with leptocystidia and the spores are hyaline, smooth and inamyloid (Hawksworth et al. 1995). Young basidiocarps are purplish in colour, becoming sullied with age. Basidiospores are produced rapidly under humid conditions (Butler and Jones 1949). Mature basidiocarps are 2 to 8 cm across with a vesicular system of hyphae which nourish the hymenium. The upper surface is tawny to brown, frequently hairy and zoned, while the lower hymenial surface is purple to lilac in colour and covered with basidia.

C. purpureum is a saprophyte with broad spectrum pathogenicity towards many broadleaved tree species (Rayner and Boddy 1986), but rarely infecting gymnosperms. Observable symptoms include sapwood discolouration, decay and sometimes death (Rayner 1977, Wall 1986, Nakasone 1990). *C. purpureum* is a primary invader of freshly wounded tissue created by pruning, felling and decay. The fungus produces basidiospores from fertile fruiting bodies (basidiocarps). The fruiting bodies form on cankered tissue and release their basidiospores during wet weather (Butler and Jones 1949, Dye 1974, Spiers 1985). Wind borne basidiospores enter fresh wounds on susceptible trees where it spreads rapidly and fruits within a year on recently deceased wood (Dye 1974). However it has low combative ability. It acts as a decay promoter and is quickly replaced by other more competitive fungi (Rayner 1978). Under suitable conditions *C. purpureum* usually fructificates for two consecutive years following infection (Wall 1997) and spores are released for several months (Dye 1974). A wounded tree may become infected by *C. purpureum* at any time although it is least susceptible during the summer months because the fungus's main source of nutrition, a carbohydrate, is at its lowest concentration (Butler and Jones 1949, Beever 1970).

Plate 3.
Chondrostereum purpureum found on a plum tree in Co. Tipperary.

C. purpureum has the potential to infect most angiosperms however host susceptibility varies from species to species. The pathogen readily infects birch spp., beech and red alder (Wall 1996, Dumas et al. 1997, Vartiamäki et al. 2009) while conifers and aspen are resistant. The fungus also causes white rot (Nakasone 1990) and it is also responsible for the silver leaf disease found in many fruit tree species such as apples, apricots, cherries and plums (Brooks and Moore 1926)(Plate 3). The silver leaf symptom is a result of translocation of a diffusible toxin produced by *C. purpureum* in the transpiration stream, which causes the palisade cells to separate from the upper epidermis, allowing the accumulation of air and the resultant silver sheen in the foliage (Butler and Jones 1949, Bishop 1979).

1.5.1.2 C. purpureum as a bio-control agent

C. purpureum has been extensively studied as a potential biological control agent for unwanted forest-based scrub weeds (Wall 1991). It is ideally suited as it is pathogenic to most broadleaved species and has a rapid colonisation ability (Wall 1991). It was first used as a vegetation management tool in the Netherlands during the 1980s to control American black cherry *Prunus serotina*. The majority of the research testing the efficacy of *C. purpureum* as a bio-herbicide has been conducted during the 1990s and 2000s, but more recent work has been carried out in Finland and Lithuania with some very positive results (Vartiamäki et al. 2009, Lygis et al 2012).

In Canada, *C. purpureum* has been tested as a myco-herbicide since the 1980s (Wall 1986, Wall 1990, Wall 1991). Myco-Tech^{TM} Paste (Myco-Forestis Corp., L'Assomption, Quebec) and Chontrol^{TM} Paste (MycoLogic Inc., University of Victoria, Victoria, British Columbia) are stump-re-sprouting inhibitors which have been registered in Canada since 2002 and 2007, respectively. They are both gel based products containing viable mycelium of *C. purpureum*. The gel is applied as a thin layer over freshly cut stumps.

These products have shown to be efficient inhibitors for the control of species such as red maple (*Acer rubrum*), sugar maple (*A. saccharum*), red alder (*Aluns ruba*), yellow birch (*Betula alleghaniensis*) and beech (*Fagus grandifolia*) (Wall 1986, Wall 1990, Wall 1991, Wall 1994, Dumas et al. 1997). Despite its use elsewhere, and differences in local efficacy between isolates (Vartiamäki et al. 2008), no studies of the potential of Irish isolates of *C. purpureum* as bio-control agents have been undertaken to date. This is despite the requirement to phase out the use of some chemical control agents associated with EU and forest certification regulations and a requirement to avoid the deliberate introduction of exotic organisms (Baars 2011).

1.6 Rhododendron seed germination

The success of rhododendron in becoming established in direct competition with native Irish scrub species is in part due to its efficiency at seedling recruitment (Erfmeier and Bruelheide 2004). Once they reach maturity, rhododendron plants produce large numbers of small wind-dispersed seeds (Cronk and Fuller 2001). The seeds are non-dormant and germinate quickly after dispersal (after five or six days in favourable conditions). Most dispersed seeds travel 10 m or less particularly in open habitat, but providing wind speed is sufficient the seeds have the potential to travel 100 m or more (Stephenson et al. 2007). Rhododendron seed production has been facilitated in Ireland by our native generalist pollinators; mainly bumblebees (*Bombus* spp.) which provide an adequate pollinator service (Stout 2007).

On a typical specimen of rhododendron each inflorescence can produce up to 5000 seeds and, on average, a 12 year old bush can produce up to 1 million seeds per annum. The capsules dehisce during the winter months and early spring, dispensing seeds roughly 0.4-1.0 mm in length and weighing 0.063 mg (Cross 1975, Mejías et al. 2002). They are similar in weight to other ericaceous plants and are regarded as some of the smallest seeds in the plant kingdom (Salisbury 1942). They are non-dormant and germinate quickly after dispersal (after five or six days in favourable conditions). They need light to germinate, however not much (Mejías et al. 2002, Erfmeier and Bruelheide 2010). The viability of seeds kept in the dark declines steadily with time and after 160 days no germination occurs (Cross 1981).

Forest litter patchwork (the mix of different vegetation types and depths) can affect rhododendron's establishment by influencing the microclimate (Fowler 1988), nutrient cycling (Proctor et al. 1983), allelopathic interactions (Rai and Tripathi 1984), or via the physical barrier created by the leaves themselves (Sydes and Grime 1981). Forest litter generally has a negative effect on germination success (Xiong and Nilsson 1999). It can also have varying effects on relative species abundance within a community (Carson and Peterson 1990). There are many reasons for this including altering moisture, light and temperature regimes for neighbouring seeds. Litter depth as a factor in seed germination and subsequent seedling establishment is considered to mostly affect small seed species and not larger seed species (Molofsky and Augspurger 1992, Myster 1994). Seeds from large seed species produce more robust seedlings and are not typically affected by forest biomass (Molofsky and Augspurger 1992, Myster 1994).

Germination of rhododendron seeds can be successful on many different types of substrate and low light levels are the most common limiting factor (Cross 1981, Mejías et al. 2002). In woodlands, disturbance of the ground cover caused by forest management practices, animal grazing or fallen trees creates gaps in the canopy allowing sufficient light to reach the forest floor for germination to occur. As the amount and distribution of forest litter can influence the establishment of rhododendron (Stephenson et al. 2006), so the management of forest litter may offer the opportunity to reduce the risk of rhododendron invasion. This scenario is more applicable in susceptible sites such as forests with an existing rhododendron infestation or plantation forests with mature rhododendron plants on neighbouring land.

1.7 Herbivore grazing

Grazing by herbivores is a limiting factor in the establishment of rhododendron, which has important implications for rhododendron management strategies (Parrott 2013). It has been reported that agricultural sites without disturbance do not support invasive species, and grazing animals affect native plant species more than invasive species (Thomson et al. 1993, Lake and Leishman 2003). In Ireland the successful establishment of rhododendron in native woodlands is reportedly due to a combination of the abundance of 'safe sites' which have arisen as a result of over-grazing and disturbance, and the shade tolerance and unpalatability of rhododendron (Cross 1982). This is because rhododendron is generally avoided by grazing animals due to its unpalatability which gives it a competitive advantage over native plants in grazed woodlands (Thomson et al. 1993, Maguire et al. 2008).

Damage caused by animal browsing can have a mortal effect on young vulnerable seedlings particularly in areas where natural regeneration is encouraged (Putman et al. 1989, Latham and Blackstock 1998, Perrin et al. 2006). Flora species diversity may be diluted by browsing animals as they feed on palatable species resulting in the more resilient species becoming dominant (Rooney and Hayden 2002). Consequently the stand structure and composition is changed as tree seedlings, understory and scrub species are replaced by grasses and unpalatable invasive species such as rhododendron (Gill et al. 1995). The most common vertebrate herbivore threats to Irish forests include deer (*Cervidae* spp), rabbit (*Oryctolagus cuniculus* L.) and domestic animals such as sheep (*Ovis aries*) (Rooney and Hayden 2002). High intensity, short-term grazing can, in some circumstances, be a valuable tool for weed control (Rosa García et al. 2012).

1.7.1 Browsing animals

1.7.1.1 Deer damage

The population of deer across Ireland is increasing mostly due the increase in suitable habitats and the general lack of natural predators (including humans) (Carden et al. 2010). The presence of deer in a habitat (primarily forest habitat) is indicated by browsing damage to young trees and incidences of faeces which are cylindrical pellets that range from black to light brown in colour (Rooney and Hayden 2002).

Damage by deer to trees is often characterised by a 'browse-line' where a deer will browse (remove buds and young shoots) a number of trees one after the other in a relatively straight line (Rooney and Hayden 2002). Browsing can result in timber defects caused by multiple leading shoots instead of one strong shoot reducing the commercial worth of that tree. Timber defects that manifest later in the life of the tree because of browsing is potentially more financially damaging to overall growth rates than if the tree was originally killed (Putman and Moore 1998). Deer may also uproot newly planted trees. Deer damage is particularly significant in broadleaf forests but when the population is high all tree species are vulnerable.

1.7.1.2 Rabbit damage

Rabbits were introduced into Ireland in the 13th century by the Normans. They are now widespread across Ireland due mostly to the persecution of potential predators such as foxes, stoats and birds of prey; the increase in available grassland and warmer climate (Sumption and Flowerdew 1985).

When populations are large their diet typically comprises of grasses supplemented by a range of other plant material. Rabbit communities are commonly found along woodland edges and field boundaries as these areas afford them a place to excavate and maintain a burrow while remaining close to a food source.

Browsing is the most common form of damage to trees caused by rabbits (Rooney and Hayden 2002). They are most active during the winter months when their main source of food is scarce. They will also browse and bite off low lying shoots leaving behind cut ends that have a characteristic clean angled cut differing from the more ragged cut made by deer (Pepper 1998). Clipping and eating shoots can occur up to a height of 0.5m (and higher during snow) and will result in substantial damage. Broadleaf species tend to be most vulnerable but all tree species may be damaged or killed when rabbit populations are high (Rooney and Hayden 2002).

1.7.1.3 Sheep damage

Sheep can be most problematic during the establishment stage of a forest plantation when newly planted seedlings are most vulnerable. They will remove ground vegetation making it easier for invasive flora to colonise the site. They will also browse and strip bark from seedlings and juvenile trees that are at pre thicket age (Rooney and Hayden 2002). Sheep grazing proved to be the limiting factor in the regeneration of an oak – birch woodland in the UK (Pigott 1983).

Fencing to protect susceptible crops from grazing damaged is extensively used as a management tool in forests and there have been many studies investigating the effects of preventing vertebrate browsing on the stand composition of important commercial and environmental tree species (Linhart and Whelan 1980, Perrin et al. 2006, Becerra and

Bustamante 2008, Newman et al. 2014). However the direct role of grazing in the establishment and survival of rhododendron in forest habitat has yet to be assessed.

1.8 Research Objectives

Effective rhododendron control can greatly increase the economic and ecological value of invaded land and this project aims to contribute to this goal by improving our understanding of the ecology and invasion dynamics of rhododendron and by assessing novel control options. An improved understanding of why the species is such an aggressive invader in certain situations will give us a better platform for developing tools for the planning of landscape level control measures.

The specific research objectives of this project were to investigate whether:

1. The native fungal pathogen *C. purpureum* can be used as a cut-stump treatment to prevent re-sprouting of both rhododendron and birch. It was decided to test birch in tandem with rhododendron as the fungus has already been proven successful on birch as an alternative to herbicide treatment. The data gathered from both the rhododendron and birch trials can be used to help determine if the fungus could become an alternative to herbicide for some of the forest vegetation management issues found in Irish forests.
2. There is a relationship between forest floor litter and the germination success of rhododendron seeds.
3. Herbivore grazing removes competitors from sites thus facilitating rhododendron invasion.

Chapter 2: Assessment of the bio-control potential of the native fungal pathogen *Chondrostereum purpureum* as a cut-stump treatment to prevent re-sprouting of Birch and Rhododendron

2.1 Background

Current guidelines on the control of rhododendron and birch in Ireland recommend manual cutting followed by chemical herbicide treatment of remaining stumps (Barron 2008, Higgins 2008, Maguire et al. 2008). However, the concept of using indigenous biological control agents in place of chemical herbicides for invasive species control has gained significant attention in recent years with a view to the production of ecologically sound biological control methods (Evans 2003, Alabouvette et al. 2006, Baars 2011, Glare et al. 2012a). *Chondrostereum purpureum* is a fungal pathogen distributed in temperate regions, which has shown significant potential for use in this regard in many parts of the world (Bishop 1979, Becker et al. 2005, De La Bastide and Hintz 2011). *C. purpureum* readily infects birch, beech and red alder (Wall 1996, Dumas et al. 1997, Vartiamäki et al. 2009) while conifers are resistant. It causes white rot (Nakasone 1990) and it is also responsible for the silver leaf disease found in many fruit tree species (Brooks and Moore 1926).

In order to have practical use as a general vegetation management tool this pathogen would have to prevent sprouting of most if not all problematic woody species and be comparable in success rates to traditional chemical treatments. In order to avoid the release of exotic organisms into the Irish environment native isolates of *C. purpureum* are required before this could be considered as a control method for rhododendron or birch in Ireland (Baars 2011). However, no studies using *C. purpureum* have been carried out in Ireland using an Irish or foreign isolate. Therefore this study set out to test its efficacy on rhododendron and birch as a cut stump treatment to replace traditional herbicide treatment.

Once the Irish isolate was found an *in vitro* experiment (a precursor to the birch and rhododendron field trials) was established that set out to compare the rate of colonisation of the recently discovered Irish *C. purpureum* isolate with a Finnish isolate (isolate P3 from Metla Culture Collection - the same isolate used in the birch cut-stump treatment (Vartiamäki et al. 2008) and an English isolate (from CABI's Microbial Culture Collection: Id code – W2494). This experiment is a preliminary investigation into whether the Irish isolate could be used for some of the vegetation management problems currently facing Irish foresters. The data from this experiment could also help identify some non-target tree species that may be at risk if the pathogen is released in close proximity.

For the in vitro experiment the efficacy of fungal colonisation on a cross section sample of sycamore *Acer pseudoplatanus*, oak *Quercus* spp, downy birch *Betula pubescens*, willow *Salix caprea*, hawthorn *Crataegus monogyna*, holly *Ilex aquifolium* and gorse *Ulex* spp, was investigated by measuring the daily rate of hyphae radial expansion.

A number of methods are available for the delivery of a fungal pathogen to birch cut stumps and this study sets out to compare three of these, including the direct application of a 14 day old culture on malt agar onto the exposed cut- stump (Wall 1990); the application of the fungus to fresh, artificially created, wounds using a small cordless drill on the sides of cut stumps (Dumas et al. 1997, Lygis et al. 2012) and delivery of the fungal pathogen using an infected timber disk (Templeton 1982). As *C. purpureum* is a primary invader of freshly wounded tissue (Dumas et al. 1997, Lygis et al. 2012), inoculating a fresh wound rather than the exposed stump could improve the effectiveness of the fungal pathogen. Acclimation of the fungal pathogen to its target species prior to field application may encourage the pathogen to colonise the field samples more readily (Templeton 1982) and so a timber disk application method, where the fungus culture is grown for two weeks prior to application on a cross-sectional sample of birch in vitro, which is then placed directly on the birch cut-stump in the field experiment, was also tested.

Only one method of fungal delivery (directly placing a culture onto exposed cut-stumps) was tested on rhododendron where the timing of application was investigated as this has previously been shown to impact the effectiveness of bio-herbicide treatments (Wall 1997, Vartiamäki et al. 2009, Lygis et al. 2012).

2.2 Methodology

2.2.1 Location and isolation of an Irish isolate of C. purpureum

According to leading experts in fungal biology and ecology there are few records of recent sightings of *C. purpureum* in Ireland. In 1978 a discovery of *C. purpureum* on the bark of a rhododendron plant (species not recorded) was made in the National Botanic Gardens, Glasnevin, Dublin. A total of six recorded sightings of the fungus are included in the National Biodiversity Database (accessed April 2013). According to the Online Atlas of Fungi hosted by the Northern Ireland Fungus Group the last recorded sighting of *C. purpureum* was eight years previous in 2001 (NIFG 2009).

The most likely place to find *C. purpureum* is on a fruit tree in a commercial orchard due to the close proximity of each tree allowing the fungus to spread to new host trees more efficiently thus increasing the likelihood that fruiting bodies would be produced each year (Rayner and Boddy 1986). A number of orchards across Ireland were contacted and one orchard, The Apple Farm in Cahir Co Tipperary reported the presence of *C. purpureum* fruiting bodies on one of their plum trees. A sample of the fruiting body was acquired and returned to the laboratory.

Tap Water Agar (TWA) and antibiotics were used to culture the raw field sample of *C. purpureum*. Organic material from the fruiting body sample was cut with a sterile scalpel and placed onto a small amount of petroleum jelly on the inside of a Petri dish lid containing the TWA. The Petri dish was sealed and once every six hours the lid was rotated 90 degrees to encourage spores to disperse over as much of the medium as possible. An optical microscope was used to verify the presence of spores and after 24 hours 10 spores were removed aseptically to culture dishes containing malt agar. The dishes were sealed and placed in an incubator at 22 °C and monitored daily for hyphae growth. On day four a colony became visible. One of the cultured samples was then sent to CABI, UK (Commonwealth Agricultural Bureaux International) for identification and confirmed to be *C. purpureum*. The fungal culture was transferred into cryo-vials containing 5% glycerol which were stored in a Styrofoam box at -80 ˚C.

2.2.2 *In vitro comparative growth rate experiment*

Twelve cross sectional disks of timber from each of the seven tree species were cut using a hand saw (three fungal treatments plus a control, each with three replications). Each sample had a diameter of 8.5 ± .4 cm in order to fit into the 9cm diameter glass Petri dishes used for the experiment. The samples were then autoclaved twice for a total of 40 minutes. The three fungal isolates (Table 1) were grown on 1.25% malt agar and incubated in the dark at 22 °C for 12 days. Plugs (5 mm in diameter) were created from each fungal colony using a sterilised hollow tube.

Table 1. Name and source of the three isolates of *C. purpureum* used in the comparative growth rate experiment.

Treatment	***Country of Origin***	***Location***	***Code***
T1	England	CABI's Microbial Culture Collection	W2494
T2	Finland	Metla Culture Collection	P3
T3	Ireland	Co. Tipperary Apple farm	Apple

Individual plugs were placed in the middle of each timber disk (with the plug end covered in fungal hyphae facing towards the disk). The control treatment consisted of a clean plug of malt agar without a pre-cultured fungal colony. Each Petri dish was then sealed with electrical insulating tape and placed into an incubator at 22 °C. The furthest extent of the hyphae spread was measured in four directions daily until the growth reached the edge of the disk (Plate 4). The data for the growth extensions were then averaged at the end of the experiment. A daily mean rate of growth was calculated to give an indication of how well the fungal growth spreads on the host tree species.

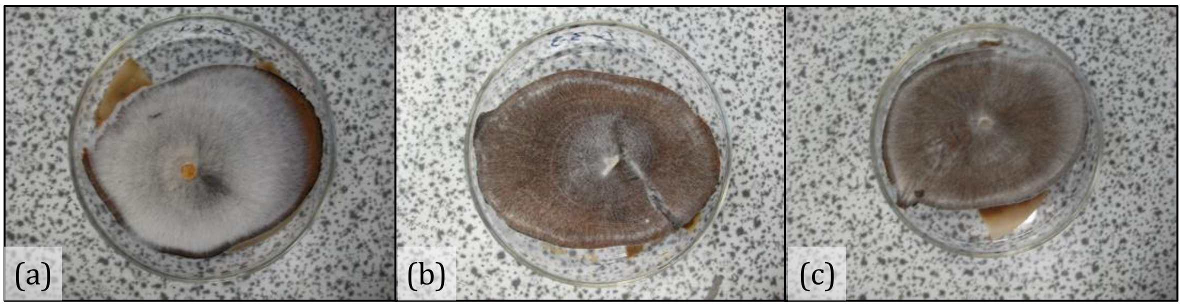

Plate 4.
The Irish (a), English (b) and Finnish (c) isolates of *Chondrostereum purpureum* on hawthorn after 8 days of growth.

Data was analysed by ANOVA in Minitab software version 16 (Minitab 2010). The effects of the three different fungal isolates and the different broadleaf tree species on the daily rate of hyphae extension were tested by a two-factorial analysis of variance. Significant differences ($P < 0.05$) among the tree species and the isolates and the daily growth rate were tested by using Tukey's post hoc test. Data are presented as means and standard errors.

2.2.3 Birch methodology

2.2.3.1 Study site

The field trial site was located on a Coillte owned forest plantation outside Lemybrien, Co. Waterford (latitude: 52.17 °N, longitude: -7.51 °W). The climate is a temperate oceanic climate characterised by mild moist and changeable weather with lack of temperature extremes. Precipitation is likely year round with an annual average of 857.4 mm (Met Eireann 2013). Average annual temperature range in this region is between max 13.8 °C and min 5.9 °C. The trial plots were established in a half hectare area of the forest that was replanted with Sitka spruce (*Picea sitchensis*) and silver birch (*Betula pendula*) following felling in 2006. There were significant occurrences of natural re-generation of birch in addition to the planted seedlings. The plantation is located near the foot of the Comeragh Mountains and is prone to water logging. The study site is even in slope and contains deep mound drains. The approximate height of the Sitka spruce was 1.50 m and of the birch was 1.65 m.

2.2.3.2 Experimental design

The trial application of *C. purpureum* for the birch trial was conducted on the 21st September 2011 taking into account the fungus is at its most active during the autumn months. Even though birch re-growth is most vigorous during spring, it was decided to establish the experiment in the autumn to give the fungus the best possible chance. On that day the maximum temperature was recorded at 14.3 °C and the minimum temperature recorded was 4.1 °C (Met Eirrean- Carraiglea weather station -accessed Feb. 2012). Fifteen experimental plots were used in this trial, consisting of five treatments replicated three times in a completely randomised design. Ten birch trees were assigned to each plot (150 specimens in total). The diameters at the base of each chosen tree ranged from 10 mm to 70 mm. The experimental plots were 2 m wide and as long as was required to contain the ten trees. A minimum buffer zone of 2 m was observed between plots.

The five treatments used were:

1. Control: no herbicide applied.
2. Chemical herbicide: Traditional glyphosate herbicide applied. (5 ml per stump of 9 g/l solution of isopropylamine salt of glyphosate applied through a spray bottle).
3. Bio-herbicide Drill: Four holes (5-10 mm diameter) were made to each side of the stump, into which a 5mm plug of the fungal herbicide *C. purpureum* was placed.
4. Bio-herbicide Direct: The fungal herb*icide C. purpureum* was placed around the rim of the stump by inverting the Petri disk insuring that the exposed cambium was completely covered by the fugal mycelium. The Petri dish was then removed.
5. Bio-herbicide Disk: Similar to the direct treatment but instead of malt agar a two week old timber disk infected with the fungus was used. The disks were inoculated in the lab as described below in Section 2.2.3.3.

Plate 5.
A treated birch stump (direct treatment) after establishment of trial. The stumps, once the treatments were applied, were covered in paper bags to protect them from rain.

2.2.3.3 Herbicide application

The inoculum of the Irish isolate of *C. purpureum* was removed from cryo-storage two weeks prior to the date of field application. The culture was grown on 1.25% malt agar and incubated in the dark at 22 °C for 12 days. The target trees (minimum diameter 10 mm; average height 1.42 ± 0.26 m) were felled using a chainsaw leaving at least a 20 cm high stump. The diameter of each stump was recorded and the assigned treatment was applied within 20 minutes of felling in order to prevent colonisation of unwanted pathogens present in the environment. Each stump was labelled after treatment application took place. The control birch stumps were left untreated. To prevent rain affecting the treatments a wax coated paper bag was placed over each treated stump and left in place for two weeks post treatment (Plate 5).

2.2.3.4 Field trial assessment

The effectiveness of each of the experimental treatments was assessed by recording the following:

1. Fruiting bodies: Cut stumps in all of the experimental plots were examined for the presence of *C. purpureum* fruiting bodies every month following application until October 2013 (25 months).

2. Stump survival: Stump survival was assessed at the conclusion of the trial. A stump was recorded as surviving if any shoot re -growth was present.

3. Shoot regeneration: The number of living shoots on each of the stumps was recorded at the conclusion of the trial in October 2013.

2.2.3.5 Data analysis

The data was analysed as a completely randomised design with five treatments and three replicate blocks. Stump survival was tested using Kruskal-Wallis non-parametric one way analysis of variance. Follow-up tests (Mann-Whitney U) were conducted to evaluate pairwise differences controlling for type 1 error using the Bonferroni approach. The effects of the five treatments on the occurrence of fruiting bodies and the number of living shoots per stump were tested using one-way analysis of variance. Significant differences between treatments were tested using Tukey's post hoc test. Data analyses were conducted using Minitab software version 16 (Minitab 2010). Data are presented as means and standard errors.

2.2.4 Rhododendron methodology.

2.2.4.1 Study site

Deerpark is a mixed conifer/broadleaf plantation forest located in Lismore, Co. Waterford (Latitude: 52.11 °N longitude: -7.91 °W). Annual temperature range for this region is a maximum of 17.7 °C and a minimum of 4.4 °C (Met Eireann 2013). This plantation was chosen for the experiment as it is representative of the type of site where rhododendron is commonly found. The climate is temperate oceanic characterised by mild moist and changeable weather without temperature extremes. The site consists of a 32 hectare forest on brown podzolic soil lying on a sandstone parent material. In this habitat the vegetation is made up of planted trees and naturally regenerated shrubs and herbaceous plants. The area of the site in which the experimental plots were established consists of mature larch (*Larix kaempferi*) with an understory of Rhododendron and some holly (*Ilex aquifolium*).

2.2.4.2 Experimental design

The field trial was established in 2011 at the two study sites to test the suitability of *C. purpureum* as a cut-stump mycoherbicide control agent for both rhododendron and birch in Ireland, and to compare 3 different application techniques. Final data collection from both field trials was planned for September 2012, but unfortunately in May 2012 contractors, conducting a thinning programme, destroyed the rhododendron study. The exclusion zone which had been established around the experiment was ignored by the contractor and the field trial was destroyed by heavy machinery. Most of the stumps were damage to such an extent that they were no longer suitable for use in the experiment. The rhododendron trial was re-established in July 2012 in a different sub-compartment at the same site while the birch trial continued as planned.

It was decided to alter the treatment application methods for the new rhododendron trial as the novel methods tested in the birch trial (drill & disk) were not showing any signs of success. Therefore only one method of fungal delivery (directly placing a culture onto exposed cut-stump) was used in the rhododendron experiment. In addition to comparing chemical herbicide to the potential bio-herbicide treatments it was decided to try and determine whether the timing of application (summer or autumn) had an effect on the success of the pathogen, as previous studies had demonstrated (Vartiamäki et al. 2009, Lygis et al. 2012).

Trial applications of *C. purpureum* were conducted at the study site on two occasions, the first, a summer application was conducted on the 12th of July 2012 and the second, an autumn application, was conducted on the 5th of October 2012. Eighteen experimental plots were used in this trial, consisting of three treatments with three replicates each on two separate application dates in a completely randomised design. Twenty rhododendron plants were assigned to each plot (360 specimens in total). The experimental plots were circular and as large as was required to contain the twenty stems. A minimum buffer zone of 2 m was observed between plots. The three treatments used were:

1. Control: no herbicide applied.

2. Chemical herbicide: Traditional glyphosate herbicide applied.

3. Bio-herbicide: Fungal herbicide *C. purpureum* applied.

On the day of the first application (12th July 2012) the temperature ranged from 11.1 °C to 20.6 °C and on the day of the second application (5th October 2012) the temperature ranged from 5.4 °C to 12.3 °C (Met Éireann, Moorepark weather station).

2.2.4.3 Herbicide application

The inoculum of the Irish isolate of *C. purpureum* was removed from cryo-storage two weeks prior to field application. The culture was grown on 1.25% malt agar and incubated in the dark at 22 °C for 14 days. From the revived fungal sample, 120 (60 per application) further cultures were created and incubated for 14 days prior to the field application. The diameter of each stump was recorded (minimum diameter 10 mm; average height 2.13 ± 0.43 m) and the assigned treatment was applied within 20 minutes of felling in order to prevent colonisation of unwanted pathogens from the immediate environment. Application of *C. purpureum* to cut rhododendron stumps in the field was conducted by inverting the Petri disk and placing it on the cut stump ensuring that the exposed cambium was completely covered (following Wall, (1990)). Each stump was then covered in plastic paraffin film and topped with aluminium foil to prevent the fungus from drying out while also keeping it in place (Plate 6). Rhododendron stumps treated with the chemical herbicide (5 ml per stump of 9 g/l solution of isopropylamine salt of glyphosate) were treated as is current best practice for dealing with problematic scrub species by applying the herbicide to cut stumps using a spray bottle. The control rhododendron stumps were left untreated.

Plate 6.
Rhododendron cut- stump at establishment treated with the fungal pathogen. Each stump was covered in plastic paraffin film and aluminium foil to prevent the fungus from drying out.

2.2.4.4 Field trial assessment

The effectiveness of each of the experimental treatments was assessed by recording the following:

1. Fruiting bodies: Cut stumps in all the experimental plots were examined for the presence of *C. purpureum* fruiting bodies every month following application until October 2013 (July application: 15 months; October application: 12 months).

2. Stump survival: Stump survival was assessed at the conclusion of the trial in October 2013. A stump was recorded as surviving if any shoot re-growth was present.

3. Shoot regeneration: The number of living shoots on each surviving stump was recorded at the conclusion of the trial in October 2013.

4. Shoot height: The height of the tallest shoot on each surviving stump was recorded at the conclusion of the trial in October 2013.

Shoot height as a metric was used on the rhododendron trial as vegetative spread (via stems touching the ground and producing roots) is a noted method of invasion (Cross 1975, Cullen 2011). Stem height was not measured in the birch trial as birch does not generally spread vegetatively (Gardiner 1968).

2.2.4.5 Data analysis

The data were analysed as a completely randomised design with three treatments and three replicate blocks applied twice (3 treatments × 2 timings × 3 replications). Data analyses were conducted using Minitab version 16 (Minitab 2010). The effects of the three treatments and the different application timings on the success of inhibiting stump regeneration were tested using a two-factorial analysis of variance. If application timing had no significant effect then the data sets for each treatment (July and October) were assessed separately. Differences in stump survival among treatments were tested using Kruskal-Wallis tests. Follow-up Mann-Whitney U tests were used to test for pairwise differences using Bonferroni corrections. The effects of the three treatments on the occurrence of fruiting bodies, number of living shoots and the height of the tallest living shoots per stump were tested using one-way analysis of variance. Significant differences between treatments were tested using Tukey's post-hoc tests. Data are presented as means and standard errors.

2.2.5 Destructive Sampling

As *C. purpureum* is a saprophyte and has a low combative ability, the fungus typically cannot survive in healthy regenerating tree stumps. Destructive sampling of the stumps treated with the fungal pathogen was carried out (25 months after the establishment of the birch trial; 15 months after the rhododendron July trial and 12 months after the October trial) to see if any *C. purpureum* mycelium persisted in the cambium of the treated stumps.

Each of the stumps previously applied with *C. purpureum* were examined for the presence of fruiting bodies. In addition samples of wood were collected from each rhododendron stump that had *C. purpureum* applied to it in both the summer and autumn treatments (both the birch and rhododendron sites were assessed in October 2013), in an attempt to re-isolate and confirm the presence of the fungus. Using a chisel, sterilised with alcohol and a hammer, small sections of wood, approximately 10 mm x 10 mm x 2 mm, were removed from the cambium/xylem of the cut stump and transferred with sterile forceps to Petri dishes containing sabouraud dextrose agar. Sabouraud dextrose agar contains antibiotics to reduce bacterial contamination allowing the fungal spores to produce unhindered. The Petri dishes were sealed and returned to the laboratory and placed in an incubator at 20 °C. The samples were examined for fungal growth and any potential *C. purpureum* was sub-cultured onto fresh agar plates which were then sent to CABI for DNA identification.

2.3 Results

2.3.1 In vitro comparative growth rate experiment

There was successful hyphae growth on all the samples with sycamore displaying the greatest daily rate (Table 2 and Figure 1). By the ninth day the growth of the hyphae had reached the edge of the timber disks on all samples. There was no significant interaction between species and treatment ($F_{2,42} = 1.16$, $p > 0.05$). Further analysis showed there was a significant difference between the three fungal strains and the daily growth rate ($F_{2,20} = 11.75$, $p < 0.01$). The Finnish isolate produced the best daily growth rate while the Irish and English isolates produced similar rates (Table 3).

Table 2. Average daily rate of fungal growth on 7 tree species is a mean from 3 replicates. Tree species with the same grouping are not significantly different from each other at the $p < 0.05$ level using Tukey's post hoc test.

Species	Growth Rate (mm/day)	Grouping
Sycamore	9.44 ± 0.52	A
Willow	8.78 ± 0.34	AB
Oak	6.95 ± 0.22	C
Holly	8.44 ± 0.38	B
Gorse	7.42 ± 0.62	C
Birch	8.75 ± 0.38	AB
Hawthorn	8.79 ± 0.93	AB

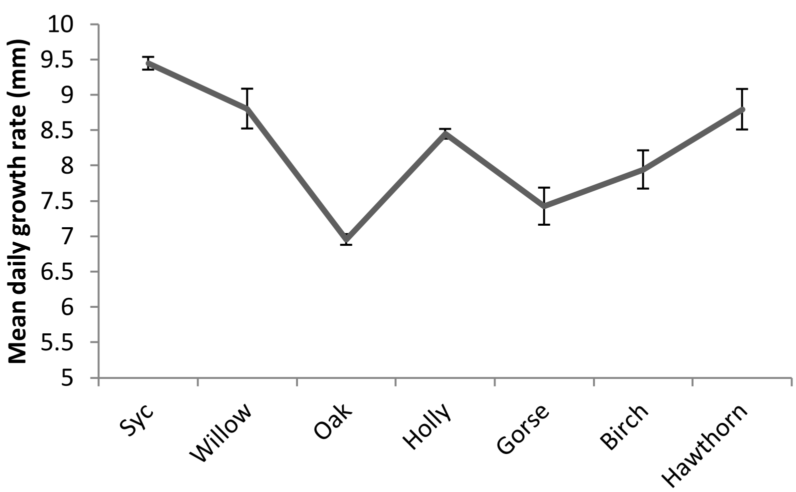

Figure 1. Daily growth rate (mm) of *C. purpureum* fungal hyphae (Combined data from English, Finnish and Irish isolates) on seven tree species. Values are means ± error of means.

Table 3. The average daily rate of three isolates of *C. purpureum* hyphae extension on disks of timber from seven broadleaf tree species. Isolates with different letters are significantly different from one another.

Fungal isolate Origin	**Growth rate mm/day**
England	9.39 ± 0.23 A
Finland	9.53 ± 0.41 B
Ireland	9.45 ± 0.38 A

2.3.2 Birch results

2.3.2.1 Fruiting bodies

Sprouting of the cut birch stumps began during the spring of 2012. No evidence of fruiting bodies was recorded on any of the birch stumps in any of the five treatment groups by the end of the trial in October 2013.

2.3.2.2 Stump survival

The survival of stumps differed significantly between the chemical herbicide treatment and the other treatments ($H = 9.22$, $DF = 4$, $p < 0.05$; Table 4). Both the untreated and disk treatment had shoot growth on 97 ± 3.3% of their stumps 24 months after treatment. The rate for the drill treatment was a little lower at 93 ± 6.7%. Even though the direct treated stumps had fewer shoots per stump than the other treatments, all the treated stumps produced some re-growth. The survival rate of the chemical herbicide treated stumps was 10 ± 5.6% leaving 90% of their stumps without re-growth. The overall effect of the *C. purpureum* was that it produced small differences in the number of dead stumps compared with the untreated stumps but significantly different to the chemical herbicide (Figure 2).

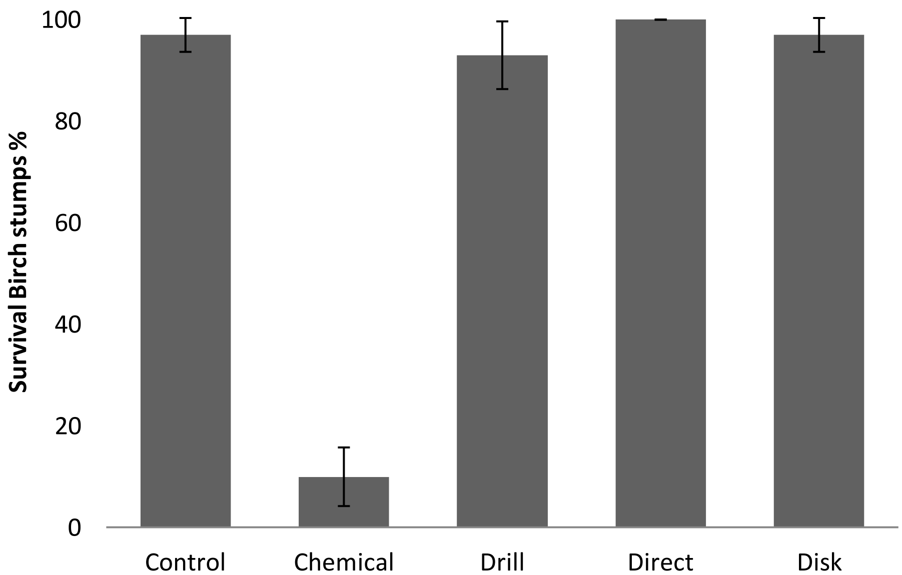

Figure 2. The percentage of surviving cut birch stumps assessed in October 2013. Values are means ± standard error of means

Table 4. Mean (± S.E.) survival (%) of birch stumps (n=3) and the number of shoots produced by stumps (n=3) at the conclusion of this trial in October 2013. Values are means and standard errors. Values in columns with the same letter were not significantly different.

Treatment	Survival (%)	Shoots per stump
Control	96.6 ± 3.3 a	3.47 ± 0.29 a
Chemical herbicide	10.0 ± 5.6 b	0.23 ± 0.14 b
Bio-herbicide Drill	93.3 ± 6.7 a	3.13 ± 0.28 ac
Bio-herbicide Direct	100.0 ± 0.0 a	2.23 ± 0.17 c
Bio-herbicide Disk	96.7 ± 3.3 a	3.03 ± 0.29 ac

2.3.2.3 Shoot regeneration

Regeneration of the cut birch stumps began during the spring of 2012 and by the time of the final assessment in October 2013 the number of regenerated shoots recorded on surviving stumps differed significantly between treatments ($F_{4,14} = 42.7$, $p < 0.001$). Fewer shoots were recorded on all of the stumps in the bio-herbicide treatments than in the control group (Table 4), though this difference was not significant. Birch stumps in the control group produced the largest number of new shoots during the trial. Of the stumps treated with the bio-herbicide the direct treatment resulted in the least amount of shoot re-growth (2.2 ± 0.17) (Plate 7), while the disk treatment, the drill treatment and the control had similar mean numbers of shoot re-growth. Birch stumps in the control group produced 3.47 ± 0.29 shoots per stump and, while not statistically different to the drill or disk treatments, this was significantly higher than the number of shoots produced by stumps in the direct treatment group (Table 4).

Plate 7.
Birch stump with moderate shoot regeneration at the conclusion of the trial having been treated with *C. purpureum* using the direct application method.

2.3.2.4 Destructive sampling

No mycelium of *C. purpureum* was found on the samples from the birch stumps sent for DNA identification. Instead bracket fungus; *Piptoporus betulinus* and basidiomycota species *Stereum hirsutum* were identified and confirmed to be active on the birch stumps.

2.3.3 Rhododendron results

2.3.3.1 Fruiting bodies

Sprouting of the cut rhododendron stumps began towards the end of the summer of 2012 for those stumps treated in July 2012. Sprouting of the October 2012 stumps began during the spring of 2013. No evidence of fruiting bodies was discovered on any of the rhododendron stumps treatment with the fungal pathogen by the end of the trial in October 2013.

2.3.3.2 Stump survival

No interaction between application dates was found for stump survival ($F_{2,17} = 3.12$, $p > 0.05$) (Table 5).

Table 5. Results of the analysis of variance (p values) for the measured variables.

Source of Variation	Observation (October 2013)
Stump survival	
Stump treatment	< 0.001
Application timing	0.413
Stump treatment × application timing	0.098
Shoot regeneration	
Stump treatment	< 0.001
Application timing	0.810
Stump treatment × application timing	0.189
Height of living shoots	
Stump treatment	< 0.001
Application timing	0.296
Stump treatment × application timing	0.929

2.3.3.2.1 July application

The per cent of surviving stumps, treated in July, at the conclusion of this trial differed significantly among treatments (H = 7.20, DF = 2, $p < 0.05$; Table 6). Stumps in the control group had a 90.0 ± 2.8% survival rate followed by a 65.0 ± 5.8% survival rate for the stumps treated with *C. purpureum* (Figure 3). The chemical herbicide treatment produced the fewest surviving stumps (38.3 ± 4.4%).

Table 6. Mean (± S.E.) survival (%) of rhododendron stumps to October 2013. Values in columns with the same letter are not significantly different (n = 3).

Treatment	July	October
Control	90 ± 2.8 a	85 ± 4.5 a
Chemical Herbicide	38 ± 4.4 b	25 ± 5.6 b
Biological Herbicide	65 ± 5.8 a	75 ± 5.6 a

2.3.3.2.2 October application

The per cent of surviving stumps at the conclusion of this trial differed significantly among treatments (H = 6.49, DF = 2, $p < 0.05$; Table 6). 85.0 ± 4.5% of rhododendron stumps in the control group survived to the end of the trial (Figure 3). The biological herbicide showed a slightly lower survival rate (75.0 ± 5.6%), while survival of stumps in the chemical herbicide group was significantly lower than either of the other two treatments (25.0 ± 5.6%).

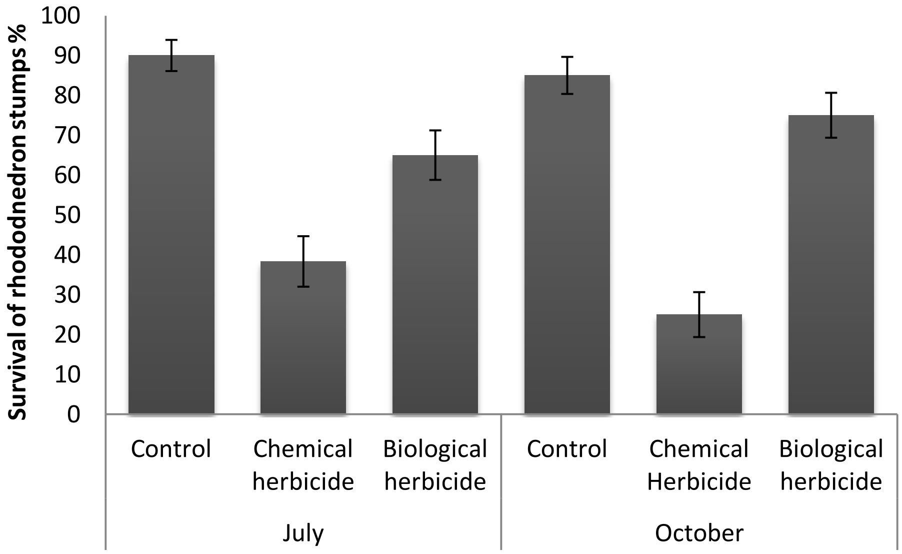

Figure 3. The percentage (± S.E.) of surviving rhododendron stumps (treated in July and October 2012) in October 2013.

Plate 8.

Cut stumps of rhododendron one year into the trial showing (a) vigorous shoot regrowth in the control group, (b) no shoot regrowth following treatment with glyphosate and (c) moderate shoot regrowth following treatment with *C. purpureum*.

2.3.3.3 Shoot regeneration

No interaction between application dates was observed (see Table 5) $F_{2,17} = 1.92$, $p > 0.05$.

2.3.3.3.1 July application

The number of regenerated shoots found on surviving stumps of rhododendron at the conclusion of the trial differed significantly among treatments ($F_{2,8} = 9.86$, $p < 0.001$). The number of living shoots was significantly greater on stumps in the control group (4.91 ± 0.87) than either of other two treatments. Rhododendron stumps treated with *C. purpureum* produced a mean of 2.32 ± 0.28 shoots per stump over the course of the experiment while the chemical herbicide treated stumps produced just 1.53 ± 0.36 shoots per stump (Table 7) with no significant difference between these two treatments (Plate 8).

Table 7. Mean (± S.E.) number of living shoots on rhododendron stumps at the conclusion of the trial. Values in columns with the same letter are not significantly different (n = 3).

Treatment	**July**	**October**
Control	4.91 ± 0.87 a	4.42 ± 0.52 a
Chemical Herbicide	1.53 ± 0.36 b	0.80 ± 0.22 b
Biological Herbicide	2.32 ± 0.28 b	3.95 ± 1.12 a

2.3.3.3.2 October application

There was a significant difference in the number of shoots found on the surviving rhododendron stumps at the conclusion of this trial among treatments ($F_{2,8} = 6.83$, $p < 0.05$). The number of living shoots on stumps in the control group was slightly higher than on stumps treated with *C. purpureum* (4.42 ± 0.52 and 3.95 ± 1.12 respectively; Table 7), with no significant difference between the two.

2.3.3.4 Shoot height

No interaction between timing was observed for shoot height, ($F_{2,17}$ = 0.07, p > 0.05).

2.3.3.4.1 July application

There was a significant difference in the mean height of the tallest regenerated rhododendron shoots among treatments ($F_{2,8}$ = 30.04, p < 0.001) at the conclusion of this trial. The tallest shoots were recorded in the control group (42.1 ± 2.9 cm) which was not statistically different from the mean height recorded on rhododendron stumps treated with *C. purpureum* (33.1 ± 3.4 cm; Table 3). The tallest shoot on rhododendron stumps treated with the chemical herbicide was 10.2 ± 1.9 cm, which was significantly shorter than either of the other two treatments (Table 8).

Table 8. Mean (± S.E.) height (cm) of tallest shoots on surviving rhododendron stumps at the conclusion of the trial (n = 3). Values in columns with the same letter are not significantly different.

Treatment	July	October
Control	42.1 ± 4.9 a	40.4 ± 2.4 a
Chemical Herbicide	10.2 ± 1.6 b	06.0 ± 1.4 b
Biological Herbicide	33.1 ± 0.9 a	30.8 ± 4.5 c

2.3.3.4.2 October application

The height of the tallest shoot on surviving rhododendron stumps differed significantly among treatments ($F_{2,8}$ = 33.37, $p < 0.001$). Stumps in the control group produced the tallest shoots (40.4 ± 2.9 cm) while shoots produced by rhododendron stumps treated with *C. purpureum* had a mean height of 30 ± 2.8 cm and by those treated with chemical herbicide had a mean height of 6.0 ± 1.4 cm; (Table 8). There was no significant difference between the height of shoots produced by rhododendron stumps in the control and *C. purpureum* groups, which was significantly taller than those produced by stumps in the chemical herbicide group.

2.3.3.5 Destructive Sampling

Samples were removed from all 120 stumps (60 from both the July and October treatment). All of the samples removed from the cambium of the stumps contained viable fungal mycelium. The samples of mycelium were carefully cultured on a malt agar medium for several weeks until it became clear that there were at most three distinct fungal species active across all the treated stumps. A sample of each of the three different species was sent for DNA analysis, and the results did confirm that one of the species was C. purpureum (the other two samples were identified as Fusarium avenaceum and Mucor hiemalis).

Active (mycelium able to reproduce on nutrient medium) *C. purpureum* was found on 10 of 60 treated stumps (July treatment) and 7 of the 60 stumps (October treatment). For the July treatment the mean number of stumps (per repetition) containing active *C. purpureum* mycelium was 3.33 ± 0.27 (16.66% ± 1.35%). There were fewer stumps with active mycelium growth found in the October treatment where a mean value of 2.33 ± 0.72 (11.66% ± 3.6%) stumps was observed.

2.4 Discussion

2.4.1 In vitro comparative growth rate experiment

The results of this study indicate that fungal hyphae extension is most efficacious on sycamore than on the remaining tree species. Oak proved to be the least susceptible to fungal growth. The three fungal isolates used in the study were English, Finnish and Irish and while the Finnish isolate produced the fastest growth rate on most of the tree species, there was no significant difference in growth rate of any of the three isolates. The spread between the best and worst performing tree species was 2.49 mm of hyphae growth a day.

For many years glyphosate has been the mainstay of vegetation management practices in Ireland ((Barron 2008, Maguire et al. 2008). Its use has become so prevalent that it is being used from invasive weed control programs, e.g. rhododendron eradication, to control of re-growth in thinned broadleaved stands. However concerns with overuse, and documented effects to the residual crop such as ‘flashback’ coupled with the certification process bodies such as the FSC demanding a reduction in pesticide use has prompted foresters to start looking at the alternatives (Mc Carthy 2005).

This study set out to discover if the Irish strain of *C. purpureum* could be used for some of the vegetation management problems facing Irish foresters in the future and to determine if it could be a viable alternative to glyphosate. The results show how susceptible some of these species are to an infection of a native wood rotting fungus in vitro. If a successful strain of *C. purpureum* is found and proven as a useful cut-stump treatment against the re-growth of rhododendron then the data gathered can be used to help draw up guidelines for the safe application of this fungus in the future.

2.4.2 Birch discussion

The results of this study demonstrate that the application of an Irish isolate of *C. purpureum* to birch following manual cutting did not reduce the adventitious re-sprouting of the treated stumps compared with the traditional chemical herbicide which is the current standard method of woody vegetation control. While the bio-herbicide treatment did reduce the emergence and growth of new shoots on treated stumps compared with untreated stumps, the difference between the treatments was not significantly different. No fruiting bodies of *C. purpureum* were discovered on treated stumps, which indicate that birch is resistant to infection from the one isolate of *C. purpureum* tested in this study. These findings differ from previous reports of the potential efficacy of *C. purpureum* as a vegetation management control tool (Wall 1994, Shamoun and Hintz 1998, Vartiamäki et al. 2009, Lygis et al. 2012).

While the chemical herbicide glyphosate treatment resulted in a 90% mortality rate on treated cut stumps, the bio-herbicide treatment resulted in no stump deaths or suppression of shoot re-growth. When comparing the three different bio-herbicide treatment methods that were investigated (Drill treatment, Direct treatment and Timber disk treatment), the direct treatment produced the most promising results, having the lowest rate of shoot regeneration on treated stumps. The direct treatment did have a significantly greater impact on shoot regeneration than the untreated control stumps; however the bio-herbicide treatment did not prevent regeneration using any of the application methods. The traditional chemical herbicide treatment resulted in a significant decrease in stump survival compared with all three bio-herbicide treatment groups and the control.

In order to confirm the presence/absence of viable mycelium of *C. purpureum* in treated stumps, samples of wood were collected (25 months after establishing the experiment) from each stump that had *C. purpureum* as its treatment. The mycelium discovered on the samples was separated into individual species and the resulting samples were sent to CABI for molecular identification. No *C. purpureum* was found on the birch stumps. A bracket fungus; *Piptoporus betulinus* and the basidiomycota species *Stereum hirsutum* were identified. Both these fungal species are commonly found on decaying birch trees (Schmidt 2006). However these species were not having a detrimental effect to the overall health of the treated birch stumps as indicated by the high percentage of stumps with regenerating shoots.

As no *C. purpureum* was discovered on any of the samples it indicates that the otherwise healthy stumps were able to compartmentalise the fungus by creating barriers in the wood preventing the fungus from spreading (Wall 1997). As *C. purpureum* is a saprophyte and has a low combative ability (Rayner 1977, Wall 1986) the fungus typically cannot survive in healthy regenerating tree stumps.

2.4.3 Rhododendron discussion

No fruiting bodies were found on rhododendron stumps treated with the bio-herbicide *C. purpureum* 15 months following application in this study, which indicates that rhododendron is resistant to infection from the isolate of *C. purpureum* tested in this study. The results of this study, the first of its kind using a native Irish bio-herbicide on rhododendron, demonstrate that the application of an Irish isolate of *C. purpureum* to rhododendron stumps following manual cutting also did not reduce the re-sprouting of the treated stumps when compared with the application of glyphosate. While the bio-herbicide tested does reduce the emergence and growth of new shoots on rhododendron stumps when compared with untreated stumps, the difference between the treatments was not statistically significantly. These findings differ from previous studies which have demonstrated the efficacy of *C. purpureum* as a substitute for chemical herbicides (Wall 1994, Shamoun and Hintz 1998, Vartiamäki et al. 2009, Lygis et al. 2012).

There was no significant difference between the survival rate of the untreated stumps and the fungal stumps when considering both the July and the October application together. However there was a small difference between the two application dates for the fungal herbicide treatment - the July application proved marginally more successful at reducing the number of shoot regrowth than the October application. This indicates that a summer application of *C. purpureum* yields more favourable results than an autumn application. An effect of application timing has also been reported previously which states that the optimum time for bio-herbicide treatment of cut stumps with *C. purpureum* is between May and July as the effectiveness of the fungal pathogen reduces towards the end of the growing season (Vartiamäki et al. 2009, Lygis et al. 2012). The resistance of both beech and birch against *C. purpureum* is greatest at the beginning of the growing season then decreases towards midsummer before increasing again towards the autumn (Wall 1991). Similar results were found when looking at shoot regeneration on surviving rhododendron stumps. Even though the stumps treated in July had more time to produce new shoot growth, they produced less than their October counterparts. This suggests that an application of *C. purpureum* during the summer would provide comparable results to the traditional chemical herbicide treatment as a method of vegetation control if suppression of potential re-growth was the focus rather than complete destruction of the plant. However, both treatments performed considerably poorer than the traditional chemical herbicide treatment in this study.

Although the July application of the fungal herbicide displayed greater efficacy in reducing shoot re-growth on treated stumps when compared with the October application, it had no effect on the height of the tallest shoot produced. The height of shoots produced by rhododendron stumps treated with the bio-herbicide in July was similar to that produced by untreated control stumps. The traditional glyphosate chemical herbicide was more effective than any of the treatments using the bio-herbicide in this study.

It should be noted that generally rhododendron requires repeated applications of herbicide over several years to be effective (Dehnen-Schmutz et al. 2004), unlike most other broadleaved species which can usually be controlled using just one application. In this study only one application was made due to time constraints (application of herbicide is typically made once every two years). The ability of rhododendron to reproduce vegetatively via suckers formed from manually cut stumps when new shoots find their way into a suitable substrate may be somewhat limited if *C. purpureum* was seen to affect the height of regenerated shoots. The fact that it does not reduce the height of new shoots negates the potential gains made by the fact that the July application produces less shoots than the control treatment which used no herbicide.

After 15 months for the July treatment and 12 months for the October treatment active mycelium was discovered on a small number of stumps treated with *C. purpureum* (17% for the July treatment and 12% for the October treatment). Previous studies have shown that there are differences in the ability of single isolates of *C. purpureum* to suppress re-growth and indeed cause death to treated broadleaf tree species (Jobidon 1998, Vartiamäki et al. 2008). The surviving mycelium found (after destructive sampling) in the inoculated stumps indicates rhododendron only has a moderate resistance to the fungus. Fruiting bodies may occur over a 4-7 year period (Wall 1997).

2.5 Conclusion

The results of this study demonstrate that the application of an Irish isolate of *C. purpureum* to rhododendron or birch following manual cutting does not reduce the adventitious re-sprouting of the treated stumps compared with the chemical herbicide. While the fungal treatment can reduce the emergence and growth of new shoots on treated stumps compared with untreated stumps, it did not perform as well in tests as the traditional chemical herbicide.

These findings for both the rhododendron and birch field trials contradict many previous studies which have demonstrated the potential efficacy of *C. purpureum* as a vegetation management tool (Wall 1994, Shamoun and Hintz 1998, Vartiamäki et al. 2009, Lygis et al. 2012). However it has been shown that there are differences in the ability of different isolates of *C. purpureum* to suppress re-growth and cause the death of treated broadleaved tree species (Jobidon 1998, Vartiamäki et al. 2008). As *C. purpureum* is a saprophyte and has a low combative ability (Rayner 1977, Wall 1986) the fungus may not survive for long in healthy regenerating tree stump as healthy stumps create barriers in the wood which prevent spread of the fungus. It would therefore be prudent to continue monitoring the trial sites used in this study for future occurrences of *C. purpureum* fruiting bodies. There are limitations with investigating just one strain of a fungal pathogen, as the efficacy of the isolate may not be indicative of other isolates that are currently active around Ireland at the present time.

Optimisation of this biological control agent through selection of isolates of the fungus that show high levels of pathogenesis, coupled with improved formulation of delivery and application techniques and timing of the application to allow rapid establishment in the host, may result in increased bio-herbicide efficacy in species which are moderately resistant to the fungus (Wall 1990). In order to fully develop *C. purpureum* as a bio-herbicide to be used as a forest vegetation management control tool in Ireland several isolates from different parts of the country, and ideally from fruiting bodies found on different plant species, should be tested. Wall (1990) discussed that a general reduction in sprouting showed *C. purpureum* could be used as an intergraded vegetation management programme. However, to have a practical use as a vegetation management control tool the most virulent isolate would have to prevent sprouting to most, if not all, problematic woody species and be comparable in success rates to the traditional treatments.

The procedure for developing a bio-control agent is specific to each individual control agent and target. There are three steps in this process: discovery, development and deployment (Watson and Wall 1995). During the development stage tests are undertaken to assess the efficacy of the pathogen as bio-control agent at landscape level including identifying host range, optimal inoculation production and monitoring disease development. During the development stage, strategies are developed that will facilitate the bio-control route to commercial market, including mass production, marketing and regulatory conditions of production (Watson and Wall 1995). Successful bio-control programmes rely on three criteria: effective production of durable and abundant inoculums; genetic stability and compatibility with the host target; and effective functioning of the bio-control agent under variable environmental conditions. Should potential bio-control fail to meet any one of these criteria then an alternate control strategy will be necessary (Templeton 1982, Wall et al. 1992, Wagner 1993, Gherardi and Angiolini 2004).

The landscape surrounding biological control has changed over the past quarter century. Research and regulation of bio-control has improved awareness of the potential of bio-control measures while also reducing tensions associated with the inherent risks of introducing foreign agents into ecosystems with the intention of controlling a malevolent pest. Barrett et al (2010) anticipates that “in the future there may be a greater emphasis on balancing efficacy with bio-safety, and that biological control agent biotypes will be selected to optimise these characteristics”. *C. purpureum* is distributed across the temperate regions of the Northern and Southern hemispheres and its population structure has been studied from regional to continental scales. The conclusion derived from these studies is that in countries where *C. purpureum* is native there may be no risk in introducing foreign isolates from other geographical regions providing they are located on the same continent (Rayner and Boddy 1986, Gosselin et al. 1995).

In the past, introductions of alien species to suppress or eradicate a naturalised pest species have often failed (Barratt et al. 2010). Can a legitimate case, therefore, be made here to introduce a foreign strain of *C. purpureum* that is proven to be as successful in reducing shoot re-growth as the more traditional methods? Can a native strain of the fungus be engineered to be more effective and more host specific? Careful consideration of course must be given to weighing up the advantages and disadvantages of genetically engineering or introduction of a foreign isolate with the continual use of chemical herbicide and the effects they have on the environment (Baars 2011).

Chapter 3: Seed germination efficacy of *Rhododendron ponticum* L. on different depths of forest litter

3.1 Introduction

The success of rhododendron as an invasive species and out-competing native Irish scrub species such as holly, gorse and heather, is in part due to its efficiency at seedling recruitment (Erfmeier and Bruelheide 2004). Once they reach maturity (around age 10) rhododendron plants produce prolific numbers of small wind-dispersed seeds (Cronk and Fuller 2001). On a typical specimen of rhododendron each inflorescence can produce up to 5000 seeds and, on average, a 12 year old bush can produce up to 1 million seeds. Seed germination can be successful on many different types of substrate with light availability the most likely limiting factor (Cross 1981, Mejías et al. 2002). In a woodland situation, soil disturbance, as caused by forest operations, animal grazing or fallen trees creates gaps in the canopy allowing sufficient light to reach the forest floor. These disturbances also encourage the colonisation of bryophytes and thus provide suitable conditions (sufficient light and moisture) for successful germination and seedling establishment of rhododendron (Cross 1981, Gray and Spies 1997). The presence of forest litter generally has a negative effect on germination success (Xiong and Nilsson 1999). There are many reasons for this, including altering moisture, light and temperature regimes for neighbouring seeds. Litter depth as a factor in seed germination and subsequent seedling establishment is considered to mostly affect small seed species such as rhododendron (Molofsky and Augspurger 1992, Myster 1994).

Many alien species do not have a detrimental effect on native ecosystems, as only a small fraction of them become insidious (Mooney and Cleland 2001). Alien species are supposed to perform best on disturbed sites due to fast germination capabilities (Hobbs and Huenneke 1992) and forest litter depth is a critical factor in seed germination success (Ahlgren and Ahlgren 1981, Tyler et al. 2006). Therefore seed germination success could be controlled directly by regulating the depth of leaf litter, with deeper litter limiting successful seed germination. An understanding of why a particular species, over another, becomes successful in establishing itself in an unfamiliar habitat is a critical factor in designing an effective control programme.

The amount and distribution of forest litter can influence the establishment of invasive species (Hobbs and Huenneke 1992, Mooney and Cleland 2001). As a successful primary invader, rhododendron is typically found in areas where recent land management activities have taken place. Disturbed substrate coverage and lack of predators provide the species with optimum regeneration conditions by creating micro-sites that allow the seeds to germinate and subsequent seedlings to establish (Cross 1975). The aim of this study was to investigate the effect of litter depth on the germination success of rhododendron seeds using seeds collected from plants in an Irish plantation forest. To investigate the germination success of rhododendron on forest litter three different depths of litter were chosen to compare varying conditions for seed germination, from a bare peat substrate up to a litter depth at which no germination is likely to occur. A conifer needle litter treatment was also used to assess the ability of rhododendron seeds to germinate on a bed of needles with no access to bare soil.

3.2 Materials and Methods

3.2.1 Seed collection and preparation

Rhododendron seeds were collected from two plants located on the edge of a stand of Sitka spruce (*Picea sitchensis*) in Deerpark, Lismore, Co. Waterford. The stand was planted in 1985 and, as rhododendron in this area is invasive, the plants were no more than 26 years old. One of the shrubs was 2.5 m in height with a crown circumference of 13.4 m and the other was 2.0 m in height and 9.5 m in circumference.

Twenty racemes were collected from each rhododendron plant on January 15th 2012. The following day each seed pod (still attached to its stalk) was separated from its raceme and placed in paper bags. On average each raceme held 15 seed pods resulting in 286 seed pods harvested from one plant and 306 from the other. The paper bags were stored in a ventilated room with natural air and at room temperature (20 ± 2 °C) for two weeks after harvesting. The seeds used were all from one shrub with the other being stored as a backup. Each bag was shaken lightly prior to the removal of the pods to encourage the release of seeds from pods that had naturally opened. The pods were then removed leaving the seeds at the bottom of the paper bag. The seeds were then counted into 15 batches of 1050, with one batch per each of three replicates of the five treatments.

3.2.2 Seed viability test

In order to assess the quality of each batch of seeds, and to ensure that there was not a difference in viability between batches, a germination test was carried out in the laboratory. The viability of the seeds was tested using 50 seeds randomly selected from each batch which were placed on filter paper in separate 12 cm Petri dishes and soaked with 8 ml of distilled water ensuring that the filter paper was completely saturated. A heated propagator (100 cm x 50 cm) was used to provide a controlled environment for the in vitro germination viability test. The propagators were kept at a constant 20 °C throughout the experiment on a constant 16 hour per day light cycle. The position of each Petri dish was swapped every three days to mitigate any effect of position on the outcome of the germination test. Distilled water was added as required to ensure that moisture was not a limiting factor in germination success. The number of successfully germinated seeds was monitored for eight weeks following sowing to assess the germination viability of rhododendron seeds on filter paper.

3.2.3 Germination experiment

The main experiment to test the germination of rhododendron seeds on different depths of forest litter was carried out in a climate controlled grow house at 20 °C with 16 hours of light per day (Plate 9). To enhance natural light high pressure sodium lights were employed when natural light fell below 18 klx (kilolux) ensuring a constant illumination over the experiment at 20 (±5) klx. Fifteen 65 L trays (0.45 m^2 base area) with holes drilled in the base to allow for adequate drainage were use in this experiment. Each tray held 8 cm depth of milled sphagnum peat substrate. Soil pH level was measured in each tray prior to sowing of seeds and ranged between 4.5 and 5.1. Each tray was divided in six equal sub-plots to facilitate the even sowing of seeds and subsequent counting of any successfully germinated seeds. Tap water (pH of 6.5) was fed through the stations irrigation system once a day ensuring that the soil was kept moist throughout. The water was stored in an open tank for at least three days before being used.

Plate 9
Germination experiment in greenhouse showing layout of trays.

Five different litter treatments were used in this experiment as described in Table 9. The broadleaf forest litter used in this experiment consisted of a mixture of litter (gathered on 23rd of January 2012) from four common tree species: beech (*Fagus sylvatica*), oak (*Quercus* spp), ash (*Fraxinus excelsior*) and holly (*Ilex aquifolium*). These were chosen to represent the range of leaf litter most commonly found in Irish broadleaved forests. The conifer needles were obtained from a stand of Sitka spruce, also on 23rd of January 2012. The forest litter was collected two weeks prior to the beginning of the experiment and stored in paper bags in a cool dry storage area to allow the litter to dry.

Table 9. Details of the five litter treatments.

Treatment	Litter type	Litter depth (cm)	Litter mass (g/m^2)	Exposed soil (%)
1	**Bare peat**	0	0	100
2	**1 cm Broadleaf**	1	150	35
3	**3 cm Broadleaf**	3	210	12
4	**5 cm Broadleaf**	5	415	0
5	**2 cm Conifer**	2	398	0

In order to standardise the amount of litter used across each replicate for each treatment the required litter mass per unit area was calculated. The weighed litter was then spread roughly over the tray prior to broadcasting of rhododendron seeds (Plate 10). The litter was spread roughly to mimic the natural state of forest litter where the lower the amount of litter found on the forest floor the higher the area of exposed soil is present. Different depths of conifer needles were not considered as they form a uniform carpet on the forest floor preventing direct access of the seed's radical to the soil. Each batch of 1000 seeds was divided into six equal parts (by weight) which were spread evenly over one of the sub-plots on the garden trays. The position of each tray on the bench was switched each week. The number of seeds that had germinated in each tray was recorded weekly until germination ceased in all trays at 16 weeks after sowing.

Plate 10.
Trays showing four of the five treatments before the rhododendron seeds were sown. Top left is the bare peat treatment, top right is the 3 cm of broadleaf litter treatment, bottom left is the 2 cm conifer needle treatment and bottom right is the 5 cm of broadleaf litter treatment.

3.2.4 Data analysis

At the end of the experiment, when the rate of germination was zero in all trays, germination success was calculated as the number of germinated seedlings expressed as a percentage of the total number of seeds sown. As the data from the germination results were non-parametric a Kruskal-Wallis test was used to test for differences among the treatments. Pair-wise comparisons were made between individual treatments using Mann–Whitney U tests, controlling for type 1 error using the Bonferroni approach. Weekly germination rates for each treatment were also recorded. Daily rates were then calculated (number of seeds germinated during one week divided by seven days). All results are presented as means and standard errors and data analysis were conducted using Minitab, version 16 (Minitab 2010).

3.3 Results.

3.3.1 Seed viability test

The mean germination success was 82.0 ± .44% after four weeks for all 15 batches of seeds. The batch with the lowest number of germinated seeds produced 38 (76%) out of 50 seeds. The batch with the highest number of seeds that germinated produced 45 (90%) seeds. The median number of seeds that successfully germinated over all the batches was 41 (82%). The standard deviation and variance for all 15 batches was 1.74 and 3.03 respectively demonstrating that the batches are not different from one another. No further germination occurred after four weeks.

3.3.2 Germination experiment

A significant difference was found among the germination success of the five treatment groups ($H = 13.5$, $DF = 4$, $p < 0.01$). The highest germination rates occurred between weeks four and six in all treatments (Figure 4). Pair-wise comparisons between treatment 1 (bare soil) and the remaining treatments resulted in $p < 0.005$ (Table 10). The same p-level was found for each pair-wise comparison as there was no overlap between the yields of each treatment. This demonstrates that each treatment is significantly different from one another.

Table 10. Pair-wise comparisons of all germination treatments (n = 3, adjusted p-value = 0.005).

Comparison	**P value**	**Comparison**	**P value**
Bare vs. 1 cm	0.002	1 cm vs. 5 cm	0.002
Bare vs. 3 cm	0.002	1 cm vs. Conifer	0.002
Bare vs. 5 cm	0.001	3 cm vs. 5 cm	0.004
Bare vs. Conifer	0.001	3 cm vs. Conifer	0.003
1 cm vs. 3 cm	0.002	5 cm vs. Conifer	0.003

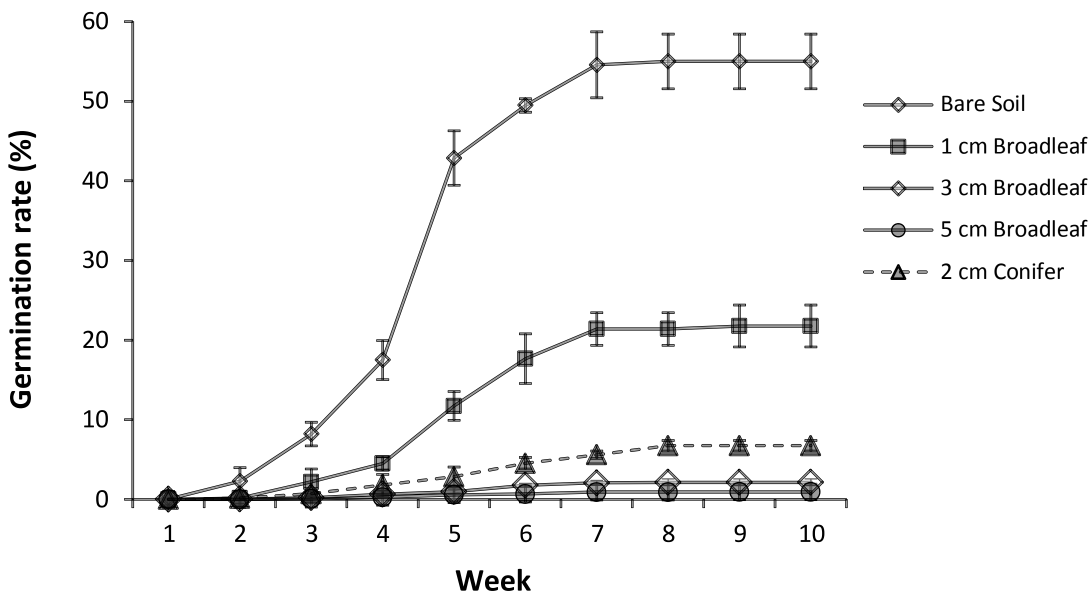

Figure 4. Mean (± S.E.) cumulative germination rate (percentage of successfully germinated seeds) of rhododendron seeds in different forest litter treatments over 10 weeks.

Over half of rhododendron seeds germinated successfully on bare peat (55.00 ± 1.98%) (Figure 5) and seed germination ceased at week eight. The germination rate in this treatment was highest during week five (36.24 ± 1.98 seeds per day). The majority of the seeds germinated during week's four to six (75.00 ± 0.31%). The overall germination rate for treatment 1 (bare peat) over the eight week period was 9.28 ± 0.61 seeds per day (Figure 4). Seed germination success on 1 cm of broadleaf litter was 21.00 ± 1.52%. The highest germination rate occurred during week five (10.33 ± 1.82 seeds per day). No further germination occurred after week nine. The mean germination rate of rhododendron seeds in this treatment over the nine week period was 3.46 ± 0.24 seeds per day.

The germination success rate of rhododendron seeds on 3 cm of broadleaf litter was 2.00 ± 0.24%. The germination rate reached a maximum of 1.14 ± 0.14 seeds per day during week six (Figure 5). Over the eight week period that rhododendron seeds emerged in this treatment, the mean rate was 0.38 ± 0.04 seeds per day. On trays with 5 cm broadleaf litter less than 1.00 ± 0.09% of seeds germinated successfully and no seeds germinated after week nine. The germination rate was highest during week five where 0.43 ± 0.18 seeds per day germinated. The mean germination rate over the nine week period was 0.16 ± 0.01 seeds per day. Rhododendron seeds on 2 cm conifer litter produced radicals on 7.00 ± 0.39% of the seeds broadcast in this treatment. The highest germination rate occurred during week six when 2.43 ± 0.43 seeds per day successfully germinated. No further germination occurred after week eight. Over the eight week period that rhododendron seeds successfully germinated, the mean rate per day was 1.20 ± 0.07 seeds.

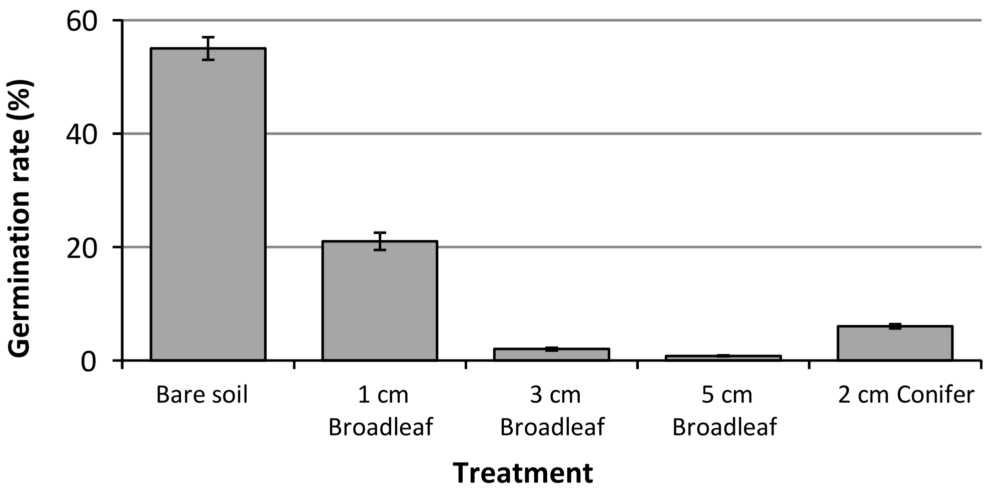

Figure 5. Mean (± S.E.) germination rate (%) of rhododendron seeds on 5 different forest litter treatments over 9 weeks (n=3).

3.4 Discussion

The seed germination success of rhododendron was high when tested in the laboratory on filter paper in this study, compared with the rates reported in published germination experiments where success rates were between 45% and 70% (Mejías et al. 2002, Stout 2007). These results indicate that poor seed viability is not a factor in recruitment failure.

Germination success in the greenhouse was highest on bare peat substrate and lowest on the deepest (5 cm) broadleaf litter indicating that litter depth influences germination success by preventing the seeds radical from reaching the substrate. Litter depth is not commonly used as a control method for invasive species, as it can be a hindrance to the establishment of desired species (Sydes and Grime 1981) or provide protection for seeds from predators (Cintra 1997). Forest litter depth is not currently a consideration for rhododendron control in Ireland (Barron

2008, Higgins 2008, Maguire et al. 2008). However rhododendron seed physiology makes this species an ideal candidate for a novel control programme involving management of forest litter depth (Stephenson et al. 2006). For example on a clearfell site sensitive to rhododendron invasion, instead of using brash to build windrows, the excess branches could be mulched and used as a litter layer to prevent rhododendron seed germination. However it should be noted that mulch may display different morphological properties than that of leaf litter, such as a greater ability to retain moisture; more prone to moss growth etc, and as such it would be prudent to examine rhododendron seed success on mulch in greater detail.

The difference in germination across depths may be due to seeds being denied access to direct sunlight by the litter, while others resting on the forest litter may produce a radical but the small seeds do not have enough energy reserve for the radical to reach the surface of the medium (Harper et al. 1970). On conifer needle beds in this study just seven per cent of the seeds germinated and it is expected that none of those seedlings would establish or develop further as the seed's radical is highly unlikely to reach the substrate surface. In the case of species that produce small seeds the ability of the seed to germinate is not the only factor of its success in producing a healthy plant (Xiong and Nilsson 1999). The establishment of the germinated seed in its substrate is equally important. The radical will need direct access to the soil and as such they will only be able to germinate on some substrates and not others. Further study into how long a radical rhododendron seeds can produce before energy reserve is depleted would be useful.

As rhododendron seeds require adequate access to bare soil, or soils covered in bryophyte carpets (Cross 1981), land disturbance plays a significant factor in the spread and recruitment of this invasive species. This experiment demonstrates that a reduction in forest litter depth, sufficient to expose bare peat substrate facilitates rhododendron seedling establishment. Conversely the delicate ephemeral nature of rhododendron seeds means that an increase in forest litter can prevent seed germination. Rhododendron seeds are small and delicate, they are non-dormant, germinate quickly and need light to do so (Cross 1981). Previous experiments on the success of rhododendron seed germination once imbibed have resulted in low germination success rates. For example Mejias (2002) shallowly buried packets of 20 seeds for nine months and, once recovered, none of the seeds germinated on filter paper. Previously Cross (1975) observed that no germination occurred on imbibed seeds kept in the dark for 161 days and then brought into the light. Erfmeire & Bruelheide (2005) while comparing the germination of Irish sourced seeds against native Spanish and Georgian seeds found that while there was no difference in optimum germination temperature or maximum germination rate, the Irish seeds did display a greater germination velocity by responding faster to the treatments. These experiments reported similar rates of germination as observed in the current study. After week eight of this experiment no seeds were observed germinating which emphasises the transient nature of these small seeds.

Differences in physical properties of litter are likely to result in different light, temperature and moisture conditions for seeds found on a forest floor (Facelli and Pickett 1991). The scope of this study was unable to include light, temperature or moisture as variables in the germination success of rhododendron seeds. However the varying depths in forest litter has in previous studies demonstrated the proportional relationship between litter depths and light available for seeds, moisture retention of the substrate and temperature of the soil underneath the substrate (Xiong and Nilsson 1999). In nearly all cases, for small seed species, the greater the depth of the forest litter the less amiable the germinating conditions are for the seeds. The conifer needle treatment, while only 2 cm deep, had a greater mass than that of the 3 cm broadleaf treatment and no resulting exposed peat surface. This highlights how a change in litter type can change the dynamics of the litter depth/germination success relationship. The conifer needle treatment had a higher germination success rate that that of the 3 cm broadleaf treatment even though there was no bare substrate exposed. It is however unlikely than these germinated seeds would reach establishment as the radical would not reach the peat surface. It would be useful to examine different depths of conifer needles to simulate the edge of the forest effect where needle depth would be shallower and rhododendron would be more likely to establish.

Using models to estimate colonising potential of an invasive species is an established tool used by ecologists (Bezrukova et al. 2012). According to Stephenson (2006) (and later demonstrated by Harris et al (2011) there is substantial potential for the development of spatial population models to predict the colonisation of rhododendron in sensitive habitats. Although many habitat requirements of rhododendron have been described in qualitative terms, quantitative information is most important when considering designing a predictive model in ecology (Guisan and Zimmermann 2000). Quantitative data, such as the data described in this paper, can be integrated into a spatial population model which in turn can aid land managers in designing optimum control strategies against the spread of rhododendron.

3.5 Conclusion

Despite a good understanding of the nature of the threat posed by invasive species few eradication programs have been a complete success. If the infestation is identified early and all individuals are removed before they have a chance to seed, then eradication can be successful (Mack and Lonsdale 2002). If, however the infestation is allowed time to establish, then complete eradication is almost impossible. The only available course of action is then containment. Both actions require significant expenditure, the heavier the infestation the greater amount of resources needed to combat it. Many alien species do not have a detrimental effect on native ecosystems, as only a small fraction of them become insidious (Mooney and Cleland 2001). An understanding of why a particular species, compared to another, becomes successful in establishing itself in an unfamiliar habitat is a critical function in designing an effective control programme.

As mentioned above, management of litter depth is not commonly used as a control method for invasive species and forest litter depth management is not currently considered as part of a targeted control program for rhododendron in Ireland. However rhododendron seed physiology makes the species an ideal candidate for a novel control program involving forest litter depth.

The establishment of rhododendron in an area is often very difficult to reverse. Established colonies of rhododendron will persist indefinitely spreading to their ecological limit particularly in sensitive ecosystems. Therefore the choice for the landowner is a pre-emptive one; prevention rather than cure. Better awareness and legislation coupled with defined identification and prevention tools will help to prevent new invasions. As rhododendron seeds need adequate access to bare soil (or soils covered in bryophyte carpets) (Cross 1981) land disturbance plays a significant factor in the spread and recruitment of this invasive species. This experiment demonstrates that a small decrease in forest litter depth, enough to expose bare substrate will facilitate seedling establishment. Conversely a small increase in forest litter can prevent significant germination. This work would benefit guidelines and standard operating procedures for future forest management plans particularly in areas which are sensitive to rhododendron invasion.

Chapter 4: Mitigation against the re-invasion of *Rhododendron ponticum* L. by limiting herbivory

4.1 Introduction

The introduction of non-native species into new habitats frequently results in their becoming invasive (Mack et al. 2000, Mooney and Cleland 2001, Gherardi and Angiolini 2004, Charles and Dukes 2007, Theoharides and Dukes 2007, Barratt et al. 2010, Stout 2011). Exotic plants may be subject to lower levels of herbivory than native plants because of the absence of specialised predators and the limited adaptability of herbivores in their introduced range. Referred to as the 'enemy release hypothesis', this theory postulates that lower levels of damage to exotic species caused by browsing animals leads to higher survival and reproductive success of the exotic species compared with local species (Wolfe 2002, Colautti et al. 2004). While the success of an invasive species is not always associated with release from herbivory (Agrawal and Kotanen 2003, Chun et al. 2010, Cooper and Mccann 2011) species that do become invasive often experience low levels of herbivory in their new environment (Cappuccino and Carpenter 2005, Cooper and Mccann 2011, Vilà et al. 2011).

Damage caused by grazing animals negatively impacts plant seedlings and reduces plant diversity as palatable species are destroyed and unpalatable species, such as rhododendron, become dominant (Cross 1981, Putman et al. 1989, Gill et al. 1995, Latham and Blackstock 1998, Rooney and Hayden 2002, Perrin et al. 2006). In some situations short-term high intensity grazing can be a valuable tool for weed control (Rosa García et al. 2012). The most common vertebrate herbivore threats to Irish forests include deer (*Cervidae spp*), rabbit (*Oryctolagus cuniculus* L.), hare (*Lepus europaeus*) and domestic animals such as sheep (*Ovis aries*) (Rooney and Hayden 2002). The population of deer across Ireland is increasing mostly due the increase in suitable habitats and the lack of natural predators (Rooney and Hayden 2002, Carden et al. 2010). Browsing impacts can result in timber defects that ultimately reduce the commercial value of plantation trees (Putman and Moore 1998). Rabbits are widespread across Ireland due mostly to the persecution of potential predators such as foxes, stoats and birds of prey and the availability of suitable grassland habitat (Sumption and Flowerdew 1985). Browsing is the most common form of damage to trees caused by rabbits (Pepper 1998, Rooney and Hayden 2002). Sheep are most problematic during establishment stage of forest plantations when newly planted seedlings are most vulnerable (Rooney and Hayden 2002).

Fencing to protect susceptible crops from browsing damage is widely used as a management tool in Ireland and there have been many studies investigating the effects of browsing on forest stand composition (Linhart and Whelan 1980, Perrin et al. 2006, Becerra and Bustamante 2008, Newman et al. 2014). However the role of grazers in the establishment and survival of invasive rhododendron has never been assessed, despite the recognised role of herbivory in facilitating rhododendron invasion (Cross 1981, Tyler et al. 2006). The role of grazers in the establishment and survival of competing native vegetation may be an important tool in the management of rhododendron in Irish woodlands and forests.

This study set out to investigate whether the prevention of grazing in woodland sites could be used to prevent the spread of rhododendron by facilitating the native vegetation (normally grazed by herbivores) to out-compete rhododendron. The presence of herbivores and others, which pose a threat to plant seedlings, can be assessed either directly by visual identification, or indirectly by observing their local activities such as occurrences of faeces, burrows and trails (Fuentes et al. 1983, Becerra and Bustamante 2008). The effect of animal browsing on the seedling survival of two common native broadleaf scrub species (silver birch (*Betula pendula*) and holly (*Ilex aquifolium*)) was investigated in two forests where rhododendron had previously become established. Birch and holly were chosen as test species as both are fast growing pioneer species commonly found in similar habitats to rhododendron. Holly is a species that has similar morphological attributes to rhododendron (think bushy plant that can cast a broad shade). Silver birch may not have the same ability to smother understory vegetation when it reaches thicket, therefore a mixture of birch and holly was investigated. Also notable is the fact that birch improves soils through de-acidification and that over time birch has the ability to increase soil pH levels (Pritchett 1979).

4.2 Materials and Methods

4.2.1 Study sites

Two typical forest plantations with rhododendron infestation were used in this experiment. Deerpark is a mixed conifer/broadleaf plantation forest located in Lismore, Co. Waterford (52.11 °N, -7.89 °W). The experimental plots were located in a 32 hectare plantation forest on brown podzolic soil lying on a sandstone parent material. The vegetation at this site was made up of tree, shrub and herbaceous plants. The area (600 m^2) used for this study comprised mature Japanese larch (*Larix kaempferi*) with an understory of rhododendron and holly. The stand had, one year previous to the establishment of the experiment, received its second thinning resulting in roughly 700 trees left per hectare.

The second site was in Nephin forest in Newport, Co. Mayo (53.59 °N, -9.33 °W). The experimental plots were located in a 12 hectare forest compartment on a blanket peat soil characterised by an acidic oligotrophic substrate. The experiment plots were located in an area that was previously a stand of Sitka spruce (*Picea sitchensis*), which has not been replanted since harvesting 14 years ago. The plots were established on a west facing slope in an area with high annual wind speeds of 7 m/s (Met Éireann: accessed 2012). Since harvesting of the commercial plantation rhododendron has invaded the site. The flora community in, and surrounding, the experimental plots was dominated by rhododendron and Sphagnum mosses with some deergrass (*Trichophorum caespitosum*) and cottongrasses (*Eriophorum spp*.) and a small population of crowberry (*Empetrum nigrum*).

The preparation of the sites at Deerpark and Newport for this study was carried out in March 2009. At each site the area used for the study was cleared of all rhododendron plants. The total area used at each site was 0.06 hectares (10 m × 60 m). Each plant was cut to its stump leaving a maximum height of 10 cm. There were more rhododendron plants at the Newport site than at the Deerpark site (142 and 17 respectively). Once cleared of rhododendron, each study area was divided into 2 plots (each measuring 5 m × 60 m). Fencing to exclude grazers (3 m high deer fence with rabbit proof mesh along the base) was erected around the perimeter of one of these plots (Plate 11). Each plot was then divided into 12 square sub-plots (5 m × 5 m) spread evenly through the rectangular plot resulting in a total of 24 sub-plots, 12 fenced and 12 unfenced. All plots were marked on the ground using rope and pegs.

Plate 11.
Deer fencing around the trial plot at the Deerpark study site.

The 24 sub-plots were distributed across 8 experimental treatments, with three replicates of each treatment (Figure 6). Of the eight treatments four were fenced and four were unfenced, with the four different treatments in the two groups comprising of a control treatment where no seedlings were planted, a birch treatment planted with birch seedlings, a holly treatment planted with holly seedlings and a mixed treatment planted with a mix of birch and holly seedlings. The mixed treatment was used to see if both pioneer species, while competing with each other for resources, would capture the site more efficiently than if planted as a single species (Kelty et al. 1992). Within the fenced and unfenced plots the experimental sub-plots were arranged linearly in three separate blocks, with each block containing one replicate of each treatment as shown in Figure 6. Five seedlings were planted 2.5 m apart in each sub-plot following the standard operating procedure recommending by the Irish Forest Service (Forest Service 2011). Planting of seedlings in this study was carried out between the 12th and 16th of December 2009. The seedlings used (bare-root stock) were sourced from Coillte's Ballintemple nursery, and had been grown from seeds collected from Irish plantation forests.

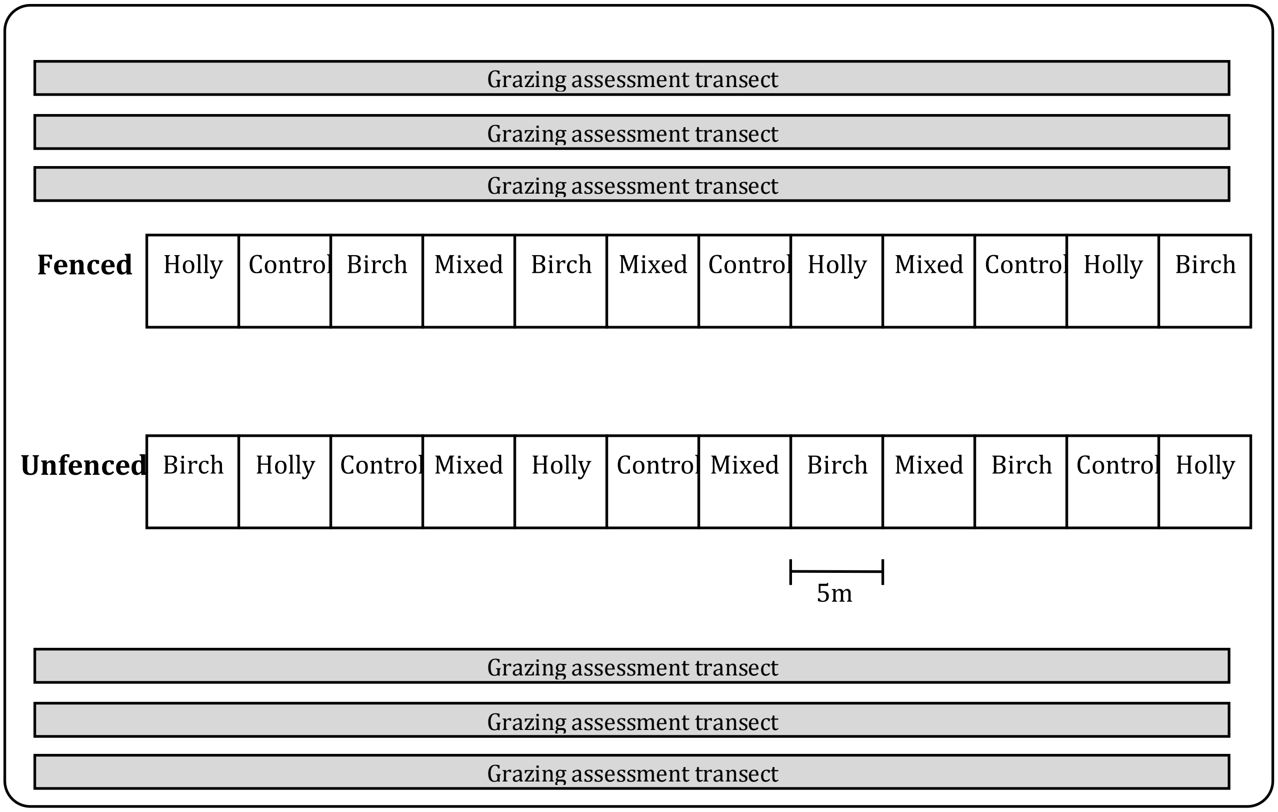

Figure 6. Experimental layout at each study site.

4.2.2 Herbivore browsing

In order to measure the presence of grazing animals at the study sites three transect lines (all unfenced), each 75 m x 2 m, were established on both sides of the experiment plots at both sites (Figure 6). Twenty sample plots, each 10 cm × 10 cm, and separated by 3.75 m, in each transect were examined for the presences of vertebrate faeces (Bailey and Putman 1981, Becerra and Bustamante 2008). The plots were established in June 2009 and assessed in October 2009.

4.2.3 Seedling survival

In order to measure the impact of grazing animals on the seedling survival of birch and holly in this study annual seedling mortality caused primarily by grazing was assessed for four years following the planting of the seedlings in December 2009. Seedling survival was assessed on four occasions in total (October 2010, October 2011, October 2012 and October 2013). During each assessment the number of surviving seedlings, the number of seedlings dead as a consequence of animal grazing, and the number of seedlings dead for reasons unrelated to grazing animals was recorded. Damage caused by vertebrate grazers to the seedling was considered browsing if the seedling did not survive (Becerra and Bustamante 2008), therefore dead seedlings with clear evidence of damage by vertebrate grazers were recorded as ‘browsed’. Living seedlings with evidence of browsing were recorded as healthy seedlings, as they still had a chance of survival. In addition to describing browsed seedlings, all dead seedlings (with no evidence of browsing along with browsed seedlings) were quantified as a percentage of planted seedlings.

During the October 2013 assessment the height of the leader branch of each of the surviving seedlings was recorded in order to investigate the effect of animal browsing on the height of the introduced seedlings. Each of the cut rhododendron plants in the study plots was monitored throughout the experiment and the height of the tallest stem was recorded in October 2013.

4.2.4. Data analysis

Differences in mean seedling survival among treatments were investigated using Kruskal-Wallis (control plots with no introduced seedlings were not included in this calculation). Pairwise comparisons using Mann-Whitney U test were employed to determine where any significant differences among the treatments occurred. Alpha level adjustment was done using a Bonferroni adjustment for multiple comparisons. Differences in mean height increases of the introduced seedlings (height at October 2013 less height when planted) among the treatments over the four year period were assessed using a one-way analysis of variance. The unplanted treatments were not assessed in this analysis. Tukey’s post-hoc test was used to determine which means differed. The height of the tallest stem from the cut rhododendron plants was compared to its original height and a mean percentage recovery value was calculated. Kruskal-Wallis test was used to test for differences among treatments and the Mann-Whitney U test was used as a pair-wise comparison for each treatment. Alpha level adjustment was done using a Bonferroni adjustment for multiple comparisons. All statistical analyses were carried out in Minitab version 16 (Minitab 2010).

4.3 Results

4.3.1 Herbivore browsing

The only grazing threat at the Deerpark study sites came from rabbits, evidence of which was recorded at 18 of the 120 sample plots (15%). No evidence of either sheep or deer was recorded at that site. By contrast, the only grazing threat at the Newport study site came from sheep, evidence of which was recorded at 48 of the 120 sample plots (40%). No evidence of rabbits or deer was recorded at the Newport study site.

4.3.2 Deerpark results

4.3.2.1 Seedling survival

Four years after establishment of this study at the Deerpark site no significant difference in seedling survival between treatments was observed ($H = 8.95$, $DF = 5$, $p > 0.05$). 24.4 ± 7.3% of all seedlings planted in unfenced treatments survived until the end of this study, while 73.3 ± 10.5% of those in fenced treatments survived (Figure 7). Mortality was consistently higher in unfenced plots across the birch, holly and mixed treatments but sample sizes were too small to allow for statistical testing of the difference in survival between fenced and unfenced treatments. Of the 75% of seedlings that died in the unfenced treatments 13.3 ± 6.7%, 13.3 ±13.3% and 20.0 ± 0.0% in Birch, Holly and Mixed treatments respectively died as a result of damage caused by grazing animals (Plate 12).

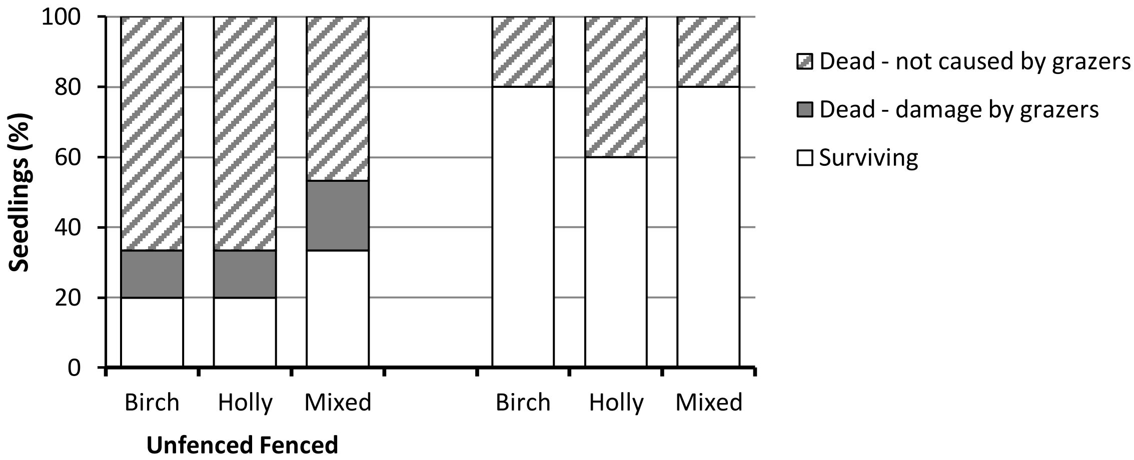

Figure 7. Proportion of seedling mortality caused by grazing damage at the Deerpark study site (n=3 per treatment).

4.3.2.2 Seedling growth

The mean height increase of the seedlings over the course of this study varied between treatments ($F_{5,17} = 6.66$, $p < 0.01$). The seedlings in the fenced treatments showed a greater increase in height than those in the unfenced treatments (Table 11). The largest increase in height was seen in the fenced birch seedling treatment and the smallest in the unfenced holly seedling treatment. Birch seedlings in the fenced treatment showed an increase of 1.01 ± 0.12 m over the four years. Comparing those values to the height increase for the unfenced birch treatments (0.07 ± 0.05 m) a significant difference was observed. The mean increase in height for the holly seedlings in the fenced treatment was 0.34 ± 0.11 m while the mean height increase for non-excluded holly was 0.06 ± 0.58 m. The comparisons for the mixed treatments showed that the unfenced treatments had a smaller growth rate than those in the fenced treatment.

Table 11. Mean (± S.E.) height increase (m) of seedlings at the Deerpark study site over the course of this study. Values with different letters were significantly different from one another (n=3).

	Height Increase (m)	
Treatment	**Fenced**	**Unfenced**
Birch	1.01 ± 0.12 a	0.07 ± 0.04 bc
Holly	0.34 ± 0.11 abc	0.06 ± 0.58 c
Birch/Holly	0.78 ± 0.12 ab	0.28 ± 0.11 bc

4.3.2.3 Rhododendron regeneration

Rhododendron regeneration, measured as percentage recovery of the cut rhododendron stumps, was significantly different among the eight treatments ($H = 15.41$, $DF = 7$, $p < 0.05$). All 11 of the rhododendron plants from outside the fenced area that were cut back at the Deerpark study site recovered 78 ± 6% of their original height by the conclusion of this study. Of the six rhododendron plants removed from inside the fenced area a mean recovery of all six plants was 56 ± 16%. Two plants recovered beyond their original height with a maximum recorded recovery of 117% of original height (Figure 8). New shoots were seen on four of the six unfenced plants, though they had not recovered beyond the height of their cut stump. Sixteen new seedlings germinated over the course of the study. Two of those rhododendron seedlings were recorded inside the fenced area while 14 were recorded outside the fenced area. Only one of these new and naturally regenerated specimens survived until the end of the study, and this was located in a plot outside the fenced area.

Plate 12.
Evidence of animal browsing damage to a birch seedling at the Deerpark study site.

The recovery rate of the rhododendron stumps per treatment produced mixed results when comparing the fenced to the unfenced treatments. Re-growth of the cut rhododendron plants was greater for both the unplanted and birch fenced treatments than that of their unfenced counterparts. Conversely both the holly and birch/holly mix fenced treatments produced less re-growth than their corresponding unfenced treatments. No paired comparisons could be made to confirm this analysis as there were insufficient numbers of rhododendron plants present per treatment.

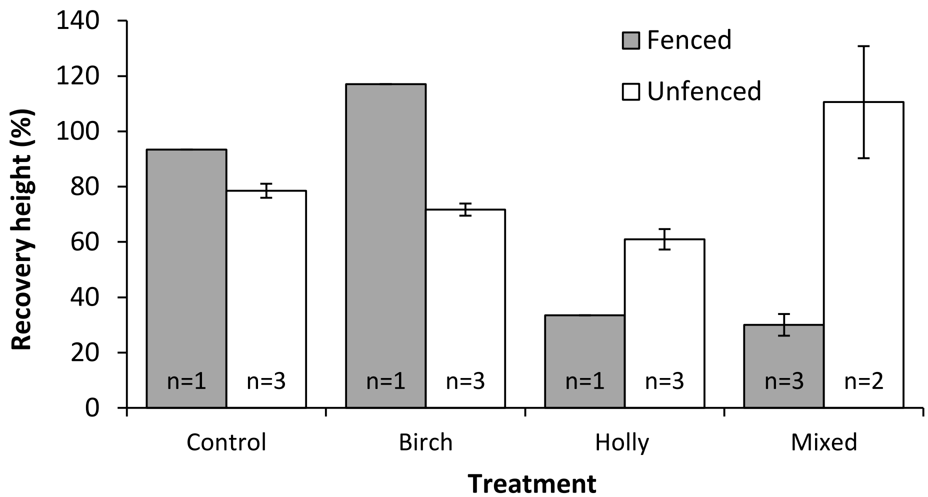

Figure 8. Mean (±S.E.) rhododendron recovery (%) in fenced and unfenced treatment plots at the Deerpark study site over the course of this study. N is the number of plots containing at least one plant per treatment.

4.3.3 Newport results

4.3.3.1 Seedling survival

The survival rates of the seedlings at the Newport study site were not significantly different from each other ($H = 5.92$, $DF = 5$, $p > 0.05$). 13.3 ± 5.8% of all seedlings planted in unfenced treatments survived until the end of this study, while 24.4 ± 7.3% of those in fenced treatments survived (Figure 9). Mortality was consistently higher in unfenced plots across the birch, holly and mixed treatments but sample sizes were too small to allow for statistical testing of the difference in survival between fenced and unfenced treatments. Of the 75% of seedlings that died in the unfenced treatments 40.0 ± 11.5%, 33.3 ± 6.7% and 26.7 ± 13.3% in birch, holly and mixed treatments respectively died as a result of damage caused by grazing animals (Figure 9). One of the deceased holly seedlings in the fenced plot was topped, the damage to the top of the stem characterised by what appeared to be a sharp bite mark, consistent with grazing. Following close inspection of the fence no obvious break was discovered ruling out the damage being caused by a ground dwelling vertebrate. The only other explanation would be that the damage may have been caused by a bird or human.

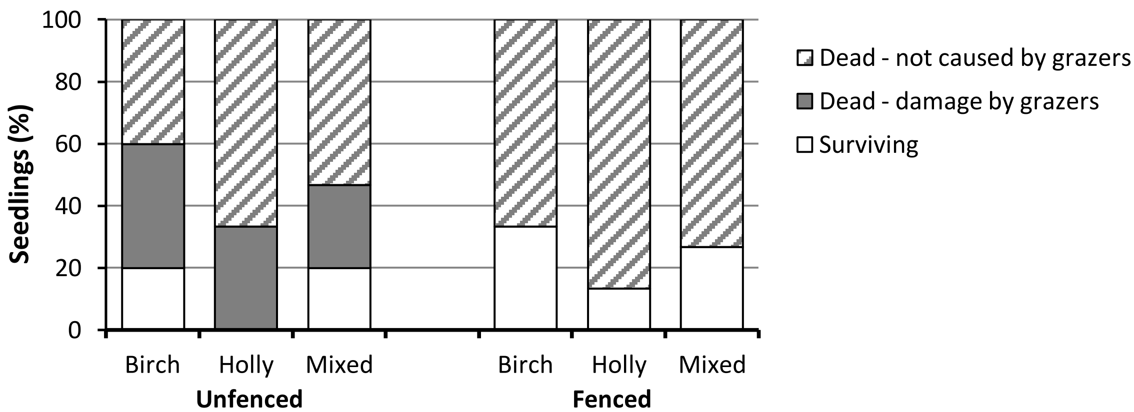

Figure 9. Proportion of seedling mortality caused by grazing damage at the Newport study site (n=3 per treatment).

4.3.3.2 Seedling height increase

The mean height increase of the seedlings varied between treatments ($F_{5,17} = 6.33$, $p < 0.01$). The seedlings protected from herbivore grazing showed a greater increase in height compared with those in the unfenced treatments (Table 11). The largest increase in height growth was produced by the birch seedlings in the fenced treatment and the smallest increase was shown by the holly seedlings in the unfenced treatment, which had a 100% mortality rate. In the case of the birch seedlings at the Newport study site, there was a significant difference between the fenced and unfenced treatments (Table 12). The mean increase in height of the holly in the fenced treatments at Newport was 0.01 ± 0.0 m while no comparison could be made with the unfenced holly treatment where a 100% mortality rate was observed. The results of the mixed treatments revealed a similar trend with seedlings in the unfenced treatment showing smaller height increases than those in the fenced treatments.

Table 12. Mean (±S.E) height increase (m) of seedlings at the Newport study site (n=3). Values in cells with different letters were significantly different from one another ($p < 0.05$).

	Height Increase (m)	
Treatment	**Fenced**	**Unfenced**
Birch	0.18 ± 0.07 a	0.02 ± 0.02 b
Holly	0.01 ± 0.01 b	0.00 ± 0.00 b
Birch/Holly	0.09 ± 0.05 ab	0.04 ± 0.02 b

4.3.3.3 Rhododendron regeneration

Analysis of the recovery of the rhododendron stumps that were cut back at the beginning of this study revealed a difference between the treatments ($H = 8.19$, $DF = 7$, $p > 0.05$). Of the 79 rhododendron plants removed inside the fenced area all have recovered over the course of the study, with a mean recovery in height of 75 cm ± 1%. Thirty one new seedlings germinated in addition to the cut rhododendron, of which 16 survived to the conclusion of the study. At least 12 of these seedlings were the result of vegetative growth from exposed roots of an existing parent plant. It is difficult to assess all the new seedlings without damaging them. The 65 rhododendron plants cut to their stumps in the unfenced area recovered a mean 67 ± 1% of their original height. 39 naturally generated rhododendron seedlings were discovered over the four years of the study, of which 15 survived to the end of the study (Figure 10).

The recovery rate of the rhododendron stumps was higher in fenced than unfenced treatments. Pairwise analysis confirmed that all the unfenced treatments had a lower recovery rate than fenced treatments although none of these differences were statistically significant.

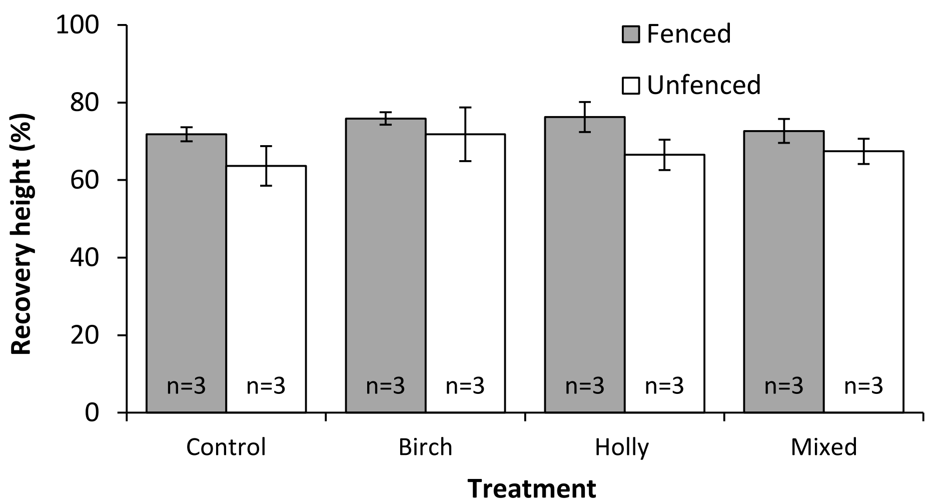

Figure 10. Mean (±S.E.) rhododendron recovery (%) in plots of fenced and unfenced plots at the Newport study site over the course of this study. N is the number of plots containing at least one plant per treatment.

Plate 13.
Rhododendron regeneration at the Newport study site. The image on the left was taken at the conclusion of the study in July 2013 and the image on the right was taken in October 2009, 6 months after establishment.

4.4 Discussion

The observations made at the Deerpark (brown podzolic) site demonstrate how fencing can increase the seedling survival of native broadleaf scrub species birch and holly. This was not the case at Newport, the peatland site where both fenced and unfenced treatments demonstrated a similar amount of dead seedlings although there was a higher rate of dead seedlings attributed to animal browsing than at Deerpark.

The high mortality rate of the holly seedlings at Newport was likely a result of its intolerance to the wet soils, its susceptibility to frost damage and also the probable low concentrations of phosphorous and nitrogen in the soil. The peat soil would be too acidic for the holly seedlings, thus not releasing the required minerals needed for optimum growth (Peterken and Lloyd 1967, Evans 1984, Aro et al. 1997, Ogilvy et al. 2006). The average increase in height of the two remaining holly seedlings in the enclosure was 0.095 m, most likely an adverse reaction to wet soils and probable low nutrient levels (Peterken and Lloyd 1967, Evans 1984).

No evidence of weevil damage was found on the damaged seedlings. The loss of 15 of the original birch seedlings, also inside the enclosure, was most likely a result of exposure and low nutrient uptake from the same acidic peaty soils. Restored open peatland sites (the Newport site was left fallow since clearfell over 14 years ago) differ considerably from forested peatland due to low pH levels, low concentrations of potassium and phosphorous and high nitrogen concentrations (Aro et al. 1997, Aro and Kaunisto 2003). Nutritional problems will be encountered when introducing new seedlings to open peatland sites that cannot be adequately addressed by fertilising as this would also encourage the recovery of the rhododendron present (Aro et al. 1997).

While the success of using native woody scrub species to suppress the re-growth of rhododendron cannot be determined until the scrub species have had a chance to form a closed canopy, possible conclusions can be inferred regarding the recovery to date of the rhododendron plants. In total 161 rhododendron plants were cut to their stump at the beginning of the study, none of the 144 plants at Newport were suppressed at the end of the study, while just four of the 17 plants at Deerpark have so far been sufficiently suppressed by competing vegetation. The majority of the birch and holly seedlings planted inside the fence at the Deerpark site survived and will likely continue to close canopy within the next ten years. The seedlings in the unfenced treatment groups did not fare as well which leaves an opportunity for the rhododendron plants that were cut back to recover and flourish. An investigation into how well rhododendron tolerates shading from native species and closed forest canopies in Ireland would be useful for future research.

The potential for reducing natural regeneration of rhododendron using birch and holly on the exposed peat site in Newport is unfortunately low. The birch and holly seedlings were not successful at the Newport site due to a combination of browsing and abiotic factors both inside and outside the fenced area. The cut rhododendron shrubs recovered nearly three quarters of their original height while 70 new rhododendron seedlings were discovered. As the introduced seedlings have not successfully captured the site to date this trend is likely to continue. It is difficult at this point to envisage the surviving scrub species forming a healthy canopy that may affect the growth of rhododendron. At the outset of this study other scrub species were considered for this site however species mostly likely to grow successfully on this soil type (for example *Calluna vulgaris*) would not be favoured by foresters. This species selection for the Newport site will need to be reviewed if the site is to remain a viable experimental site to test the original hypothesis.

The modern plantation forest in Ireland is required to set aside at least 15% of its land cover to biodiversity enhancement which essentially means that these designated areas are not managed intensively. Should rhododendron establish itself in these areas, it can significantly reducing the biodiversity viability of the immediate area. The effect of the rhododendron spreading throughout the plantation undermines the philosophy of regenerative schemes like the Native Woodland Scheme. These biodiversity areas promote animal movement through forests by creating habitat corridors facilitating the movement of browsing animals through a plantation. This increases the risk of browsing damage to native tree and scrub species, species which may have the ability to outcompete rhododendron. Using fencing would suit a small area such as the aforementioned biodiversity plots found in plantation forests (Becerra and Bustamante 2008). However this plan would not be feasible on large scale landscape control programs not only due to the immense cost of establishing fencing around the site but also the effect such an undertaking would have on non- target species affected by the closing of specific biodiversity corridors. Low levels of grazing are essential for biodiversity conservation in forests (Newman et al. 2014) and planning a sustainable forest will need to balance both functions.

Recognising the threat is critical to successfully managing an invasive species. The best control strategies first begin with the identification of a threshold density of the invader at which the benefit of control equals its costs (Gherardi and Angiolini 2004). A preliminary evaluation of the costs/benefits relationship of a control program and an evaluation of the potential success of such a program can help elevate financial worries landowners may have. These assessments can also be used to demonstrate and justify the funding necessary for such projects. Typical rhododendron control programs are quantified in terms of cost as ‘number of stems to be removed’ or ‘area /scale of infestation’ and control programs are designed around these terms. The problem with this method, especially in plantation forests, is that it is difficult to quantify the full extent an infestation of rhododendron would have on native species and the local ecosystem. It is for this reason that control programs should be designed around the positive response of the management actions to the aforementioned native species and ecosystem and not on size of area treated or number of individuals to be removed.

4.5 Conclusion

Rhododendron coppices readily thus rendering mechanical cutting ineffective and often requires multiple herbicide applications to kill it. The most common method of control is a combination of manual cutting and herbicide application (Edwards 2006, Barron 2008, Maguire et al. 2008). In areas such as Killarney and Snowdonia National Parks it is possible to enlist voluntary help organised by various charities. This unfortunately is not a solution for forest plantations.

Herbicide use, while quite efficient and cost effective, has caused increasing problems that might be seen to outweigh its usefulness (Mc Carthy et al. 2011). These problems include public concern over contamination of water sources and harmful effects to non-target flora and fauna. Also negative effects can arise caused by over application and soil persistence of some herbicides. Herbicide resistance has also been noted in many weed species (Heap 2009). There is particular concern about using chemicals in environmentally sensitive sites. This study assesses fencing to protect native scrub species, which in turn could out-complete invasive rhododendron, as opposed to mechanical and chemical eradication of rhododendron plants. If successful a similar control program would limit if not remove the need to use chemical herbicides.

The observations made over the four years of the experiment (2010- 2013) demonstrates a negative effect that animal browsing has on the establishment and growth of birch and holly. These native scrub species, protected from animal browsing and once they close canopy may have a better opportunity to prevent rhododendron re-growth and invasion. This is the case for Deerpark, the brown podzolic site but unfortunately, to date, the experiment was inconclusive for Newport, the peatland site. While the current experiment, to assess the ability of fencing to protect and encourage native scrub species to out-complete rhododendron, was monitored for four years it was constructed so that it can easily become a long-term study from which data can be collected for many years to come. The dead seedlings at both sites will be replaced in the autumn of 2014 and at the conclusion of the second phase of the study (expected when the native species close canopy) it should be clearer if preventing animal browsing does increase native tree and scrub/vegetation species and can this species successfully outperform rhododendron?

5. Overall Conclusions and Recommendations

Rhododendron ponticum L. was first introduced to Ireland during the 19th century as an ornamental garden plant and has since become an established invasive species throughout country (Cross 1975, Gritten 1995, Dehnen-Schmutz and Williamson 2006, Barron 2008, Maguire et al. 2008). As a successful invasive alien species rhododendron out-competes native plants and poses a serious threat to native biodiversity in Ireland, particularly to our native woodlands and peatlands. Rhododendron has also, in recent decades, become a significant management issue in plantation forests in Ireland (Harris et al. 2009, Cullen 2011). Rhododendron negatively impacts both the economic and the ecological value of invaded land and its control is essential to the conservation of native communities (Manchester and Bullock 2000, Kelly 2007, Parrott 2013). Best practice for rhododendron management relies on an understanding of its population biology and community ecology (Peterken 2001, Dehnen-Schmutz and Williamson 2006).

In Ireland for over the past 50 years rhododendron has been studied extensively in an effort to find a method of controlling it effectively and economically (Dehnen-Schmutz et al. 2004, Kelly 2007, Maguire et al. 2008, Baars 2011). Many researchers have toiled with different aspects of the plant's biology to try and find some weakness that could be exploited in a way to enable land managers to control it in forest habitats (Brown 1953, Cox and Hutchinson 1963, Robinson 1980, Edwards et al. 2000, Esen 2000, Wong et al. 2002, Evans 2003, Dehnen-

Schmutz et al. 2004, Tyler et al. 2006, Higgins 2008, Erfmeier and Bruelheide 2010, Parrott 2013). This project set out to further enhance our knowledge of this invasive weed species by investigating some areas that were never previously studied in Ireland. In this context the study set out to improve our understanding of the auto-ecology and invasion dynamics of rhododendron in Irish forests and to investigate control options to inform rhododendron management plans.

The first part of this thesis (**Chapter 2**), which involved studying the bio-control of rhododendron using an indigenous fungus *Chondrostereum purpureum*, was both novel and a first in an effort to control the species in Ireland using a bio-herbicide. Traditional management of rhododendron in forests in Ireland is based on manual control using cutting and/or chemical herbicides (Barron 2008, Maguire et al. 2008). However the implementation of strict EU and Forest Stewardship Council certification regulations requires the exploration of alternative, ecologically sound, control methods (Forest Service 2000). One potentially suitable control method is biological control, which is based on the use of indigenous organisms to control the spread of rhododendron. Using this method, a suitable pathogen such as a fungus, is mass produced and applied to target vegetation with the goal of repressing the target species. *C. purpureum* is a fungal pathogen with the potential to repress or eliminate problematic broadleaved trees species such as rhododendron, and is currently available commercially for this purpose in other countries including Finland and Lithuania (Vartiamäki et al. 2009, Lygis et al. 2012). No studies on the potential for biological control of rhododendron using native pathogenic fungi have been conducted in Ireland to date, and so we tested the usefulness of an Irish isolate of *C. purpureum* for rhododendron control. An extensive survey of woodlands and orchards was undertaken and an Irish isolate of *C. purpureum* was located on a plum tree (*Prunus domestica*) at a commercial orchard in Co. Tipperary in 2009. Following molecular identification and rigorous laboratory testing an experiment was set up to test its usefulness in the prevention of re-growth of cut stems of both rhododendron and birch (*Betula pendula*). The efficacy of both summer and autumn applications was assessed for one year following application using four parameters: Presence/absence of fungal fruiting bodies; Stump survival; Number of living shoots on surviving stumps; and Height of the tallest shoot on surviving stumps. The results of the study demonstrated that a combination of mechanical cutting and the subsequent application of *C. purpureum* is not an effective method of vegetation management for either rhododendron or birch. Stumps treated with the fungal pathogen in this study displayed fewer shoots per stump than those left untreated, but further treatment would be required to completely eradicate the invasive shrub. Although the results were not conclusive the fungus did show some control effects by reducing re-growth, though it was still not as effective as the traditional herbicide against which it was compared. Fruiting bodies may occur over a 4-7 year period, therefore continued monitoring of the trial sites for future occurrences of fruiting bodies is recommended (Wall 1990).

This study was limited to testing just one strain of the fungus due to difficulties in sourcing different strains and thus further comparable studies with different Irish isolates are necessary to completely rule out any future prospects of *C. purpureum* becoming part of a forest vegetation management tool in Ireland. Success in other countries with *C. purpureum* on woody vegetation has demonstrated the potential of this fungus as a bio-control agent (Canada has even produced a commercial product) (Vartiamäki et al. 2009, Lygis et al. 2012). As only one isolate was found and tested in this study further research therefore should be undertaken to find, identify and test the virulence and efficacy of other Irish isolates of *C. purpureum*. Molecular comparisons should be carried out of European isolates of the fungus with the Irish isolates to identify any differences. If none are found then maybe some of the European isolates should be tested under Irish conditions. This would have to include rigorous testing on non-target species as well.

Forest floor litter negatively impacts the germination success of many plant species, and both the amount and distribution of forest litter can influence the establishment of invasive alien species (Hobbs and Huenneke 1992, Mooney and Cleland 2001). Many land management practices, particularly in forestry, disturb ground cover, leading to soil exposure, which may increase the risk of rhododendron invasion. The effect of forest floor litter on the germination of rhododendron seeds was measured by assessing germination rates in five different litter types: Bare soil; 1 cm broadleaved litter; 3 cm broadleaved litter; 5 cm broadleaved litter; and 2 cm conifer litter (**Chapter 3**). The results showed a clear relationship between litter type and seed germination success with seed having poorer success rates in deeper forest litter. This study demonstrated that even small decreases in forest litter depth, sufficient to expose bare soil, facilitates rhododendron seedling establishment.

Previous research also found that rhododendron produced a prolific amount of seed, however this seed only remained viable for approximately 160 days (Mejías et al. 2002). As rhododendron seeds need adequate access to bare soil (or soils covered in bryophyte carpets), land disturbance plays a significant factor in the spread and recruitment of this invasive species (Cross 1981). This finding has important implications for the management of litter, brash, and soil exposure in the management plans of Irish forests along with a recommendation to include rhododendron control as an annual cost in future forest planning. Rhododendron seed physiology makes the species an ideal candidate for a novel control program involving forest litter/brash depth after harvesting operations. This control program could be integrated into improved management practices in areas where rhododendron is a problem.

The spread of rhododendron is negatively related to herbivore grazing and its successful establishment is a result of un-palatability to herbivore animals. This has potential implications for the control of rhododendron in Ireland and this study (**Chapter 4**) set out to investigate whether the prevention of grazing in woodland sites could be used to prevent the spread of rhododendron and test the hypothesis that the native vegetation normally grazed by herbivores could out-compete rhododendron in the absence of grazers. Study sites were manually cleared of all rhododendron, and fencing was used to exclude grazers from half of the cleared sites. Within fenced and unfenced study sites we used control areas where no seedlings were introduced, areas planted with birch seedlings, areas planted with holly seedlings and areas planted with a birch/holly mix in order to compare the re-invasion rates of rhododendron in each of the treatment sites. The findings suggest that in some of the plots the holly and holly/ birch mix are successfully suppressing the re-growth of rhododendron. It is also clear that without fencing to exclude herbivores the holly and birch seedlings have little chance of survival and are being out-competed by rhododendron which, following this initial phase of the study will potentially spread uninhibited and free from competition. Although the data obtained from this study may be largely qualitative in its current format, we expect that the results obtained during the current study will form part of a long-term monitoring study, the first of its kind, thus forming part of a robust dataset to which value will continue to be added in the future.

Therefore it would be prudent to undertake measures to reduce grazing pressures and allow existing vegetation to grow unchecked to minimise the invasion potential of the rhododendron. This will be especially relevant in woodland areas where natural regeneration is preferred and also in ensuring the success of Continuous Cover Forestry (CCF) in the future. Using fencing as a means to prevent grazing would suit a small area such as the biodiversity plots found in plantation forests. This control process would not work on a large scale due to the immense cost of establishing fencing around the site. In addition there is the question about the effect such an undertaking would have on non-target species affected by the closing of specific biodiversity corridors.

This thesis presents research which will inform control and management practices for rhododendron in Irish forests. Improved management and control practices are required in areas where rhododendron has become a problem, and prevention is required in areas where it is a potential future problem. Rhododendron seed viability is poor and can potentially be exploited in its control, which merits further research. In areas identified as being sensitive to invasion by rhododendron, forestry operations should be limited during the optimum time for rhododendron seed dispersal (December to February). Forestry operations should aim to reduce disturbance of the litter layer in order to minimise the amount of bare soil exposed. Potential for the use of bio-control agents in the control of rhododendron should be fully investigated using a wide range of native Irish isolates of *C. purpureum*. Finally, in sensitive areas measures should be undertaken to protect existing flora from grazing pressures in order to allow native vegetation to grow unchecked and enable it to better compete with the invasive rhododendron.

Rhododendron and the ecological and economic problems that it causes, should serve as a warning to us of the importance of prevention of invasions of alien species in the future. Control measures are currently not implemented until a species has become a problem, but prevention, where possible, is favourable to controlling these species after they have become established. In the short term some areas of current forest management practices require attention, including the productive areas of the forest, the biodiversity areas, riparian buffer zone management and biodiversity corridors. While the importance of each of these for the maintenance of biodiversity is recognised, they also provide optimal corridors for invasive species and therefore management guidelines must recognise this and incorporate these considerations if mitigation is to be successful. Specifically the following guidelines should be integrated into a typical forest management plan:

a. Identification and mapping of rhododendron infested areas in the forest area.

b. Rhododendron (and any other invasive weed species) control should be included as an annual cost and included in the forest management plans of each forest.

c. Minimise as much as possible operations in and around these areas during the optimum time for rhododendron seed dispersal i.e. December-February in an attempt to:

    i. Minimise disturbance of the litter layer and

    ii. Reduce the amount of bare soil exposed

d. Aim to control or remove the rhododendron invasion sources prior to carrying out harvesting operations in nearby compartments.

In conclusion rhododendron has become a huge problem in plantation forest habitats and in order to control it effectively and economically an integrated pest management strategy will have to be employed utilising a mixture of novel bio-controls, innovative management strategies exploiting weaknesses such as seed longevity and viability, litter depth, and ensuring that other plant species can compete free of grazing pressures. The establishment of rhododendron in an area is often very difficult to reverse. The choice for the landowner is a pre-emptive one; prevention rather than cure. Better awareness and legislation coupled with defined identification and prevention tools will help to prevent new invasions.

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