UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Biological Sciences

The mechanisms used by the invasive shrub *Rhododendron ponticum* to inhibit the growth of surrounding vegetation

by

Benjamin Davis

Thesis for the degree of Doctor of Philosophy

August 2013

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Biological Sciences

Doctor of Philosophy

THE MECHANISMS USED BY THE INVASIVE SHRUB *RHODODENDRON PONTICUM* TO INHIBIT THE GROWTH OF SURROUNDING VEGETATION

by Benjamin Davis

In the United Kingdom, *Rhododendron ponticum* is one of our most invasive plant species, and yet there have been few published scientific studies compared with many other invasive species. Changes in environmental conditions are often implicated as being responsible for its impact on the native vegetation, and this study demonstrated that light availability, temperature, water availability, organic matter and soil pH were all different beneath stands of *R. ponticum*, compared to areas of open grassland where growth of the native species was not limited. Studies in the New Forest highlighted that light availability and soil pH were the two environmental conditions most likely to explain the impact of *R. ponticum*. However, glasshouse experiments testing the effect of these changes on the germination and growth of two native species, *Lolium perenne* (perennial rye grass) and *Trifolium repens* (white clover), revealed that the low light conditions only reduced the root elongation and leaf appearance of *T. repens*, and the soil pH had no inhibitory effect on either species. *R. ponticum* was also shown to release allelopathic compounds into the soil. However, on their own these compounds had no inhibitory effect on the germination or growth of *L. perenne*, and germination and leaf appearance of *T. repens* were reduced by less than 60%, indicating that other factors are involved in the inhibition of growth. Light and nutrient stress were shown to increase the susceptibility of the test species to allelopathic compounds, and the light and pH conditions found in uninvaded woodland in the New Forest increased the synthesis and accumulation of allelopathic compounds in the soil beneath the rhododendron. These findings demonstrate the importance of pre-existing conditions and the presence of other species in the success of invasive species, and that the inhibition of growth of the native species is due to a complex combination of biotic and abiotic factors.

Contents

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of Figures

List of Tables

Declaration of Authorship

I, **Benjamin Davis**, declare that the thesis entitled '**The mechanisms used by the invasive shrub *Rhododendron ponticum* to inhibit the growth of surrounding vegetation**' and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission.

Signed: ……

Date: ……

Acknowledgements

There are a number of people without whom this study would not have been possible. Firstly, I must thank my supervisors Professor Guy Poppy and Dr. Malcolm Hudson for the help and guidance they have provided throughout this study. I would like to thank Dr. Patrick Doncaster for his statistics advice, and also Pilar, Dave and Mike for all their help in the lab and glasshouse. I would like to thank the staff at Minstead Lodge for allowing me to use their grounds, and Simon Weymouth at the Forestry Commission for providing maps and information about study sites in the New Forest. I am particularly grateful to Dr. Phil Stevenson at Kew Gardens for performing the chemical analysis of the leaf samples.

I would like to offer my special thanks to everyone at Exbury Gardens, particularly John Anderson and Lucy Cartledge, for not only allowing me to use the gardens and glasshouse facilities, but also for providing me with compost and pots, for looking after all my plants, for letting me use the golf buggy for field work, and for all the other help that they have provided. Finally, I wish to thank Kate Halliwell, for funding this PhD and for all the proof reading that she has done, and I would like to thank my friends, particularly Amelia, for all the support and encouragement.

Chapter 1

General introduction

1.1 Invasive species

Invasive species are those species living outside their native range, which have an adverse effect on the habitats they invade, either economically or environmentally (Global Invasive Species Database, 2013). It is non-native plants that can have the biggest impact on habitats and economies, and many have been shown to reduce the diversity of native species (Shaw & Tanner, 2008). A number of studies have shown that invasive plant species can affect environmental conditions, either through resource competition, the alteration of ecosystem processes or allelopathy (i.e. any direct or indirect harmful effect by one plant on another through the production of chemical compounds (Singh *et al*., 2001)), and these changes are often implicated as the cause for the inhibition of native plant growth (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). However, few studies have thoroughly tested the effect of these changes on plant growth, and whether they alone could account for the change in ecosystem structure and function (Levine *et al*., 2003). In the United Kingdom *Rhododendron ponticum* is one of the most rapidly expanding invasive plant species (DAISIE, 2012), and yet this is the first study to investigate the influence of a range of environmental factors on the growth of native species beneath *R. ponticum*, and specifically, whether the combination of environmental stresses and allelopathy is sufficient to explain the inhibition of growth.

Non-native species now occur in almost every inhabited part of the world (Global Invasive Species Database, 2013). Not all introduced species cause problems, and most are beneficial, such as the wide variety of imported plant species which grow in our gardens and on agricultural land (Pimentel *et al*., 2001; Shaw & Tanner, 2008). However, when an uncontrollable species does invade, the effect can be devastating. These invasive species can become very abundant in their introduced range, causing serious problems and costing billions of pounds in control and eradication programs across the world (Pimentel *et al*., 2001; Bossdorf *et al*., 2005; De Bruxelles, 2010). They are known to cause considerable costs to the economy, with impacts ranging from loss of crops and building damage, to the loss of livelihoods and ecosystem services, as well as affecting industries, including agriculture, forestry, construction and tourism (CABI, 2012). They can also have a significant environmental impact. They are considered the second greatest threat to biodiversity after habitat loss, and

are a major cause of global change (De Bruxelles, 2010; Pyšek & Richardson, 2010). Although invasive species are not a new phenomenon (Fry & Goodwin, 1997), climate change and the expansion of world trade have led to a rapid increase in numbers, and Europe is currently home to over 10,000 invasive alien species (Shaw & Tanner, 2008; DAISIE, 2012).

It is non-native plants that can have the biggest impact on habitats and economies, accounting for a third of the economic costs of invasive alien species worldwide (Shaw & Tanner, 2008). They can out-compete the native species, forming dense monocultures and changing the structure and functioning of native communities and ecosystems (Bossdorf *et al*., 2005). What makes these invasive species such a serious problem is their ability to rapidly invade large areas (Pimentel *et al*., 2005), and the spread rates of some invasive plants are very impressive (Shaw & Tanner, 2008). Since its introduction into Florida in 1906, *Melaleuca quinquenervia* (broad-leaved paper bark) has displaced over 202,000 hectares of the native vegetation (Turner *et al*., 1997), and *Lythrum salicaria* (purple loosestrife) has been spreading at a rate of 115,000 hectares per year since it was introduced into North America in the early 19th century (Pimentel *et al*., 2005). The most common method that these species use to invade areas is with seed, and many invasive plants produce large numbers of seeds, which are easily dispersed (Andersen & Calov, 1996; Rayamajhi *et al*., 2002; Stout *et al*., 2006).

Previous work has also found that in general there is a reduction in the diversity of specialist natural enemies in the introduced range compared with the native range, and this 'enemy release hypothesis' is one of the most cited reasons why invasive species are so successful (Keane & Crawley, 2002; Callaway & Ridenour, 2004). It is thought that as the plants are no longer under the pressure of these natural enemies, they are able to evolve higher rates of growth, germination and seedling recruitment than the native species, allowing them to have a much greater impact on the structure and function of native ecosystems (Callaway & Ridenour, 2004; Erfmeier & Bruelheide, 2004). Hybridization and genetic mutation allows these species to adapt to their new environment and to tolerate conditions which other species might not be adapted to survive (Milne & Abbott, 2000; Prentis *et al*., 2008), and as most of the expensive defensive chemicals that the plant produces in its native habitat are no longer needed, natural selection should favour less defended but more competitive

genotypes (Callaway & Ridenour, 2004; Bossdorf *et al*., 2005). However, recent work has shown that the non-native species are often no less affected by enemies than native species in the invaded community (Colautti *et al*., 2004). Although the loss of their natural enemies might allow them to become more successful than in their native range, it is likely that is is a complex combination of processes that underlie biological invasions and further work is needed to identify the relative importance of this in the success of the species (Colautti *et al*., 2004).

Controlling the spread of invasive species is difficult as there are so many of them (DAISIE, 2012). Often a combination of manual, mechanical and herbicidal controls are required to attempt to manage the species (DAISIE, 2012). However, due to the number of seeds produced, and their vigorous growth, any disturbance caused by their removal quickly results in reinvasion (Lozon & MacIsaac, 1997). The environmental hazards also have to be taken into account. *Heracleum mantegazzianum* (giant hogweed) produces phytotoxic sap, containing photosensitizing furanocoumarins, which in contact with human skin and combined with UV radiation causes dermatitis (Thiele & Otte, 2007). Therefore, the danger to human health makes eradication efforts very difficult.

It is possible that re-associating co-evolved natural enemies with the plant species might provide some level of control, and it is this principle that is the basis for biological control research (Shaw & Tanner, 2008). However, identifying a suitable enemy for biological control is difficult. It involves identifying the very few natural enemies in the native range of the plant that are able to provide permanent weed suppression in a single release without further interference or cost (Shaw & Tanner, 2008). Despite being extremely active in biological control research, the release of *Aphalara itadori* (Japanese knotweed psyllid) into the UK in 2010 and *Stenopelmus rufinasus* (Azolla weevil) in 2012 were the only times a biological control approach has been used in Europe against an invasive plant (Shaw *et al*., 2011; CABI, 2013).

The effect of invasive plant species on community structure is well studied. Many invasive plant species have been shown to reduce the diversity of native species, and there has been a substantial amount of work investigating the ecological mechanisms responsible for this impact (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Many invasive plant species affect environmental conditions, either

through resource competition, the alteration of ecosystem processes or allelopathy, and these changes are often implicated as the cause for the inhibition of native plant growth (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003; Dukes & Mooney, 2004). However, a review of over 150 papers studying the mechanisms underlying the impacts of exotic plant invasions found that less than 5% of the studies actually tested the effect of these changes on plant growth, and whether they alone could account for the change in ecosystem structure and function (Levine *et al*., 2003). Furthermore, a study by Gliessman and Muller (1978) into the allelopathic mechanisms of dominance in bracken (*Pteridium aquilinum*) in Southern California is still considered to be one of the most thorough studies of an invasive plant, and although they assessed the contribution of various environmental conditions on the establishment and maintenance of bracken dominance, they did not consider the interactions between them (Gliessman & Muller, 1978). Understanding these processes is essential in order to successfully control the spread of invasive species, and we need to know which processes have to be overcome if native species are to re-establish in invaded ecosystems (Levine *et al*., 2003). However, this is difficult as they do not act independently, and there are often interactions between them (Matlack, 1993; Stark & Firestone, 1995; Yan *et al*., 1996; Ste-Marie & Paré, 1999).

1.2 Effect on environmental conditions

Competition for light has been implicated in a number of studies as the key factor responsible for the impact of invasive plants (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Light availability was significantly reduced beneath stands of *Pinus nigra* (European black pine), *Mimosa pigra* (giant sensitive tree) and *Lonicera tatarica* (Tatarian honeysuckle), compared to native stands, and it was suggested that light limitation was responsible for the inhibition of native plant growth (Braithwaite *et al*., 1989; Woods, 1993; Leege & Murphy, 2001), and *Lonicera maackii* (Amur honeysuckle) was shown to have the greatest impact on the survival of shade-intolerant annuals, suggesting that the reduced light environments created by the *Lonicera maackii* canopy also played an important role in the lower survival of the native species (Gould & Gorchov, 2000).

Despite these findings that some invasive non-native species can reduce light to levels that can suppress native species, very few studies have tested this experimentally (Gorchov & Trisel, 2003). Reinhart *et al*. (2006) showed that stands of *Acer platanoides* (Norway maple) reduced both the light quantity and light quality reaching the forest floor. They went on to show that light conditions representative of the understory of *Acer platanoides* invaded forest decreased the survival of five of the seven species tested, relative to seedlings growing in light conditions similar to that of the native understory, suggesting that light quantity drives native suppression, although they did not test the importance of this mechanism relative to other mechanisms.

Studies have indicated that changes in vegetation types can alter local or regional climatic patterns (Hoffman & Jackson, 2000), and many invasive species, such as *Laretia acaulis* and *Azorella monantha* (cushion plants) and *Ammophila arenaria* (European marram grass), will alter the microclimate beneath them (Dukes & Mooney, 2004; Cavieres *et al*., 2007). Changes in vegetation can also affect water availability, and this is often the key factor responsible for the reduced diversity of native plants in invaded areas (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Several species, including *Argopyron spicatum* (bluebunch wheatgrass), *Carpobrotus edulis* (iceplant) and *Artemisia tridentate* (sagebrush), alter water use, either by differences in evapotranspiration, due to differences in the time when the plants are photosynthetically active, or by differences in root depth (Cline *et al*., 1977; D'Antonio & Mahall, 1991). Invasive plants can also reduce the amount of rainfall reaching the ground, which is the case with *Orbea variegate* (African carrion flower) (Dunbar & Facelli, 1999), and a number of species have been shown to alter the organic matter input into an ecosystem (Hobbie, 1992; Ehrenfeld, 2003), which will affect the water holding capacity of the soil (Hudson, 1994).

Many non-native plants have been shown to affect nutrient availability (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Several invasive species, including *Myrica faya* (fire tree) and *Berberis thunbergii* (Japanese barberry), produce highly decomposable leaf litter, which alters ecosystem nutrient cycling (Allison & Vitousek, 1998; Ehrenfeld *et al*., 2001), and changes in water availability will affect nutrient availability, either through uptake, or due to leaching of basic nutrients. Changes in temperature, water availability, soil pH and nutrient availability

can all affect the activity of soil organisms (Killham, 1994), which will in turn affect nutrient cycling (Ste-Marie & Paré, 1999), particularly nitrification and decomposition (Stark & Firestone, 1995). Plants can also directly alter the nutrient retention of ecosystems by affecting erosion of nutrient-rich topsoil, or by sequestering available soil nutrients, thus reducing leaching losses (Gholz *et al*., 1985). The effect of invasive species on the nitrogen cycle is the most widely studied (Levine *et al*., 2003). Nitrogen-fixing invaders, such as *Acacia* species and *Myrica faya*, can increase the rate of nitrogen input to a system (Vitousek & Walker, 1989; Stock *et al*., 1995), while other plant invaders, including *Populus balsamifera* (balsam poplar), *Andropogon virginicus* (broomsedge bluestem) and *Carduus nutans* (musk thistle), might decrease nitrogen input by leaching chemicals that reduce the ability of other species to fix nitrogen (Rice, 1992; Wardle *et al*., 1994).

Many invasive species also have an effect on the pH of the soil. They can do this indirectly, by altering microclimate, water availability, organic matter or nutrient cycling (Stark & Firestone, 1995; Yan *et al*., 1996; Hagedorn *et al*., 1997). A number of invasive species have also been shown to have a direct effect on the soil pH. *Berberis thunbergii* and *Microstegium vimineum* (Japanese stiltgrass) can change the pH of the soil through the uptake of compounds (Nye, 1981; Ehrenfeld *et al*., 2001), while *Salsola tragus* (Russian thistle) releases compounds into the soil (Cannon *et al*., 1995). This includes oxalic acid, which is able to solubilise unavailable soil phosphorus, making it available for uptake, and increasing the growth of the native species (Cannon *et al*., 1995).

Although all these changes can be very important on the native species, individually they are often not sufficient to explain the inhibition of growth (Catovsky & Bazzaz, 2000; Lei *et al*., 2006). However, there are interactions between environmental factors, and multiple environmental stresses will often have a much greater impact together, than individually (Mittler, 2006). Elevated CO_2 and elevated precipitation have been found to increase the effect of temperature on invasion of California grassland by woody species, and elevated CO_2, increased temperature, precipitation, and nitrogen deposition were found to have a much greater effect on forb diversity in California annual grasslands when in combination than individually (Zavaleta *et al*., 2003). Water stress was shown to have a much greater effect on photosynthesis in *Nerium oleander* (Mediterranean rose bay) when grown in full sun (Björkman &

Powles, 1984), and water stress was also shown to increase the effect of high light intensity and decrease the effect of heat stress on photosynthesis in *Solanum nigrum* (European black nightshade), *Solanum tuberosum* (potato) and *Solanum lycopersicum* (tomato) (Havaux, 1992). Low nutrient availability has also been found to reduce the survival of *Viburnum opulus* (guelder rose) and *Betula* spp (birch), but only in low light intensities (Grubb *et al*., 1996; Catovsky & Bazzaz, 2000), and the effect of water availability on the germination and survival of both *Quercus michauxii* (swamp chestnut oak) and *Liquidambar styraciflua* (sweetgum) was shown to be different, depending on the light availability (Battaglia *et al*., 2000).

1.3 Allelopathy

Many invasive species have also been shown to exert an allelopathic effect on the surrounding vegetation (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Allelopathy refers to any direct or indirect harmful effect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment (Singh *et al*., 2001). The main point involving allelopathy is that its effect depends upon a chemical compound being released into the environment by an allelopathic agent, and differs from resource competition, which involves the removal or reduction of some factor from the environment that is needed by some plants sharing the same habitat (Rice, 1974). These compounds are selectively toxic, affecting some species but not others (Chase *et al*., 1991), and can persist in the soil, affecting neighbouring plants, as well as those planted in succession (Souto *et al*., 1994). Recent studies investigating allelopathy have focused on the possibility of using these compounds as natural weed killers (Macías *et al*., 2001; Bhadoria, 2011) as a way of minimizing the dependency on synthetic herbicides, which are becoming a worldwide problem, not only as they create environmental pollution, but also due to the increasing number of herbicide resistant weeds (Bhadoria, 2011).

More than thirty different classes of primary and secondary compounds have been identified as allelopathic compounds (Singh *et al*., 2001). Although several primary compounds have been shown to have an allelopathic effect (Elmore, 1980), secondary compounds are far more common (Singh *et al*., 2001). These compounds

usually belong to one of three large chemical classes; terpenes, phenols and alkaloids (Singh *et al*., 2001). Terpenes occur in all plants, and a number of monoterpenes (Schulz *et al*., 2007), sesquiterpenes (Abdelgaleil & Hashinaga, 2007), and triterpenes (Macías *et al*., 1994) have been shown to have an allelopathic effect. Phenols are another large class of secondary metabolites, and several flavonoids (Fiorentino *et al*., 2008), tannins (Salminen *et al*., 2004), courmarins, such as scopolin (Razavi, 2011), and cinnamic acid and derivatives, including caffeic acid, chlorogenic acid, ferulic acid, and p-coumaric acid (Patterson, 1981) have all been found to be involved in allelopathy. Alkaloids are nitrogenous compounds that are found in many vascular plants, and a number of alkaloids, including caffeine (Isfahan & Shariati, 2007), nicotine (Rizvi *et al*., 1989), and mustard oil glycosides (Bialy *et al*., 1990) have also been shown to have an allelopathic effect.

Leaves are the most common source of allelopathic compounds, and many species, including *Brassica nigra* (black mustard), *Carex distachya* (sedge) and *Helianthus annuus* (sunflower), have been found to contain high concentrations of toxins in their leaves (Turk & Tawaha, 2003; Ashrafi *et al*., 2008; Fiorentino *et al*., 2008). These are often involved in protecting the plant against herbivores and pathogens (Wink, 1988). However, they can also be involved in pigmentation, UV protection, plant development and signaling (Gould & Lister, 2006), and roots, stems and flowers have also been shown to contain inhibitors (Carballeira, 1980; Turk & Tawaha, 2003), and although they are not so widely studied, so have many fruits (Donnelly *et al*., 2008) and seeds (Friedman & Waller, 1983).

Allelopathic compounds can be released into the environment in a number of ways. Volatiles from *Eucalyptus globulus* (Tasmanian blue gum), *Eucalyptus citriodora* (lemon-scented gum) (Kohli & Singh, 1991) and *Quercus ilex* (holm oak) (Bertin & Staudt, 1996), leachates from *Vaccinium myrtillus* (common bilberry) (Gallet, 1994), *Cymbopogon citratus* (lemon grass) (Fujii *et al*., 2004) and *Eupatorium adenophorum* (sticky snakeroot) (Zhao *et al*., 2009), and root exudates from *Juglans nigra* (black walnut) (Bertin *et al*., 2003) and *Oryza sativa* (rice) (Kato-Noguchi *et al*., 2002) have all been shown to have an allelopathic effect on the surrounding vegetation. The compounds can also be released during residual decomposition, although this is very difficult to prove (Singh *et al*., 2001). It is possible that microorganisms are changing non toxic compounds to toxic ones, or synthesising

inhibitors (Singh *et al*., 2001), and water soluble inhibitors should easily leach out of plant residues after death when the various membranes generally lose their differential permeability (Yamamoto, 2009). There are however many potent inhibitors which are only slightly soluble in water, such as those found in *Medicago sativa* (alfalfa), *Piper methysticum* (kava) (Xuan *et al*., 2005), *Helianthus annuus* (Wilson & Rice, 1968), *Eucalyptus globulus* and *Acacia melanoxylon* (Australian blackwood) (Souto *et al*., 1994), and these are only released into the soil by decomposition (Singh *et al*., 2001).

Allelopathy can affect many aspects of plant ecology, including growth and productivity, as well as species diversity and the structure of plant communities (Muller, 1966; Einhellig, 1994). There is no common mode of action or physiological target site for allelochemicals (Inderjit & Duke, 2003). They can affect photosynthesis (Mersie & Singh, 1993), respiration (Kohli *et al*., 1998) and transpiration (Schulz *et al*., 2007), as well as inhibiting cell division (Nishida *et al*., 2005), and DNA and protein synthesis (Mersie & Singh, 1993; Nishida *et al*., 2005). They can also have an effect on enzyme activity (Friebe *et al*., 1997), membrane permeability and nutrient uptake (Baziramakenga *et al*., 1997; Wink *et al*., 1998), metabolism of food reserves (Levitt *et al*., 1984) and plant defence (Glinwood *et al*., 2003). However, commonly cited effects of allelopathy include reduced seed germination and seedling growth (Angiras *et al*., 1988). This means that allelopathy can be extremely important on the success of non-native species, helping to explain why some exotic species are so successful at invading natural plant communities (Callaway & Aschehoug, 2000; Bais *et al*., 2003).

1.4 Environmental conditions and allelopathy

Although allelopathy is commonly implicated as being responsible for the impact of invasive plants, (see 1.3), on its own it is often unable to explain the inhibition of growth of the native species (Gliessman & Muller, 1978; Inderjit & Del Moral, 1997). Sixteen plant species common in western Washington, including *Acer macrophyllum* (bigleaf maple), *Cornus nuttallii* (Pacific dogwood), *Sambucus racemosa* (red elderberry) and *Sorbus sitchensis* (Sitka mountain-ash) were shown to have an inhibitory effect on *Hordeum vulgare* (barley), *Bromus tectorum* (drooping

brome) and *Pseudotsuga menziesii* (Douglas-fir) under laboratory conditions, but this effect was not observed in the field (Del Moral & Cates, 1971). Reducing root competition was also found to reduce the allelopathic effect of *Empetrum hermaphroditum* (mountain crowberry) on *Pinus sylvestris* (Scots pine) (Nilsson, 1994), and although *Centaurea maculosa* (spotted knapweed) was shown to have an allelopathic effect on *Festuca idahoensis* (Idaho fescue), *Centaurea maculosa* outperformed *Festuca idahoensis* even in the presence of activated carbon, demonstrating the importance of the combined roles of resource competition and allelopathy (Ridenour & Callaway, 2001). Many invasive plant species also affect environmental conditions, either through resource competition or the alteration of ecosystem processes (Levine *et al*., 2003), and plants of the same species growing close together have been found to vary greatly in their allelopathic effects, suggesting that differences in stress conditions play an important role in determining the amounts of inhibitor produced by a plant, as well as affecting the the sensitivity of the plant to various allelopathic compounds (Singh *et al*., 2001).

Light availability is often found to be altered beneath invasive species (see 1.2), and this can be extremely important in affecting allelopathic weed suppression. Visible light has been found to increase the concentration of allelopathic compounds in *Zea mays* (maize) (Kato-Noguchi, 1999), *Liriodendron tulipifera* (tulip poplar), *Cornus florida* (dogwood) (Dudt & Shure, 1994), *Potamogeton amplifolius* (largeleaf pondweed) (Cronin & Lodge, 2003) and *Dittrichia viscose* (false yellowhead) (Karageorgou *et al*., 2002), and decrease the concentration of allelopathic compounds in *Zingiber officinale* (Malaysian ginger) (Ghasemzadeh *et al*., 2010) and *Prymnesium parvum* (Granéli & Salomon, 2010). Ultraviolet radiation has been shown to induce the synthesis of a number of phenolic compounds (Daniel *et al*., 1999), and the concentration of allelopathic compounds in *Betula pendula* (silver birch) (Tegelberg *et al*., 2004) and *Flavocetraria nivalis* (Bjerke *et al*., 2002) increased when exposed to UV radiation. The red: far-red ratio has been shown to affect the concentrations of phenols in the leaves of *Betula pendula* (Tegelberg *et al*., 2004) and *Brassica napus* (rapeseed) (Gerhardt *et al*., 2008), X-radiation has been found to increase the scopolin concentrations in the leaves of *Nicotiana tabacum* (tobacco) (Koeppe *et al*., 1970), and concentrations of furanocoumarin in seeds of *Psoralea corylifolia* (babchi) were increased by gamma irradiation (Jan *et al*., 2012). Day length has also been found to be important, and the rate of root exudation by

Cucumis sativus (Japanese cucumber) increased with the elongation of photoperiod (Pramanik *et al*., 2000).

Under light-limited growth conditions, some species have also been shown to be more susceptible to allelopathic compounds, particularly those that interfere with photosynthesis (Von Elert & Jüttner, 1996), and inhibitory effects of allelochemicals of *Vicia villosa* (hairy vetch) increased significantly under shading conditions (Teasdale, 1993). However, the effect of light conditions on the sensitivity of *Desmodesmus quadrispina*, *Nitzschia palea*, and *Nostoc* PCC 7120 to compounds produced by *Desmodesmus quadrispina*, *Monoraphidium dybowski*, or *Uronema confervicolum* varied among target species (Leflaive & Ten-Hage, 2009), and *Cistus ladanifer* (gum rockrose) exudates were shown to demonstrate the greatest phytotoxicity in inhibiting both germination and seedling development under long photoperiods (Lobón *et al*., 2002).

Temperature can also be very important, and the concentration of toxins in *Vaccinium myrtillus* (Laine & Henttonen, 1987) and *Cucumis sativus* (Pramanik *et al*., 2000) increased with the elevation of temperature. Toxin production has also been found to increase dramatically in temperature stressed *Prymnesium parvum* cells (Granéli & Salomon, 2010), and chilling has been found to affect the concentrations of chlorogenic acid and scopolin in the tissues of *Nicotiana tabacum* (Koeppe *et al*., 1970). The inhibition threshold concentration for ferulic acid to affect seedling growth of *Glycine max* (soybeans) and *Sorghum bicolor* (sorghum) was also found to be reduced by growth temperatures at the higher end of the normal range for the species (Einhellig & Eckrich, 1984; Einhellig, 1996), and the inhibitory effects of allelochemicals of *Cistus ladanifer* were found to increase significantly under unfavourable temperature conditions (Lobón *et al*., 2002).

Moisture availability has also been shown to affect the concentration of allelopathic compounds, and drought stress increased the concentrations of alkaloids in *Papaver somniferum* (opium poppy) (Szabó *et al*., 2003) and phenolics in *Echinacea purpurea* (purple coneflower) (Gray *et al*., 2003a), *Cyperus rotundas* (purple nutsedge) (Tang *et al*., 1995), *Hypericum perforatum* (St. John's wort) (Gray *et al*., 2003b) and *Solanum lycopersicum* (English-Loeb *et al*., 1997). The concentration of leaf exudate phenolics in *Dittrichia viscose* were also increased under water stress,

although due to the decrease in leaf area, the total leachable phenolics were lower (Karageorgou *et al*., 2002). Drought stress was also found to reduce the inhibition threshold concentration for ferulic acid to affect seedling growth of *Glycine max* and *Sorghum bicolor* (Einhellig & Eckrich, 1984; Einhellig, 1996).

Nutrient availability can affect the concentrations of allelopathic compounds produced by the plants. Toxin production increased dramatically in *Prymnesium parvum* cells under nitrogen or phosphorus deficient conditions (Granéli & Johansson, 2003), and phosphorus limitation also led to a change in the composition, as well as a significant increase, in the released organic compounds from *Trichormus doliolum* (Von Elert & Jüttner, 1996). The concentration of leaf exudate phenolics in *Dittrichia viscosa* were also increased in nutrient deficient conditions, although due to the decrease in leaf area, the total leachable phenolics were lower (Karageorgou *et al*., 2002). Increased nutrient availability can also have an effect, and leaf phenolics in *Potamogeton amplifolius* were increased by high nutrient availability (Cronin & Lodge, 2003). Nutrient availability has also been found to affect the sensitivity of plants to various allelopathic compounds, and under nutrient-deficient conditions, *Arachis hypogaea* (peanut), *Amaranthus retroflexus* (redroot amaranth), *Cucumis sativus* and *Lolium multiflorum* (Italian ryegrass) were more susceptible to volatiles produced by *Ageratum conyzoides* (Kong *et al*., 2002), and the diatom *Thalassiosira weissflogii* was more susceptive to allelopathic compounds produced by *Prymnesium parvum* (Fistarol *et al*., 2005). However, nutrient-depleted conditions were shown to decrease the sensitivity of *Desmodesmus quadrispina*, *Nitzschia palea*, and *Nostoc* PCC 7120 to compounds produced by *Desmodesmus quadrispina*, *Monoraphidium dybowski*, or *Uronema confervicolum* (Leflaive & Ten-Hage, 2009).

Soil texture can be very important as allelochemicals bind to the soil particles, and generally, loose and sandy soils exhibit a more inhibitory effect than heavy and loamy soils, as allelochemicals fail to be sorbed on the former (Dalton *et al*., 1989; Singh *et al*., 2001). This sorption of the allelochemicals is greatly influenced by the pH of the soil and the organic matter content (Dalton *et al*., 1989; Singh *et al*., 2001). Microbes can also have a significant effect on the allelopathic activity, particularly of phenolic acids, as they can metabolize the released phenolic acids, and the transformed or newly synthesised phenolics might differ in their phytotoxicity from the original ones (Singh *et al*., 2001). Many microbes also produce their own

allelopathic compounds. These are generally non-specific and have been shown to inhibit the growth of several annual and perennial species (Barazani & Friedman, 2001; Singh *et al*., 2001).

The presence of allelopathic agents and other compounds have also been found to be important. Concentrations of scopoletin and scopolin were higher in the tissues of *Helianthus annuus* seedlings treated with scopoletin or 2,4-dichlorophenoxyacetic acid (Dieterman *et al*., 1964; Einhellig *et al*., 1970), and synthesis of pisatin was initiated in *Pisum sativum* (pea) tissue by the drugs tilorone, chloroquine, imipramine and quinacrine (Hadwiger, 1972). The synthesis of phenolic compounds in plants has also been found to be affected by the application of herbicides and, to a lesser extent, insecticides and fungicides (Daniel *et al*., 1999), and non-toxic plant species have been found to incorporate active portions of inhibitory compounds into their tissues (Lawrence *et al*., 1991). Under natural conditions, inhibitory effects of allelochemicals are often observed at concentrations well below their individual inhibitory levels (Singh *et al*., 2001). This suggests that these compounds can also act synergistically, and mixtures of allelopathic chemicals, as well as other organic compounds, have been shown to have a much greater inhibitory effect than the individual compounds alone (Blum, 1996; Reigosa *et al*., 1999). The crude water extract of *Empetrum hermaphroditum* was found to have a much greater inhibitory effect than the purified substance on the germination of *Populus tremula* (aspen) (Odén *et al*., 1992), and additive inhibition resulted from the joint action of ferulic acid (Einhellig, 1996) or water extracts of *Sorghum bicolor*, *Helianthus annuus* and *Morus alba* (mulberry) (Khaliq *et al*., 2012), with low levels of herbicides.

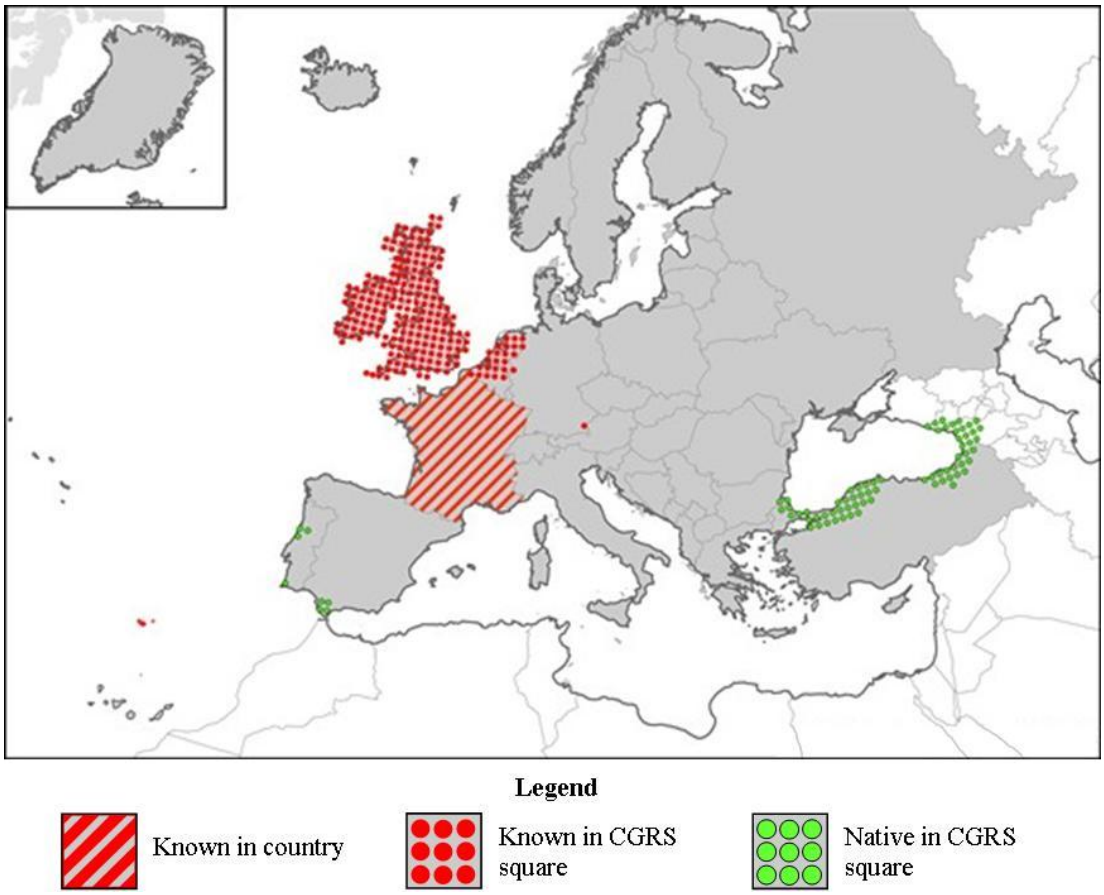
1.5 Rhododendrons

Rhododendron is a large genus with approximately 850 natural species, and well over 30,000 hybrids and cultivars (Cullen, 2005). They belong to the order *Ericales*, family *Ericaceae*, which includes *Erica*, *Calluna*, *Kalmia* and *Vaccinium*. They can be evergreen or deciduous, ranging in size from small shrubs to large trees, and can be found throughout most of the Northern Hemisphere except for dry areas, and extending into the Southern Hemisphere in Australia and south-east Asia (Cullen, 2005; Discover Life, 2013). Although the majority of these species are slow growing

and are valued for their large, showy flowers (Cullen, 2005), several, including *R. ponticum* and *R. maximum*, have become a problem, inhibiting the growth of the native vegetation and altering community structure and function (Nilsen *et al*., 1999; Dehnen-Schmutz *et al*., 2004).

1.5.1 *Rhododendron ponticum*

R. ponticum, often called 'common rhododendron', is the species that is most acclimatized in Britain (Milne & Abbott, 2000). Although it has two subspecies, the first being *R. ponticum* subspecies *ponticum*, which is native to Turkey, Lebanon, Bulgaria and the Caucasus, and the second being *R. ponticum* subspecies *baeticum*, which is native to Spain and Portugal (Erfmeier & Bruelheide, 2005), it is subspecies *baeticum* from which almost all UK populations are derived (Milne & Abbott, 2000). It was introduced into Britain in 1763, becoming especially popular in parks, gardens, and on country estates, providing ornamental value, as well as cover for game (Rotherham, 2001), although fossil evidence suggests that the range of *R. ponticum* was much greater in Europe during an interglacial period between 302,000 and 428,000 years ago, and might have been native in the United Kingdom under very different climatic conditions (Coxon *et al*., 1994). Subsequent widespread introductions have also occurred, aiding its spread (Milne & Abbott, 2000). Where they were not used for the plant itself, they were used as a rootstock onto which other more attractive rhododendrons could be grafted (Dehnen-Schmutz *et al*., 2004). However, it is so vigorous that the roots readily send up suckers from below the graft, often allowing it to overtake and kill the intended grafted rhododendron (Dehnen-Schmutz *et al*., 2004).



CGRS = Common European Chorological Grid Reference System used for species distribution mapping.

Figure 1. 1. Map showing the distribution of *R. ponticum* across Europe (reproduced from DAISIE, 2012).

Genetic diversity can be considerably reduced during invasions following founder effects and genetic drift. However, analysis of native and invasive populations of *R. ponticum* showed that although the highest level of genetic diversity was found within native Georgian populations, native Spanish and invasive Irish populations showed reduced levels of genetic diversity but displayed no further reduction in the invasive range (Erfmeier & Bruelheide, 2011). Analysis of *R. ponticum* populations in Britain found evidence of interbreeding with *R. catawbiense* and *R. maximum*, both of which are native to the eastern United States, (Milne & Abbott, 2000). This hybridity is widely distributed throughout invasive United Kingdom populations (Milne & Abbott, 2000), and preservation of sufficient genetic variation in invasive *R. ponticum* populations in Britain has been vital, allowing the species to adapt to its new environment (Erfmeier & Bruelheide, 2011). Invasive populations have also been shown to differ from non-invasive ones in their growth patterns and have evolved much higher rates of annual shoot growth germination and seedling

recruitment (Erfmeier & Bruelheide, 2004). Erfmeier & Bruelheide (2010) found that rhododendron cuttings had low survival rates in Georgia and Spain, and despite being such an invasive species, in its native habitat in the southern Iberian Peninsula it is actually at risk from extinction (Almeida *et al*., 2005).

R. ponticum is a large, hairless evergreen shrub with spreading branches, which tends to form large patches (Erfmeier & Bruelheide, 2005). It thrives in mild, wet climatic conditions, where there are poor, acidic soils with a pH of approximately 3.5 - 5.0 (Cross, 1975). It can establish in heath and moorland, as well as bogs, mires, riverbanks, grassland and salt marsh (Dehnen-Schmutz *et al*., 2004). However, it is woodland that is most affected (Dehnen-Schmutz *et al*., 2004), with deciduous woodland providing the most suitable conditions for its spread (Harris *et al*., 2011). It is a serious problem as it spreads easily into these habitats, threatening the native biodiversity (Cross, 1981).

1.5.2 Methods of invasion

There are two methods that *R. ponticum* uses to invade surrounding areas. The first is by sending out horizontally growing branches, which will root where they touch the ground (personal observation). This can result in a single plant covering large areas with thickly interlaced branches, and allows the rhododendron to spread into areas which would otherwise be unsuitable for their growth (Cross, 1981). However, the most predominant method is using sexually produced seeds (Stout, 2007b). *R. ponticum* seeds are tiny and are wind dispersed. The seeds are similar in weight to other ericaceous species, and therefore are some of the smallest in the plant kingdom (Cross, 1981). Each flower head can produce about five thousand viable seeds, so that a large plant can produce over a million seeds per year (Cross, 1981).

Light is essential for the successful germination of *R. ponticum*, and in dark conditions the seeds rapidly lose their viability. However, due to their small size the seeds are at serious risk from desiccation. To compensate for this, seedlings have developed shade tolerance, which allows them to grow in darker conditions, but where there is more moisture (Cross, 1981), and there is genotypic evidence for

higher growth rates in the invasive species, particularly at low light concentrations (Erfmeier & Bruelheide, 2005).

Seedlings have difficulty becoming established in areas where there is already continuous ground cover from native plants (Cross, 1981). Establishment is best in areas with a thin bryophyte or leaf litter layer, which is able to hold a large amount of water (Stephenson *et al*., 2006). Seedlings do not germinate well in areas where the bryophyte or leaf litter layer is thicker than 1cm because the roots fail to reach the soil and the seedlings are unable to survive during a dry spell (Cross, 1981). They will also grow in disturbed areas. In undisturbed woodland, *R. ponticum* would probably be limited to naturally unstable areas and therefore its success is largely due to habitat disturbance, particularly woodland and forestry management (Stephenson *et al*., 2006). This creates gaps in the canopy allowing sufficient light penetration for seedling germination, while the exposure of bare ground and the subsequent colonisation by bryophytes might provide ideal conditions for seedling establishment due to reduced competition (Stephenson *et al*., 2006). However, very few seedlings occur on bare soil. This might be due to bare mineral soil and humus being unsuitable for growth as the surface layer dries out rapidly and the seedlings become subject to extreme water stress. There is also evidence that heavy drips from the canopy can physically destroy seedlings by uprooting them or burying them (Cross, 1981). Under a thin canopy, seedlings might occasionally occur on bare soil but they are limited to moist sites (Cross, 1981).

1.5.3 Mycorrhizal association

Mycorrhizal associations have been found to develop within the roots of *R. ponticum*. They can be found in a number of genera, including *Rhododendron*, *Erica*, *Calluna* and *Vaccinium*, but are specific to ericaceous plants (Pearson & Read, 1973). The mycorrhizal associations have adapted to a broad range of habitats, from moist humus soils of the northern hemisphere to dry sandy soils occurring in the southern hemisphere (Mitchell & Gibson, 2006). The isolates capable of forming mycorrhizal associations have been identified as free-living in the soil, even in areas without ericaceous plants (Pearson & Read, 1973). However, they do appear to be limited to soils which are either high in organic matter or sandy, where nutrient

availability is low (Mitchell & Gibson, 2006). They encourage the development of a densely branched fibrous root system which has a high capacity for dissolved mineral uptake (Read, 1996), and they are able to use complex forms of nitrogen and phosphorus, allowing the plants to thrive in nutrient-poor soils (Mitchell & Gibson, 2006).

The ericoid mycorrhizal associations which develop within the roots of *R. ponticum* also have the ability to protect the plants against toxic conditions. They regulate the uptake of toxic compounds from the soil, and they also have a high affinity for iron, as well as for many other metals including aluminium, manganese, copper and zinc (Mitchell & Gibson, 2006).

All these mechanisms give the plant an advantage over other non-ericaceous plants and allow them to establish in wide range of habitats (Mitchell & Gibson, 2006). It also means that they are extremely successful at colonizing heathlands, as the heathers which exist there have already established suitable mycorrhizal networks (Mitchell & Gibson, 2006).

1.5.4 Pollinators

Pollination of *R. ponticum* is due primarily to insects, particularly bumble bees. Reliance on insect visitation for seed production should be unusual among invasive species as they lack their mutualistic insect pollinators. However, there are a number of alien species that are entirely entomophilous (Stout, 2007b). This might be an advantage as a lack of pollinators in species with a mixed mating system can lead to increased self fertilization, which can reduce the plant's fitness (Stout, 2007a, 2007b), and this generalization might be one of the reasons why *R. ponticum* is such a successful invasive species (Stout *et al*., 2006). *R. ponticum* produces a large brightly coloured floral display (Figure 1. 2), and flowers also secrete profuse volumes of sugar-rich nectar (Stout, 2007b). This makes them very attractive to insects, and during the main flowering season of May and June, they dominate the attentions of pollinating insects (Stout *et al*., 2006, 2007a). This means that the flowers of native plant species in the area could suffer from a lack of pollinating

insects, and as a result they might not successfully produce seeds (Chittka & Schürkens, 2001; Bjerknes *et al*., 2007).

Figure 1. 2. *R. ponticum* in flower at Exbury Gardens in the New Forest.

1.5.5 Toxins

Toxic chemicals occur in significant amounts in the tissues of *R. ponticum*, meaning that there are few animal species associated with it in Britain, and it has relatively few natural enemies compared to other species (Judd & Rotherham, 1992). Grayanotoxin I, or acetylandromedol, is a diterpene specific to ericaceous plants that is responsible for rhododendron toxicity (Özhan *et al*., 2004). Although it is not produced by all *Rhododendron* species, it has been found in the leaves, flowers and nectar of *R. ponticum* (Sütlüpmar *et al*., 1993; Shrestha *et al*., 2009). These can bind to sodium channels in cell membranes, thereby increasing the permeability of sodium ions and inhibiting inactivation of sodium channels (Li *et al*., 2013). Phenols have also been found in *R. ponticum*. These are most concentrated in the young tissues, especially developing leaves and buds, and provide an initial deterrent against herbivores, before the tissues have obtained the added defence of physical toughness found in older tissues (Rotherham, 2001). Young emergent leaf buds also have the protection of a sticky exudate that also contains phenols, which discourages small

invertebrates from eating the buds because they get stuck in the secretion (Rotherham, 2001).

1.5.6 Effects on biodiversity

The suitability of the climate and soil, the absence of serious pests and diseases, habitat disturbance and profuse seed production, has led to *R. ponticum* becoming invasive in the United Kingdom, Ireland, Belgium, France and Netherlands and New Zealand (DAISIE, 2012; NZflora, 2013). Once it has invaded an area, few native plants are able to survive (Cross, 1981; Eşen & Zedaker, 2004). It forms dense thickets which inhibit the growth of the native species (Cross, 1981). In woodlands, only those trees which succeed in growing above the level of the *R. ponticum* canopy will persist, and once these die they will not be replaced because their seedlings cannot become established (Cross, 1981). Once the native plants have disappeared, the animals which rely upon them for food or shelter, cannot survive, and even where trees remain above the *R. ponticum* canopy, species disappear because they rely on a complex habitat which is no longer available (Tews *et al*., 2004).

R. ponticum has been found to be the host plant for two very serious diseases of hardwood and coniferous trees. *Phytopthora ramorum* was first discovered in the UK in 2002, and is responsible for the highly damaging tree disease known as Sudden Oak Death (Webber, 2007). It has been found to affect a number of species growing in close proximity to infected *R. ponticum*, especially those in the family *Fagaceae*, including *Fagus*, *Quercus* and *Castanea* (Webber, 2007). On some species only foliage and shoots are affected, but on other hosts the infection is a lethal stem canker (Webber, 2007). *Phytopthora kernoviae* is another disease with symptoms which are similar to those of *Phytopthora ramorum*. It was first discovered in 2003 in woodlands in Cornwall, and although it mainly affects rhododendrons, especially *R. ponticum*, a number of other species have been found to be infected, including *Fagus* and *Quercus* (Brasier *et al*., 2005).

It has, therefore, become essential to manage *R. ponticum* populations, not only for their invasive properties but also in order to reduce the spread of these diseases (CABI, 2012). The cost of controlling the spread of *R. ponticum* is dependent upon

the control method employed, the size and density of the bushes, and site accessibility, although it has been estimated to be as much as £10,000 per hectare (Dehnen-Schmutz *et al*., 2004). However, despite being one of our most rapidly expanding plant species, *R. ponticum* remains popular with large groups of society due to its ornamental value (Dehnen-Schmutz *et al*., 2004). This means that it is not suitable for biological control as the conflicts of interest are too great. The other problem is that even after clearance, the remaining humus layer prevents natural regeneration, and even after removal, re-infestation can easily occur from the millions of seeds left in the soil (Cross, 1975).

1.6 Aims of this study

In order to successfully control the spread of invasive species, it is essential to understand the ecological mechanisms responsible for their inhibitory effect on the native species (Levine *et al*., 2003). Many invasive species have been studied in great detail, including other *Rhododendron* species, particularly *R. maximum* in America (Nilsen *et al*., 1999, 2001), and yet despite *R. ponticum* being such a serious problem, there have been few published scientific studies compared with many other invasive species. Previous studies into *R. ponticum* have focused on its biology, the factors affecting its success and methods of controlling its spread (Cross, 1975; Milne & Abbott, 2000; Dehnen-Schmutz *et al*., 2004; Erfmeier & Bruelheide, 2004; Stephenson *et al*., 2006). Although several studies have suggested that it is the reduced light availability beneath *R. ponticum* that is responsible for the inhibition of growth (Stephenson *et al*., 2006; Stout *et al*., 2006), other environmental conditions, including water and nutrient availability, have also been found to be altered by rhododendron (Nilsen *et al*., 2001), but there have been no studies testing the influence of these changes on the native species. Previous studies have also suggested that allelopathy is involved in the impact of rhododendrons. However, a study by Rotherham and Read (1988) appears to be the only one to investigate the influence of allelopathy on the inhibition of growth beneath *R. ponticum*, and although they found that soil which had supported *R. ponticum* under controlled conditions had an inhibitory effect on the growth of *Festuca ovina*, but did not test the influence of allelopathy compared to other environmental conditions, and there has been no work testing the interactions between the two. The overarching aim of

this study was to investigate the influence of a range of environmental factors on the growth of the native species beneath *R. ponticum* in the New Forest, Hampshire, UK, and to specifically test whether the interactions between environmental conditions and allelopathy can explain the inhibiton of growth beneath *R. ponticum* in the New Forest.

In chapter 3, environmental conditions were measured beneath *R. ponticum* in invaded woodland in the New Forest, to identify the key environmental factors most likely to be involved in the inhibition of growth of the native vegetation, and to establish a baseline for subsequent experimental manipulation. In chapter 4, the effect of these conditions were then tested on the germination and growth of two species naturally occurring with *R. ponticum*, while controlling for other environmental differences between invaded and uninvaded sites, to determine whether differences in environmental conditions are enough to explain the inhibition of growth beneath *R. ponticum* in the New Forest. Chapter 4 also tested the effect of growth and litter decomposition of *R. ponticum* on soil pH under controlled conditions, to determine whether *R. ponticum* is having an effect, or whether conditions were already unsuitable for the native species.

Main hypothesis tested: Changes in environmental conditions are responsible for the inhibition of growth beneath *R. ponticum* in the New Forest.

As well as testing the effects of aqueous leachates (i.e. the solution resulting from leaching soluble constituents from a solid) and root exudates from *R. ponticum* on the germination and growth of two test species to determine whether allelopathy alone is enough to explain the inhibition of growth beneath *R. ponticum*, chapter 5 also tested soil which had supported *R. ponticum* to determine whether allelopathic compounds build up over time or persist in the soil.

Main hypothesis tested: *R. ponticum* releases allelopathic compounds which persist in the soil and have an inhibitory effect on native species in the New Forest.

In chapter 6, the combination of key environmental conditions and allelopathic compounds were tested on the germination and growth of two test species, to determine whether the environmental conditions found beneath *R. ponticum* enhance

the allelopathic effect on the native vegetation. Chapter 6 also investigated whether different light and pH conditions affect the synthesis or accumulation of allelopathic compounds beneath *R. ponticum*, and whether compounds produced by *Quercus robur* (English oak) and *Pinus sylvestris* (Scots pine) enhance the inhibitory effect of *R. ponticum*.

Main hypothesis tested: The inhibition of growth beneath *R. ponticum* in the New Forest is due to the combination of allelopathic compounds and environmental factors.

Chapter 2

General methods

2.1 Study sites

The New Forest, southern England (Figure 2. 1), covers 145 square miles, and supports a complex variety of wildlife habitats, formally common in lowland Western Europe, but now rare and fragmented (New Forest Life, 2007). It contains some of the largest areas of lowland northern Atlantic wet heathland and lowland European dry heathland in the United Kingdom. The New Forest also contains extensive areas of coniferous woodland, as well as the largest area of mature, semi-natural beech (*Fagus sylvatica*) woodland in Britain, and the most extensive area of active oak (*Quercus* spp.) and beech wood-pasture in north-west Europe (Defra, 2013). These habitats support a wide range of plants and animals, including a number of unique and scarce species (New Forest Life, 2007; Defra, 2013). However, *R. ponticum* can now be found in all 10 km^2 grid cells in the New Forest (National Biodiversity Network's Gateway, 2013), and is posing a serious threat to these native habitats, and the diversity of species that they support (Mitchell *et al*., 1997).

Figure 2. 1. Map of the New Forest showing the location of the selected study sites (Reproduced from Wikimedia, 2013).

In chapter 3, field work was conducted at two study sites in the New Forest, in order to measure the environmental conditions beneath *R. ponticum*. Site 1 was Exbury Gardens (50.8025^{o} N, 1.3983^{o} W), a 200 acre site, world-famous for its collection of rhododendrons, azaleas, camellias and rare trees and shrubs. Site 2 was Minstead Lodge (50.8996^{o} N, 1.5961^{o} W), a large Victorian country house in 17 acres of grounds. Both sites had areas of *R. ponticum* and areas of open grassland, and they had restricted access so little human disturbance (Figure 2. 1). Ideally data would have been collected at more sites, but with less repeats at each. However, this was

financially difficult, and when measuring all the environmental conditions it was not logistically possible for one person to collect data from more than two sites due to the number of samples that would have been required. However, as the main aim of chapter 3 was to establish a set of baseline conditions as reference points for subsequent experimental studies, two sites were sufficient to determine which of the environmental conditions are likely to be having an inhibitory effect on native plant growth beneath *R. ponticum* (Reinhart *et al*., 2006). If the principal purpose had been to investigate patterns and correlations between the environmental conditions, then more sites would have been necessary (Kikvidze *et al*., 2005).

In chapter 4, light availability and soil pH were the only factors being measured, so more sites could be used as more confidence was needed. Because environmental conditions can vary naturally with site due to a number of factors, such as geological substrate, hydrology, or disturbance history (Reinhart *et al*., 2006), measurements were taken at three deciduous and three evergreen woodland sites which had been invaded by *R. ponticum* to take into account possible site effects. Deciduous woodland was dominantly *Q. robur* and *Fagus sylvatica* (European beech), although there were also occasional *Fraxinus excelsior* (European ash) and *Betula pendula*. Evergreen woodlands were composed entirely of *P. sylvestris*. The three deciduous woodland sites were Exbury Gardens (50.8025^{o} N, 1.3983^{o} W), Poundhill Inclosure (50.8430^{o} N, 1.6193^{o} W) and Turf Hill Inclosure (50.9530^{o} N, 1.7266^{o} W). The three evergreen woodland sites were Copythorne Common (50.9327^{o} N, 1.5673^{o} W), Poundhill Inclosure (50.8484^{o} N, 1.6221^{o} W) and Blackhill (50.9624^{o} N, 1.5671^{o} W) (Figure 2. 1). These sites were chosen as they had large areas of *R. ponticum* in either oak or pine woodland, as well as neighbouring areas of open grassland, and they also had limited access so little human disturbance. Measurements could not be taken at Minstead Lodge as large areas of the rhododendrons had been cleared. By having both a deciduous woodland site and an evergreen woodland site at Poundhill Inclosure, it reduced spatial variation (Farley & Fitter, 1999). However, no other suitable sites were found that had both woodland types.

In order to test whether *R. ponticum* has an allelopathic effect on the germination and growth of native species in chapters 5 and 6, one site would have been sufficient (Jose & Gillespie, 1998; Nilsen *et al*., 1999). However, because environmental conditions can vary naturally with site (Reinhart *et al*., 2006), which can affect the

concentration of allelopathic compounds produced by a plant, as well as the time it remains in the soil (Leflaive & Ten-Hage, 2009), samples were collected from two sites for each woodland to take into account possible site effects. The two deciduous woodland sites were Exbury Gardens and Poundhill Inclosure, and the two evergreen woodland sites were Copythorne Common and Poundhill Inclosure (Figure 2. 1, Figure 2. 2).

(continued)

Figure 2. 2. a & b) *R. ponticum* and open grassland at Exbury Gardens. c & d) *R. ponticum* and open grassland at Copythorne Common. e & f) *R. ponticum* in deciduous and evergreen woodland at Poundhill Inclosure. g & h) Open grassland at Poundhill Inclosure.

(Figure 2. 2 continued)

At each of the sites, 1 m^2 plots were selected beneath *R. ponticum* plants which were fully established, at least 180 cm tall, and were at least 5 m from any other large plants, which might have affected the environmental conditions, or might have released their own allelopathic compounds (Kuiters & Sarink, 1986). *Q. robur* and *P. sylvestris* were not included in this due to the difficulty in finding sufficient suitable sites that were more than 5 m from either of the species. As *R. ponticum* is commonly found in oak or pine woodland (personal observation), it is also possible that that the presence of these species plays an important role in the inhibition of growth of native species in the New Forest, as well as affecting the concentration of toxins produced by the rhododendron (Leflaive & Ten-Hage, 2009). The sites were still open to grazing from herbivores, including deer, rabbits and various insects (Tubbs, 2001). However, preliminary experiments demonstrated that this was not sufficient to explain the inhibition of growth of the native species (unpublished data, 2009), although due to the unpalatability of *R. ponticum* (Rotherham, 2001), might have helped the rhododendron to become so successful. In chapters 3 and 4, fifteen plots were selected at each site to take into account variation in environmental conditions within each site (Reinhart *et al*., 2006). However, in chapters 5 and 6, eight plots were sufficient to determine the importance of allelopathy on the inhibition of native plant growth (Nilsen *et al*., 1999; Sisodia & Siddiqui, 2009). Plots were selected in different areas around each site, to avoid localized differences.

At each of the sites, plots were also selected in neighbouring areas of open grassland (where grasses were the predominant species, and very few trees or shrubs were found). Plots were selected which had limited human disturbance, were at least 10 m from any large plants (greater than 1 m tall), and where growth was not limited. This meant that environmental conditions (i.e. light and water availability) could be

compared to identify which of them were different in areas invaded by *R. ponticum*. By having areas of open grassland and *R. ponticum* in close proximity to each other, it ruled out factors associated with site as the cause for differences in growth. These could have included atmospheric composition (such as CO_2 concentrations), heavy metal contamination, or plants in different areas having altered growth rates due to genetic differences (Raven *et al*., 2005). Any differences in environmental conditions between the two areas could either have been caused by the presence of *R. ponticum*, or could have been different already and been the reason why *R. ponticum* was able to become established in those areas (Cross, 1975), and this will be investigated in chapter 4.

In chapters 4, 5 and 6, one plot was also selected 5 m from each of the chosen rhododendrons, so that light availability and soil pH could be compared between areas to identify if they were altered by the rhododendron, or if they were already different before the arrival of the rhododendron, and also so the soil could be compared to determine whether the rhododendron was releasing allelopathic compounds into the soil, or whether the soil already had an inhibitory effect on the native species. By selecting plots close to the rhododendron, it reduced spatial variation in environmental conditions (Reinhart *et al*., 2006). Plots were selected 5 m from the chosen rhododendron as it was difficult to get any further from the plant without getting too close to another rhododendron, although previous work has shown that at this distance, the concentration of allelopathic compounds in the soil was significantly reduced (Jose & Gillespie, 1998).

2.2 Soil sampling

In order to collect soil samples, the top 5 cm of soil was removed from each field plot to remove the partially decomposed leaf litter (Nilsen *et al*., 1999). Samples were then collected from the soil below. Samples were collected from the top 5 - 10 cm of soil, as previous studies found that the highest concentration of allelochemicals within this layer (Ponder & Tadros, 1985). Each sample was stored separately in a plastic bag, and then transported in a cool box to reduce aeration and evaporation (Allen *et al*., 1974). When measuring the moisture, organic matter, pH and nitrate in the soil, samples were stored in a cool room at 4^{o}C, until they could be analysed.

However, when aqueous leachates (i.e. the solution resulting from leaching soluble constituents from a solid) were being collected, the samples were immediately air dried on paper towels at approximately 20^{o}C for 24 hours. This reduced microbial activity, as well as condensation, both of which could have affected the results, and could have altered any allelopathic effects of the samples (Singh *et al*., 2001).

2.3 Leachates

To test the effect of soil which had supported *R. ponticum* on the germination and growth of native species, aqueous leachates were collected based on the method used by Inderjit and Dakshini (1994). Soil leachates were collected by placing 150 g of air dried soil in a 250 ml plastic beaker containing 150 ml of distilled water. This concentration has been used a number of previous studies investigating allelopathy (Lodhi & Killingbeck, 1982; Zhu & Mallik, 1994). Distilled water was used as the solvent as aqueous extraction is more ecologically relevant than organic extraction (Ballester *et al*., 1977). Beakers were also left with just distilled water to use as a control. Beakers were left for 24 hours, and then the solution was filtered through Whatman No. 1 filter paper to remove the soil material.

2.4 Native study species

As *R. ponticum* is most commonly found in woodland (personal observation), woodland species would have been more relevant, and preliminary trials were conducted on a number of species to determine whether they were suitable for use in this study (unpublished data, 2009). *Hyacinthoides non-scripta* (bluebell), *Digitalis purpurea* (foxglove), *Galanthus nivalis* (snowdrop), *Rumex acetosa* (sorrel) and *Rumex sanguineus* (wood dock) are all common in the New Forest, were all found growing in the study sites around *R. ponticum* (personal observation), and seeds could be easily obtained. However, the seeds of *Digitalis purpurea* were too small to efficiently count, and all species had poor germination. Tree species, including *Fagus sylvatica*, *Q. robur* and *P. sylvestris* are often used, especially when demonstrating allelopathy (Nilsson, 1994; Löf, 2000). However, germination and growth of these species is often slow, and percentage germination is poor (Nilsson *et al*., 1996; Oleskog & Sahlén, 2000; Kolářová *et al*., 2010). There has also been a

large amount of work using *Triticum aestivum* (wheat) and other crop species (Khan *et al*., 2004; Hussain *et al*., 2007), and therefore a great deal is known about their optimum conditions for growth. However, wheat had poor germination in preliminary trials (unpublished data, 2009), and none of these crop species were ecologically relevant, so could not be used to demonstrate allelopathy in subsequent chapters (Romeo & Weidenhamer, 1998).

Lolium perenne (perennial rye grass) and *Trifolium repens* (white clover) were chosen as the two study species. Although they are both grassland species, they are commonly used as model organisms, and as they have been used in previous studies, a great deal is already known about their optimum conditions for growth. Both are shade tolerant and both will tolerate acidic conditions (Turkington & Burdon, 1983; Hannaway *et al*., 1999), so should be able to establish beneath *R. ponticum*. Both are commonly found in the south of England (Sterry, 2006; personal observation) meaning they are ecologically relevant, so could be used to demonstrate allelopathy, and not just phytotoxicity (Romeo & Weidenhamer, 1998), and previous studies have also shown that both species are affected by allelopathic compounds (Carballeira & Cuervo, 1980). Preliminary trials also demonstrated that both species germinate quickly, and there is high percentage germination, meaning they are easy to use (unpublished data, 2009).

2.5 Germination and growth

Germination, root elongation and leaf appearance are widely used measures of plant fitness, and these were measured based on the methods used by Anjum and Bajwa (2005), Thomas (1981) and Streck *et al*. (2003). Germination was measured by calculating the proportion of seeds in each Petri dish that had germinated after 7 days for *L. perenne* and after 3 days for *T. repens*. Preliminary experiments demonstrated that this was the time taken for 50% of the seeds to have germinated (unpublished data, 2009). Root growth of *L. perenne* was measured by calculating the proportion of seedlings in each Petri dish that had germinated within 7 days, which had roots greater than 10 mm after 8 days (time taken in preliminary experiments for 50% of the seedlings to have roots greater than 10 mm (unpublished data, 2009)). Root growth of *T. repens* was measured by calculating the proportion of seedlings in each

Petri dish that had germinated within 3 days, which had roots greater than 5 mm after 5 days (time taken in preliminary experiments for 50% of the seedlings to have roots greater than 5 mm (unpublished data, 2009)). Leaf appearance of *L. perenne* was measured by calculating the proportion of seedlings in each Petri dish that had germinated within 7 days, which had primary leaves after 9 days (time taken in preliminary experiments for 50% of the seedlings to have primary leaves (unpublished data, 2009)). Leaf appearance of *T. repens* was measured by calculating the proportion of seedlings in each Petri dish that had germinated within 3 days, which had primary leaves after 7 days (time taken in preliminary experiments for 50% of the seedlings to have primary leaves (unpublished data, 2009)).

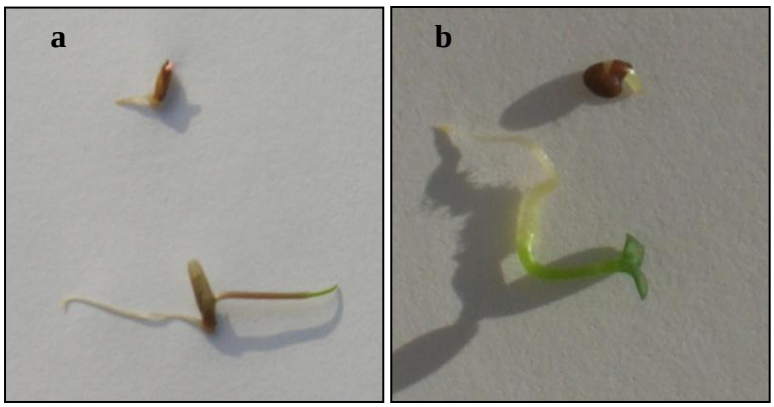


Figure 2. 3. Germination, root elongation and primary leaf appearance of a) *L. perenne* and b) *T. repens*.

Chapter 3

Identifying the key environmental differences beneath *Rhododendron ponticum* compared to open grassland in the New Forest

3.1 Introduction

R. ponticum is one of the United Kingdom's most rapidly expanding invasive alien species (DAISIE, 2012). It has become a severe problem throughout the British Isles, as once it has invaded an area, few native plants are able to survive (Dehnen-Schmutz *et al*., 2004). Numerous studies have investigated the mechanisms behind the impact of other invasive species, and changes in environmental conditions, either through resource competition or the alteration of ecosystem processes, are often implicated as the cause for the inhibition of native plant growth (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003).

Competition for light has been implicated in a number of studies as the key factor responsible for the impact of other invasive plants (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003), and many *Rhododendron* species, including *R. ferrugineum*, *R. hodgsonii*, *R. maximum* and *R. ponticum* have been shown to alter light availability differently to native vegetation (Cross, 1981; Filella & Peñuelas, 1999; Beckage *et al*., 2000; Nilsen *et al*., 2001; Gratzer *et al*., 2004). However, changes in vegetation types can also alter the microclimate (Hoffman & Jackson, 2000) and water availability (Nilsen *et al*., 2001), and these are often thought to be involved in the inhibition of native plants in other invaded areas (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Soil and air temperature were both found to be reduced beneath *Rhododendron* species, including *R. caucasicum* and *R. maximum* (Clinton, 2003; Akhalkatsi *et al*., 2006), and several species, including *R. maximum* and *R. periclymenoides* have been shown to affect water use (Knox, 1989; Lipscomb & Nilsen, 1990). Plants with dense canopies, such as rhododendrons, have been found to reduce the amount of rainfall reaching the ground (Fleischbein *et al*., 2005), and their densely branched shallow root system allows rhododendrons to take up more water than native species (Read, 1996). *Rhododendron* species, including *R. maximum* and *R. ponticum*, have also been shown to increase the organic matter input into an ecosystem (Cross, 1981; Wurzburger & Hendrick, 2007), which will affect the water holding capacity of the soil (Hudson, 1994).

Changes in nutrient cycling have often been implicated as being responsible for the reduced diversity beneath other invasive plants (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003), and a number of *Rhododendron* species have been found to reduce the availability of nutrients, particularly cations, beneath them (Cross, 1975; Arunachalam *et al*., 1998; Nilsen *et al*., 2001). Although the concentration of nutrients in the leaves of rhododendrons is generally low, slow leaf turnover rate allows them to sequester large quantities of available nutrients, meaning they are not available for other plants (Monk *et al*., 1985), and even when they do lose their leaves, due to their high lignin content, decomposition, and therefore the release of nutrients, is slow (Arunachalam *et al*., 1998). *Rhododendron* species, including *R. arboreum, R. arboretum* and *R. maximum,* have also been found to reduce nitrogen and phosphorus mineralization (the process by which organic molecules are converted to inorganic forms which are available for plants) (Arunachalam *et al*., 1998; Kraus *et al*., 2004) and increase the immobilization of nitrogen (Maithani *et al*., 1998), and *R. maximum* has also been shown to alter nitrogen cycling through the formation of recalcitrant polyphenol–organic N complexes (Wurzburger & Hendrick, 2007). Changes in water availability can also affect nutrient availability, either through uptake, or due to leaching of basic nutrients (Raven *et al*., 2005), and erosion can directly alter the nutrient retention of ecosystems (Gholz *et al*., 1985), although rhododendrons have been found to reduce erosion (Tiwari & Chauhan, 2006).

Changes in temperature, water availability, soil pH and nutrient availability will all affect the activity of soil organisms (Killham, 1994), and earthworm densities (Boettcher & Kalisz, 1991) and microbial populations (Sutton & Wilkinson, 2007) were both found to be reduced beneath rhododendrons. A number of *Rhododendron* species, including *R. arboretum* and *R. dauricum* have also been shown to leach chemicals into the soil that reduce the ability of other species to fix nitrogen (Rice & Pancholy, 1974; Cao *et al*., 2002; Swaroop *et al*., 2005). This will not only affect nutrient cycling, but can also alter the pH of the soil (Yan *et al*., 1996).

As well as being affected indirectly by changes in environmental conditions, many other invasive species can directly alter the pH. Species that absorb nitrogen as ammonia, which includes *Rhododendrons*, tend to lower the pH in the rhizosphere, due to the uptake of excess cations over anions (Nye, 1981), and a number of

Rhododendron species, including *R. dauricum* and *R. formosanum*, have also been shown to release phenolic acids into the soil which could lower the pH (Cao *et al*., 2002; Chou *et al*., 2009). This change in the pH of the soil can have a direct effect on nutrient availability (Haynes & Swift, 1986).

Many plant species alter environmental conditions beneath them, which is not unique to invasive species (Arrieta & Suárez, 2005; Bossuyt *et al*., 2007; Delgado *et al*., 2007). However, what makes invasive species such a serious threat to native biodiversity is their ability to readily invade large areas (Pimentel *et al*., 2005). Higher rates of growth, germination and seedling recruitment than native species (Erfmeier & Bruelheide, 2005), as well as the suitability of the climate and soil, the absence of serious pests and diseases, habitat disturbance and profuse seed production, can allow invasive plants to have a much greater impact on the structure and function of native ecosystems (Cross, 1981).

Environmental conditions have been shown to be altered beneath *Rhododendron* species, including *R. ponticum*, compared to uninvaded woodland, and it is likely that this plays an important role in the inhibition of growth (Nilsen & Horton, 2002). However, previous work testing the effect of these changes on the diversity and cover of native plants has focused on *R. maximum*, an American species, and despite being such a serious problem, there appears to have been no work investigating the role of environmental conditions in the impact of *R. ponticum*. Reduced light availability beneath *R. maximum* was also the only environmental factor previously tested on the growth and survival of native plants (Clinton & Vose, 1996; Lei *et al*., 2002, 2006). Although it was shown to be important, it was found not to be solely responsible, and other mechanisms were thought to be involved in the inhibition of growth (Clinton & Vose, 1996; Lei *et al*., 2006), but there was no further work investigating the influence of other stresses. In this chapter, environmental conditions were measured beneath *R. ponticum* at two sites in the New Forest. These conditions were compared to those in open grassland, where there was continuous ground cover, to identify which were likely to be important in the inhibition of growth beneath *R. ponticum*, and to use as a baseline for experimental manipulation in chapter 4 to identify whether differences in environmental conditions alone are enough to cause the inhibition of growth of native species. Light availability, soil and air temperature, rainfall, water availability, organic matter, soil pH and nitrate were chosen as they

are the factors that are most commonly altered by the presence of invasive species (Levine *et al*., 2003), and, based on previous work, are likely to be involved in the inhibition of growth beneath *R. ponticum* (Nilsen *et al*., 2001; Nilsen & Horton, 2002).

Aims:

1. To identify the interactions between different environmental conditions beneath *R. ponticum* in the New Forest.

2. To establish a baseline for subsequent experimental manipulation to identify whether differences in environmental conditions alone are enough to cause the inhibition of growth of native species.

3.2 Methods

In order to measure the environmental conditions beneath *R. ponticum*, field work was conducted in 2009 during the start of the growing season, at Exbury Gardens and Minstead Lodge in the New Forest (see 2.1). Although both these sites are gardens, they are not highly cultivated, and the grassland is only mowed once a year. At each site, environmental conditions were measured at fifteen plots beneath *R. ponticum* and at fifteen plots in neighbouring areas of open grassland where growth was not limited so that environmental conditions could be compared to identify which are likely to be involved in the inhibition of growth beneath *R. ponticum*.

3.2.1 Light, temperature and rainfall

Measurements for light intensity, soil temperature and air temperature were taken at the start of the growing season, on the 13th March, 9th April, 7th May and 4th June 2009 (Smith *et al*., 2000). All measurements were collected on dry, sunny days, between 09.00 and 15.00 hrs, and results for both sites were collected on the same day. This reduced differences due to weather and sampling time. It was not possible for one person to collect data from both sites at the same time, so measurements were taken at Exbury Gardens in the morning and at Minstead Lodge in the afternoon. Light intensity was measured using an LX-1020B digital LUX meter (Sinometer Instruments, Shenzhen, China). Air temperature was measured 30 cm above ground level, and soil temperature was measured 10 cm below ground level, using a digital thermometer (Rapid Electronics Ltd, Essex, UK). All measurements were taken twice at each plot. Rainfall was measured using a rain gauge on the 18th March, 9th April, 7th May and 4th June 2009, and the rainfall per day was calculated.

To take into account differences between sites due to daily cycling, EL-USB-2 data loggers (Lascar Electronics Ltd, Wiltshire, UK) were set up at two plots in each area. Measurements were taken at 12.00 every day, between the 7th March and the 23rd September 2009, and the average temperature was calculated. This allowed comparisons to be made between sites, as all measurements were recorded at the same time every day.

3.2.2 Soil sampling

To measure the moisture, organic matter, pH and nitrate in the soil, samples were collected from Exbury Gardens on the 30th March 2009 and from Minstead Lodge on the 1st April 2009. Four 200 g soil samples were collected from each plot, and each sample was transported in a cool box, and immediately stored in a cool room at 4°C, until they could be analysed (Allen *et al*., 1974) (see 2.2).

3.2.3 Moisture

To measure the moisture in the soil, 100 g of soil was placed in a 250 ml glass beaker. The beaker containing soil was then placed in an oven at 40°C for 60 hours. Drying at 40°C has been shown to have less effect on the nutrient availability and organic matter content of the sample compared to drying at higher temperatures, and allows the sample to be stored for longer before analysis (Allen *et al*., 1974). The dried sample was weighed and the percentage of moisture was calculated. The dry soil was then placed back in cold storage at 4°C for further analysis.

3.2.4 Soil pH

The pH of the soil was measured by making a soil suspension using the standard 1:1 soil to water ratio method used by the U.S. Department of Agriculture. Thirty grams of dried soil and 30 ml of distilled water were added to a 250 ml beaker and stirred vigorously (U.S. Department of Agriculture, 2004). When measuring soil pH, fresh samples are recommended as drying will affect microbial activity, nutrient availability and surface acidity (Bartlett & James, 1980). However, due to the time constraints, analysis of fresh samples was not possible. The mixture was allowed to stand for 10 minutes. It was then stirred well immediately before immersing electrodes to measure pH. The pH of each sample was measured using an HI-98127 digital pH meter (Hanna Instruments Ltd, Bedfordshire, UK). To ensure accuracy and consistency, two separate soil suspensions were made for every tenth sample, and the pH of each was measured.

3.2.5 Organic matter

Organic matter was measured in one of the samples from each plot, using the standard loss on ignition method (Santisteban *et al*., 2004). Eight grams of dry soil was placed in a 15 ml crucible. The crucible containing the soil was then placed in a furnace, and heated to 550^{o}C for 4 hours. After cooling, the sample was weighed again and the amount of organic matter lost on ignition was calculated.

3.2.6 Nitrate

The levels of nitrate in the soil were measured in two of the samples from each plot using a Nitrachek 404 (KPG products Ltd, East Sussex, UK) with Mercoquant test strips (Merck KGaA, Darmstadt, Germany). Ten grams of dry soil was mixed with 20 ml of distilled water. When measuring nitrate concentration, fresh samples are recommended (Allen *et al*., 1974), but again, due to the time constraints, this was not possible. This was filtered to ensure that the solution was clear. The concentration was then measured by following the instructions provided by the manufacturer (KPG products Ltd, East Sussex, UK).

3.2.7 Data analysis

Analysis of variance (ANOVA) requires the residuals to be normally distributed. Apart from moisture and organic matter, all other data were transformed using the logarithmic transformation ($\log_{10}(x + 0.1)$), to allow this parametric test. The arcsin transformation ($\arcsin(\sqrt{(x/100)})$) was used for moisture and organic matter. The residuals were then tested for normality using the Shapiro–Wilk test, to ensure that they met the assumptions of parametric statistical tests (Doncaster & Davey, 2007). SPSS v.20.0 (SPSS Inc., Chicago, Illinois) was used for all statistical procedures and significance evaluated using $\alpha = 0.050$.

Principal component analysis (PCA) was used to summarize the pattern of interactions among variables, by grouping together variables that were highly correlated with each other into components that explained the total variance in the

environmental conditions beneath *R. ponticum* and in open grassland, at the two sites in the New Forest. Results were pooled across vegetation types and all data were included in a single analysis. Varimax rotation was used to reduce the complexity of the components by making the large loadings larger and the small loadings smaller within each component. As the Kaiser-Meyer-Olkin (KMO) statistic was greater than 0.5 (0.673), the data were suitable for PCA. Any variables that were not correlated with each other were deleted prior to PCA (Wuensch, 2004; Bollen *et al*., 2009).

The effects of site (Exbury Gardens and Minstead Lodge) and vegetation type (open grassland and woodland which had been invaded by *R. ponticum*) on light intensity, soil temperature, air temperature and rainfall (Y), and how this varies with month (March, April, May and June), were examined using the following three-factor nested ANOVA model with repeated measured, with site, vegetation type, and their interaction as fixed factors, and month as a random factor:

$$Y = \text{Month}'_4 \mid S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$$

All four environmental conditions were shown to differ over time (Appendix I), which was expected due to changes in weather through the seasons, as well as increases in shade by surrounding trees due to leaf growth (Kato & Komiyama, 2002). Further analysis was conducted on the average measurements over the four months, which gave the average conditions for the main growing season between March and June, and took into account the differences between each month.

The effects of site (Exbury Gardens and Minstead Lodge) and vegetation type (open grassland and woodland which had been invaded by *R. ponticum*) on the different environmental conditions were examined using the following two-factor fully cross-factored ANOVA models (hence-forth referred to as Models 1a, 1b, 1c, 1d, 1e, 1f, 1g and 1h), with site, vegetation type, and their interaction as fixed factors:

1a) $\log_{10}(\text{Light intensity}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1b) $\log_{10}(\text{Air temperature}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1c) $\log_{10}(\text{Soil temperature}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1d) $\arcsin(\text{Soil moisture}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1e) $\log_{10}(\text{Rainfall}) = S'_{5}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1f) $\log_{10}(\text{Soil pH}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1g) $\arcsin(\text{Organic matter}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

1h) $\log_{10}(\text{Nitrate availability}) = S'_{15}\ (\text{Vegetation type}_2 \mid \text{Site}_2)$

3.3 Results

3.3.1 Correlations between environmental conditions

Strong positive correlations were found between light and soil temperature (0.805), light and air temperature (0.617), and organic matter and moisture (0.742). There was also a positive correlation between pH and nitrate (0.448). Strong negative correlations were found between light and moisture (-0.639), and light and organic matter (-0.636). Negative correlations were also found between pH and organic matter (-0.414), pH and air temperature (-0.334) and between nitrate and air temperature (-0.338), although these were not as strong (Table 3. 1). As there were correlations between all the variables, they were all included in the principal component analysis (Wuensch, 2004; Bollen *et al*., 2009).

The PCA components were ranked according to the degree by which they explain the variances in measure correlations (Wuensch, 2004; Bollen *et al*., 2009). Retention of the first two components produced a model that covered 73.24% of variance. A third component was not included as it only covered an additional 9.93% of variation in measure correlations, and had an eigenvalue of less than 1, so represents less variance than a single variable (Appendix II) (Wuensch, 2004; Bollen *et al*., 2009).

The first component, principal component 1, explains 47.22% of the variation in environmental conditions (Appendix II). It has high positive loadings from light (0.932), soil temperature (0.810) and air temperature (0.664), and high negative loadings from moisture (-0.809) and organic matter (-0.791) (Table 3. 2). Component 1 appears to explain the variation due to vegetation type (i.e. beneath *R. ponticum* versus open grassland), suggesting that variation in light, temperature, moisture and organic matter was due to the presence of *R. ponticum* (Figure 3. 1).

The second component, principal component 2, explains a further 26.02% of the variation in environmental conditions (Appendix II). It has high positive loadings from pH (0.827) and nitrate (0.800), and a high negative loading from air temperature (-0.602) (Table 3. 2). Component 2 appears to explain the variation due to site (i.e. Exbury versus Minstead), suggesting that the variation in soil pH and

nitrate was due more to site, than the presence of *R. ponticum* (Figure 3. 1). Air temperature was found to load on both components, suggesting that although it was affected by the presence of *R. ponticum*, it was also affected by site (Figure 3. 1, Table 3. 2).

Table 3. 1. Correlation matrix, showing the correlations between each of the environmental factors, pooled across vegetation types (n= 60).

	Soil temperature	Air temperature	Light	Moisture	pH	Organic matter	Nitrate
Soil temperature	-	-	-	-	-	-	-
Air temperature	0.488**	-	-	-	-	-	-
Light	0.805**	0.617**	-	-	-	-	-
Moisture	-0.490**	-0.391**	-0.639**	-	-	-	-
pH	0.213	-0.334**	0.260*	-0.170	-	-	-
Organic matter	-0.430**	-0.328**	-0.636**	0.742**	-0.414**	-	-
Nitrate	-0.013	-0.338**	-0.050	-0.102	0.448**	-0.194	-

** P <0.010.

* P <0.050.

Table 3. 2. Rotated component matrix, using varimax rotation, showing the measured loadings of each environmental factor to the first two principal components.

	Component	
	1	2
Light	0.932	-0.034
Moisture	-0.809	-0.134
Organic matter	-0.791	-0.337
Soil temperature	0.810	-0.039
Air temperature	0.664	-0.602
pH	0.245	0.827
Nitrate	0.010	0.800

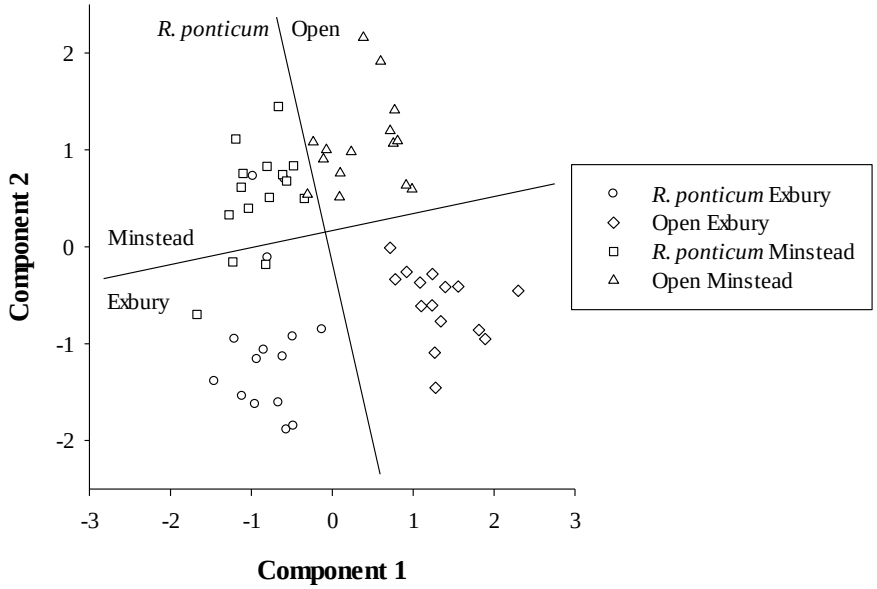


Figure 3. 1. Map of measures, showing all the plots in relation to the first two principle components.

3.3.2 Light intensity

Light availability was reduced beneath stands of *R. ponticum* at both the sites (Vegetation type main effect $F_{1,56}$ = 685.93, P <0.001 from Model 1a analysis). Differences were also observed between sites (Site main effect $F_{1,56}$ = 37.42, P <0.001 from Model 1a analysis), with light availability being lower at Minstead Lodge than at Exbury Gardens, although this was only observed in open grassland

(Site * Vegetation type interaction $F_{1,56}$ = 40.45, P <0.001 from Model 1a analysis), and there was no difference in light availability beneath the rhododendron between the two sites. Average light intensity beneath *R. ponticum* was found to be 95% lower than in open grassland (Figure 3. 2).

3.3.3 Air temperature

Air temperature was lower beneath the *R. ponticum* canopy (Vegetation type main effect $F_{1,56}$ = 55.06, P <0.001 from Model 1b analysis), although this difference was only seen at Exbury Gardens (Site * Vegetation type interaction $F_{1,56}$ = 31.24, P <0.001 from Model 1b analysis). Air temperature was also found to differ between sites (Site main effect $F_{1,56}$ = 239.34, P <0.001 from Model 1b analysis), with higher temperatures observed at Exbury Gardens. Average air temperature under *R. ponticum* was found to be 12.4^{o}C, compared to 13.5^{o}C in open grassland, a difference of only 1.1^{o}C (Figure 3. 3).

The same pattern was observed for the average temperature between the 7th March and the 23rd September, although the average temperature under *R. ponticum* was 15.9^{o}C, compared to 21.1^{o}C in open grassland (Appendix III).

3.3.4 Soil temperature

Soil temperature was reduced beneath *R. ponticum*, compared with that in open grassland at both sites (Vegetation type main effect $F_{1,56}$ = 94.81, P <0.001 from Model 1c analysis). There was no difference between the sites for either rhododendrons or for open grassland (Site main effect $F_{1,56}$ = 2.60, P = 0.113 from Model 1c analysis), and there was no interaction between site and vegetation type (Site * Vegetation type interaction $F_{1,56}$ = 0.31, P = 0.58 from Model 1c analysis). Average soil temperature under *R. ponticum* was found to be 10.0^{o}C, compared to 11.8^{o}C in open grassland, a difference of only 1.8^{o}C (Figure 3. 4).

3.3.5 Water availability

Soil moisture was higher beneath *R. ponticum*, compared with open grassland (Vegetation type main effect $F_{1,56}$ = 34.15, P <0.001 from Model 1d analysis), despite the finding that rhododendrons reduced the rainfall reaching the ground (Vegetation type main effect $F_{1,15}$ = 12.36, P = 0.003 from Model 1e analysis), although these differences were only observed at Exbury Gardens (Site * Vegetation type interaction $F_{1,56}$ = 5.75, P = 0.02 and $F_{1,15}$ = 4.91, P = 0.04 from Model 1d and Model 1e analysis for soil moisture and rainfall respectively) (Figure 3. 5, Figure 3. 6). However, neither soil moisture (Site main effect $F_{1,56}$ = 1.10, P = 0.299 from Model 1d analysis) or rainfall (Site main effect $F_{1,15}$ = 3.20, P = 0.093 from Model 1e analysis) were shown to be affected by site.

3.3.6 Soil pH

Soil pH was lower beneath *R. ponticum*, compared with open grassland (Vegetation type main effect $F_{1,56}$ = 16.08, P <0.001 from Model 1f analysis). Soil pH was also found to be affected by site for both rhododendrons and open grassland (Site main effect $F_{1,56}$ = 40.94, P <0.001 from Model 1f analysis), with pH being more acidic at Exbury Gardens, although there was no interaction between site and vegetation type (Site * Vegetation type interaction $F_{1,56}$ = 1.13, P = 0.29 from Model 1f analysis). Average soil pH under *R. ponticum* was found to be 4.50, compared to 4.99 in open grassland (Figure 3. 7).

3.3.7 Organic matter

Organic matter was higher beneath rhododendrons, compared with open grassland at both sites (Vegetation type main effect $F_{1,56}$ = 30.77, P <0.001 from Model 1g analysis). There were no differences between the two sites (Site main effect $F_{1,56}$ = 0.54, P = 0.47 from Model 1g analysis), and there was no interaction between site and vegetation type (Site * Vegetation type interaction $F_{1,56}$ = 2.34, P = 0.13 from Model 1g analysis) (Figure 3. 8).

3.3.8 Nitrate availability

The presence of *R. ponticum* had no effect on nitrate at either sites (Vegetation type main effect $F_{1,56} = 0.08$, $P = 0.78$ from Model 1h analysis). Nitrate was found to be affected by site (Site main effect $F_{1,56} = 22.87$, $P < 0.001$ from Model 1h analysis), with nitrate availability higher at Minstead Lodge, although there was no interaction between site and vegetation type (Site * Vegetation type interaction $F_{1,56} = 1.44$, $P = 0.24$ from Model 1h analysis) (Figure 3. 9).

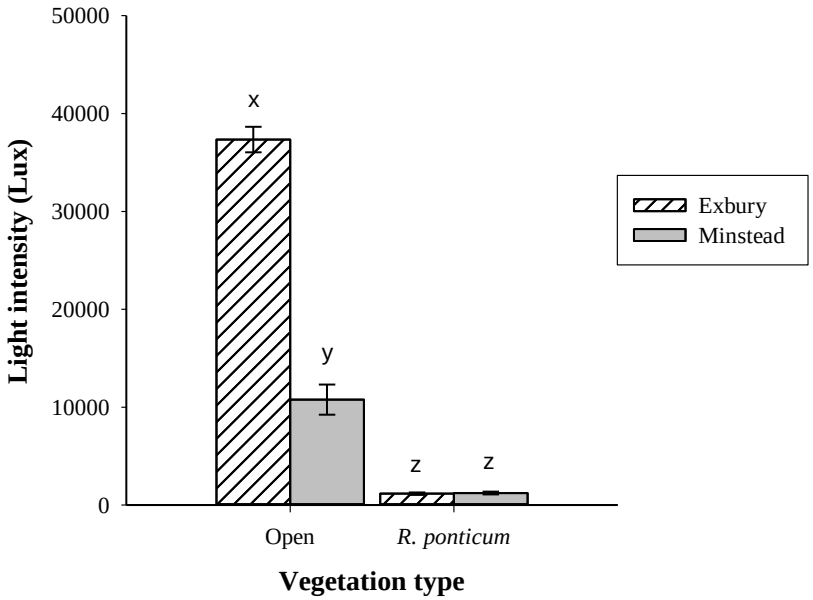


Figure 3. 2. Differences in mean light intensity (LUX) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other ($P < 0.050$).

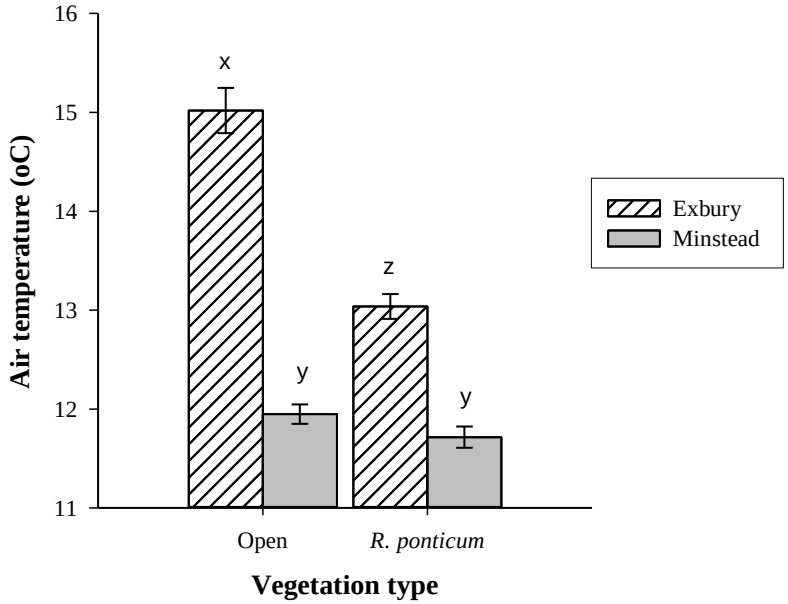


Figure 3. 3. Differences in mean air temperature (^{o}C) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

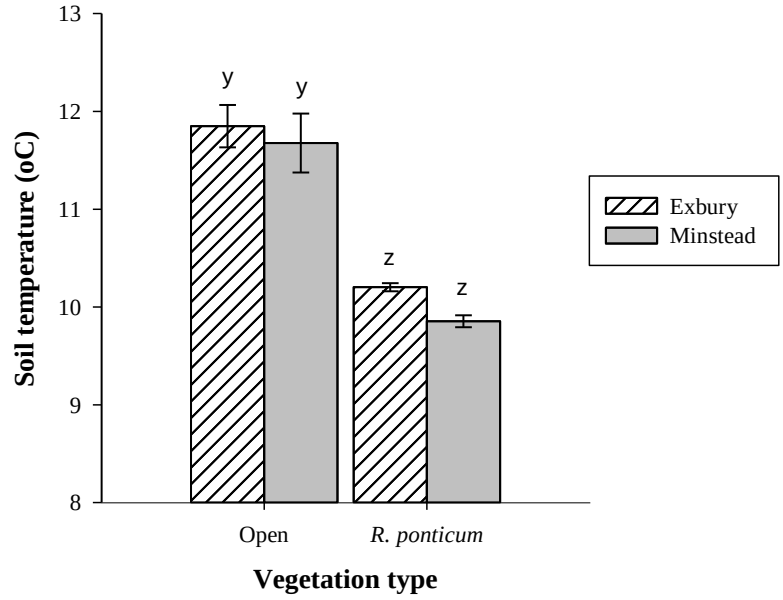


Figure 3. 4. Differences in mean soil temperature (^{o}C) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

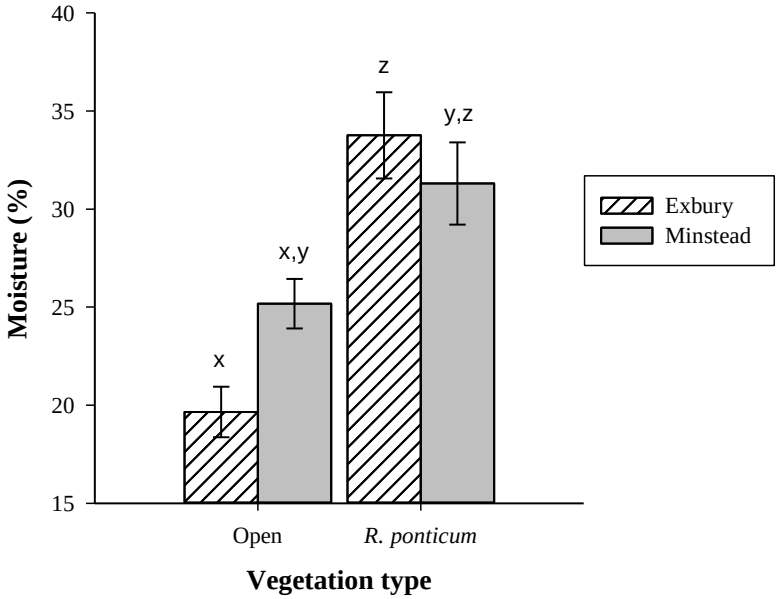


Figure 3. 5. Differences in mean soil moisture (%) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

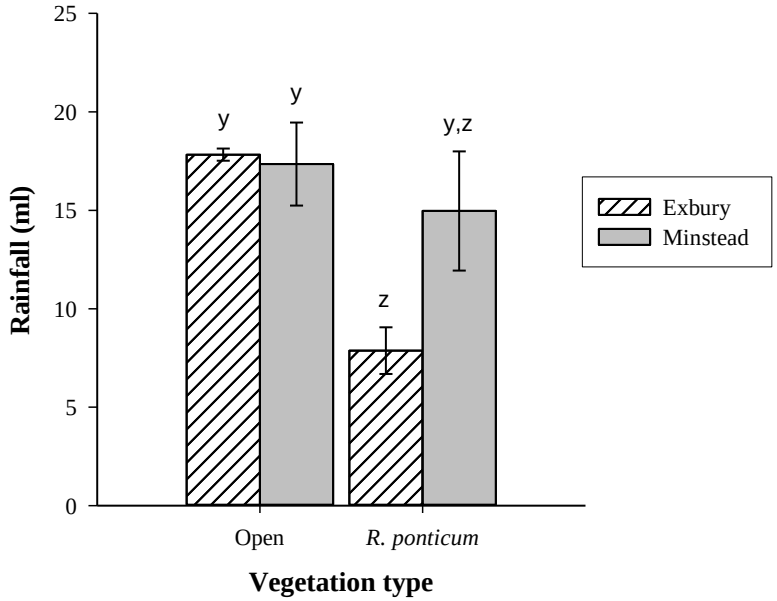


Figure 3. 6. Differences in mean rainfall (ml) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 5). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

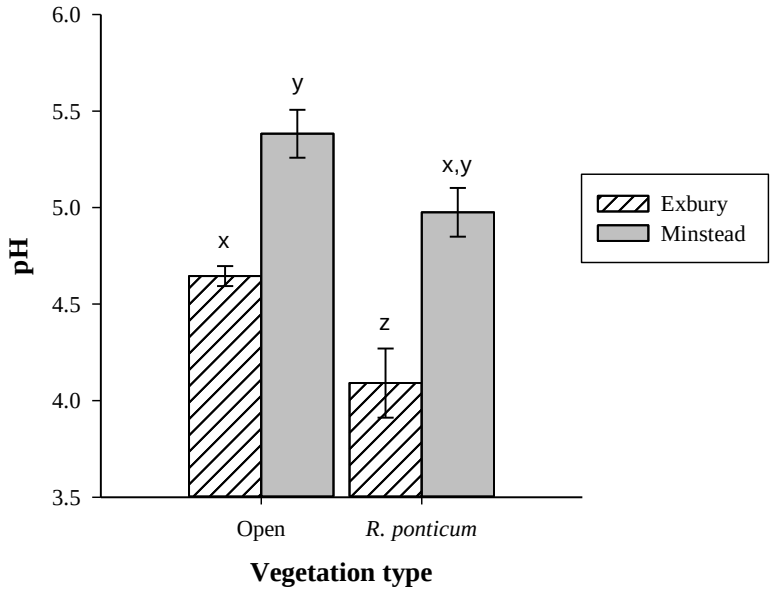


Figure 3. 7. Differences in mean soil pH between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

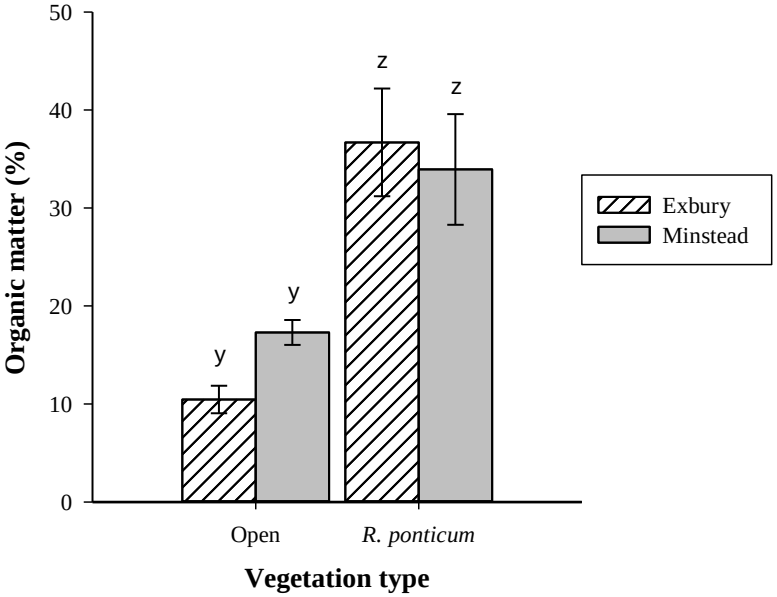


Figure 3. 8. Differences in mean organic matter (%) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

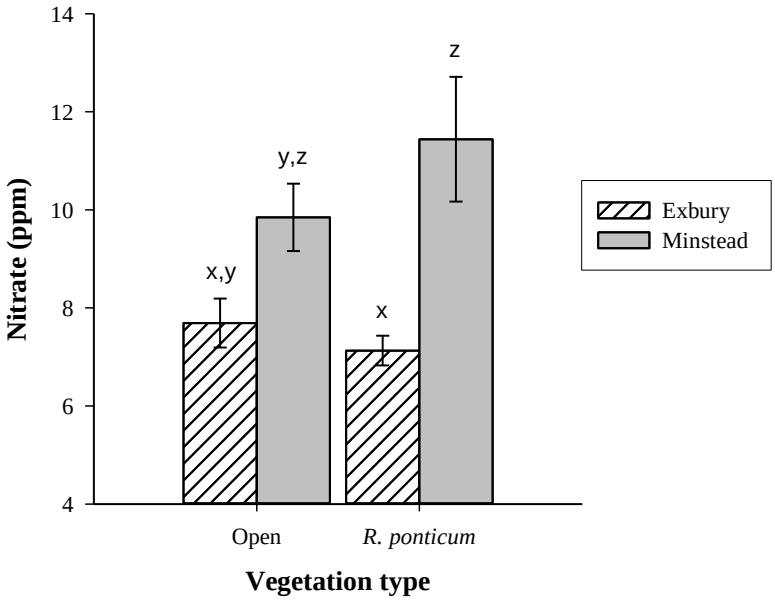


Figure 3. 9. Differences in mean soil nitrate (ppm) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 15). Letters show which areas and sites Tukey's post-hoc analysis identified as being different from each other (P <0.050).

3.4 Discussion

As expected, environmental conditions were different beneath stands of *R. ponticum*, compared to that in open grassland. However, the degree of difference between many of the environmental conditions did not appear to be great enough to have a significant inhibitory effect on native species. It was also expected that light availability would be an important factor, and this was supported by the statistical analysis, which showed that the reduced light availability accounted for the greatest proportion of variation in the environmental conditions.

The effect of rhododendrons on light availability has been widely studied, and competition for light is commonly implicated as being responsible for the impact of *Rhododendron* species on the diversity and cover of native plants (Cross, 1981; Lei *et al*., 2006; Clinton, 1995). The average light intensity under *R. ponticum* at the two sites in the New Forest was 95% lower than in open grassland. Light intensity is extremely important, affecting germination, growth and biomass allocation (Poorter & Nagel, 2000; Raven *et al*., 2005), as well as having a direct effect on photosynthesis and respiration (Gordon, 1969; Lichtenthaler *et al*., 1981). Plant growth is known to be reduced in 95% shade (Leishman & Westoby, 1994; Sack & Grubb, 2002), and several studies testing the effect of reduced light availability beneath rhododendrons have shown that it does play an important role in the inhibition of growth (Clinton & Vose, 1996; Lei *et al*., 2002). Principal component analysis revealed that the reduced light availability beneath *R. ponticum* explained a high proportion of the variation in environmental conditions, with many of the other environmental conditions measured being strongly correlated to light availability. As *R. ponticum* is evergreen, it also means that light availability will be reduced throughout the year, so is likely to have a greater impact. It is therefore likely that the reduced light availability is one of the main reasons why the growth of surrounding vegetation is inhibited.

Soil pH is very important, affecting growth and influencing the dominance of different plant species (Goldberg, 1985; Tilman & Olff, 1991), and the pH of the soil was lower beneath *R. ponticum* than that in open grassland at both sites. Rhododendrons have been shown to prefer acid soils (Cross, 1975), and the principal

component analysis indicated that the soil pH was due more to site, and not due to the presence of the rhododendron, suggesting that the acidic conditions might have been present before the arrival of the rhododendron, which will be investigated in chapter 4. However, even if this is the case, soil pH can have a significant influence on the availability of nutrients (Haynes & Swift, 1986), and root length and density are adversely affected by soil acidity, reducing root-soil contact and affecting nutrient and water uptake (Haling *et al*., 2010). It is therefore likely that the acidic conditions play an important role in the inhibition of growth, although it is not clear whether the difference in pH beneath *R. ponticum* alone could account for the inhibition of growth.

Although other environmental conditions were different beneath *R. ponticum* compared to open grassland, they were unlikely to be responsible for the inhibition of growth of native species for a variety of reasons. Both soil and air temperature were lower beneath *R. ponticum*, which supported previous findings with other *Rhododendron* species (Clinton, 2003). This difference was expected as temperature is directly affected by light availability (Matlack, 1993), and the statistical analysis did show that there was a strong correlation between the two factors, indicating that the reduced temperature was a result of the reduced light availability. Temperature is known to be an important factor, having a direct effect on photosynthesis, respiration and transpiration (Gifford, 1995; Mark & Tevini, 1996) and affecting the germination and establishment of plants (Went, 1953). Soil temperature also affects water and nutrient uptake (Karlsson & Nordell, 1996), herbivore activity (Veteli *et al*., 2002), as well as the activity of soil organisms such as nitrifying bacteria (Fdz-Polanco *et al*., 1994), which can in turn affect the availability of nutrients, and alter the pH (Yan *et al*., 1996). However, the average difference in air temperature and soil temperature between open grassland and areas invaded by *R. ponticum* was 1.1^{o}C and 1.8^{o}C respectively, and previous work has shown that temperature differences of this size are too small to have a significant effect on plant growth (Frank *et al*., 1973).

Organic matter was higher beneath *R. ponticum* than in open grassland. As well as leaves from the rhododendrons, leaves from other species, especially *Q. robur*, were found to collect beneath *R. ponticum*. Leaves of *R. ponticum* have a high lignin content (Arunachalam *et al*., 1998) which means that decomposition is slow, and *R.*

ponticum has been shown to reduce the activity of soil organism, including decomposers (Sutton & Wilkinson, 2007), fungi (Ertürk *et al*., 2009) and earthworms (Boettcher & Kalisz, 1991), by altering environmental conditions (Killham, 1994) and by leaching compounds which have an inhibitory effect (Rice & Pancholy, 1974; Cao *et al*., 2002; Swaroop *et al*., 2005; Ertürk *et al*., 2009). This could result in a build up of undecomposed organic matter. High leaf litter generally increase seed and seedling survival, except for small seeds which might not be able to penetrate the litter layer (Everham *et al*., 1996). It is possible that the thickness of the leaf litter means that seeds cannot become established, although several native plant species have been shown to be capable of growing in the thick leaf litter, and removal of the leaf litter from under *R. ponticum* had no effect on the growth of native species (personal observation). This increase in organic matter can also affect the availability of nutrients by altering ecosystem nutrient cycling (Hobbie, 1992), and can affect the soil pH due to changes in decomposition and nitrification (Van Miegroet & Cole, 1984; Yan *et al*., 1996). Increases in organic matter beneath *R. ponticum* might also lead to an increase in allelopathic compounds (Jalal & Read, 1983), and such compounds will be investigated in chapter 5.

Clinton and Vose (1996), Nilsen *et al*. (2001) and Nilsen and Horton (2002) found that water availability was lower under rhododendrons compared to uninvaded woodland, and proposed that in addition to reduced light availability, this played an important role in the inhibition of growth of native species. One of the ways in which invasive species do this is by reducing the amount of rainfall reaching the ground (Dunbar & Facelli, 1999), and *R. ponticum* in the New Forest was shown to have the same effect. However, soil moisture under *R. ponticum* in the New Forest was higher than that in open grassland. This was likely due to lower evaporation in the shade beneath *R. ponticum* (Matlack, 1993), and the statistical analysis did show that there was a strong negative correlation between water availability and light intensity. Organic matter will also increase the water availability by increasing the water holding capacity of the soil (Hudson, 1994), and again the statistical analysis did show a strong correlation between the two. Water availability has been found to affect nutrient availability, either through uptake (Day *et al*., 1978), due to leaching of basic nutrients (Hagedorn *et al*., 1997), or by increasing microbial activity, which can affect nitrification and decomposition (Stark & Firestone, 1995). These changes in microbial activity, as well as leaching of basic nutrients, can also affect soil pH

(Yan *et al*., 1996). Water availability can also directly affect the rate of growth, and will affect photosynthesis, respiration and transpiration (Puritch, 1973). However, as the moisture at both sites was below field capacity (personal observation), water logging effects would not have been involved (Bramley *et al*., 2007), and rainfall was lower under *R. ponticum*, suggesting that the inhibition of growth could not be due to heavy rainfall damaging or burying seedlings (Cross, 1981).

Although Nilsen *et al*. (2001) and Nilsen and Horton (2002) showed that nutrient resources, particularly cations, were reduced beneath *Rhododendron* species, it was expected that nutrient availability beneath *R. ponticum* would have been higher, as increases in organic matter would increase the nutrient input into the soil (Hobbie 1992). However, there was not a significant difference in nitrate availability between areas of *R. ponticum* and open grassland. *R. ponticum* has been found to have an inhibitory effect on soil organisms (Boettcher & Kalisz, 1991; Sutton & Wilkinson, 2007), resulting in a reduction in decomposition and nitrification. The high lignin content of rhododendron leaves also means that decomposition, and therefore the release of nutrients, is slow (Arunachalam *et al*., 1998), and the statistical analysis showed that there was not a significant correlation between nitrate availability and organic matter beneath *R. ponticum* in the New Forest, and that nitrate availability was more dependent on site than on the presence of the rhododendron. Previous work showed that the availability of other nutrients were reduced (Nilsen *et al*., 2001), which can have a significant effect on the native species, affecting growth and biomass allocation (Ericsson, 1995), community composition (Bedford *et al*., 1999), and plant defence (Coley *et al*., 1985). However, field trials demonstrated that none of these differences in nutrients were associated with inhibition of growth of the species tested (Nilsen *et al*., 2001), and addition of NPK fertilizer to rhododendron humus resulted in no significant difference in germination (Cross, 1975). The increased water availability beneath *R. ponticum* should also increase nutrient uptake (Day *et al*., 1978), suggesting that nutrient availability is unlikely to be responsible for the inhibition of growth of the native species.

Invasive species have been found to affect a number of other environmental conditions, including composition of the atmosphere; either through altered rate of CO_2 uptake and storage (Stratton & Goldstein, 2001) or the emission of nitrogenous gases (Cheng *et al*., 2007), stability of the soil; either by decreasing the soil bulk

density or by leaving the ground bare and liable to erosion (Thiele & Otte, 2007), and fire regime characteristics, such as frequency, intensity and the extent of the fire (D'Antonio & Vitousek, 1992), as well as biotic factors, including pollinator activity (Chittka & Schürkens, 2001), prevalence of disease (Pimentel *et al*., 2005) and herbivory (Gliessman & Muller, 1978). However, these factors are unlikely to be responsible for the inhibition of growth of native species beneath *R. ponticum* in the New Forest (D'Antonio & Vitousek, 1992; Lei *et al*., 2002; Tiwari & Chauhan, 2006; Webber, 2007; Dietzsch *et al*., 2011).

Many of the environmental conditions measured were found to differ between the two sites. Although it would have been better to treat 'site' as a random variable, as only two sites were used, it would have only given one error degree of freedom so the variance could not be calculated. However, by having sub-site replication within each site, this took into account the fixed effects. Light availability and air temperature were both lower at Minstead Lodge compared to Exbury Gardens. Although it is possible that these differences were due to increased cloud cover in the afternoon (Hardy *et al*., 1982) when Minstead Lodge was measured, the same pattern was observed when air temperature was measured at the same time at each site using data loggers. This suggests that the differences were due to the site rather than sampling time. Cloud cover tends to develop inland (Hardy *et al*., 1982), which could explain why light and temperature were reduced at Minstead Lodge, compared to Exbury Gardens which is near the coast. Exbury Gardens also had larger areas of open grassland, whereas at Minstead Lodge the areas were smaller and the surrounding trees could have reduced the light reaching the ground. Water availability and rainfall were also found to be affected by site. If this was due to sampling time then water availability should decrease throughout the day due to evaporation, but water availability was found to be higher at Minstead Lodge. This suggested that it was a site difference, and was due to the reduced light availability at Minstead Lodge. Rainfall was also found to be higher beneath *R. ponticum* at Minstead Lodge compared to Exbury Gardens, although there were no differences between open grassland. This suggested that the difference was not due differences in rainfall, but due to the woodland in which *R. ponticum* was found growing at Minstead Lodge being more open compared to Exbury Gardens, which affected the amount of rainfall reaching the ground (Dunbar & Facelli, 1999). Soil pH and nitrate availability were also found to be higher at Minstead Lodge compared to Exbury

Gardens. Neither of these conditions are likely to be significantly affected by sampling time, but have been found to vary between areas (Cambardella *et al*., 1994; Wilson *et al*., 1997), due to differences in environmental conditions (Yan *et al*., 1996) and soil type (Raven *et al*., 2005). In the following chapter, measurements will be taken at three deciduous and three evergreen sites to take into account site differences, and will alternate between the deciduous and evergreen sites to reduce the chance that any differences between areas are due to sampling time.

Although a number of environmental conditions were shown to be different beneath stands of *R. ponticum* compared to open grassland, based on previous work and a fundamental knowledge of plant growth, light availability and soil pH are the two environmental conditions that were most likely to be responsible. It was expected that light availability would be reduced beneath *R. ponticum*, and reduced light availability alone has been shown to be important in the inhibition of growth beneath other *Rhododendron* species. However, it was not sufficient to explain the inhibition of growth, and previous work suggests that other mechanisms were involved (Clinton & Vose, 1996; Lei *et al*., 2006). Many plants commonly found in the study sites will also tolerate the acidic conditions found beneath *R. ponticum* (Turkington & Burdon, 1983; Hannaway *et al*., 1999), and therefore, on its own, soil pH is unlikely to explain the inhibition of growth. However, there are often interactions between environmental factors, and multiple environmental factors will often have a much greater impact together, than individually (Mittler, 2006). Therefore, it is possible that the combination of the reduced light availability and the acidic pH beneath *R. ponticum* is sufficient to explain the inhibition of growth of native species. By ruling out many of the environmental conditions, fewer variables need to be tested, and in the next chapter the research can focus on the interactions between light availability and soil pH.

Chapter 4

Determining the influence of reduced light availability and soil pH on the inhibition of growth beneath *Rhododendron ponticum*

4.1 Introduction

Changes in environmental conditions, either through resource competition or alteration of ecosystem processes, are often implicated as the cause for the inhibition of native plant growth beneath the invasive shrub *R. ponticum* (Cross, 1981; Nilsen & Horton, 2002). However, few studies have thoroughly tested the effect of these changes on plant growth, and whether they alone could account for the change in ecosystem structure and function (Levine *et al*., 2003).

Many non-native plant species alter light availability differently to the native species (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003), and light availability has been shown to be reduced beneath *Rhododendron* species. Leaves of rhododendrons were found to contain high concentrations of UV-B radiation absorbing pigments, as well as having high UV reflectance (Filella & Peñuelas, 1999), which will affect the amount of radiation reaching the ground. *R. maximum* was shown to reduce direct radiation by 50% and diffuse radiation by over 12% compared to forest without the shrub layer (Lei *et al*., 2006), and the frequency and duration of sunflecks beneath *R. maximum* were reduced by 96% (Nilsen *et al*., 2001). During the growing season, photosynthetically active radiation beneath *R. maximum* was on average 77% lower than in areas without *R. maximum*, and light levels beneath the rhododendron were observed to be less than 2% full sun (Clinton, 1995). However, despite these findings that some rhododendrons can reduce light to levels that can suppress native species, few studies have tested this experimentally.

Lei *et al*. (2002) found that canopy tree survival beneath *R. maximum* in its native range was consistent with the shade tolerance of the species, and Lei *et al*. (2006) showed that the presence of *R. maximum* suppressed average mid-day photosynthesis of both *Quercus rubra* (northern red oak) and *Prunus serotina* (black cherry) seedlings, and daily carbon gain in *Quercus rubra* seedlings was lower in forest with *R. maximum*, consistent with the reduced light availability. Clinton and Vose (1996) also showed that light availability representative of conditions found beneath *R. maximum* in its native range decreased the survival of *Acer rubrum* (red maple), relative to seedlings growing in the open understory, demonstrating that light quantity is important in the regeneration of overstory species. However, while

reduced light availability certainly plays a major role in the inhibition of growth of other native species, other factors must also be involved (Nilsen *et al*., 2001; Lei *et al*., 2006), and Clinton and Vose (1996) showed that percentage germination and survival of *Acer rubrum* in *R. maximum* plots were lower than beneath the shade cloth, suggesting that in addition to the light limitation associated with *R. maximum*, edaphic effects such as low soil moisture or allelopathic compounds might inhibit growth beneath the rhododendron canopy.

Many invasive species can also have an effect on the pH of the soil. Changes in microclimate, water availability, organic matter and nutrient cycling can all have an indirect effect on soil pH (Yan *et al*., 1996), and rhododendrons have been shown to affect all those factors (Nilsen *et al*., 2001). A number of invasive species have also been shown to have a direct effect on the soil pH. Rhododendrons use nitrogen in the form of ammonium more efficiently than nitrate, which will lower the pH in the rhizosphere due to the uptake of excess cations over anions (Nye, 1981), and several *Rhododendron* species, including *R. dauricum* and *R. formosanum*, have been found to release phenolic acids into the soil which can also lower the pH (Cao *et al*., 2002; Chou *et al*., 2010).

Soil pH is very important for plant growth, affecting water and nutrient uptake (Haynes & Swift, 1986; Haling *et al*., 2010). Litter decomposition is reduced beneath rhododendrons, mainly due to the lower soil pH associated with the presence of rhododendron (Sariyildiz & Küçük, 2009), and leachates from rhododendrons were found to contain phenolic acids, which can affect microbial activity and nutrient availability (Grayston *et al*., 1997). It is therefore likely that that changes in the pH of the soil plays an important role in the alteration of community composition. However, there is insufficient evidence as to whether differences in pH beneath invasive species are enough to account for the inhibition of growth of native species.

Other abiotic conditions, including nutrient and water availability, have also been shown to be different beneath rhododendrons compared to uninvaded woodland (Cross, 1975, 1981; Nilsen *et al*., 2001). However, based on the findings in chapter 3, most of these conditions are unlikely to be responsible for the impact on native communities, and light availability and soil pH are the two environmental factors most likely to be responsible for the inhibition of growth beneath *R. ponticum* in the

New Forest. Although on their own these conditions are unlikely to explain the inhibition of growth of native plants (Clinton & Vose, 1996; Nilsen *et al*., 2001; Lei *et al*., 2006), there are often interactions between environmental factors, and multiple environmental stresses will often have a much greater impact together, than individually (Mittler, 2006). Despite this, there has been insufficient work investigating the interactions between light availability and soil pH, and it is possible that the combination of the reduced light availability and the acidic pH beneath *R. ponticum* is sufficient to explain the inhibition of growth of native species.

Although many environmental conditions were found to be different beneath *R. ponticum* compared to uninvaded woodland (Cross, 1975, 1981), rhododendrons have difficulty becoming established in areas where there is already continuous ground cover from native plants (Cross, 1981). A number of studies have looked at the biotic interactions between the exotic and the native species, and have suggested that native species are limited or excluded by competition from the exotic dominants (MacDougall & Turkington, 2005). However, recent work has suggested that exotic dominance could have more to do with altered disturbance regimes, which are more limiting for native species than they are for invasive species (MacDougall & Turkington, 2005). Therefore, it is possible that conditions were already unsuitable for the growth of native plants, but provided conditions which allowed *R. ponticum* to become such a serious problem. Understanding whether the environmental conditions are actually being altered by the arrival of the species is essential from a restoration standpoint, if native species are to re-establish in exotic-dominated systems (Levine *et al*., 2003).

In this chapter, the effect of simulated light quantity and soil pH representing conditions in the understories of native deciduous and evergreen woodland and deciduous and evergreen woodland invaded by *R. ponticum* were tested on the germination and growth of two naturally occurring species, while controlling for other differences between invaded and uninvaded sites (e.g. water availability, allelopathy, etc.). The effect of plant growth and litter decomposition of *R. ponticum* and three other naturally occurring species was tested on soil pH, under controlled conditions.

Aims:

1. To determine whether light and pH conditions in areas where *R. ponticum* are found growing were unsuitable for the growth of native plants before the arrival of the rhododendron.

2. To determine the extent to which *R. ponticum* reduces the light availability in the understory.

3. To determine whether *R. ponticum* is lowering the pH, either actively during growth or through the decomposition of organic matter.

4. To determine whether light availability and soil pH beneath *R. ponticum* can explain the inhibition of growth of native species.

4.2 Methods

4.2.1 Light availability and soil pH beneath *R. ponticum* in the New Forest

Light availability and soil pH were measured in March 2011 in three deciduous and three evergreen woodlands in the New Forest which had been invaded by *R. ponticum*. Based on chapter 3, these were the two environmental conditions that were most likely to be involved in the inhibition of growth beneath *R. ponticum*. At each of the sites, measurements were taken at fifteen plots beneath *R. ponticum*, 5 m from each of the chosen rhododendrons, and at fifteen plots in neighbouring areas of open grassland (see 2.1).

Light availability was measured as photosynthetically active radiation (PAR), taken 50 cm above ground level at each of the plots. To reduce differences due to weather, measurements for light were all collected on a cloudless day, on the 16th March 2011, between 08.00 and 16.00. Although these measurements were collected before the deciduous trees were fully in leaf, light availability was still lower than in open grassland, and any inhibitory effects of the reduced light availability on the native species would be increased as the growing season progressed. To reduce the chance that any differences between areas were due to sampling time, measurements were taken alternating between the deciduous and evergreen sites, and at each site, measurements were collected alternating between the three different treatments (i.e. open grassland, beneath *R. ponticum*, and 5 m from *R. ponticum*). PAR was measured using the SKP 200 PAR meter with the SKP 210/S sensor (Skye Instruments Ltd, Powys, UK). All measurements were taken twice at each plot, and an average was calculated.

To measure the pH of the soil, approximately 300 ml of soil was collected from each plot on the 18th or 19th March 2011 (see 2.2). The pH of the sample was then measured on the 21st March 2011 by making a soil suspension using the standard 1:1 soil to water ratio method used by the U.S. Department of Agriculture (see 3.2.5).

4.2.2 Effect of light availability and soil pH on native species

In order to determine the role of light availability and soil pH beneath *R. ponticum* on native plant growth, a glasshouse experiment was conducted in April 2011. Field research often involves too many interactive factors to be able to assess the influence of any one environmental factor on plant growth (Short, 1987). Therefore, a mesocosm experiment was conducted using passive greenhouse apparatus. Although this can have an effect on light transmittance, temperature, gas exchange and humidity (Debevec & MacLean, Jr., 1993; Kennedy, 1995a), it could still be used to provide an experimental ecosystem with close to natural conditions, but in which environmental factors can be realistically manipulated (Debevec & MacLean, Jr., 1993; Kennedy, 1995b). This allowed the effect of simulated light intensity and soil pH environments representative of conditions beneath *R. ponticum* on the germination and growth of native species to be assessed, while controlling for other variables such as rainfall and herbivores, which could affect the results. Light availability (PAR) and pH were manipulated to simulate the conditions found in open grassland, in deciduous and evergreen woodland, and beneath *R. ponticum*, using the method used by Reinhart *et al*. (2006). To make the growing conditions similar to natural conditions, the glasshouse was naturally lit, as artificial light can have an effect on plant growth (Raven *et al*., 2005), and factors such as temperature and day length were not controlled.

Twenty seeds of *L. perenne* or *T. repens* (see 2.4) were placed in 9 cm Petri-dishes lined with a 9 cm by 7 cm oval cotton wool pad. Seeds were obtained from Emorsgate Seeds (Norfolk, UK - www.wildseed.co.uk). PAR levels were reduced to 57% shade, 75% shade or 97% shade, using shade cloth, which was tested to ensure that it was accurate. Petri dishes were also left in full sun. This was to simulate light conditions found in deciduous woodland, evergreen woodland, beneath *R. ponticum*, and in open grassland, respectively (see 4.3.1). The cotton wool pad was moistened with 15 ml of distilled water, the pH of which was adjusted to pH 4.62, pH 4.16 or pH 3.66, using sulphuric acid, a method commonly used in hydroponics (Rolot & Seutin, 1999). This was to simulate pH conditions found in open grassland, deciduous woodland and evergreen woodland respectively (see 4.3.1). *R. ponticum* was found to have no effect on the pH of the soil in either of the woodland types (see 4.3.1), so pH under rhododendrons was not tested separately. Six repeats were

conducted for each treatment, and the experiment was repeated twice. Petri dishes were arranged randomly within the greenhouse to avoid differences in light intensity and temperature. Air temperature was measured under each of the shade treatments to validate the experiment, although Tukey's post-hoc analysis revealed no difference in temperature between treatments. Germination, root elongation and leaf appearance were measured based on the methods used by Anjum and Bajwa (2005), Thomas (1981) and Streck *et al*. (2003) (see 2.6).

4.2.3 Effect of *R. ponticum* on soil pH

In order to test the effect of *R. ponticum* on soil pH, another mesocosm experiment was conducted in a naturally lit glasshouse between March and June 2011 so that conditions could be maintained as close to natural as possible, but in which environmental factors, such as herbivory and rainfall which could have affected the concentration of compounds in the soil (Jefferson & Pennacchio, 2003; Thelen *et al*., 2005), could be controlled. To make the growing environment similar to natural conditions, other factors (such as temperature and day length) were not controlled. *R. ponticum* plants were collected from Cadnam Common (50.9445^{o} N, 1.5886^{o} W) and *R. ponticum* leaves were collected from Exbury Gardens (50.8025^{o} N, 1.3983^{o} W) on the 3rd March. *Q. robur*, *P. sylvestris* and *Prunus rotundifolia* (common laurel) were used as controls to test whether *R. ponticum* has a greater effect on pH than other species. *Q. robur* and *P. sylvestris* were chosen as they are both commonly found growing with *R. ponticum* in the New Forest. *P. rotundifolia* was chosen as it is an invasive, evergreen shrub, similar in size and habit to *R. ponticum*, making it an ideal species to use as a comparison (RHS, 2006; personal observation). *P. sylvestris* was purchased from Country Homes and Gardens (Norfolk, UK - www.stores.ebay.co.uk/countryhomesandgardens) and *Q. robur* and *P. rotundifolia* were purchased from Hedgehogs Nursery (Fife, UK - www.scotplantsdirect.co.uk). All plants were between 30 and 60 cm tall. Leaves from *Q. robur* were collected from Exbury Gardens, needles from *P. sylvestris* were collected from Turf Hill Inclosure (50.9530^{o} N, 1.7266^{o} W), and leaves from *P. rotundifolia* were collected from Brook (50.9311^{o} N, 1.6001^{o} W).

Twenty three cm plastic pots were filled with 5 litres Levington ericaceous compost (Fargro Ltd, West Sussex, UK), and pots were assigned 1 of the 13 treatments (see Table 4. 1). To test the effect of plant growth on the pH of the soil, one of the four species was planted into pots numbered 1, 4, 7 and 10. The roots of the plants were washed before planting to remove any soil, which could have affected the results. To test the effect of decomposition on the pH of the soil, 30 g of chopped leaves (approximately 1-3 cm^2) from one of the four species was mixed in with the compost in pots 2, 5, 8 and 11. To test the combined effect of growth and decomposition on pH, one of the four species was planted into pots numbered 3, 6, 9 and 12, and 30 g of chopped leaves of that species was mixed in with the compost. Pot number 13 was left with just compost, to use as a negative control.

Table 4. 1. Pot number with the assigned treatment, testing the individual and combined effect of growth and decomposition of the four species, on soil pH.

Number	Species	Treatment
1	*R. ponticum*	Plant
2	*R. ponticum*	Leaves
3	*R. ponticum*	Plant + Leaves
4	*Q. robur*	Plant
5	*Q. robur*	Leaves
6	*Q. robur*	Plant + Leaves
7	*P. sylvestris*	Plant
8	*P. sylvestris*	Leaves
9	*P. sylvestris*	Plant + Leaves
10	*P. rotundifolia*	Plant
11	*P. rotundifolia*	Leaves
12	*P. rotundifolia*	Plant + Leaves
13	Control	

Forty repeats were conducted for each treatment. The pots were randomly arranged in the glasshouse to avoid differences in light intensity and temperature, and were watered with rain water when required. Soil samples were collected from each pot after 100 days and the pH was measured by making a soil suspension using the standard 1:1 soil to water ratio method used by the U.S. Department of Agriculture (see 3.2.5). After measuing the pH, plants were left in the glasshouse to use in

subsequent experiments testing the effect of *R. ponticum* on soil under controlled conditions.

In order to test the effect of rhododendron leaf decomposition on pH while controlling for environmental variables, a second experiment was conducted based on the method used by Ibrahima *et al*. (1995). On the 29th February 2012, *R. ponticum* leaves were collected from two deciduous woodlands (Exbury Gardens and Poundhill Inclosure) and two evergreen woodlands (Copythorne Common and Poundhill Inclosure) (see 2.1). Eight plots were selected at each site, and approximately 20 g of rhododendron leaves were collected from each of the plots. The leaves were chopped to 0.5-2 cm^2 and then oven dried at 70°C for 24 hours, to speed up decomposition. Two grams of dried leaves were then placed in a glass jar with 400 ml of rain water, with one repeat per sample. Jars were left with just rain water to use as a control. The jars were stored in the dark at 20°C, and the pH of each sample was measured after 1 hour, 3 hours, 24 hours, 72 hours, 168 hours, 240 hours and 360 hours, using an HI-98127 digital pH meter (Hanna Instruments Ltd, Bedfordshire, UK). To check for accuracy and consistency, the pH of each sample was measured twice, and an average was taken.

4.2.4 Data analysis

As analysis of variance (ANOVA) requires the residuals to be normally distributed, the data were transformed using the logarithmic transformation ($\log_{10}(x + 0.1)$) to allow this parametric test. The residuals were then tested for normality using the Shapiro–Wilk test, to ensure that they met the assumptions of parametric statistical tests (Doncaster & Davey, 2007). SPSS v.20.0 (SPSS Inc., Chicago, Illinois) was used for all statistical procedures and significance evaluated using $\alpha = 0.050$.

The effect of vegetation type (open grassland (deciduous), open grassland (evergreen), deciduous woodland, evergreen woodland, deciduous woodland which had been invaded by *R. ponticum* and evergreen woodland which had been invaded by *R. ponticum*) on light availability (PAR) and soil pH was examined using the following one-factor ANOVA models (hence-forth referred to as Models 2.1a and

2.1b), with vegetation type as a fixed factor:

$$\text{2.1a)}\quad \log_{10}(\text{PAR}) = S'_{45}(\text{Vegetation type}_6)$$

$$\text{2.1b)}\quad \log_{10}(\text{pH}) = S'_{45}(\text{Vegetation type}_6)$$

Tukey's post-hoc analysis was conducted on 'vegetation type' to identify which groups differ from which others. As site was shown to have no effect (P >0.050), this was not included in the statistical analysis.

The effects of light availability (full sun, 57% shade, 75% shade and 97% shade) and pH (3.66, 4.16 and 4.62) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 2.2), with light availability (PAR), pH, and their interaction as fixed factors:

$$\log_{10}(\text{Y}) = S'_{12}(\text{PAR}_4 \mid \text{pH}_3)$$

Tukey's post-hoc analysis was conducted on both 'PAR' and 'pH' to identify which groups differ from which others.

The effect of *R. ponticum* (growth, decomposition, growth and decomposition, and compost control) on soil pH was examined using the following one-factor ANOVA model (hence-forth referred to as Model 2.3), with *R. ponticum* as a fixed factor:

$$\log_{10}(\text{pH}) = S'_{40}(R.\ ponticum_4)$$

Tukey's post-hoc analysis was conducted on '*R. ponticum*' to identify which groups differ from which others.

The effect of a) growth, b) decomposition, or c) growth and decomposition of different species (*R. ponticum*, *Q. robur*, *P. sylvestris* and *P. rotundifolia*) on soil pH was examined using the following one-factor ANOVA models (hence-forth referred to as Models 2.4a, 2.4b and 2.4c), with species as a fixed factor:

$$\log_{10}(\text{pH}) = S'_{40}(\text{Species}_4)$$

Tukey's post-hoc analysis was conducted on 'species' to identify which groups differ from which others.

The effect of decomposition of *R. ponticum* leaves (with *R. ponticum* (n= 32) and without *R. ponticum* (n= 8)) on soil pH, and how this changes over time (1 hour, 6 hours, 24 hours, 72 hours, 168 hours, 240 hours and 360 hours), was examined using

the following two-factor repeated-measures ANOVA model (hence-forth referred to as Model 2.5), with *R. ponticum* as a fixed factor and time as a random factor:

$$\log_{10}(\text{pH}) = \text{Time}_{7} \mid S'_{8\text{-}32}\ (R.\ ponticum_{2})$$

Tukey's post-hoc analysis was conducted on 'time' to identify which groups differ from which others. As site and woodland type were shown to have no effect ($P > 0.050$), these were not included in the statistical analysis.

4.3 Results

4.3.1 Light availability and soil pH beneath *R. ponticum* in the New Forest

i. Light availability (PAR)

Light availability differed between vegetation type (Vegetation type main effect $F_{5,264}$ = 199.09, P <0.001 from Model 2.1a analysis), with differences between all vegetation types, except between the two open sites (P = 0.532) and beneath *R. ponticum* in the two woodlands (P = 0.588). Average light availability (PAR) was 389.94 μmol·m^{-2}·s^{-1} in open grassland. Average light availability (PAR) was 168.66 μmol·m^{-2}·s^{-1} in deciduous woodland and 97.48 μmol·m^{-2}·s^{-1} in evergreen woodland, a reduction of 56.8% and 75.0% respectively. Average light availability beneath *R. ponticum* was 10.31 μmol·m^{-2}·s^{-1} irrespective of woodland type, a reduction of 97.4% relative to open grassland (Figure 4. 1).

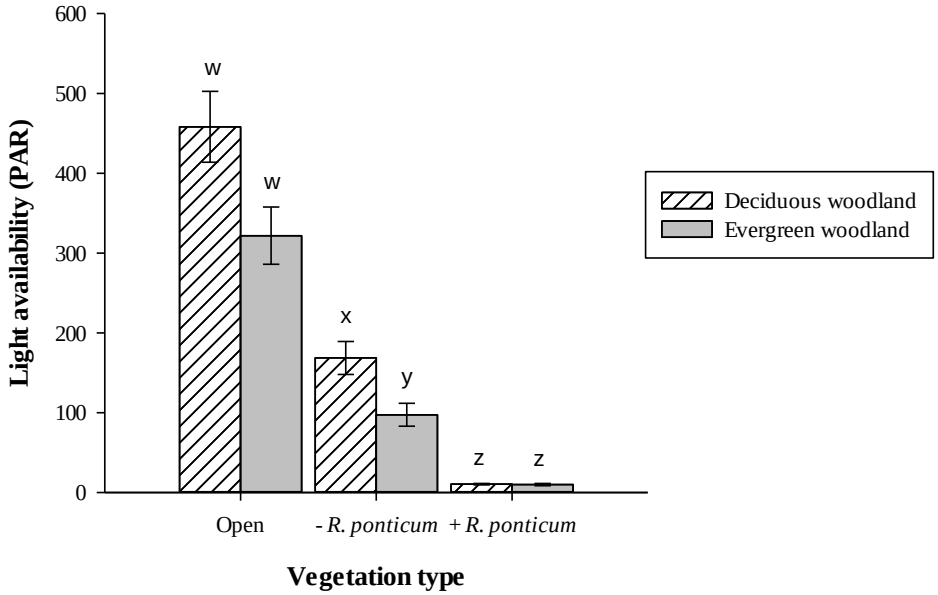


Figure 4. 1. Differences in mean light availability (photosynthetically active radiation (PAR)) in open grassland adjacent to deciduous and evergreen woodland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 45). Letters show which vegetation types Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Soil pH

Soil pH differed between vegetation types (Vegetation type main effect $F_{5,264}$ = 25.02, P <0.001 from Model 2.1b analysis). However, there was no difference between the two open sites (P = 0.379) or between *R. ponticum* in deciduous woodland and the neighbouring area of open grassland (P = 0.400). The presence of *R. ponticum* was also found to have no effect on the pH of the soil in either of the woodlands (P = 0.723 and P = 0.990 for deciduous and evergreen woodlands respectively). Average soil pH in open grassland was 4.62, average pH in deciduous woodland was 4.16, and average pH in evergreen woodland was 3.66 (Figure 4. 2).

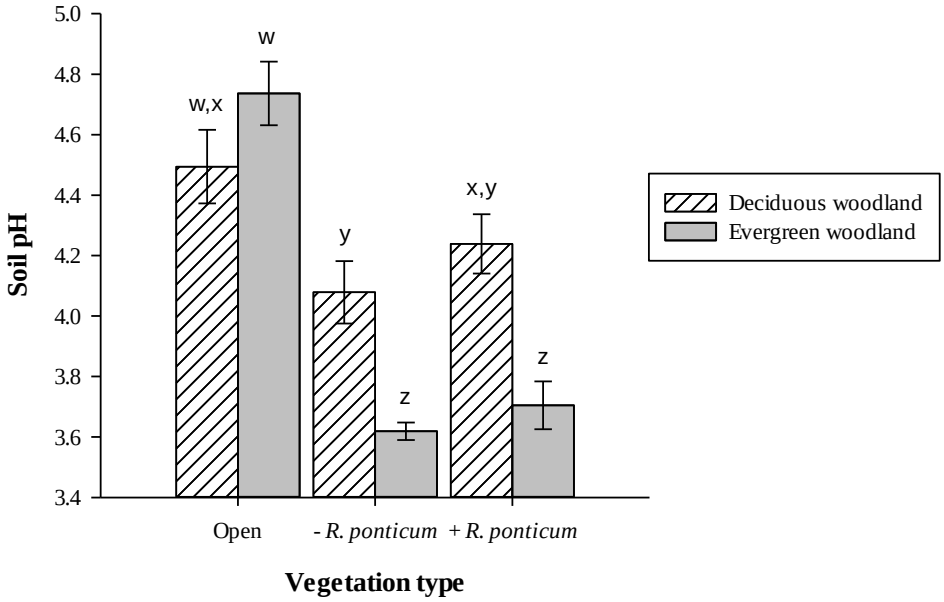


Figure 4. 2. Differences in mean soil pH in open grassland adjacent to deciduous and evergreen woodland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 45). Letters show which vegetation types Tukey's post-hoc analysis identified as being different from each other (P <0.050).

4.3.2 Effect of light intensity and soil pH on the germination and growth of native species

i. Germination

Light availability was shown to have an effect on the germination of *L. perenne* (PAR main effect $F_{3,132} = 3.51$, $P = 0.017$ from Model 2.2 analysis), although the only difference was between seeds in full sun and in 75% shade ($P = 0.010$), with higher germination in 75% shade, conditions found in evergreen woodland (Figure 4. 3). Light availability also had an effect on *T. repens* (PAR main effect $F_{3,132} = 77.92$, $P < 0.001$ from Model 2.2 analysis), with germination increasing with increasing shade treatments, although there was no difference between 57% shade and 75% shade ($P = 0.216$) (Figure 4. 4). pH was also found to have an effect on germination of *T. repens* (pH main effect $F_{2,132} = 3.32$, $P = 0.039$ from Model 2.2 analysis), although the only difference was between seeds in pH 4.16 and pH 4.62 ($P = 0.050$), with higher germination in pH 4.62, conditions found in open grassland (Figure 4. 4). However, pH had no effect on the germination of *L. perenne* (pH main effect $F_{2,132} = 0.93$, $P = 0.398$ from Model 2.2 analysis), and there was no interaction between light availability and pH for either of the species tested (PAR * pH interaction $F_{6,132} = 1.40$, $P = 0.221$ and $F_{6,132} = 0.84$, $P = 0.543$ from Model 2.2 analysis for *L. perenne* and *T. repens* respectively).

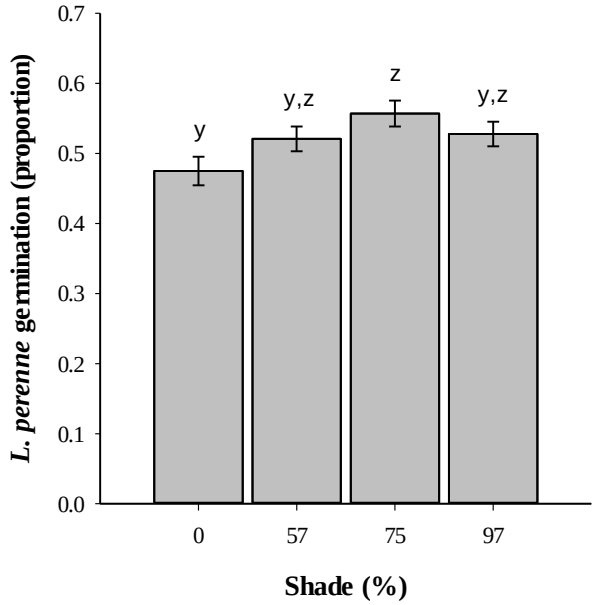


Figure 4. 3. Differences in mean germination of *L. perenne* in different light (PAR) conditions, representative of conditions in open grassland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 36). pH was not shown as it was found to have no effect. Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

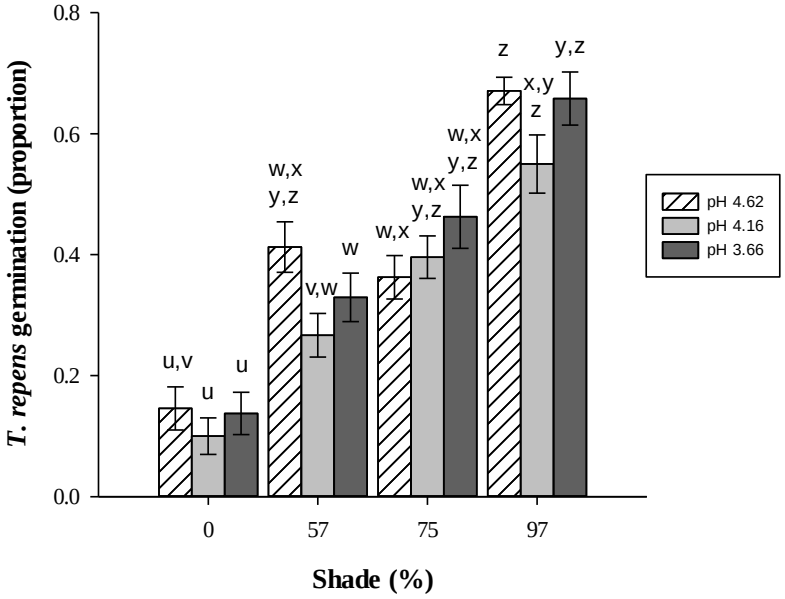


Figure 4. 4. Differences in mean germination of *T. repens* in different light (PAR) and pH conditions, representative of conditions in open grassland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 12) . Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Light availability was shown to have no effect on root elongation of *L. perenne* (PAR main effect $F_{3,132} = 1.77$, $P = 0.157$ from Model 2.2 analysis). However, it did have an effect on *T. repens* (PAR main effect $F_{3,132} = 8.32$, $P < 0.001$ from Model 2.2 analysis), with root elongation being higher in the full sun treatment compared to any of the others ($P < 0.010$), although there was no difference between any of the shade treatments ($P > 0.050$) (Figure 4. 5). pH was found to have no effect on either of the species tested, either on its own (pH main effect $F_{2,132} = 1.33$, $P = 0.268$ and $F_{2,132} = 1.63$, $P = 0.200$ from Model 2.2 analysis for *L. perenne* and *T. repens* respectively) or in combination with light availability (PAR * pH interaction $F_{6,132} = 0.14$, $P = 0.990$ and $F_{6,132} = 1.15$, $P = 0.336$ from Model 2.2 analysis for *L. perenne* and *T. repens* respectively).

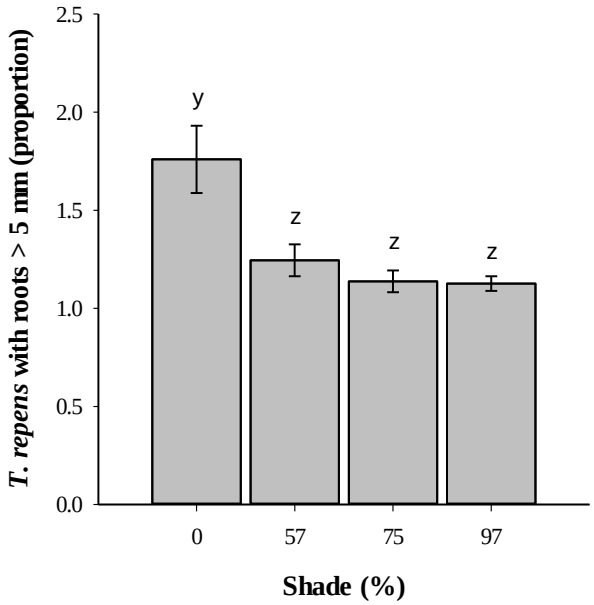


Figure 4. 5. Differences in mean root elongation of *T. repens* in different light (PAR) conditions, representative of conditions in open grassland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 36). pH was not shown as it was found to have no effect. Letters show which treatments Tukey's post-hoc analysis identified as being different from each other ($P < 0.050$).

iii. Leaf appearance

Light availability was found to have an effect on leaf appearance of *L. perenne* seedlings (PAR main effect $F_{3,132} = 15.53$, $P < 0.001$ from Model 2.2 analysis), with leaf appearance faster in the higher shade treatments, although there were no differences between full sun and 57% shade (open grassland and deciduous woodland) ($P = 0.999$) or between 75% shade and 97% shade (evergreen woodland and beneath *R. ponticum*) ($P = 0.073$) (Figure 4. 6). Light availability also had an effect on *T. repens* seedlings (PAR main effect $F_{3,132} = 26.23$, $P < 0.001$ from Model 2.2 analysis), with rate of leaf appearance decreasing with increasing shade. However, there were no differences between full sun and 57% shade (open grassland and deciduous woodland) ($P = 0.235$), or between 57% shade and 75% shade (deciduous woodland and evergreen woodland) ($P = 0.645$) (Figure 4. 7). On its own, pH was found to have no effect on leaf appearance of either of the species tested (pH main effect $F_{2,132} = 1.25$, $P = 0.289$ and $F_{2,132} = 1.29$, $P = 0.279$ from Model 2.2 analysis for *L. perenne* and *T. repens* respectively), and there was no combined effect of light and pH on *L. perenne* (PAR * pH interaction $F_{6,132} = 1.80$, $P = 0.104$ from Model 2.2 analysis). However, there was an interaction between light and pH on *T. repens* (PAR * pH interaction $F_{6,132} = 2.33$, $P = 0.036$ from Model 2.2 analysis), with reduced light availability having less of an inhibitory effect in the pH 4.16 and pH 3.66 treatments (the pH conditions found under *R. ponticum* in deciduous and evergreen woodland) (Figure 4. 7).

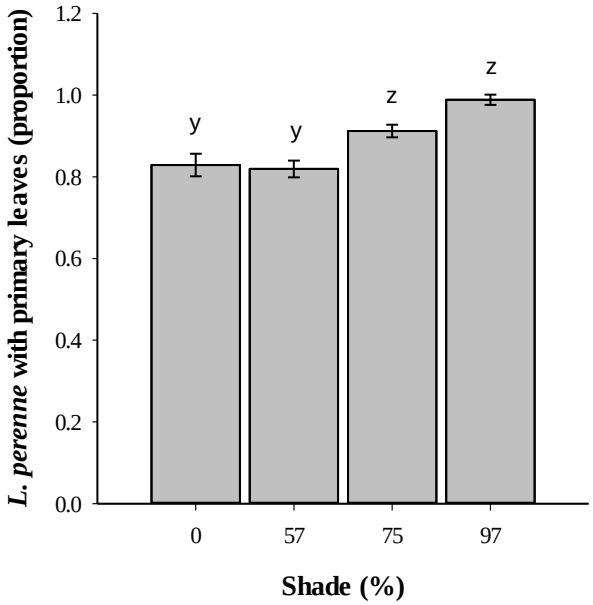


Figure 4. 6. Differences in mean leaf appearance of *L. perenne* in different light (PAR) conditions, representative of conditions in open grassland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 36). pH was not shown as it was found to have no effect. Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

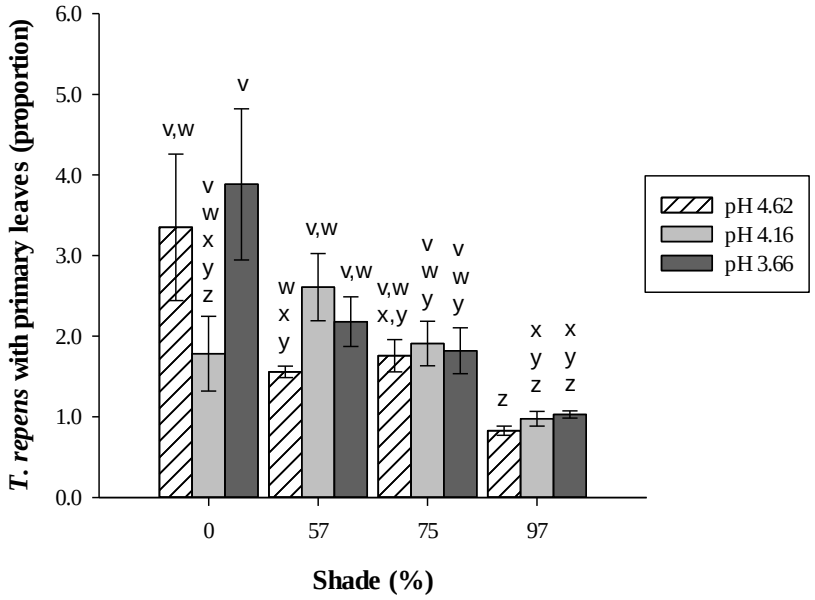


Figure 4. 7. Differences in mean leaf appearance of *T. repens* in different light (PAR) and pH conditions, representative of conditions in open grassland, in native deciduous and evergreen woodland, and in deciduous and evergreen woodland that had been invaded by *R. ponticum* (+/- 1 SE) (n= 12). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

4.3.3 Effect of *R. ponticum* on soil pH

On their own, neither growth nor decomposition of *R. ponticum* was shown to have an effect on soil pH (P >0.050) (Figure 4. 8). Although decomposition was no different from any of the other species tested (Species main effect $F_{3,156}$ = 0.45, P = 0.720 from Model 2.4b analysis), growth of the other three species was shown to increase the pH, which meant that soil which had supported *R. ponticum* was more acidic than any of the other species (Species main effect $F_{3,156}$ = 14.83, P <0.001 from Model 2.4a analysis) (Figure 4. 9). There was however an additive effect, and the combination of growth and decomposition of *R. ponticum* increased the pH of the soil (*R. ponticum* main effect $F_{3,156}$ = 3.73, P = 0.013 from Model 2.3 analysis) (Figure 4. 8), although this was no different from any of the other species tested (P >0.050) (Figure 4. 10).

However, the pH of rain water containing decomposing *R. ponticum* leaves was lower than the rain water control throughout the experiment (*R. ponticum* main effect $F_{1,38}$ = 384.67, P <0.001 from Model 2.5 analysis). pH was also found to change over time (Time main effect $F_{6,228}$ = 5.14, P <0.001 from Model 2.5 analysis), although this differed between treatments (*R. ponticum* * Time interaction $F_{6,228}$ = 53.91, P <0.001 from Model 2.5 analysis), with pH increasing over time in the water without rhododendron leaves, but initially decreasing over time in water containing rhododendron leaves, but then increasing (Figure 4. 11).

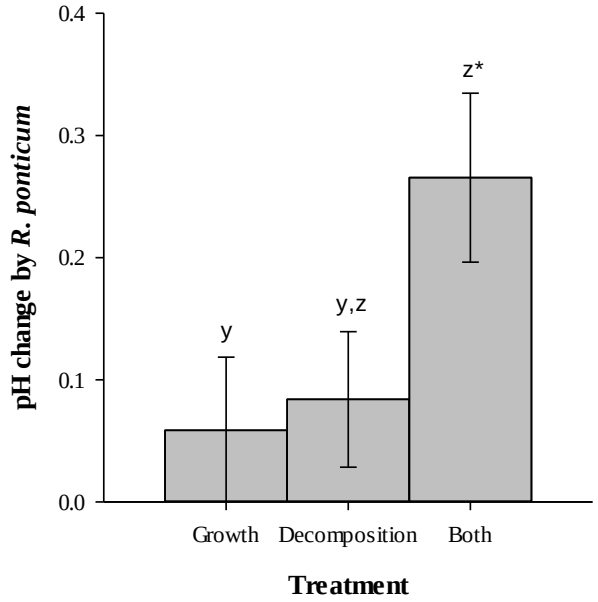


Figure 4. 8. Differences between the mean change in soil pH after 100 days by growth of *R. ponticum*, decomposition of *R. ponticum* and the combination of the two (+/- 1 SE) (n= 40). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (*P* <0.050). * indicates which treatments Tukey's post-hoc analysis identified as being different from control (zero) (n= 40) (*P* <0.050).

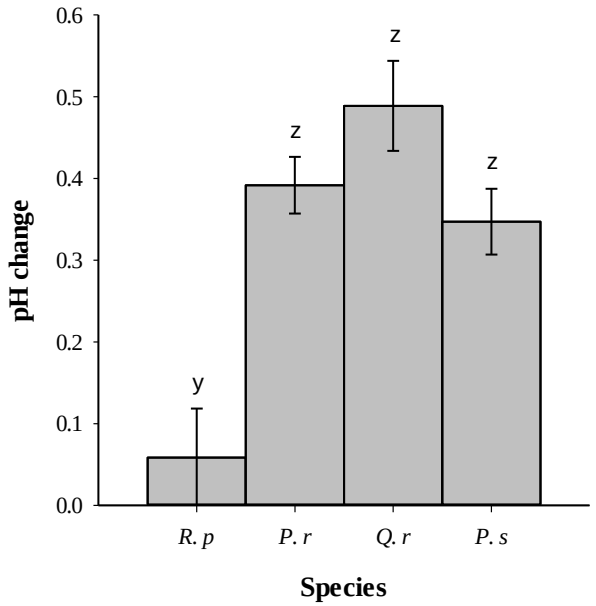


Figure 4. 9. Differences between the mean change in soil pH after 100 days by growth of *R. ponticum* (*R. p*), *P. rotundifolia* (*P. r*), *Q. robur* (*Q. r*) and *P. sylvestris* (*P. s*) (+/- 1 SE) (n= 40). Letters show which species Tukey's post-hoc analysis identified as being different from each other (P <0.050).

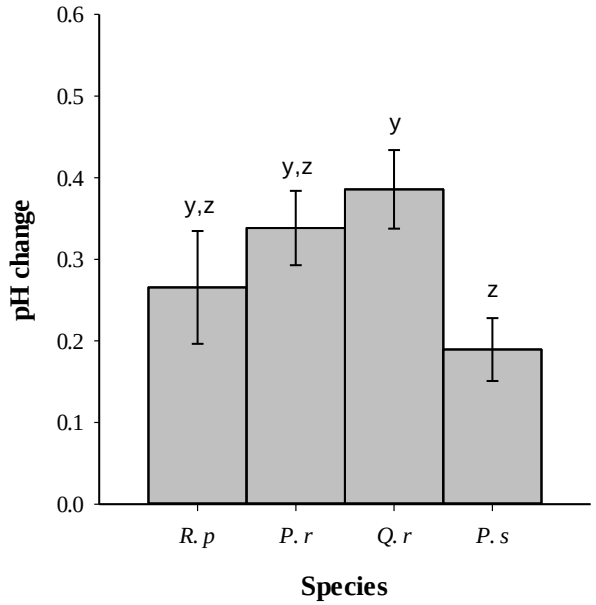


Figure 4. 10. Differences between the mean change in soil pH after 100 days by the combination of growth and decomposition of *R. ponticum* (*R. p*), *P. rotundifolia* (*P. r*), *Q. robur* (*Q. r*) and *P. sylvestris* (*P. s*) (+/- 1 SE) (n= 40). Letters show which species Tukey's post-hoc analysis identified as being different from each other (P <0.050).

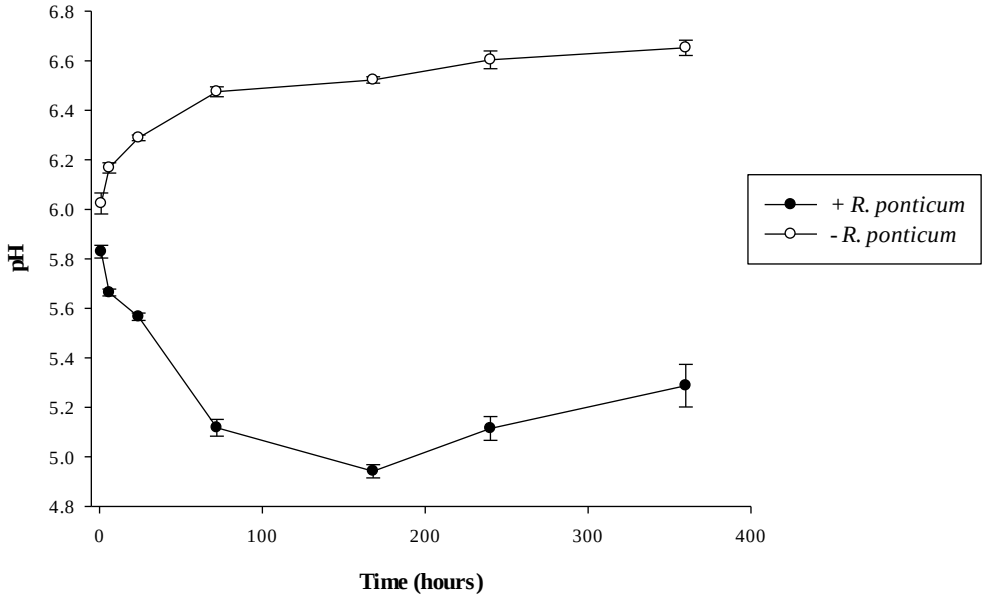


Figure 4. 11. Mean change in pH over time in rain water containing decomposing *R. ponticum* leaves (n= 32) and in rain water without rhododendron leaves (n= 8) (+/- 1 SE).

4.4 Discussion

Although light availability and soil pH were both different beneath *R. ponticum* compared to open grassland, neither of them could explain the inhibition of growth of the native species, either individually or in combination. Environmental conditions were also shown to already be different in uninvaded woodland compared to open grassland before the arrival of the rhododendron, and although they were not limiting for the native species, they might have provided the ideal conditions allowing *R. ponticum* to establish.

Average light availability beneath *R. ponticum* in the New Forest was found to be 97% lower than in neighbouring areas of open grassland. Light availability is extremely important, affecting germination, growth and biomass allocation (Poorter & Nagel, 2000), and reduced light availability beneath invasive species is often implicated as the key factor responsible for the inhibition of growth of native vegetation (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). However, the reduced light availability beneath *R. ponticum* had no effect on the germination of *L. perenne* compared to that in full sun, and germination of *T. repens* was actually found to be faster in the shaded treatment. Germination of a number of species of plants has been shown to be faster in lower light availabilities (McLaren & McDonald, 2003). This could be due to due to higher soil moisture availability in the shade due to lower evaporation (Webster & Day, 1993), and as seeds of *T. repens* are small and at risk from desiccation (Tow & Lazenby, 2001), this could explain why germination was faster in the shade.

Plants have evolved complex sensing mechanisms which trigger morphological changes to avoid shade. The shade-avoidance response redirects resources and growth potential into increased growth to optimize light capture by plants, resulting in plants with a reduced root system (Lichtenthaler *et al*., 1981; Morelli & Ruberti, 2000), and root elongation of *T. repens* was shown to be reduced. As *R. ponticum* has adapted to grow in shaded conditions (Cross, 1981), this could allow the rhododendron to outcompete native species for water and nutrient resources as it would have a more extensive root system (Erfmeier & Bruelheide, 2005). However, light availability was shown to have no inhibitory effect on the root development of

L. perenne. The shade avoidance response also results in plants which are taller with larger leaves (Eriksen & Whitney, 1981; Lei *et al*., 2002), and leaf development of *L. perenne* was shown to be faster in the shaded treatments. However, the reduced light availability beneath *R. ponticum* was found to have an inhibitory effect on leaf appearance of *T. repens*. These findings demonstrate that although the reduced light availability beneath *R. ponticum* is important in affecting the growth of the native species, on its own it is not sufficient to explain the inhibition of growth.

Although some species are capable of survival and growth under very low light conditions, irrespective of their shade tolerance (Metcalfe, 1996; Hastwell & Facelli, 2003; McLaren & McDonald, 2003), as *R. ponticum* is evergreen, light availability will be reduced throughout the year, so is likely to be one of key factors responsible for the impact on the native species. Light availability can have a significant effect on plant mortality (Saverimuttu & Westoby, 1996), and Lei *et al*. (2002) demonstrated that seedling survival rate was lower under rhododendrons, with order of mortality consistent with the shade tolerance of the species. Therefore, it is possible that over a longer period of time, the reduced light availability found beneath *R. ponticum* could be sufficient to inhibit survival of the native species, and further work is needed to test whether this is the case. However, even in an evergreen understory there are still 'safe sites' where light availability is higher (Nilsen *et al*., 2001), and although previous work has shown that although light availability often plays an important role in the inhibition of growth of native species, it was rarely found to be solely responsible, and often other mechanisms were involved (Clinton & Vose, 1996; Levine *et al*., 2003; Lei *et al*., 2006).

The soil beneath *R. ponticum* in the New Forest was found to be more acidic compared to neighbouring areas of open grassland, which can affect growth and influence nutrient availability (Haynes & Swift, 1986; Tilman & Olff, 1991). Root length and density are also adversely affected by soil acidity (Haling *et al*., 2010). Although *L. perenne* and *T. repens* prefer pH conditions between about 5.5 and 7.0, both have been shown to have extremely broad edaphic tolerances, growing on soils ranging from markedly acidic to highly calcareous (Turkington & Burdon, 1983; Hannaway *et al*., 1999), and the pH conditions beneath *R. ponticum* had no effect on the germination, root elongation or leaf appearance of *L. perenne* or on root elongation or leaf appearance of *T. repens*. Only germination of *T. repens* was found

to be reduced. Although this might have given *R. ponticum* the advantage, and allowed it to outcompete the native species, only the pH beneath *R. ponticum* in deciduous woodland had any inhibitory effect, indicating that on its own, pH could not explain the inhibition of growth beneath *R. ponticum*.

Multiple environmental stresses have often been shown to have a greater impact on plant growth, than individual stresses (Mittler, 2006). However, the only observed effect of the combination of light availability and soil pH on the two species tested, was that in the pH conditions found beneath *R. ponticum*, shade was found to have less of an inhibitory effect on the leaf appearance of *T. repens*. Although a number of other environmental conditions were different beneath stands of *R. ponticum* compared to open grassland, it was not possible to test the influence of them all. This chapter focussed on light availability and soil pH as the two conditions that, based on the findings in chapter 3, were thought most likely to be responsible for the inhibition beneath *R. ponticum* in the New Forest. It is possible that multiple environmental stresses, such as competition for light, water and nutrients, and biotic factors, such as increased herbivory and competition for pollinators, which, based on previous work and a fundamental knowledge of plant growth, were considered unlikely to have a significant effect, could have been having a far greater effect on the native species when they were subjected to the combination of stresses. However, allelopathy is often implicated as being responsible for the impact of invasive species (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003), and previous work suggested that *R. ponticum* might be having an allelopathic effect on the native species (Rotherham & Read, 1988), which will be investigated in the following chapters.

Although light availability and soil pH were both found to be lower beneath *R. ponticum*, recent work has suggested that rather than these changes in environmental conditions being due to the arrival of the invasive species, the conditions were already present and were more limiting for native species than they were for the invasive species, which therefore dominate (MacDougall & Turkington, 2005). Seedlings of *R. ponticum* have difficulty becoming established in areas where there is already continuous ground cover from native plants (Cross, 1981), which suggests that conditions in the areas invaded by *R. ponticum* were already limiting for the native species.

As seeds of *R. ponticum* are at serious risk from desiccation, they are limited to shaded sites where there is more moisture (Cross, 1981). Therefore, the reduced light availability in the woodland could already have been unsuitable for the growth of native species before the arrival of the rhododendron. However, only root elongation and leaf appearance of *T. repens* were shown to be reduced by the light availability in the two woodlands, compared to full sun. Although this might have given *R. ponticum* a competitive advantage over some species, the reduced light availability in the two woodlands had no inhibitory effect on the germination, root elongation and leaf appearance of *L. perenne*, or the germination of *T. repens*, compared to that in full sun, suggesting that the shaded conditions in the woodlands were still suitable for the growth of native species.

R. ponticum has also been shown to prefer acid soils, and growth in soils with a pH greater than 5.0 is poor, with the plants showing signs of mineral deficiencies (Cross, 1975). *Q. robur* and *P. sylvestris* were the most dominant species in the areas that had been invaded by *R. ponticum*, and both have been found to lower the soil pH (Duryea *et al*., 1999), suggesting that the acidic conditions might already have been present. Previous studies have shown that soil in the New Forest is generally acidic (Tubbs, 2001; West, 2010), and Wilson *et al*. (1997) found that average pH in woodland in the New Forest was 4.0 or lower. This is not suprising considering the large areas of heathland and the presence of other calcifuge plants, including *Ulex europaeus* (gorse) and *P. sylvestris*, which are known to prefer acidic sites (Tubbs, 2001). However, rhododendrons have been found to grow in magnesium-rich soils with a pH as high as 8.6 (Tod, 1959, cited in Cross, 1975). Not only that, but plants that absorb nitrogen as ammonia, which includes rhododendrons, tend to lower the pH in the rhizosphere, due to the uptake of excess cations over anions (Nye, 1981), so it would therefore be expected that *R. ponticum* would have an effect on soil pH. Although the decomposition of *R. ponticum* leaves in water caused a decrease in the pH, decomposition of *R. ponticum* under controlled conditions had no significant effect on soil pH. Under controlled conditions, growth of *R. ponticum* was also found to have no significant independent effect on soil pH. However, the combination of the two was found to increase the pH of the soil, although this was no different from any of the other species tested (*Q. robur*, *P. sylvestris* or *P. rotundifolia*). There was also no difference in pH between native woodland and woodland invaded by *R. ponticum*, for either of the woodland types. This suggested that the soil beneath *R.*

ponticum was already more acidic, and although pH conditions in the two woodland types did not explain the inhibition of growth of the native species, it might have been one of the reasons why the rhododendron was able to become established.

Although the reduced light availability and soil pH beneath *R. ponticum* were found to have an effect on the germination and growth of *T. repens* and *L. perenne*, the effects were small, and environmental conditions alone appear unable to explain the inhibition of growth of native species in the New Forest. Many of the species found growing near *R. ponticum* are also better adapted to growth in shaded conditions than the two test species (Sterry, 2006), and many are found growing in the acidic conditions found beneath *R. ponticum* (personal observation), so environmental conditions are even less likely to have a significant effect on the native woodland species. *R. ponticum* is known to inhibit the growth of the native species (Dehnen-Schmutz *et al*., 2004), and this raises the question of allelopathic interaction. Many invasive species have been shown to release compounds into the soil which exert an allelopathic effect on the surrounding vegetation (Callaway & Aschehoug, 2000; Bais *et al*., 2003), which is often shown to be responsible for the inhibition of growth. However, previous work has also demonstrated the importance of environmental factors (Nilsen *et al*., 1999). It is possible that the effects of the allelopathic compounds are being enhanced by the environmental conditions found under rhododendrons, and it is the combination of the two that is responsible for the inhibition of growth of the native species (Nilsen *et al*., 1999; Singh *et al*., 2001), and this will be the focus of the research in the next chapters.

Chapter 5

Determining the influence of allelopathy on the inhibition of growth beneath *Rhododendron ponticum*

5.1 Introduction

R. ponticum has become a serious threat to biodiversity across the United Kingdom, inhibiting the growth of the native vegetation and altering community structure (Dehnen-Schmutz *et al*., 2004). Several studies have shown that environmental conditions are different beneath rhododendrons compared to uninvaded woodland (Cross, 1975; Nilsen *et al*., 2001). However, chapters 3 and 4 have indicated that environmental conditions do not explain the inhibition of growth beneath *R. ponticum* in the New Forest, suggesting that it might be having an allelopathic effect on the native species. Allelopathy refers to any direct or indirect harmful effect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment (Singh *et al*., 2001). It can affect many aspects of plant ecology (Muller, 1966; Einhellig, 1994), and can be extremely important on the success of non-native species, helping to explain why some exotic species are so successful at invading natural plant communities (Callaway & Aschehoug, 2000; Bais *et al*., 2003).

The *Ericaceae* are known to be a particularly inhibitory family (Del Moral & Cates, 1971). *Empetrum hermaphroditum* has been shown to have an inhibitory effect on the germination of *Populus tremula* (Nilsson *et al*., 1998), and on the germination, growth and survival of *P. sylvestris* (Nilsson *et al*., 2000). This was thought to be due to the bibenzyl batatasin-III, which was isolated from *Empetrum hermaphroditum* and shown to have an inhibitory effect (Nilsson *et al*., 2000), although a number of other allelopathic compounds, including phenolic acids and condensed tannins, were also found in the leaves and humus (Gallet *et al*., 1999).

Vaccinium myrtillus has also been shown to produce compounds, which prevent the natural regeneration of *Picea abies* (subalpine spruce) and *Picea mariana* (black spruce) (Mallik & Pellissier, 2000). Fresh leaves, leaf leachate, humus, and humus leachate all reduced the dry weight of primary roots of both *Picea mariana* and *Picea abies*, and germination of the two spruces were reduced in soil which had supported *Vaccinium myrtillus* (Gallet, 1994; Mallik & Pellissier, 2000). High concentrations of triterpenoid compounds known to have allelopathic effects were identified in the fruits and leaves of *Vaccinium myrtillus* (Szakiel & Mroczek, 2007).

Water soluble extracts of fresh leaves and soil of *Kalmia angustifolia* (sheep laurel) were also found to have an inhibitory effect on the primary root and shoot growth of *Picea mariana*, and a number of allelopathic compounds were identified, including *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, syringic acid and ferulic acid (Zhu & Mallik, 1994).

Aqueous extracts of *Calluna vulgaris* (common heather), *Daboecia cantabrica* (Irish heath), *Erica arborea* (tree heath), *Erica australis* (Spanish heath) and *Erica scoparia* (besom heath) were all found to inhibit root and hypocotyl growth of *Trifolium pratense* (red clover) (Ballester *et al*., 1977, 1979, 1982), and intact flowers of *Daboecia cantabrica* inhibited germination (Ballester *et al*., 1982). Aqueous extracts obtained from soils dominated by *Erica australis* were also shown to have inhibitory effects on the germination of *Trifolium pretense* and *T. repens* (Carballeira & Cuervo, 1980). A number of phenolic hydrosoluble compounds, including protocatechuic acid, vanillic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid, were identified as possible phytotoxins (Ballester *et al*., 1977; Carballeira, 1980).

Rhododendron species have also been shown to produce compounds which have an allelopathic effect on the native vegetation. Leachates from the flowers, leaves, litter and organic matter of *R. formosanum* were shown to inhibit the radicle growth of *Ocimum basilicum* (basil), *Brassica chinensis* (Chinese cabbage), *Amaranthus inamoenus* (Chinese spinach), *Bidens pilosa* (cobbler's pegs), *Ageratum houstonianum* (flossflower) and *Lactuca sativa* (lettuce) (Chou *et al*., 2009, 2010), and leachates from *R. maximum* litter had an inhibitory effect on the germination and root elongation of *Lepidium sativum* (cress) and *Lactuca sativa* (Nilsen *et al*., 1999). Aqueous extracts from the stems and leaves of *R. nivale* were also shown to have an inhibitory effects on plumule and radicle growth of *Rhodiola fastigiata* (clustered rhodiola) (Zheng *et al*., 2009), and extracts from leaves of *R. arboretum* reduced the germination of *T. pretense* (Modgil & Kapil, 1990). Extracts from the leaves and litter of *R. albiflorum* were also found to inhibit *Hordeum vulgare*, *Bromus tectorum* and *Pseudotsuga menziesii* (Del Moral & Cates, 1971).

Rhododendron species have been shown to contain a number of phytotoxic compounds, including diterpenoids and phenolics (Klocke *et al*., 1991; Bao *et al*., 2003; Swaroop *et al*., 2005; Kemertelidze *et al*., 2007; Sharma *et al*., 2010). Flavonoids, such as quercetin and leucoanthocyanidins, have been identified in over two hundred and six *Rhododendron* species, while gossypetin, another flavonoid that is generally rare in the angiosperms, has been found in over one hundred and fifty six species (Harborne & Williams, 1971). Several other phytotoxic phenolics known to have inhibitory effects, including ferulic acid, *p*-coumaric acid, syringic acid, vanillic acid and p-hydroxybenzoic acid were identified in the leaves of *R. dauricum* and *R. formosanum* (Cao *et al*., 2002; Chou *et al*., 2010), tannins have been identified in the leaves of *R. macrophyllum* (Kraus *et al*., 2004), and several courmarins, including umbelliferone and scopoletin, have been isolated from the leaves of *R. luteum* (Komissarenko *et al*., 1973).

Although allelopathy refers to the interaction between plants, many of the same compounds are involved in different types of interaction (Rice, 1974). It is thought that many of the compounds produced by plants which have an allelopathic effect are primarily involved in defending the plant against herbivores and pathogens (Wink, 1988), and previous work has shown that many compounds which play an important role in plant defence, either by affecting feeding behaviour or inhibiting insect growth (Klocke *et al*., 1991; Liu *et al*., 2008; Tripathi *et al*., 2011), also have allelopathic properties. These compounds are also involved in pigmentation, UV protection, plant development and signaling (Gould & Lister, 2006). It is likely that the compounds then leach into the soil during rainfall or are released during decomposition (Singh *et al*., 2001), and although they might give the plant an advantage over other species, the allelopathic effect does not appear to be an intentional process. Although it is difficult to prove whether plants do actively produce alleopathic compounds as a way of out-competing the native species, it is accepted that allelopathy can be extremely important on the success of non-native species, helping to explain why some exotic species are so successful at invading natural plant communities (Callaway & Aschehoug, 2000; Bais *et al*., 2003). Several compounds, such as grayanoid diterpenes (including andromedotoxin), which are toxic to the native fauna, have been identified in the tissues of *R. ponticum* (Koca & Koca, 2007; Li *et al*., 2013), and leachates from the leaves and flowers of *R. ponticum* have been shown to possess antibacterial and antifungal properties (Erturk

et al., 2009). Therefore, it is possible that the same compounds are having an allelopathic effect on native plant species beneath *R. ponticum*.

Although it is likely that allelopathy is involved in the impact of rhododendron, a study by Rotherham and Read (1988) appears to be the only one to investigate the influence of allelopathy on the inhibition of growth beneath *R. ponticum*. They found that soil which had supported *R. ponticum* under controlled conditions had an inhibitory effect on the growth of *Festuca ovina*, but did not determine how the compounds were released into the environment (i.e. whether it was leachates or root exudates, during decomposition, or whether they were being modified in the soil by microorganisms). There was also no work to determine whether the compounds persist in the natural environment in concentrations sufficient to affect the native plant species (Rotherham & Read, 1988). In this chapter, *R. ponticum* was assayed for its allelopathic properties, while controlling for differences in environmental conditions between invaded and uninvaded sites (e.g., light availability, soil pH, etc.). Aqueous leachates from the above ground parts of the plant were tested, as well as root exudates, on the germination and growth of two naturally occurring species. Soil which had supported *R. ponticum* was also tested to determine whether allelopathic compounds build up over time and persist in the soil.

Aims:

1. To determine whether aqueous leachates from new leaves, old leaves, decaying leaves or flowers of *R. ponticum* have an allelopathic effect on native species.
2. To determine whether root exudates from *R. ponticum* have an allelopathic effect on native species.
3. To determine whether soil which has supported *R. ponticum* has an allelopathic effect on native species.

5.2 Methods

5.2.1 Plant leachates

In order to test whether leachates from the above ground parts of *R. ponticum* have an allelopathic effect on the germination and growth of native species, plant material was collected in two deciduous and two evergreen woodland sites in the New Forest, which had been invaded by *R. ponticum* (see 2.1). Approximately 10 g of new leaves (less than one year old), 10g of old leaves (more than one year old) and 10 g of flowers were collected from four *R. ponticum* plants at each site on the 17th May 2011 (see 2.1). All plant material was transported in a cool box in separate plastic bags, and immediately air dried on paper towels at approximately 20°C for 24 hours, before aqueous leachates were collected. Approximately 20 g of old leaves were also collected from each rhododendron on the 16th February 2011 to test whether plants release leachates during decomposition. The leaves were chopped to speed up decomposition, and then placed in 5 x 10 cm 1 mm mesh bags. The bags were then buried 10 – 15 cm below the surface in open grassland (50.9283° N, 1.5717° W) for 90 days to allow the microorganisms to break down the leaves (Santos *et al*., 1984), and then the decaying plant material was removed from the bags. Two percent aqueous leachates from the new leaves, old leaves, decaying leaves and flowers of *R. ponticum* were then collected following the method used by Cruz-Ortega *et al*. (2002), by placing 0.5 g of air dried plant material in a 100 ml plastic beaker containing 25 ml of distilled water. This concentration has been used in previous studies investigating the allelopathic effects of plant leachates (Zhang & Fu, 2010). Beakers were also left with just distilled water to use as a control. Beakers were left for 24 hours, and then the solution was filtered through Whatman No. 1 filter paper to remove the plant material.

In a second experiment to test whether higher concentrations of leaf leachates have a greater inhibitory effect on the germination and growth of the two test species, approximately 50 g of leaves were collected from eight *R. ponticum* plants at each site on the 3rd April 2012. Forty percent aqueous leachates from the leaves of *R. ponticum* were then collected by placing 40 g of air dried plant material in a 250 ml plastic beaker containing 100 ml of distilled water. Serial dilutions were made to

give 50 ml of 40%, 20%, 10% and 5% concentration leaf leachate solutions (Jose & Gillespie, 1998). Beakers were also left with 100% distilled water to use as a control.

5.2.2 Root exudates

To test the effect of exudates from the roots of *R. ponticum*, *R. ponticum* seed (Nickys Nursery, Kent, UK) were sown into a plastic seed tray (38 x 24 x 6 cm) containing J. Arthur Bowers ericaceous compost (Totton allotments, Hampshire, UK) on the 8th April 2010. The tray was placed in a naturally lit glasshouse, and was watered with distilled water once a week using capillary matting. Once the seeds had germinated, seedlings were transplanted into individual 6 cm pots filled with J. Arthur Bowers ericaceous compost. The pots were placed back in the glasshouse, and as well as being watered once a week with distilled water, they were also fed once a month using 0.5% Miracle-Gro azalea, camellia and rhododendron liquid plant food (Morrisons, Hampshire, UK). Plants (between 10 and 15 cm tall) were carefully removed from the compost on the 27th February 2012. To reduce damage to the root system, which would have affected the amount of exudate released, any remaining soil was carefully removed by rinsing with 0.1% Miracle-Gro all purpose concentrated liquid plant food (Morrisons, Hampshire, UK). Nutrient solution was used, as 100% distilled water would affect the turgor of the root cells, resulting in an increase in the amount of exudates (Aulakh *et al*., 2001).

Root exudates were collected hydroponically based on the method used by Subbarao *et al*. (2006). Twenty 350 ml plastic beakers were filled with 250 ml of distilled water containing 0.1% Miracle-Gro all purpose concentrated liquid plant food (Morrisons, Hampshire, UK). A *R. ponticum* plant was placed in half of the beakers so the roots were submerged, and the other half were left with just nutrient solution to use as a control. Beakers were left for 24 hours, and then the plants were removed from the solution.

5.2.3 Soil

In order to test the effect of leachates from soil from beneath *R. ponticum* on the germination and growth of native species, approximately 200 g of soil was collected from each plot in the two deciduous and the two evergreen woodland sites in the New Forest on the 30th January 2012 (see 2.1). Approximately 200 g of soil was also collected from the ten pots which had supported to *R. ponticum* under controlled conditions and from the ten pots containing the control soil (see 4.2.3) on the 20th June 2011. After air drying, aqueous leachates were then collected based on the method used by Inderjit and Dakshini (1994) (see 2.3). Another 5 g of soil was collected from eight of the pots which had supported *R. ponticum* under controlled conditions and from eight of the pots containing the control soil on the 3rd September 2012, in order to test whether there was a greater effect when seeds were sown directly into soil which had supported *R. ponticum*.

5.2.4 Separating the above ground and below ground effects

In order to separate the above ground and below ground effects of *R. ponticum*, a field experiment based on the methods used by Nilsson (1994) and Cahill, Jr. and Casper (2000) was conducted at Exbury Gardens and Minstead Lodge (see 2.1). On the 27th February 2009, two 30 litre (23 x 47 x 36 cm) plastic boxes were sunk into the ground in eight plots beneath *R. ponticum* at the two sites. As a control, two boxes were also sunk into the ground in eight open grassland plots at each site. Each box had four 19 mm drainage holes in the bottom, but half also had ten 50 mm holes around the outside to allow contact with the surrounding soil. Three litres of 20 mm gravel were placed in the bottom of each box to aid drainage. Each box was then filled with a 1:4 mix of sharp sand (B&Q, Hampshire, UK) and J. Arthur Bowers multipurpose compost (Golden Acres Garden Centre, Hampshire, UK), and the boxes were then left for three years to allow time for the concentration of any allelochemicals that might be produced to build up in the soil. To test whether the inhibitory effect beneath *R. ponticum* was due to an above ground effect or a below ground effect, soil samples were collected from each box on the 5th March 2012 (see 2.2), and aqueous leachates were collected (see 2.3).

5.2.5 Germination and growth of plants

In order to determine whether *R. ponticum* has an allelopathic effect on the germination and growth of native species, mesocosm experiments were then conducted using passive greenhouse apparatus (Debevec & MacLean, Jr., 1993; Kennedy, 1995b). This provided an experimental ecosystem with close to natural conditions, in which the allelopathic potential of *R. ponticum* could be tested, while controlling for environmental variables, such as rainfall and herbivores, which would not have been possible to control in the field (see 4.2.2).

Twenty seeds of *L. perenne* or *T. repens* (see 2.4) were placed in 9 cm Petri dishes lined with a 9 cm by 7 cm oval cotton wool pad. The cotton wool pad was moistened with 15 ml of one of the leachate solutions, with one Petri dish for each sample. Two Petri dishes were used for leachates from soil which had supported *R. ponticum* under controlled conditions, and an average was taken. The pH of each sample was measured to ensure that any differences in growth were not due to pH (Tilman & Olff, 1991). Although there were differences in pH between the treatments, in chapter 4 it was demonstrated that these differences were too small to have a significant effect on the species tested. Seeds were also grown in soil which had supported *R. ponticum*, based on the method used for litter by Sarah *et al*. (2011). Fifteen grams of air dried soil was placed in a Petri dish, and then the soil was covered with a layer of Whatman No. 1 filter paper. Twenty seeds of *L. perenne* or *T. repens* were placed on the filter paper, and soil was moistened with 15 ml of distilled water. Germination, root elongation and leaf appearance were then measured based on the methods used by Anjum and Bajwa (2005), Thomas (1981) and Streck *et al*. (2003) (see 2.6).

5.2.6 Data analysis

As analysis of variance (ANOVA) requires the residuals to be normally distributed, the data were transformed using the logarithmic transformation ($\log_{10}(x + 0.1)$) to allow this parametric test. The residuals were then tested for normality using the Shapiro–Wilk test, to ensure that they met the assumptions of parametric statistical

tests (Doncaster & Davey, 2007). SPSS v.20.0 (SPSS Inc., Chicago, Illinois) was used for all statistical procedures and significance evaluated using $\alpha = 0.050$.

The effect of aqueous leachates from the above ground parts of *R. ponticum* (old leaves, new leaves, decomposing leaves, flowers and dH_2O control) on the germination and growth of the study species (Y) was examined using the following one-factor ANOVA model (hence-forth referred to as Model 3.1), with plant part as a fixed factor:

$$\log_{10}(Y) = S'_{16}(\text{Plant part}_5)$$

Tukey's post-hoc analysis was conducted on 'plant part' to identify which groups differ from which others. As site and woodland type were shown to have no effect ($P > 0.050$), these were not included in the statistical analysis.

The effect of different concentrations of aqueous leachates from leaves of *R. ponticum* (5%, 10%, 20%, 40% (n= 32) and 0% control (n= 8)) on the germination and growth of the study species (Y) was examined using the following one-factor ANOVA model (hence-forth referred to as Model 3.2), with concentration as a fixed factor:

$$\log_{10}(Y) = S'_{8\text{-}32}(\text{Concentration}_5)$$

Tukey's post-hoc analysis was conducted on 'concentration' to identify which groups differ from which others. As site and woodland type were shown to have no effect ($P > 0.050$), these were not included in the statistical analysis.

The effect of exudates from the roots of *R. ponticum* (root exudate and dH_2O control) on the germination and growth of the study species (Y) was examined using the following one-factor ANOVA model (hence-forth referred to as Model 3.3), with root exudate as a fixed factor:

$$\log_{10}(Y) = S'_{10}(\text{Root exudate}_2)$$

The effect of aqueous leachates from soil which had supported *R. ponticum* under controlled conditions (with and without *R. ponticum*) on the germination and growth of the study species (Y) was examined using the following one-factor ANOVA model (hence-forth referred to as Model 3.4), with *R. ponticum* as a fixed factor:

$$\log_{10}(Y) = S'_{8}(R.\ ponticum_2)$$

The effect of soil which had supported *R. ponticum* under controlled conditions (with and without *R. ponticum*) on the germination and growth of the study species (Y) was examined using the following one-factor ANOVA model (hence-forth referred to as Model 3.5), with *R. ponticum* as a fixed factor:

$$\log_{10}(Y) = S'_8 (R.\ ponticum_2)$$

The effect of vegetation type (open grassland, woodland and woodland which had been invaded by *R. ponticum*) on the germination and growth of the study species (Y) were examined using the following one-factor ANOVA model (hence-forth referred to as Model 3.6), with vegetation type as a fixed factor:

$$\log_{10}(Y) = S'_{32} (\text{Vegetation type}_3)$$

Tukey's post-hoc analysis was conducted on 'vegetation type' to identify which groups differ from which others. As site and woodland type were shown to have no effect (P >0.050), these were not included in the statistical analysis.

The effects of vegetation type (open grassland and woodland which had been invaded by *R. ponticum*) and soil contact (contact and no contact with the surrounding soil) on germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 3.7), with vegetation type, soil contact, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{16} (\text{Soil contact}_2 \mid \text{Vegetation type}_2))$$

As site was shown to have no effect (P >0.050), this was not included in the statistical analysis.

5.3 Results

5.3.1 Effect of leachates from the above ground parts of *R. ponticum*

i. Germination

Although 2% aqueous leachates from above ground parts of *R. ponticum* had no effect on the germination of *L. perenne* (Plant part main effect $F_{4,75} = 1.30$, $P = 0.277$ from Model 3.1 analysis), at higher concentrations, *R. ponticum* leaf leachate was found to have an effect (Concentration main effect $F_{4,131} = 2.74$, $P = 0.031$ from Model 3.2 analysis), with germination of *L. perenne* faster in the 5%, 20% and 40% leachates from *R. ponticum* leaves compared to the 0% control ($P < 0.050$). There was no difference between the 10% leachate and the 0% control ($P = 0.084$), or between the 10% leachate and the other concentrations ($P > 0.050$) (Figure 5. 1).

Two percent aqueous leachates from above ground parts of *R. ponticum* had an effect on the germination of *T. repens* (Plant part main effect $F_{4,75} = 6.95$, $P < 0.001$ from Model 3.1 analysis), with germination faster in leachates from old leaves and decaying leaves ($P < 0.050$). There was no difference between new leaves and flowers compared to the distilled water control ($P > 0.050$) (Figure 5. 2). The same pattern was observed in higher concentrations of *R. ponticum* leaf leachate (Concentration main effect $F_{4,131} = 11.79$, $P < 0.001$ from Model 3.2 analysis), with germination faster in leachates from *R. ponticum* leaves compared to the 0% control, although there was no difference between the different concentrations ($P > 0.050$) (Figure 5. 3).

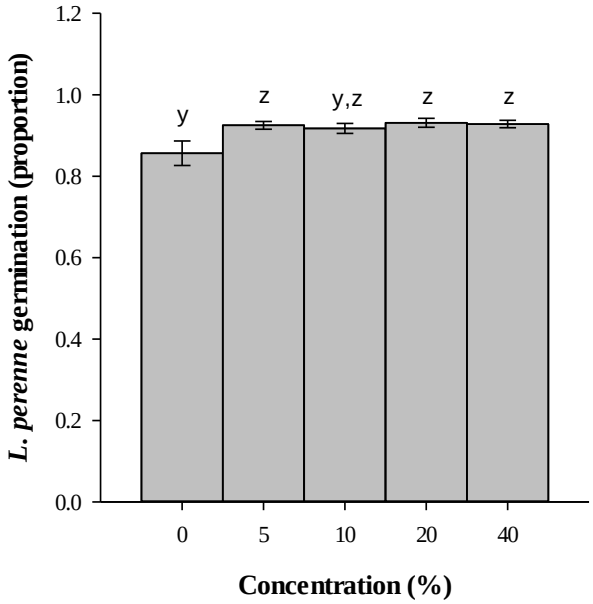


Figure 5. 1. Differences in mean germination of *L. perenne* in different concentrations of aqueous leachates from leaves of *R. ponticum* in the New Forest (+/- 1 SE) (n= 32, except for the 0% control, which n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (*P* <0.050).

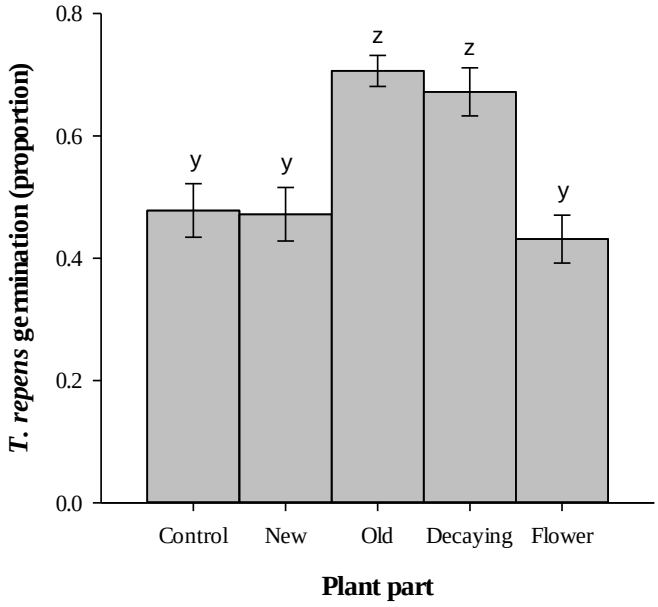


Figure 5. 2. Differences in mean germination of *T. repens* in 2% aqueous leachates from new leaves, old leaves, decaying leaves and flowers of *R. ponticum* in the New Forest (+/- 1 SE) (n= 16). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (*P* <0.050).

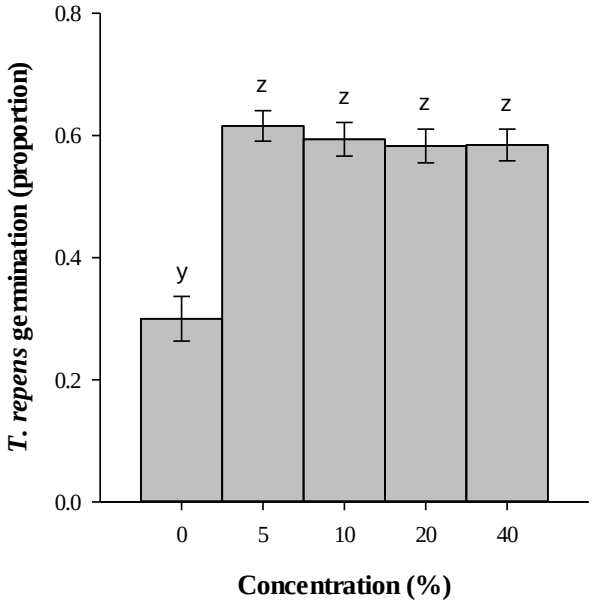


Figure 5. 3. Differences in mean germination of *T. repens* in different concentrations of aqueous leachates from leaves of *R. ponticum* in the New Forest (+/- 1 SE) (n= 32, except for the 0% control, which n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Two percent aqueous leachates from above ground parts of *R. ponticum* had no effect on the root elongation of *L. perenne* (Plant part main effect $F_{4,75} = 1.34$, $P = 0.262$ from Model 3.1 analysis) or *T. repens* (Plant part main effect $F_{4,75} = 0.78$, $P = 0.545$ from Model 3.1 analysis). Higher concentrations of *R. ponticum* leaf leachates were also found to have no effect on the root elongation of either species (Concentration main effect $F_{4,131} = 0.73$, $P = 0.575$ and $F_{4,131} = 1.28$, $P = 0.282$ from Model 3.2 analysis for *L. perenne* and *T. repens* respectively).

iii. Leaf appearance

Two percent aqueous leachates from above ground parts of *R. ponticum* had no effect on the leaf appearance of *L. perenne* (Plant part main effect $F_{4,75} = 0.81$, $P = 0.522$ from Model 3.1 analysis) or *T. repens* (Plant part main effect $F_{4,75} = 1.38$, $P = 0.249$ from Model 3.1 analysis). Higher concentrations of *R. ponticum* leaf leachates were also found to have no effect on the leaf appearance of *L. perenne* (Concentration main effect $F_{4,131} = 0.59$, $P = 0.673$ from Model 3.2 analysis). However, higher concentrations were found to have an effect on *T. repens* (Concentration main effect

$F_{4,131}$ = 3.45, P = 0.010 from Model 3.2 analysis), with rate of leaf appearance increasing with increasing concentrations, although the only differences were between 5% and 40% (P = 0.009) and between 10% and 40% (P = 0.026), and none of the different concentrations of leaf leachates differed from the 0% control (P >0.050) (Figure 5. 4).

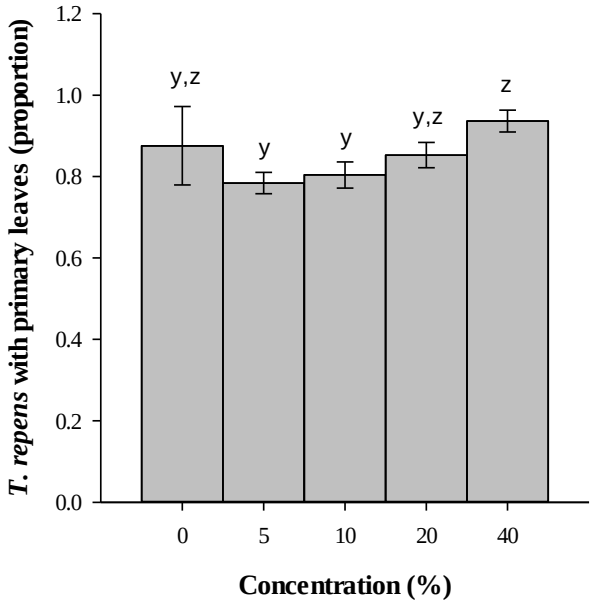


Figure 5. 4. Differences in mean leaf appearance of *T. repens* in different concentrations of aqueous leachates from leaves of *R. ponticum* in the New Forest (+/- 1 SE) (n= 32, except for the 0% control, which n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

5.3.2 Effect of root exudates from *R. ponticum*

Exudates from the roots of *R. ponticum* had no effect on the germination of *L. perenne* (Root exudate main effect $F_{1,18}$ = 0.03, P = 0.871 from Model 3.3 analysis) or *T. repens* (Root exudate main effect $F_{1,18}$ = 2.69, P = 0.118 from Model 3.3 analysis). Exudates from the roots of *R. ponticum* were also found to have no effect on the root elongation (Root exudate main effects $F_{1,18}$ = 0.003, P = 0.954 and $F_{1,18}$ = 2.56, P = 0.127 from Model 3.3 analysis for *L. perenne* and *T. repens* respectively), or leaf appearance (Root exudate main effects $F_{1,18}$ = 0.79, P = 0.387 and $F_{1,18}$ =

3.07, $P = 0.097$ from Model 3.3 analysis for *L. perenne* and *T. repens* respectively) of either of the species tested.

5.3.3 Effect of soil which had supported *R. ponticum*

i. Germination

Soil which had supported *R. ponticum* under controlled conditions had no effect on the germination of *L. perenne* (*R. ponticum* main effects $F_{1,18} = 1.65$, $P = 0.215$ and $F_{1,14} = 2.26$, $P = 0.155$ from Model 3.4 and Model 3.5 analysis for soil leachates and soil respectively). However, it did have an effect on the germination of *T. repens* (*R. ponticum* main effects $F_{1,18} = 11.75$, $P = 0.003$ and $F_{1,14} = 19.74$, $P = 0.001$ from Model 3.4 and Model 3.5 analysis for soil leachates and soil respectively), with germination slower in soil which had supported *R. ponticum* (Figure 5. 5, Figure 5. 6).

There was no difference in germination of *T. repens* between soil leachates from woodland with or without *R. ponticum* or from open grassland (Vegetation type main effect $F_{2,93} = 2.66$, $P = 0.076$ from Model 3.6 analysis). However, germination of *L. perenne* was slower in soil leachates from woodland with *R. ponticum* than from open grassland (Vegetation type main effect $F_{2,93} = 3.58$, $P = 0.032$ from Model 3.6 analysis), although there was no difference in germination between woodland with or without *R. ponticum* ($P = 0.164$), or between woodland without *R. ponticum* and open grassland ($P = 0.659$) (Figure 5. 7).

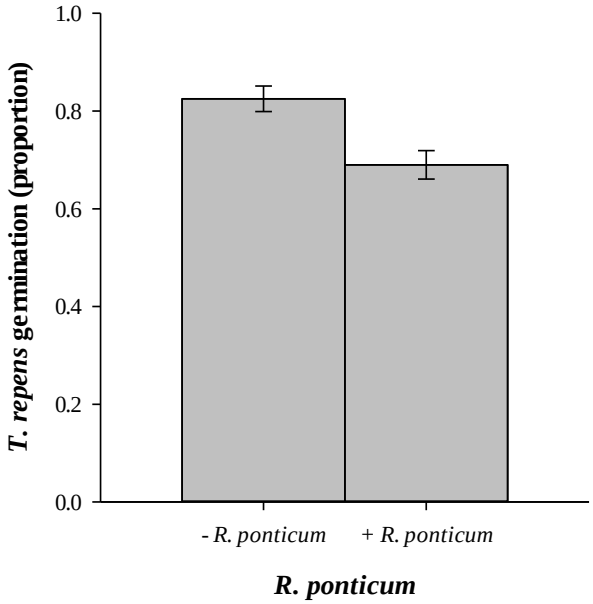


Figure 5. 5. Differences in mean germination of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions (+/- 1 SE) (n= 10).

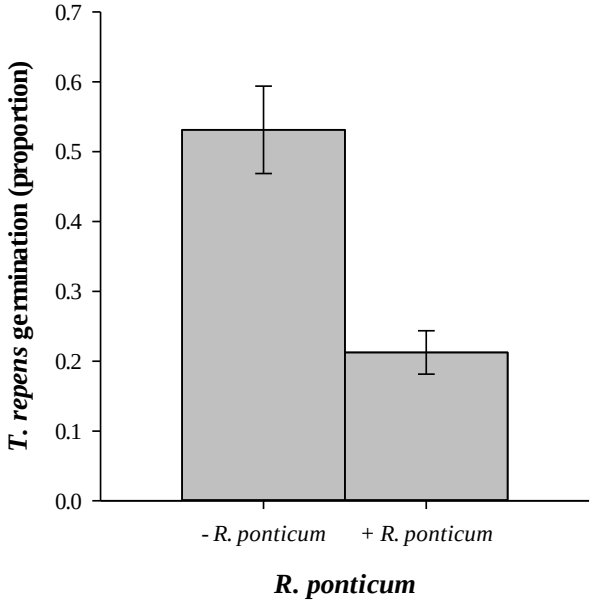


Figure 5. 6. Differences in mean germination of *T. repens* in soil which had supported *R. ponticum* under controlled conditions (+/- 1 SE) (n= 8).

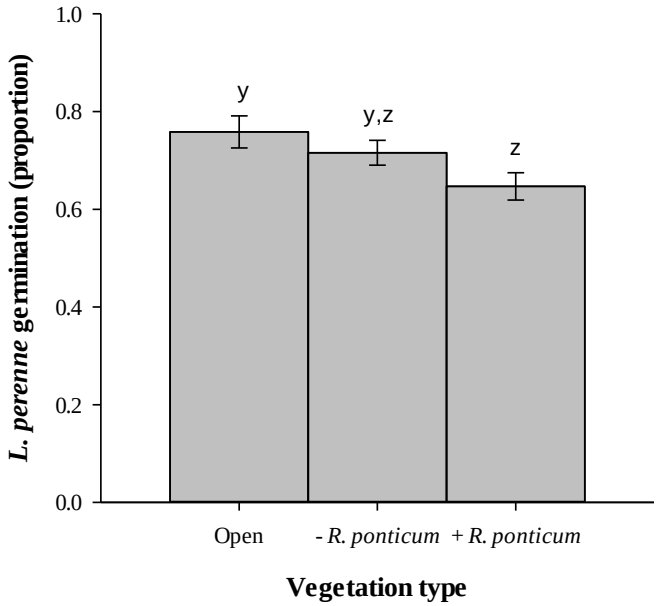


Figure 5. 7. Differences in mean germination of *L. perenne* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum* (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Soil which had supported *R. ponticum* under controlled conditions had no effect on the root elongation of *L. perenne* (*R. ponticum* main effects $F_{1,18} = 0.72$, $P = 0.408$ and $F_{1,14} = 3.74$, $P = 0.073$ from Model 3.4 and Model 3.5 analysis for soil leachates and soil respectively). However, leachates from soil which had supported *R. ponticum* under controlled conditions did have an effect on *T. repens* (*R. ponticum* main effect $F_{1,14} = 7.26$, $P = 0.017$ from Model 3.4 analysis), with root elongation faster in soil which had supported *R. ponticum* (Figure 5. 8), although there was no effect when seedlings were grown directly in soil which had supported *R. ponticum* (*R. ponticum* main effect $F_{1,14} = 0.35$, $P = 0.566$ from Model 3.5 analysis).

Root elongation of *L. perenne* and *T. repens* were both slower in soil leachates from woodland without *R. ponticum* than from open grassland (Vegetation type main effect $F_{2,93} = 11.21$, P <0.001 and $F_{2,93} = 5.05$, $P = 0.009$ for *L. perenne* and *T. repens* respectively from Model 3.6 analysis). However, this effect was not observed when *R. ponticum* was present, and there was no difference between root elongation in soil leachates from beneath *R. ponticum* than from open grassland ($P = 0.153$ and $P = 0.999$ for *L. perenne* and *T. repens* respectively) (Figure 5. 9, Figure 5. 10).

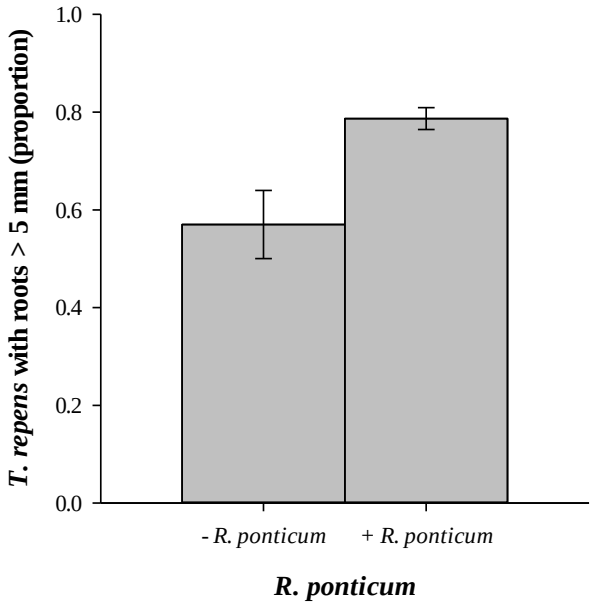


Figure 5. 8. Differences in mean root elongation of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions (+/- 1 SE) (n= 10).

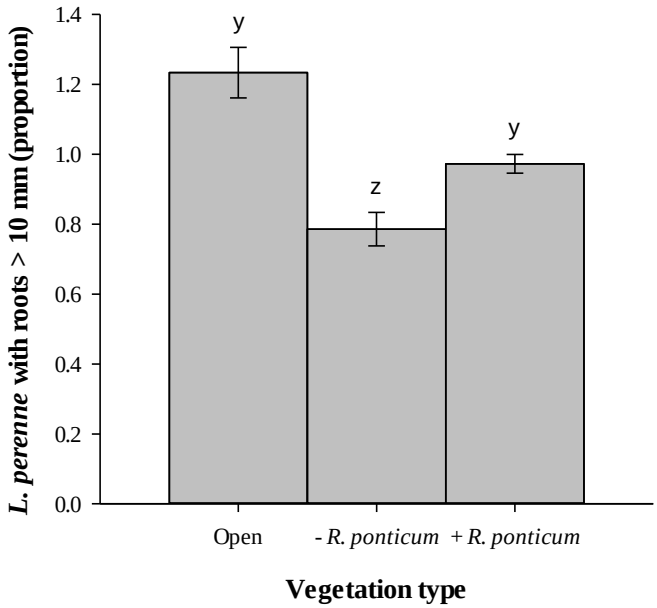


Figure 5. 9. Differences in mean root elongation of *L. perenne* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum* (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

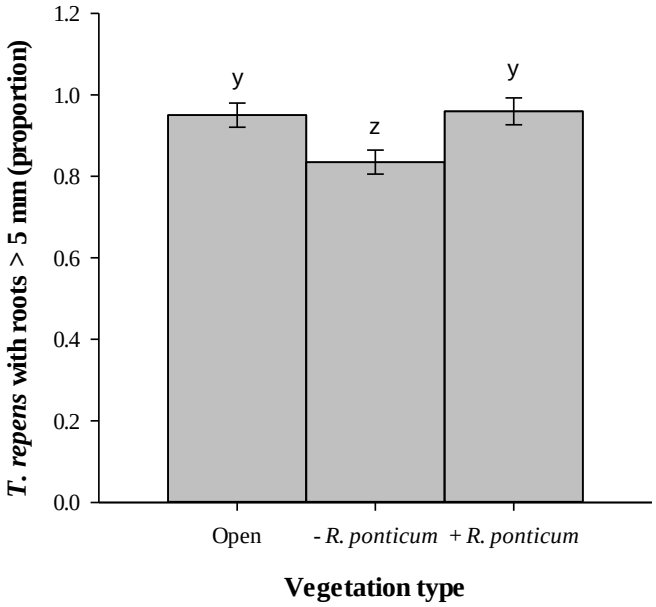


Figure 5. 10. Differences in mean root elongation of *T. repens* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum* (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

iii. Leaf appearance

Soil which had supported *R. ponticum* under controlled conditions had no effect on the leaf appearance of *L. perenne* (*R. ponticum* main effects $F_{1,18}$ = 2.61, P = 0.124 and $F_{1,14}$ = 1.50, P = 0.241 from Model 3.4 and Model 3.5 analysis for soil leachates and soil respectively). However, it did have an effect on *T. repens* (*R. ponticum* main effects $F_{1,14}$ = 24.10, P <0.001 and $F_{1,14}$ = 9.91, P = 0.007 from Model 3.4 and Model 3.5 analysis for soil leachates and soil respectively), although when seedlings were grown in leachates from soil which had supported *R. ponticum*, rate of leaf appearance of *T. repens* was reduced (Figure 5. 11), and when seedlings were grown directly in soil which had supported *R. ponticum*, rate of leaf appearance of *T. repens* was increased (Figure 5. 12).

There was no difference in leaf appearance of *L. perenne* between soil leachates from woodland with or without *R. ponticum* or from open grassland (Vegetation type main effect $F_{2,93}$ = 1.84, P = 0.166 from Model 3.6 analysis). However, leaf appearance of *T. repens* was slower in soil leachates from woodland than from open grassland (Vegetation type main effect $F_{2,93}$ = 7.68, P = 0.001 from Model 3.6 analysis),

although this effect was not affected by the presence of *R. ponticum* (*P* = 0.560) (Figure 5. 13).

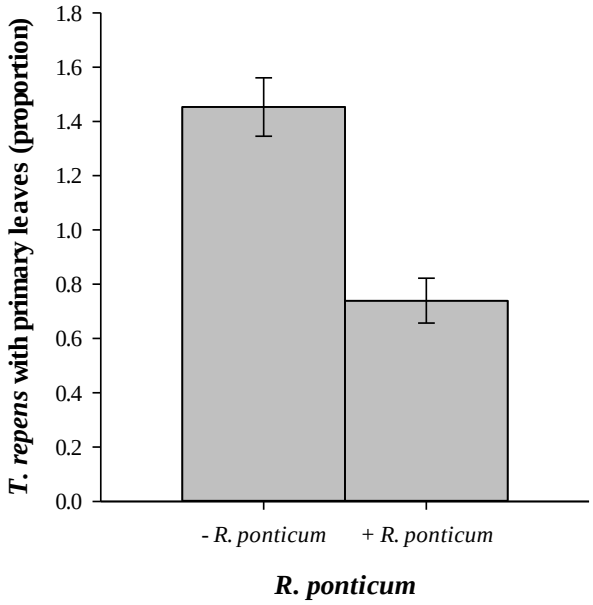


Figure 5. 11. Differences in mean leaf appearance of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions (+/- 1 SE) (n= 10).

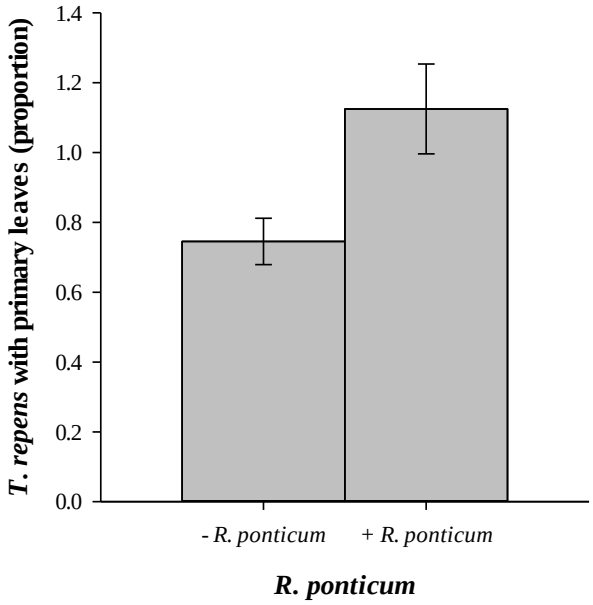


Figure 5. 12. Differences in mean leaf appearance of *T. repens* in soil which had supported *R. ponticum* under controlled conditions (+/- 1 SE) (n= 8).

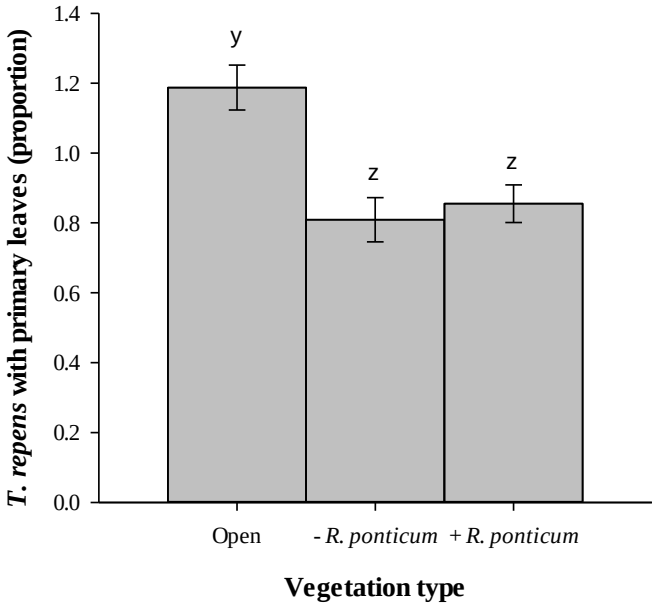


Figure 5. 13. Differences in mean leaf appearance of *T. repens* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum* (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

5.3.4 Separating the above ground and below ground effects of *R. ponticum*

i. Germination

Leachates from soil from beneath *R. ponticum* had no effect on the germination of *L. perenne* (Vegetation type main effect $F_{1,60}$ = 1.31, P = 0.258 from Model 3.7 analysis) or *T. repens* (Vegetation type main effect $F_{1,60}$ = 2.79, P = 0.100 from Model 3.7 analysis). Contact with the surrounding soil was also found to have no effect on the germination of *T. repens* (Soil contact main effect $F_{1,60}$ = 0.90, P = 0.347 from Model 3.7 analysis), although it did have an effect on the germination of *L. perenne* (Soil contact main effect $F_{1,60}$ = 4.04, P = 0.049 from Model 3.7 analysis), with germination faster when soil had been in contact with surrounding soil. However, this effect was observed whether *R. ponticum* was present or not, with no interaction between the two (*R. ponticum* * Soil contact interaction $F_{1,60}$ = 1.54, P = 0.219 from Model 3.7 analysis) (Figure 5. 14). There was however an interaction between *R. ponticum* and soil contact on *T. repens* (*R. ponticum* * Soil contact interaction $F_{1,60}$ = 8.54, P = 0.005 from Model 3.7 analysis), with germination slower

in leachates from soil from beneath *R. ponticum*, but only when it had been in contact with the surrounding soil (Figure 5. 15).

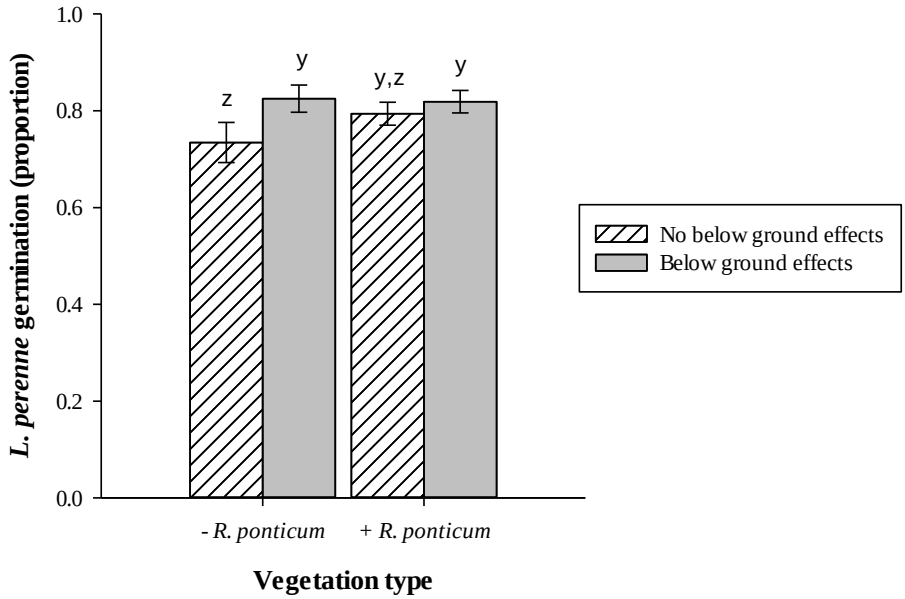


Figure 5. 14. Differences in mean germination of *L. perenne* in aqueous leachates from open grassland and woodland which had been invaded by *R. ponticum*, with and without below ground effects (+/- 1 SE) (n= 16). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

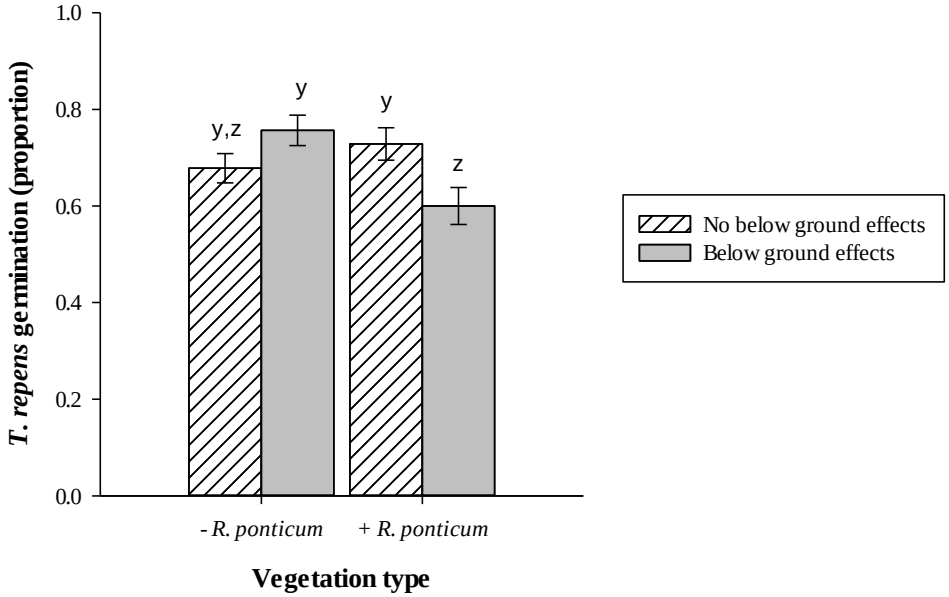


Figure 5. 15. Differences in mean germination of *T. repens* in aqueous leachates from open grassland and woodland which had been invaded by *R. ponticum*, with and without below ground effects (+/- 1 SE) (n= 16). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Leachates from soil from beneath *R. ponticum* had no effect on the root elongation of *L. perenne* (Vegetation type main effect $F_{1,60} = 0.16$, $P = 0.692$ from Model 3.7 analysis) or *T. repens* (Vegetation type main effect $F_{1,60} = 0.53$, $P = 0.471$ from Model 3.7 analysis). Contact with the surrounding soil was also found to have no effect on the root elongation of either species (Soil contact main effects $F_{1,60} = 1.49$, $P = 0.227$ and $F_{1,60} = 0.05$, $P = 0.829$ from Model 3.7 analysis for *L. perenne* and *T. repens* respectively), and there was no interaction between the presence of *R. ponticum* and contact with the surrounding soil for either species (*R. ponticum* * Soil contact interactions $F_{1,60} = 3.04$, $P = 0.087$ and $F_{1,60} = 1.15$, $P = 0.288$ from Model 3.7 analysis for *L. perenne* and *T. repens* respectively).

iii. Leaf appearance

Leachates from soil from beneath *R. ponticum* had no effect on the leaf appearance of *L. perenne* (Vegetation type main effect $F_{1,60} = 0.002$, $P = 0.961$ from Model 3.7 analysis) or *T. repens* (Vegetation type main effect $F_{1,60} = 0.02$, $P = 0.903$ from Model 3.7 analysis). Contact with the surrounding soil was also found to have no effect on the leaf appearance of either species (Soil contact main effects $F_{1,60} = 0.07$, $P = 0.789$ and $F_{1,60} = 0.42$, $P = 0.519$ from Model 3.7 analysis for *L. perenne* and *T. repens* respectively), and there was no interaction between the presence of *R. ponticum* and contact with the surrounding soil for either species (*R. ponticum* * Soil contact interactions $F_{1,60} = 0.15$, $P = 0.703$ and $F_{1,60} = 1.30$, $P = 0.258$ from Model 3.7 analysis for *L. perenne* and *T. repens* respectively).

5.4 Discussion

Although germination of *L. perenne* and germination and leaf appearance of *T. repens* were reduced in soil which had supported *R. ponticum*, neither species were completely inhibited, demonstrating that allelopathy alone could not explain the inhibition of growth of the native species beneath *R. ponticum*. Allelopathic compounds produced by soil microorganisms or native tree species (such as *Q. robur* and *P. sylvestris*) could also have already been present in the soil before the arrival of the rhododendron, and although *R. ponticum* might have been able to tolerate these conditions, the inhibition beneath *R. ponticum* might be due to the species already present.

Reduced seed germination, root growth and leaf appearance are commonly cited effects of allelopathy (Patterson, 1981; Angiras *et al*., 1988; Anjum & Bajwa, 2005), and many ericaceous species, including *Rhododendrons*, have been shown to produce compounds that reduce root or leaf growth (Blum *et al*., 1985; Gallet, 1994; Zhu & Mallik, 1994). This study demonstrated that although soil leachates from beneath *R. ponticum* reduced the rate of leaf appearance of *T. repens*, it had no inhibitory effect on the leaf appearance of *L. perenne*, or on the root elongation of either of the species tested. However, many ericaceous species have also been shown to release allelopathic compounds into the soil which have an inhibitory effect on the germination of native species (Carballeira & Cuervo, 1980; Mallik & Pellissier, 2000), and germination of both *L. perenne* and *T. repens* were reduced in soil which had supported *R. ponticum*, indicating that the rhododendron is having an allelopathic effect. This supports previous work by Rotherham and Read (1988), which showed that growth of *Festuca ovina* (sheep's fescue) was suppressed when grown either in soil with living *R. ponticum* plants, or in that which had recently supported *R. ponticum*. It is possible that this effect was not due to the presence of the rhododendron specifically, but rather due to the soil having supported a plant which reduced the availability of below ground resources, such as nutrients (Raven *et al*., 2005). However, Rotherham and Read (1988) found that the effect was not removed by the addition of nutrients, and many ericaceous species have been shown to release allelopathic compounds (Carballeira & Cuervo, 1980; Mallik & Pellissier, 2000), suggesting that the inhibition of growth beneath *R. ponticum* is, at least in

part, due to allelopathy. Rotherham and Read (1988) also found that root growth of *Festuca ovina* (sheep's fescue) was totally inhibited in soil with living *R. ponticum* plants and in soil which had recently supported *R. ponticum*, whereas this study found that *R. ponticum* had no inhibitory effect on the root elongation of either *L. perenne* or *T. repens*. This suggests that *R. ponticum* might affect some species differently to others (Chase *et al*., 1991), and although *Festuca ovina* is more commonly found growing on calcareous grassland (Sterry, 2006), it is possible that the rhododendron does have a greater allelopathic effect on the native woodland species.

Preliminary experiments identified a number of compounds produced by *R. ponticum*, including grayanotoxin 1 and grayanotoxin 3, as well as several flavonoids (Appendix IV). Due to time constraints it was not possible to carry out a detailed analysis of all the compounds produced, although this preliminary work suggests that the inhibitory effect could be due to a combination of compounds. Given more time, further work could be conducted to isolate the compounds produced and to identify which are responsible for the inhibitory effect on the native species. However, although they were not identified in the analysis performed in this study, ferulic acid is a common allelopathic compound, which has been identified in a number of ericaceous species, including *Erica scoparia* (Ballester *et al*., 1977), *Vaccinium myrtillus* (Gallet, 1994), *Empetrum hermaphroditum* (Gallet *et al*., 1999), *Kalmia angustifolia* (Zhu & Mallik, 1994), and *Erica australis* (Spanish heath) (Carballeira, 1980), as well as several *Rhododendron* species, including *R. dauricum* and *R. formosanum* (Cao *et al*., 2002; Chou *et al*., 2010), and quercetin is another known allelopathic compound, which has been identified in over two hundred and six *Rhododendron* species (Harborne & Williams, 1971). Therefore it is likely that these compounds are involved in the inhibition of growth beneath *R. ponticum*, and the effect of these will be tested in the final chapter.

These compounds can be released into the environment in a number of ways. Although volatiles from *Rhododendron* species have been shown to have an allelopathic effect (Del Moral & Cates, 1971), in general volatile inhibitors are most ecologically relevant under arid and semiarid conditions (Qasem & Foy, 2001), so it is unlikely that volatile inhibitors are responsible for the inhibition of growth beneath *R. ponticum* in the New Forest. Many ericaceous species have also been shown to

produce leachates which are responsible for their inhibitory effect on the native species (Mallik & Pellissier, 2000; Chou *et al*., 2010). However, this study demonstrated that aqueous leachates from *R. ponticum* had no inhibitory effect on the germination or growth of either species. The inhibitory effect of soil which had supported *R. ponticum* on the germination of *T. repens* was also shown to be removed by reducing the below ground effects of *R. ponticum*, indicating that the inhibition is due to the presence of toxins in the soil. Although it is possible that the concentration of leachates builds up in the soil over time (Carballeira & Cuervo, 1980; Muscolo & Sidari, 2006), this study demonstrated that even at higher concentrations well above those commonly used in allelopathy studies (Caamal-Maldonado *et al*., 2001; Cruz-Ortega *et al*., 2002) these leachates could not explain the inhibition of growth. Roots from a number of ericaceous species have also been shown to contain allelopathic compounds (Carballeira, 1980; Nisar *et al*., 2011), although this study demonstrated that on their own, root exudates from *R. ponticum* had no effect on the germination or growth of either species. However, allelochemicals can act synergistically, and mixtures of allelopathic chemicals have been shown to have a much greater inhibitory effect than the individual compounds alone (Blum, 1996), and it is possible that the long-term continuous influx of toxins from root exudates and leachates might be responsible for the inhibition of plant growth (Nilsen *et al*., 1999; Muscolo & Sidari, 2006).

Microbes present in the soil can also have a significant effect on the activity of allelochemicals, particularly of phenolic acids. It is possible that microbes in the soil, such as bacteria (Schmidt, 1988), fungi (Turner & Rice, 1975) or macroinvertebrates (Hunter *et al*., 2003), are metabolizing phenolic acids released by *R. ponticum*, and these transformed or newly synthesised phenolics differ in their phytotoxicity from the original ones (Singh *et al*., 2001). However, *R. ponticum* was shown to have an inhibitory effect in soil which was collected from the field and in compost. Although the compost was not sterilized, it was unlikely that the same microbes would be present in the different soils (Berg and Smalla, 2009), and although the inhibitory effect was shown to vary with soil type, Rotherham and Read (1988) showed that *R. ponticum* suppressed the growth of *Festuca ovina* seedlings even in partially sterilized soil. Although this suggests that microbes cannot be entirely responsible, many microbes, including rhizobacteria and fungi, have been shown to produce their own allelopathic compounds (Bush *et al*., 1997; Barazani & Friedman, 2001), and it

is possible that the inhibition of growth is due to the combination of these compounds with allelochemicals produced by *R. ponticum*.

Previous work has also shown that many of the species commonly found in woodland in the New Forest produce their own allelopathic compounds (Kuiters & Sarink, 1986), and root elongation of *L. perenne* and *T. repens*, and leaf appearance of *T. repens* were all shown to be reduced in soil leachates from woodland without *R. ponticum*. Seedlings of *R. ponticum* have difficulty becoming established in areas where there is already continuous ground cover from native plants (Cross, 1981). Therefore, it is possible that the allelopathic compounds were already present in the soil before the arrival of the rhododendron, preventing the growth of native species and providing the ideal conditions for *R. ponticum*, which are able to tolerate the compounds due to mycorrhizal associations which develop with the roots of *R. ponticum* and regulate the uptake of toxic compounds from the soil (Mitchell & Gibson, 2006). However, this study has indicated that *R. ponticum* does produce its own allelopathic compounds, although on their own these were not sufficient to explain the inhibition of growth of the native species. It is possible that other species are releasing their own compounds into the soil which are enhancing the allelopathic effect of the rhododendron (Blum, 1996), and this will be investigated in chapter 6.

Although *R. ponticum* is known to inhibit the growth of native species (Dehnen-Schmutz *et al*., 2004) and this study suggests that *R. ponticum* does have an allelopathic effect on the native species, neither of the species tested were completely inhibited by *R. ponticum*, and the presence of *R. ponticum* was shown to reduce the inhibitory effect of soil leachates from woodland on the root elongation of *L. perenne* and *T. repens*. Leachates from soil which had supported *R. ponticum* were also found to increase the root elongation of *T. repens*, and leaf appearance of *T. repens* seedlings grown directly in soil which had supported *R. ponticum* under controlled conditions was found to be increased. These findings suggest that *R. ponticum* might be releasing compounds into the soil which are increasing growth. It is possible that the rhododendron mycorrhizal associations are mobilizing nutrients making them available for other plants (Read & Perez-Moreno, 2003), or that nutrients are leaching from the leaves (Ostman & Weaver, 1982). Many ericaceous species have also been found to produce compounds, including *p*-coumaric acid, vanillic acid and quercetin, which at low concentrations can stimulate growth, but are toxic at higher

concentrations (Mersie & Singh, 1993; Fries *et al*., 1997), and aqueous leachates from above ground parts of *R. ponticum* had a stimulatory effect on the germination of *L. perenne* and *T. repens* and on the leaf appearance of *T. repens*. However, this study also found that root exudates and aqueous leachates from above ground parts of *R. ponticum* had no effect on the root elongation of either species. This suggests that allelopathy alone is unable to explain the inhibition of growth of native species in the New Forest, and previous studies have shown that several of the native species commonly found growing in uninvaded woodland in the New Forest are capable of tolerating similar allelopathic compounds (Donath & Eckstein, 2008; Baltzinger *et al*., 2012).

Many other ericaceous species have also been shown to produce allelopathic compounds, which on their own are often not sufficient to explain the inhibition of growth (Inderjit & Mallik, 2002). Eppard *et al*. (2005) showed that although allelopathic compounds produced by *Kalmia latifolia* reduced the root, shoot, and total biomass of *Pinus rigida* seedlings, this effect was not significant, and Nilsen *et al*. (1999) found that allelopathy on its own was not likely to be an important cause for the inhibition of seedling survival within thickets of *R. maximum*. However, previous work has also demonstrated the importance of environmental factors on the inhibition of growth (Nilsen *et al*., 1999; Nilsen & Horton, 2002). Although on their own these differences in environmental conditions beneath *R. ponticum* were not sufficient to inhibit growth (see chapters 3 and 4), plants of the same species growing close together have been found to vary greatly in their allelopathic effects, suggesting that environmental stresses can exacerbate the effects of allelopathic compounds, as well as playing an important role in determining the amounts of inhibitor produced by a plant (Singh *et al*., 2001). Therefore it is likely that environmental conditions beneath *R. ponticum* are enhancing the allelopathic effect, and this will be the focus of the research in the final chapter.

Chapter 6

Determining the influence of key environmental conditions on the allelopathic potential of *Rhododendron ponticum*

6.1 Introduction

The *Ericaceae* are known to be a particularly inhibitory family, with many species releasing allelopathic compounds into the soil (Del Moral & Cates, 1971), and several studies have suggested that allelopathy is responsible for the inhibition of growth of the native species beneath the invasive shrub *R. ponticum* (Cross, 1981; Rotherham & Read, 1988). However, on their own, these compounds are often unable to explain the inhibition of growth of the native species (Nilsen *et al*., 1999; Eppard *et al*., 2005), and in chapter 5 it was demonstrated that although *R. ponticum* does have an allelopathic effect on the native species, allelopathy alone is not sufficient to explain the inhibition of growth beneath *R. ponticum* in the New Forest. *Rhododendron* species have also been shown to alter environmental conditions beneath them (Cross, 1975; Nilsen *et al*., 2001), and changes in environmental conditions beneath invasive species are often implicated as the cause for the inhibition of native plant growth (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). However, chapters 3 and 4 suggested that most of these differences were unlikely to inhibit the native species, and although reduced light availability is likely to be involved in the inhibition of growth beneath *R. ponticum,* based on the findings in these chapters, light availability alone was also not sufficient to explain the impact of *R. ponticum* on the native species in the New Forest.

However, many species known to produce allelopathic compounds have also been shown to alter the environmental conditions beneath them, and it is often thought that it is the combination of the two that is responsible for the inhibition of growth. Although *Kalmia angustifolia* and *Kalmia latifolia* (mountain laurel) are known to produce allelopathic compounds that interfere with nutrient uptake (Bradley *et al*., 2000; Eppard *et al*., 2005), on their own these chemicals were shown not to have a significant effect on the growth of *Pinus mariana* or *Pinus rigida* (pitch pine) seedlings (Inderjit & Mallik, 2002; Eppard *et al*., 2005). However, *Kalmia angustifolia* and *Kalmia latifolia* are often limited to low nutrient areas and it was suggested that the allelopathic effect could be exacerbated in the field when seedlings are exposed to multiple stressors, such as reduced nutrient availability (Inderjit & Mallik, 2002; Eppard *et al*., 2005). *R. maximum* has also been shown to produce allelopathic compounds, although Nilsen *et al*. (1999) showed that on its

own this was not likely to be an important cause for the inhibition of seedling survival. However, reduced light availability was also not sufficient to explain the high mortality beneath *R. maximum*, suggesting that the inhibition of the native species might be due to the allelopathic compounds in combination with the light limitation (Clinton & Vose, 1996). A number of allelopathic compounds known to be released from many ericaceous species have also been shown to have a much greater effect under environmental stresses (Einhellig, 1987; Lobón *et al*., 2002).

One of the only studies to separate the importance of allelopathy and resource competition was by Nilsson (1994), who showed that although below ground competition and allelopathy by *Empetrum hermaphroditum* were both important factors in retarding the growth of the native species, reducing root competition reduced the allelopathic effect of *Empetrum hermaphroditum* on *P. sylvestris* seedlings. Michelsen *et al*. (1995) also showed that while *Empetrum hermaphroditum* released allelopathic agents from the leaves which reduced the germination and growth of *P. sylvestris*, this effect was greater when nutrient availability was low.

In chapters 3 and 4 it was demonstrated that reduced light availability was the environmental condition most likely to explain the impact of *R. ponticum* on the native species, and although it was not sufficient to explain the inhibition of growth, a number of studies have shown light availability to be an important factor in affecting the sensitivity of plants to different allelopathic compounds (Singh *et al*., 2001). *Rhododendron* species have been shown to produce a number of allelopathic compounds, including ferulic acid, *p*-coumaric acid, vanillic acid and quercetin (Cao *et al*., 2002; Chou *et al*., 2010), all of which interfere with photosynthesis (Moreland & Novitzky, 1987; Mersie & Singh, 1993). Therefore, it is likely that these allelochemicals will have a much greater inhibitory effect in the light limited growth conditions found beneath *R. ponticum* (Von Elert & Jüttner, 1996).

Light availability has also been found to play an important role in determining the amount of inhibitor produced by a plant (Dudt & Shure, 1994; Kato-Noguchi, 1999). Solar radiation has been shown to activate the biosynthesis of ferulic acid, *p*-coumaric acid and quercetin in *Vaccinium myrtillus* (Jaakola *et al*., 2004), and far-red light increased the synthesis and accumulation of phenolic acids, including quercetin,

in plant tissues (Beggs *et al*., 1987). Ultraviolet radiation has been shown to increase the accumulation of phenolics acids, including ferulic acid, *p*-coumaric acid (Luthria *et al*., 2006) and quercetin (Gerhardt *et al*., 2008). However, Ghasemzadeh *et al*. (2010) showed that synthesis of quercetin increased when light intensity decreased, and Gerhardt *et al*. (2008) found that accumulation of quercetin was suppressed by increasing the amount of far-red light.

A number of *Rhododendron* species have also been found to reduce the availability of nutrients beneath them (Cross, 1975; Nilsen *et al*., 2001), either by sequestering large quantities of available nutrients (Monk *et al*., 1985), or by altering nutrient cycling (Wurzburger & Hendrick, 2007). In chapter 3 it was demonstrated that nitrate availability was not significantly different between areas of *R. ponticum* and areas of open grassland, and suggested that on their own, the availability of other nutrients was unlikely to be sufficient to explain the inhibition of growth of native species beneath *R. ponticum* in the New Forest. However, a number of the species commonly found in the New Forest, such as *Fagus sylvatica* and *Molinia caerulea* (purple moor grass), are good competitors for nutrients (Aerts *et al*., 1991; Bolte & Villanueva, 2006). Sandy acidic soils, like those typically found in the New Forest (Tubbs, 2001; West, 2010), are generally low in nutrients (Raven *et al*., 2005), and in chapter 3 it was demonstrated that nitrate availability was already low before the arrival of the rhododendron (Powers, 1980). Mycorrhizal associations develop with the roots of *R. ponticum* plants, which encourage the development of a densely branched fibrous root system, have a high capacity for dissolved mineral uptake, and are able to use complex forms of nitrogen and phosphorus. This allows the rhododendrons to thrive in nutrient-poor soils (Mitchell & Gibson, 2006), and might allow them to invade areas which are unsuitable for native species. Therefore it is possible that nutrient availability is low throughout the New Forest, and although this is not sufficient to explain the inhibition of growth on its own, it is possible that low nutrient conditions are enhancing the allelopathic effect of *R. ponticum* (Kong *et al*., 2002).

R. ponticum has been found to affect the pH of the soil. Although in this study it was not possible to test the combination of pH and allelopathic compounds on the germination and growth of the native species, as it is likely that any compounds which affect the pH would also interfere with the allelopathic compounds (Wium-

Andersen *et al*., 1983; Blum, 1996), in chapter 4 it was demonstrated that the difference in pH was not sufficient to account for the inhibition of growth of the native species. However, pH can also be extremely important in affecting the synthesis of allelochemicals (Northup *et al*., 1995) and their accumulation in the soil (Friedman & Jürgens, 2000). Although it is therefore likely that the reduced light availability and acidic conditions play an important role in the accumulation of allelochemicals beneath *R. ponticum*, there appears to be no work investigating this.

Under natural conditions, inhibitory effects of allelochemicals are often observed at concentrations well below their individual inhibitory levels (Singh *et al*., 2001). This suggests that in addition to being affected by environmental conditions, allelochemicals act synergistically, and mixtures of allelopathic chemicals, as well as other organic compounds, have been shown to have a much greater inhibitory effect than the individual compounds alone (Blum, 1996). In the New Forest, *R. ponticum* is often found growing in deciduous and evergreen woodland, and many of the tree species commonly found in these woodlands, including *Betula pendula*, *Fagus sylvatica*, *Fraxinus excelsior*, *P. sylvestris* and *Q. robur*, have been shown to produce their own allelopathic compounds (Kuiters & Sarink, 1986). Therefore, it is possible that as well as providing the ideal environmental conditions allowing *R. ponticum* to become established (Cross, 1981), these species might also be releasing their own compounds into the soil that enhance the allelopathic effect of the rhododendron and allows it to inhibit the growth of the native species. However, there appear to be no studies investigating the effect of these species on the impact of *R. ponticum*.

Previous studies into the impact of invasive species tend to focus on the influence of one individual factor (i.e. light availability or allelopathy), and yet on their own these conditions are often not sufficient to explain the inhibition of growth of the surrounding vegetation (Clinton & Vose, 1996; Eppard *et al*., 2005). Previous work has shown that environmental stresses can exacerbate the effects of allelopathic compounds, as well as playing an important role in determining the amounts of inhibitor produced by a plant (Singh *et al*., 2001). However, this work tends to focus on crops and other model species (Teasdale, 1993; Kato-Noguchi, 1999; Pramanik *et al*., 2000), and few studies have investigated the influence of these interactions on the impact of invasive species (Tang *et al*., 1995; Karageorgou *et al*., 2002; Lobón *et al*., 2002; Thelen *et al*., 2005). There appears to have been no work investigating

whether multiple environmental stresses explain the impact of rhododendrons, either by affecting the susceptibility of the native species to allelopathic compounds or by increasing the synthesis of allelopathic compounds by the rhododendron. Previous work has also shown that mixtures of allelopathic compounds, as well as other organic compounds, can have a greater inhibitory effect than the individual compounds alone (Blum, 1996; Reigosa *et al*., 1999). However, these studies focus on mixtures of compounds produced by the same species (Odén *et al*., 1992), or on the joint action of allelopathic compounds with herbicides (Einhellig, 1996; Khaliq *et al*., 2012), and there has been insufficient work investigating the impact of allelopathic compounds produced by invasive species in combination with compounds produced by the native trees or microorganisms. While previous chapters have focused on the individual effects of environmental conditions or allelopathy, this final chapter will specifically test the interactions between the two, and the role these play in the inhibition of growth beneath *R. ponticum* in the New Forest.

In this chapter, two known allelopathic compounds (see 6.2.1), as well as soil which had supported *R. ponticum*, were tested on the germination and growth of two naturally occurring species grown under different light conditions, to determine whether the reduced light availability beneath the rhododendron combined with allelopathic compounds is sufficient to explain the effect of *R. ponticum*. Nutrients were added to soil which had supported *R. ponticum*, to determine whether nutrient stress enhances the allelopathic effect of the rhododendron on the germination and growth of two naturally occurring species. Soil which had supported *R. ponticum* grown under different light and pH conditions was also assayed for its allelopathic properties, to determine whether the environmental conditions found in areas invaded by *R. ponticum* can increase the synthesis or accumulation of the allelopathic compounds in the soil. Finally, two known allelopathic compounds, as well as soil which had supported *Q. robur* or *P. sylvestris*, which are commonly found growing with *R. ponticum* in the New Forest (personal observation), were tested to determine whether compounds produced by these species increase the allelopathic effect of *R. ponticum*.

Aims:

1. To determine whether the reduced light conditions found beneath *R. ponticum* enhance the allelopathic effect of *R. ponticum* on native species.

2. To determine whether nutrient stress enhances the allelopathic effect of *R. ponticum* on native species in the New Forest.

3. To determine whether *R. ponticum* growing in different light and pH conditions produce different concentrations of allelopathic compounds.

4. To determine whether compounds produced by *Q. robur* and *P. sylvestris* enhance the inhibitory effect of *R. ponticum* on native species.

6.2 Methods

6.2.1 Effect of reduced light availability on the allelopathic effect of *R. ponticum* and known allelopathic compounds

In order to determine whether the reduced light availability found beneath *R. ponticum* in the New Forest enhance its allelopathic effect on native species, approximately 200 g of soil was collected (see 2.2) from the two sites at Poundhill Inclosure (see 2.1) on 14th February 2012, and from Exbury Gardens and Copythorne Common (see 2.1) on the 14th March 2012. After air drying, aqueous leachates were then collected based on the method used by Inderjit and Dakshini (1994) (see 2.3).

A glasshouse experiment was also conducted to determine whether the reduced light conditions found beneath *R. ponticum* in the New Forest enhances the effect of two known allelopathic compounds. Ferulic acid has been identified in a number of ericaceous species, including *Erica scoparia* (Ballester *et al*., 1977), *Vaccinium myrtillus* (Gallet, 1994), *Empetrum hermaphroditum* (Gallet *et al*., 1999), *Kalmia angustifolia* (Zhu & Mallik, 1994), and *Erica australis* (Spanish heath) (Carballeira, 1980), as well as several *Rhododendron* species, including *R. dauricum* and *R. formosanum* (Cao *et al*., 2002; Chou *et al*., 2010). It has been shown to reduce germination, root elongation, leaf expansion, water and nutrient uptake and photosynthesis (Blum & Rebbeck, 1989; Lyu & Blum, 1990; Reigosa *et al*., 1999). Quercetin has been identified in over 206 *Rhododendron* species (Harborne & Williams, 1971), and has been shown to reduce germination, shoot and root growth, nutrient uptake, photosynthesis and respiration (Moreland & Novitzky, 1987; Fries *et al*., 1997; Takahashi *et al*., 1998; Basile *et al*., 2000; Parvez *et al*., 2004). On the 27th September 2012, 485 mg of ferulic acid or 755 mg of quercetin were placed in 750ml plastic beakers containing 500 ml of distilled water. The solution was stirred for 5 minutes until the ferulic acid and quercetin had dissolved. This was to make up 5 mM concentration solutions, which previous work, as well as preliminary trials (Appendix V), has shown was enough to exert an allelopathic effect on germination and growth (Devi & Prasad, 1992; Ćurković-Perica & Ježić, 2010). Distilled water was used as a control.

6.2.2 Effect of nutrient availability on the allelopathic effect of *R. ponticum*

In order to determine whether nutrient availability affects the allelopathic effect of *R. ponticum* on native species, approximately 200 g of soil was collected from each plot in the two deciduous and the two evergreen woodland sites in the New Forest on the 30th January 2012 (see 2.1). Approximately 200 g of soil was also collected from the ten pots which had supported to *R. ponticum* under controlled conditions and from the ten pots containing the control soil (see 4.2.3) on the 20th June 2011. After air drying, aqueous leachates were then collected (see 2.3). Leachates were divided into two 100 ml plastic beakers, and Miracle-Gro all purpose concentrated liquid plant food (Morrisons, Hampshire, UK) was then added to half the beakers to make up a concentration of 0.3% NPK nutrient solution. Preliminary trials demonstrated that this concentration should be enough to reduce any nutrient deficiencies, without having a significant effect on pH and without having an inhibitory effect on the two study species (Appendix VI). Another 5 g of soil was collected from eight of the pots which had supported *R. ponticum* under controlled conditions and from eight of the pots containing the control soil on the 3rd September 2012, in order to test whether there was a greater effect when seeds were sown directly into soil which had supported *R. ponticum*.

6.2.3 Allelopathic effect of *R. ponticum* grown under different light and pH conditions

In order to determine whether environmental conditions enhance the allelopathic potential of *R. ponticum*, rhododendrons were grown in different light availability (PAR) and pH conditions, which were manipulated using the method used by Reinhart *et al*. (2006) (see 4.2.2). *R. ponticum* plants collected from Cadnam Common on the 3rd March 2011 were grown in full sun in a naturally lit glasshouse, and were watered with rain water when required (see 4.2.3). Pots filled with just compost were used as a control. Thirty two pots were used for each, to give eight repeats for each treatment. On the 13th September 2011, PAR levels to half the pots were reduced to 75% shade, using shade cloth, and the other half of the pots were left in full sun. This was to simulate light conditions found in evergreen woodland and open grassland respectively (see 4.3.1). Half the pots in each shade treatment were

then watered every two days with distilled water, the pH of which was adjusted to pH 3.66 or pH 4.62, using sulphuric acid, a method commonly used in hydroponics (Rolot & Seutin, 1999). This was to simulate pH conditions found in evergreen woodland, and open grassland respectively (see 4.3.1). On the 11th June 2012, approximately 200 g of soil was collected from each pot (see 2.2). After drying, aqueous leachates were collected (see 2.3).

6.2.4 Effect of *Q. robur* or *P. sylvestris* leachates and known allelopathic compounds on the allelopathic effect of *R. ponticum*

In order to determine whether *Q. robur* or *P. sylvestris* produce compounds which enhance the allelopathic effect of *R. ponticum*, plants were grown under controlled conditions. *R. ponticum*, *Q. robur* and *P. sylvestris* plants collected from the New Forest on 3rd March 2011 were grown in a naturally lit glasshouse, and were watered with rain water when required (see 4.2.3). Pots filled with just compost were used as a control. Sixteen repeats were conducted for each treatment. On the 16th April 2012, approximately 400 g of soil was collected from each pot containing *R. ponticum*, approximately 300 g of soil was collected from each pot containing *Q. robur* or *P. sylvestris*, and approximately 200 g of soil was collected from the pots containing just compost (see 2.2).

After drying, aqueous leachates were collected based on the method used by Inderjit and Dakshini (1994) (see 2.3). To test whether *Q. robur* or *P. sylvestris* produce compounds which enhance the allelopathic effect of *R. ponticum*, 250 ml beakers were filled with a mixture of 75 g of soil which had supported *R. ponticum* and either 75 g of soil which had supported *Q. robur* or 75 g of soil which had supported *P. sylvestris*. Beakers were also filled with 150 g of soil which had supported either *R. ponticum*, *Q. robur* or *P. sylvestris* to test whether they had an allelopathic effect on their own, and beakers were also left with just distilled water to use as a control. Soil was soaked in 150 ml of distilled water for 24 hours, and then the solution was filtered through Whatman No. 1 filter paper to remove the soil.

To test whether known allelopathic compounds enhance the toxicity of soil which has supported *R. ponticum*, approximately 200 g of soil was collected (see 2.2) from eight pots which has supported *R. ponticum* under controlled conditions (see 4.2.3) on the 26th September 2012, and approximately 200g of soil was collected (see 2.2) from half of the plots at the four sites in the New Forest (see 2.1) on the 8th October 2012. After drying, aqueous leachates were collected (see 2.3). Ferulic acid or quercetin was then dissolved in the leachates, with 97 mg of ferulic acid or 151 mg of quercetin per 100 ml of soil leachates. Preliminary experiments found that this concentration was sufficient to exert an allelopathic effect (Appendix V). Soil leachates which did not contain ferulic acid or quercetin were used as a control.

6.2.5 Germination and growth of plants

Mesocosm experiments were then conducted using passive greenhouse apparatus (Debevec & MacLean, Jr., 1993; Kennedy, 1995b). This provided an experimental ecosystem with close to natural conditions, in which the allelopathic effect of *R. ponticum* could be tested, while controlling for environmental variables, such as rainfall and herbivores, which would not have been possible to control in the field (see 4.2.2).

Twenty seeds of *L. perenne* or *T. repens* (see 2.4) were placed in 9 cm Petri dishes lined with a 9 cm by 7 cm oval cotton wool pad. The cotton wool pad was moistened with 15 ml of one of the leachate solutions, with one Petri dish for each sample. To test whether the reduced light availability beneath *R. ponticum* enhances the allelopathic effect, two Petri dishes were used for each sample collected in 6.2.1. Petri dishes were also set up containing 15ml of 1 mM quercetin or ferulic acid solutions (see 6.2.1), with sixteen Petri dishes for each chemical. The pH of each sample was measured to ensure that any differences in growth were not due to pH (Goldberg, 1985; Tilman & Olff, 1991; Tang & Yu, 1999; Haling *et al*., 2010). Although there were differences in pH between the treatments, in chapter 4 it was demonstrated that these differences were too small to have a significant effect on the species tested. Petri dishes were arranged randomly within the naturally lit glasshouse to avoid differences in light intensity and temperature.

Seeds were also grown in soil which had supported *R. ponticum*, based on the method used by Sarah *et al*. (2011). Fifteen grams of air dried soil was placed in Petri dishes, with two Petri dishes for each sample, and then the soil was covered with a layer of Whatman No. 1 filter paper. Twenty seeds of *L. perenne* or *T. repens* were placed on the filter paper, and soil was moistened with 15 ml of distilled water. Miracle-Gro all purpose concentrated liquid plant food (Morrisons, Hampshire, UK) was added to the distilled water in half the Petri dishes containing glasshouse soil to make up a concentration of 0.3% nutrient solution.

To test whether the reduced light availability beneath *R. ponticum* enhances the allelopathic effect, light availability (PAR) was then manipulated using the method used by Reinhart *et al*. (2006) (see 4.2.2). PAR levels in half the Petri dishes containing the samples collected in 6.2.1 or the 5 mM quercetin or ferulic acid solutions were reduced to 97% shade to simulate light conditions found beneath *R. ponticum* (see 4.3.1). The other half were left in full sun to simulate the conditions found in open grassland. Germination, root elongation and leaf appearance were measured based on the methods used by Anjum and Bajwa (2005), Thomas (1981) and Streck *et al*. (2003) (see 2.6).

6.2.6 Data analysis

As analysis of variance (ANOVA) requires the residuals to be normally distributed, the data were transformed using the logarithmic transformation ($\log_{10}(x + 0.1)$) to allow this parametric test. The residuals were then tested for normality using the Shapiro–Wilk test, to ensure that they met the assumptions of parametric statistical tests (Doncaster & Davey, 2007). SPSS v.20.0 (SPSS Inc., Chicago, Illinois) was used for all statistical procedures and significance evaluated using $\alpha = 0.050$.

The effects of vegetation type (open grassland, woodland and woodland which had been invaded by *R. ponticum*) and light availability (full sun and 97% shade) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.1), with vegetation type, light availability (PAR), and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{32}\ (\text{Vegetation type}_3 \mid \text{PAR}_2)$$

Tukey's post-hoc analysis was conducted on 'vegetation type' to identify which groups differ from which others. As site and woodland type were shown to have no effect (P >0.050), these were not included in the statistical analysis.

The effects of known allelopathic compounds (ferulic acid, quercetin and dH_2O control) and light availability (full sun and 97% shade) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.2), with allelopathic compound, light availability (PAR), and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{8}\ (\text{Allelopathic compound}_3 \mid \text{PAR}_2)$$

Tukey's post-hoc analysis was conducted on 'allelopathic compound' to identify which groups differ from which others.

The effects of aqueous leachates from soil which had supported *R. ponticum* under controlled conditions (with and without *R. ponticum*) and addition of nutrients (with and without nutrients) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.3), with *R. ponticum*, nutrients, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{10}\ (\textit{R. ponticum}_2 \mid \text{Nutrients}_2)$$

The effects of soil which had supported *R. ponticum* under controlled conditions (with and without *R. ponticum*) and addition of nutrients (with and without nutrients) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.4), with *R. ponticum*, nutrients, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{8}\ (\textit{R. ponticum}_2 \mid \text{Nutrients}_2)$$

The effects of vegetation type (open grassland, woodland and woodland which had been invaded by *R. ponticum*) and addition of nutrients (with and without nutrients) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.5), with vegetation type, nutrients, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{32}\ (\text{Vegetation type}_3 \mid \text{Nutrients}_2)$$

Tukey's post-hoc analysis was conducted on 'vegetation type' to identify which groups differ from which others. As site and woodland type were shown to have no effect (P >0.050), these were not included in the statistical analysis.

The effects of light availability (PAR) (full sun and 75% shade) and pH (3.66 and 4.62) on the allelopathic potential of soil which had supported *R. ponticum* under controlled conditions, on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.6), with light availability (PAR), pH, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{8}\ (\text{PAR}_2 \mid \text{pH}_2)$$

The effects of soil which had supported *R. ponticum* under controlled conditions (with and without *R. ponticum*) and soil which had supported *P. sylvestris* or *Q. robur* under controlled conditions (with and without *P. sylvestris* or *Q. robur*) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA models (hence-forth referred to as Models 4.7a and 4.7b), with *R. ponticum*, *P. sylvestris* or *Q. robur*, and their interaction as fixed factors:

4.7a) $\log_{10}(Y) = S'_{16}\ (R.\ ponticum_2 \mid P.\ sylvestris_2)$

4.7b) $\log_{10}(Y) = S'_{16}\ (R.\ ponticum_2 \mid Q.\ robur_2)$

The effects of soil which had supported *R. ponticum* under controlled conditions (and without *R. ponticum*) and known allelopathic compounds (ferulic acid, quercetin and dH_2O control) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.8), with *R. ponticum*, allelopathic compound, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{8}\ (R.\ ponticum_2 \mid \text{Allelopathic compound}_3)$$

Tukey's post-hoc analysis was conducted on 'allelopathic compounds' to identify which groups differ from which others.

The effects of vegetation type (open grassland, woodland and woodland which had been invaded by *R. ponticum*) and known allelopathic compounds (ferulic acid,

quercetin and dH_2O control) on the germination and growth of the study species (Y) were examined using the following two-factor fully cross-factored ANOVA model (hence-forth referred to as Model 4.9), with vegetation type, allelopathic compound, and their interaction as fixed factors:

$$\log_{10}(Y) = S'_{16}(\text{Vegetation type}_3 \mid \text{Allelopathic compound}_3)$$

Tukey's post-hoc analysis was conducted on both 'vegetation type' and 'allelopathic compound' to identify which groups differ from which others. As site and woodland type were shown to have no effect ($P > 0.050$), these were not included in the statistical analysis.

6.3 Results

6.3.1 Summary of main findings

Environmental conditions were shown to have a significant effect on the susceptibility of *L. perenne* and *T. repens* to allelopathic compounds, and on the allelopathic potential of *R. ponticum*. However, the effects were found to differ on germination, root elongation and leaf appearance, and to also vary between the two species, with *T. repens* appearing to be more susceptible to environmental stresses (Table 6. 1).

Table 6. 1. Summary table of main findings from chapter 6.

	Germination	**Root elongation**	**Leaf appearance**
Light availability + *R. ponticum*	n.s	n.s	*R. ponticum* increased inhibitory effect of shade on *T. repens*
Light availability + known allelopathic compounds	n.s	Shade reduced inhibitory effect of ferulic acid on *T. repens*	n.s
Nutrient availability + lab soil leachates	Nutrients reduced inhibitory effect of *R. ponticum* on *T. repens*	*R. ponticum* reduced inhibitory effect of nutrients on both species	n.s
Nutrient availability + field soil	Additions of nutrients increased inhibitory effect of *R. ponticum* on *T. repens*	Nutrients reduced inhibitory effect of woodland on *L. perenne*. Nutrients increased *T. repens*, but only in woodland without *R. ponticum*.	Nutrients reduced the inhibitory effect of woodland on *T. repens*
Light availability and soil pH on allelopathic potential	When grown in shade, *R. ponticum* had a greater allelopathic effect on both species	n.s	*T. repens* was slower in leachates from *R. ponticum* grown in pH 4.62 in full sun, but slower in leachates from *R. ponticum* grown in pH 3.66 in shade

(continued)

(Table 6. 1 continued)

Q. robur* / *P. sylvestris* + *R. ponticum	*Q. robur* reduced stimulatory effect of *R. ponticum* on *T. repens*	n.s	n.s
Known allelopathic compounds + lab soil	*R. ponticum* reduced inhibitory effect of ferulic acid on *T. repens*	n.s	Quercetin increased inhibitory effect of *R. ponticum* on *T. repens*
Known allelopathic compounds + field soil	*R. ponticum* increased inhibitory effect of ferulic acid on *T. repens*	n.s	n.s

n.s = no interaction (P >0.050)

6.3.2 Effect of reduced light availability on the allelopathic effect of *R. ponticum* and known allelopathic compounds

i. Germination

The reduced light availability found beneath *R. ponticum* did not enhance the effect of *R. ponticum* (Vegetation type * PAR interactions $F_{2,186} = 1.41$, $P = 0.248$ and $F_{2,186} = 0.46$, $P = 0.634$ from Model 4.1 analysis for *L. perenne* and *T. repens* respectively) or of the known allelopathic compounds (Allelopathic compound * PAR interactions $F_{2,42} = 0.11$, $P = 0.892$ and $F_{2,42} = 0.78$, $P = 0.467$ from Model 4.2 analysis for *L. perenne* and *T. repens* respectively) on the germination of either of the species tested.

ii. Root elongation

The reduced light availability found beneath *R. ponticum* did not enhance the effect of *R. ponticum* on the root elongation of either species (Vegetation type * PAR interactions $F_{2,186} = 1.38$, $P = 0.255$ and $F_{2,186} = 0.67$, $P = 0.513$ from Model 4.1 analysis for *L. perenne* and *T. repens* respectively), or of the known allelopathic compounds on the root elongation of *L. perenne* (Allelopathic compound * PAR interaction $F_{2,42} = 0.59$, $P = 0.558$ from Model 4.2 analysis). However, it was shown to reduce the inhibitory effect of ferulic acid on root elongation of *T. repens* (Allelopathic compound * PAR interaction $F_{2,42} = 10.51$, P <0.001 from Model 4.2 analysis) (Figure 6. 1).

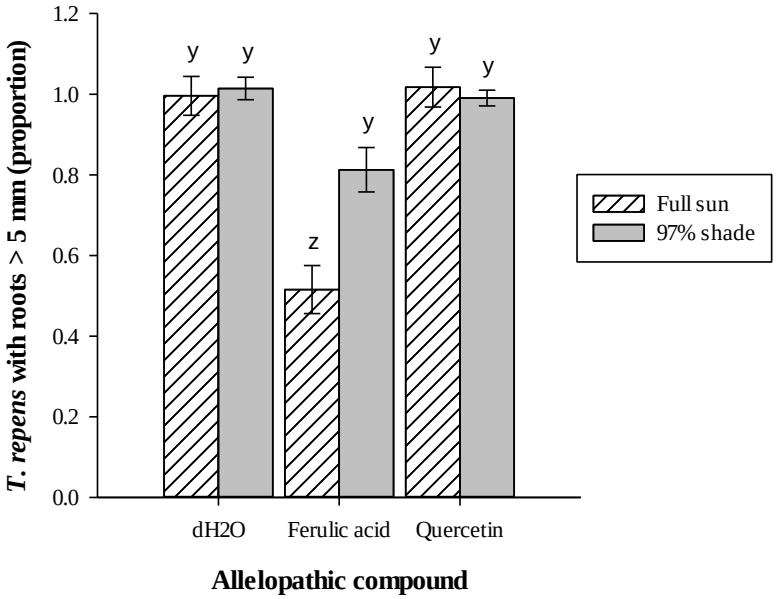


Figure 6. 1. Differences in mean root elongation of *T. repens* in distilled water, ferulic acid and quercetin, in full sun and 97% shade (+/- 1 SE) (n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

iii. Leaf appearance

The reduced light availability found beneath *R. ponticum* did not enhance the effect of the known allelopathic compounds on the leaf appearance of either species (Allelopathic compound * PAR interactions $F_{2,42}$ = 1.17, P = 0.321 and $F_{2,42}$ = 1.37, P = 0.266 from Model 4.2 analysis for *L. perenne* and *T. repens* respectively), or of *R. ponticum* on the leaf appearance of *L. perenne* (Vegetation type * PAR interaction $F_{2,186}$ = 1.95, P = 0.146 from Model 4.1 analysis). However, the reduced light availability was shown to have a greater inhibitory effect on leaf appearance of *T. repens* in leachates from soil which had supported *R. ponticum* (Vegetation type * PAR interaction $F_{2,186}$ = 3.06, P = 0.049 from Model 4.1 analysis) (Figure 6. 2).

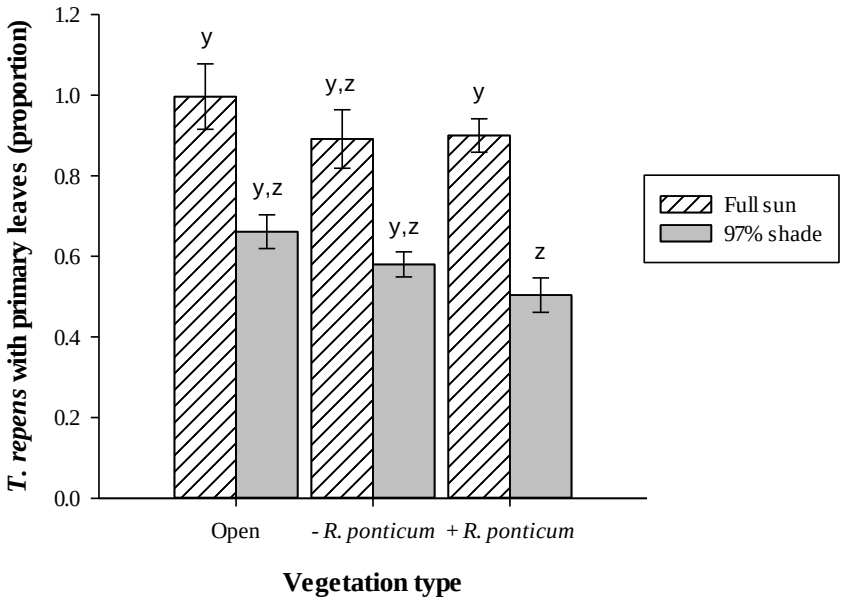


Figure 6. 2. Differences in mean leaf appearance of *T. repens* in aqueous leachates from open grassland, uninvaded woodland and woodland which had been invaded by *R. ponticum*, in full sun and 97% shade (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

6.3.3 Effect of nutrient availability on the allelopathic effect of *R. ponticum*

i. Germination

Addition of nutrients did not reduce the effect of soil which had supported *R. ponticum* under controlled conditions on the germination of either species (*R. ponticum* * Nutrients interactions $F_{1,28} = 0.01$, $P = 0.942$ and $F_{1,28} = 0.20$, $P = 0.657$ from Model 4.4 analysis for *L. perenne* and *T. repens* respectively), and it was also shown not to reduce the effect of leachates from soil which had supported *R. ponticum* under controlled conditions on *L. perenne* (*R. ponticum* * Nutrients interaction $F_{1,36} = 0.22$, $P = 0.644$ from Model 4.3 analysis). However, addition of nutrients to leachates from soil which had supported *R. ponticum* under controlled conditions removed the inhibitory effect of *R. ponticum* on the germination of *T. repens* (*R. ponticum* * Nutrients interaction $F_{1,36} = 5.36$, $P = 0.026$ from Model 4.3 analysis) (Figure 6. 3), and addition of nutrients to field soil was actually found to increase the inhibitory effect of *R. ponticum* on the germination of *T. repens* (Vegetation type * Nutrients interaction $F_{2,186} = 5.73$, $P = 0.004$ from Model 4.5

analysis) (Figure 6. 4), although it did not reduce the effect of *R. ponticum* on *L. perenne* (Vegetation type * Nutrients interaction $F_{2,186} = 2.82$, $P = 0.063$ from Model 4.5 analysis).

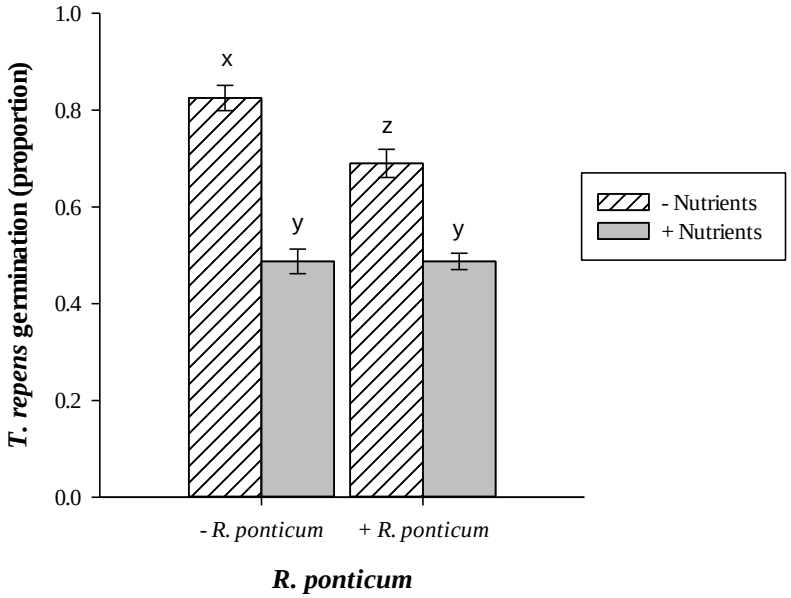


Figure 6. 3. Differences in mean germination of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions, with and without the addition of nutrients (+/- 1 SE) (n= 10). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

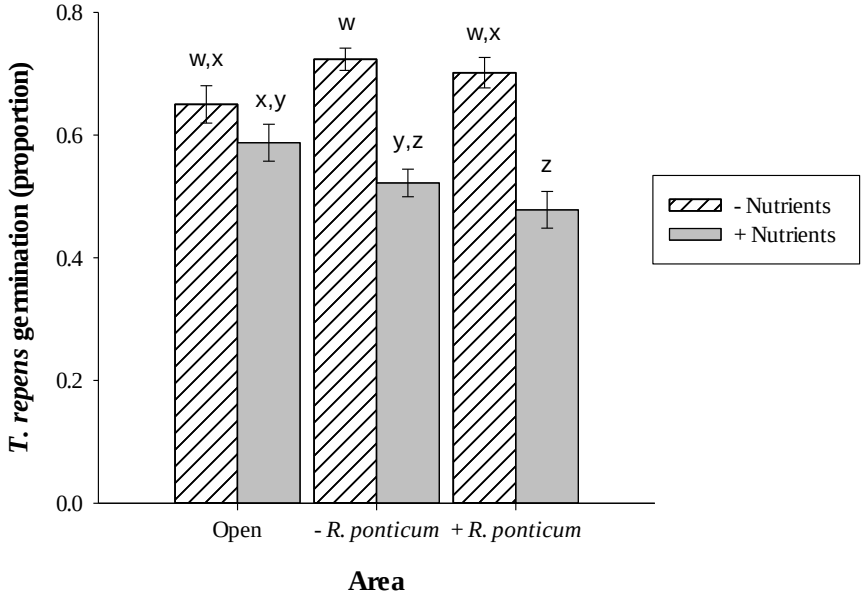


Figure 6. 4. Differences in mean germination of *T. repens* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum*, with and without the addition of nutrients (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Addition of nutrients reduced the root elongation of *L. perenne* (Nutrients main effect $F_{1,36}$ = 23.54, P <0.001 from Model 4.3 analysis) and *T. repens* (Nutrients main effect $F_{1,36}$ = 5.95, P = 0.020 from Model 4.3 analysis), although this effect was removed by leachates from soil which had supported *R. ponticum* under controlled conditions (*R. ponticum* * Nutrients interactions $F_{1,36}$ = 6.02, P = 0.019 and $F_{1,36}$ = 4.28, P = 0.046 for *L. perenne* and *T. repens* respectively from Model 4.3 analysis) (Figure 6. 5, Figure 6. 7), and nutrients had no effect when seedlings were grown directly in soil which had supported *R. ponticum* under controlled conditions (*R. ponticum* * Nutrients interactions $F_{1,28}$ = 0.01, P = 0.939 and $F_{1,28}$ = 0.002, P = 0.962 from Model 4.4 analysis for *L. perenne* and *T. repens* respectively). However, nutrients were found to reduce the inhibitory effect of woodland on the root elongation of *L. perenne* (Vegetation type * Nutrients interaction $F_{2,186}$ = 8.31, P <0.001 from Model 4.5 analysis), although it had no effect on the effect of *R. ponticum* (Figure 6. 6), and they were also found to increase the root elongation of *T. repens*, but only in woodland without *R. ponticum* (Vegetation type * Nutrients interaction $F_{2,186}$ = 5.56, P = 0.005 from Model 4.5 analysis) (Figure 6. 8).

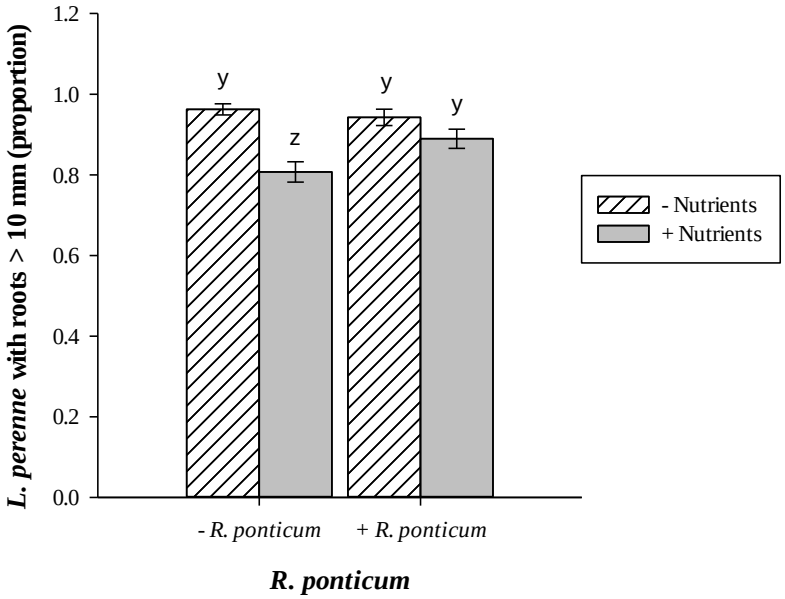


Figure 6. 5. Differences in mean root elongation of *L. perenne* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions, with and without the addition of nutrients (+/- 1 SE) (n= 10). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

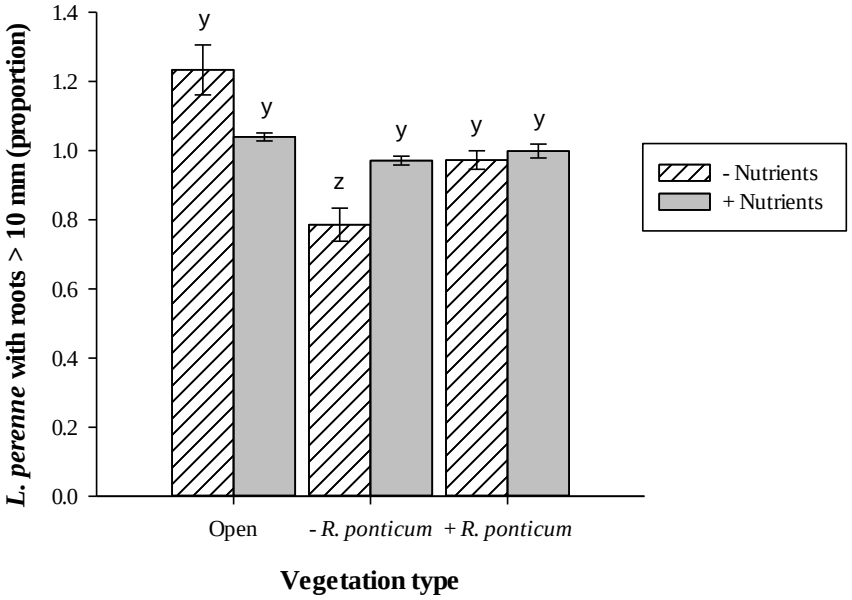


Figure 6. 6. Differences in mean root elongation of *L. perenne* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum*, with and without the addition of nutrients (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

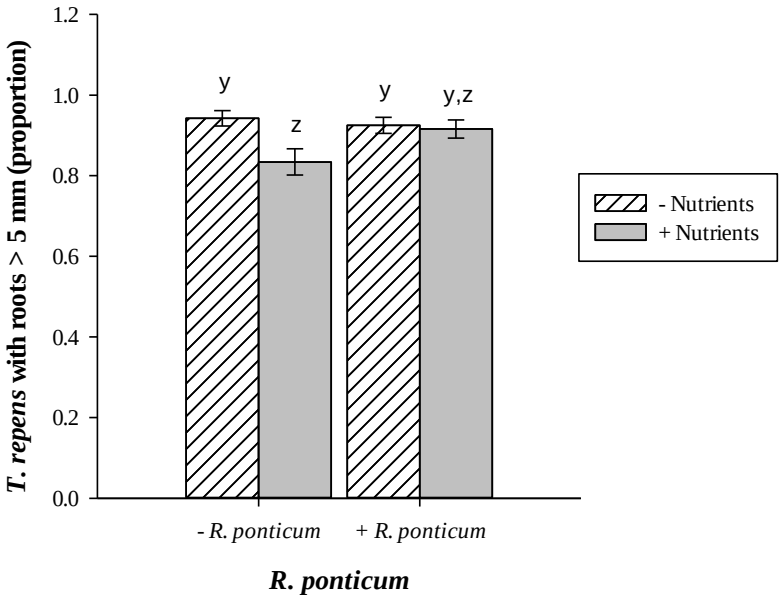


Figure 6. 7. Differences in mean root elongation of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions, with and without the addition of nutrients (+/- 1 SE) (n= 10). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

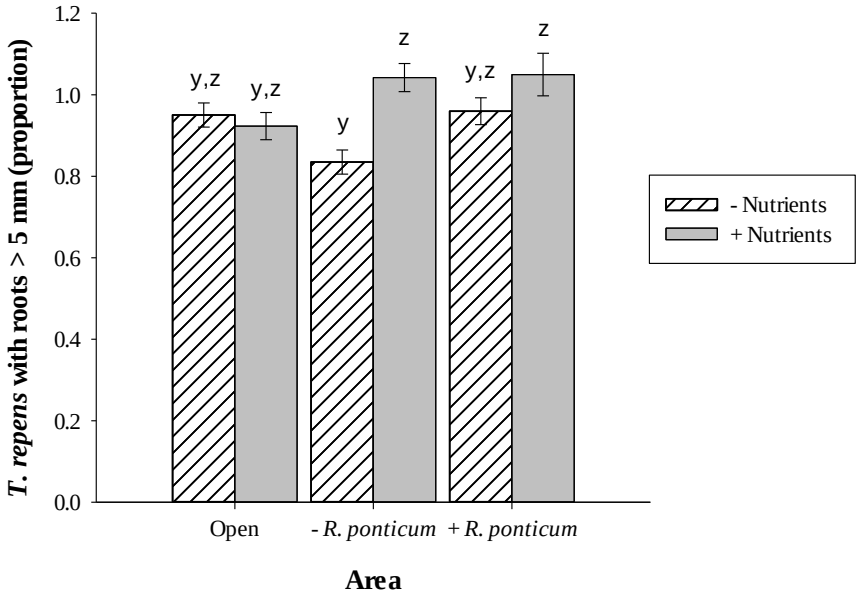


Figure 6. 8. Differences in mean root elongation of *T. repens* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum*, with and without the addition of nutrients (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

iii. Leaf appearance

Addition of nutrients did not reduce the effect of soil which had supported *R. ponticum* under controlled conditions on the leaf appearance of *L. perenne* (*R. ponticum* * Nutrients interactions $F_{1,36} = 3.31$, $P = 0.077$ and $F_{1,28} = 0.42$, $P = 0.525$ from Model 4.3 and Model 4.4 analysis for soil leachates and soil respectively) or *T. repens* (*R. ponticum* * Nutrients interactions $F_{1,36} = 1.03$, $P = 0.318$ and $F_{1,28} = 2.76$, $P = 0.108$ from Model 4.3 and Model 4.4 analysis for soil leachates and soil respectively). Addition of nutrients to field soil was also found not to reduce the effect of *R. ponticum* on the leaf appearance of *L. perenne* (Vegetation type * Nutrients interaction $F_{2,186} = 1.82$, $P = 0.166$ from Model 4.5 analysis), although it did reduce the inhibitory effect of woodland on the leaf appearance of *T. repens* (Vegetation type * Nutrients interaction $F_{2,186} = 9.20$, $P < 0.001$ from Model 4.5 analysis) (Figure 6. 9).

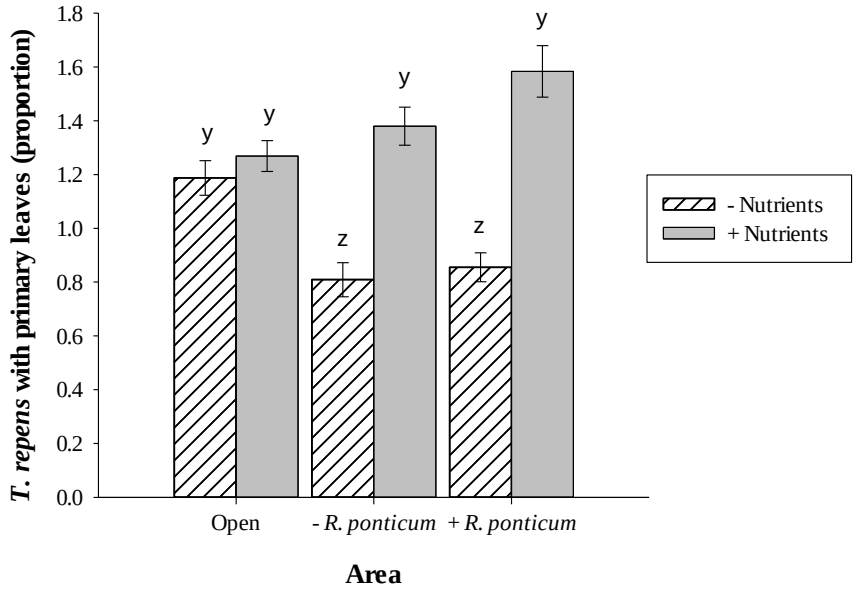


Figure 6. 9. Differences in mean leaf appearance of *T. repens* in aqueous leachates from open grassland, uninvaded woodland, and woodland which had been invaded by *R. ponticum*, with and without the addition of nutrients (+/- 1 SE) (n= 32). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other ($P < 0.050$).

6.3.4 Allelopathic effect of *R. ponticum* grown under different light and pH conditions

i. Germination

When grown under reduced light conditions, *R. ponticum* was found to have a greater allelopathic effect on the germination of *L. perenne* (PAR main effect $F_{1,28} = 10.40$, $P = 0.003$ from Model 4.6 analysis) and *T. repens* (PAR main effect $F_{1,28} = 17.64$, $P < 0.001$ from Model 4.6 analysis) (Figure 6. 10, Figure 6. 11). However, growing *R. ponticum* under different pH conditions did not enhance the allelopathic effect of the rhododendron on the germination of the two species, either on its own (pH main effect $F_{1,28} = 0.23$, $P = 0.636$ and $F_{1,28} = 0.90$, $P = 0.350$ from Model 4.6 analysis for *L. perenne* and *T. repens* respectively) or in combination with the reduced light availability (PAR * pH interactions $F_{1,28} = 0.12$, $P = 0.734$ and $F_{1,28} = 2.56$, $P = 0.121$ from Model 4.6 analysis for *L. perenne* and *T. repens* respectively).

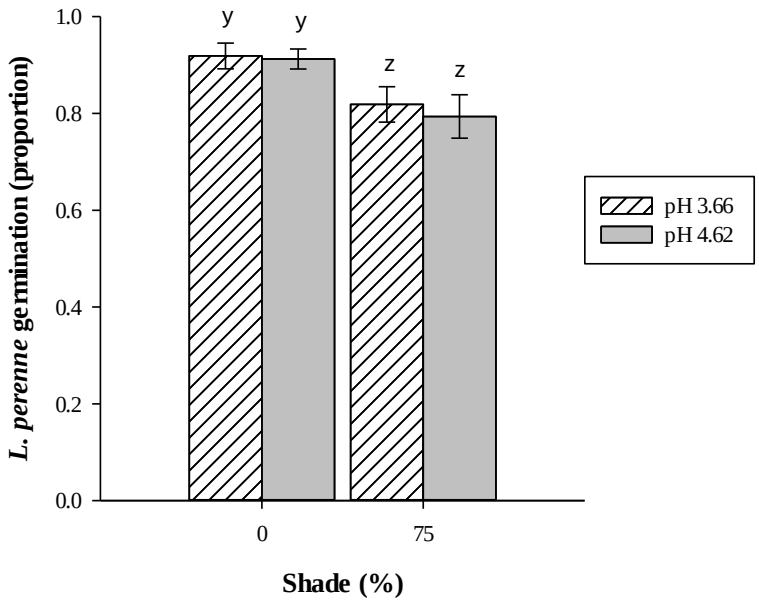


Figure 6. 10. Differences in mean germination of *L. perenne* in aqueous leachates from soil which had supported *R. ponticum* growing under different light (PAR) and pH conditions (+/- 1 SE) (n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other ($P < 0.050$).

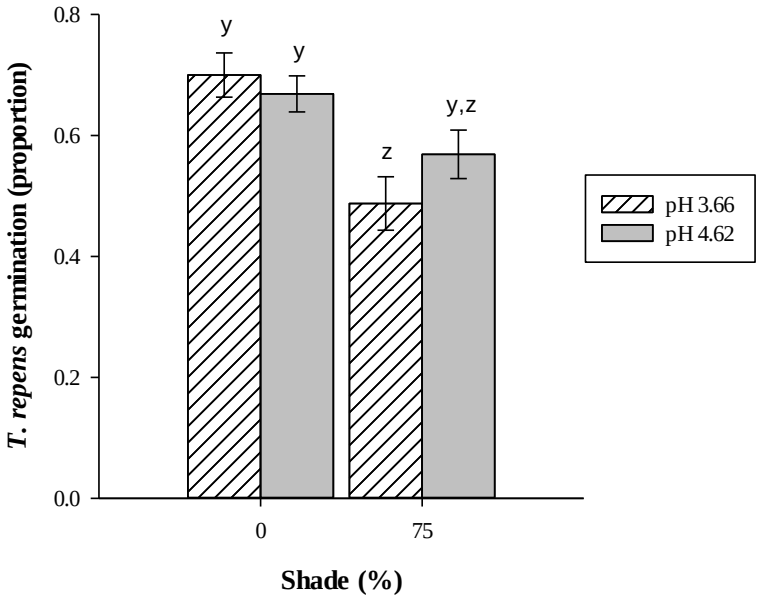


Figure 6. 11. Differences in mean germination of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* growing under different light (PAR) and pH conditions (+/- 1 SE) (n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Growing *R. ponticum* under different light (PAR main effect $F_{1,28}$ = 0.83, P = 0.370 and $F_{1,28}$ = 1.95, P = 0.174 from Model 4.6 analysis for *L. perenne* and *T. repens* respectively) or pH conditions (pH main effect $F_{1,28}$ = 0.989, P = 0.328 and $F_{1,28}$ = 0.01, P = 0.925 from Model 4.6 analysis for *L. perenne* and *T. repens* respectively) were shown not to increase the allelopathic effect of the rhododendron on the root elongation of either of the species tested. There was also no interaction between light availability and pH on the allelopathic effect of *R. ponticum* on the root elongation of either species (PAR * pH interactions $F_{1,28}$ = 3.95, P = 0.057 and $F_{1,28}$ = 2.90, P = 0.100 from Model 4.6 analysis for *L. perenne* and *T. repens* respectively).

iii. Leaf appearance

Growing *R. ponticum* under different light (PAR main effect $F_{1,28}$ = 0.46, P = 0.503 and $F_{1,28}$ = 0.32, P = 0.578 from Model 4.6 analysis for *L. perenne* and *T. repens* respectively) or pH conditions (pH main effect $F_{1,28}$ = 1.40, P = 0.247 and $F_{1,28}$ = 0.03, P = 0.863 from Model 4.6 analysis for *L. perenne* and *T. repens* respectively) were shown not to increase the allelopathic effect of the rhododendron on the leaf

appearance of either of the species tested. There was also no interaction between light availability and pH on the allelopathic effect of *R. ponticum* on the leaf appearance of *L. perenne* (PAR * pH interaction $F_{1,28} = 2.60$, $P = 0.118$ from Model 4.6 analysis), although leaf appearance of *T. repens* was slower in soil leachates from rhododendrons which had been growing in pH 4.62 in full sun, but slower in leachates from rhododendrons which had been growing in pH 3.66 in the shade (PAR * pH interaction $F_{1,28} = 8.95$, $P = 0.006$ from Model 4.6 analysis) (Figure 6. 12).

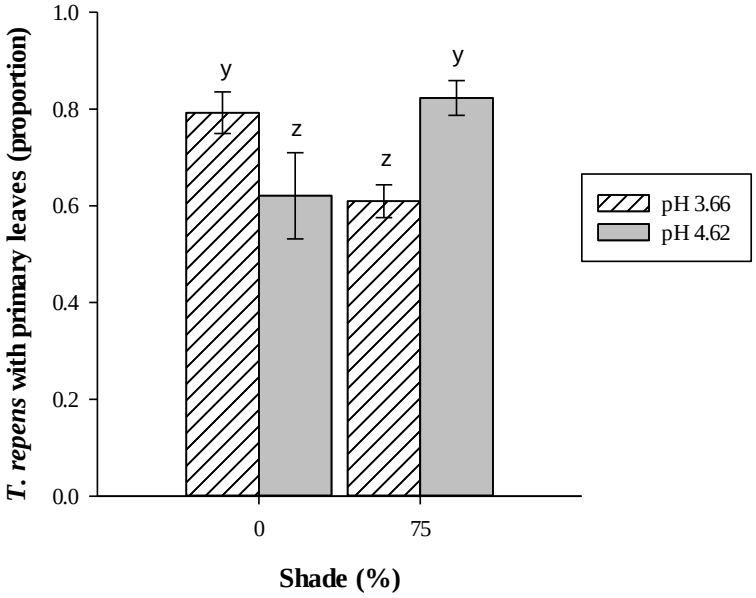


Figure 6. 12. Differences in mean leaf appearance of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* growing under different light (PAR) and pH conditions (+/- 1 SE) (n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

6.3.5 Effect of *Q. robur* or *P. sylvestris* leachates and known allelopathic compounds on the allelopathic effect of *R. ponticum*

i. Germination

Leachates from soil which had supported *P. sylvestris* or *Q. robur* under controlled conditions had no effect on the germination of *L. perenne* (*P. sylvestris* and *Q. robur* main effects $F_{1,60} = 0.67$, $P = 0.416$ and $F_{1,60} = 0.38$, $P = 0.539$ from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively) or *T. repens* (*P. sylvestris*

and *Q. robur* main effects $F_{1,60} = 0.74$, $P = 0.394$ and $F_{1,60} = 0.41$, $P = 0.523$ from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively). *P. sylvestris* leachates were also shown not to enhance the effect of *R. ponticum* leachates on either of the species tested (*R. ponticum* * *P. sylvestris* interaction $F_{1,60} = 0.07$, $P = 0.793$ and $F_{1,60} = 2.12$, $P = 0.150$ from Model 4.7a analysis for *L. perenne* and *T. repens* respectively), or between *R. ponticum* and *Q. robur* leachates on *L. perenne* (*R. ponticum* * *Q. robur* interaction $F_{1,60} = 0.38$, $P = 0.4539$ from Model 4.7b analysis). However, leachates from *Q. robur* were found to remove the effect of *R. ponticum* (*R. ponticum* * *Q. robur* interaction $F_{1,60} = 4.93$, $P = 0.030$ from Model 4.7b analysis) (Figure 6. 13).

Addition of known allelopathic compounds did not to enhance the effect of leachates from soil which had supported *R. ponticum* on the germination of *L. perenne* (*R. ponticum* * Allelopathic compound and Vegetation type * Allelopathic compound interactions $F_{2,42} = 0.77$, $P = 0.470$ and $F_{4,135} = 2.02$, $P = 0.095$ from Model 4.8 and Model 4.9 analysis for lab soil and field soil respectively). However, ferulic acid was shown to have an inhibitory effect on the germination of *T. repens*, but only in leachates from field soil which had supported *R. ponticum* (Vegetation type * Allelopathic compound interaction $F_{4,135} = 2.77$, $P = 0.030$ from Model 4.9 analysis) (Figure 6. 15), but leachates from soil which had supported *R. ponticum* under controlled conditions were found to reduce the inhibitory effect of ferulic acid on germination (*R. ponticum* * Allelopathic compound interaction $F_{2,42} = 3.46$, $P = 0.041$ from Model 4.8 analysis) (Figure 6. 14).

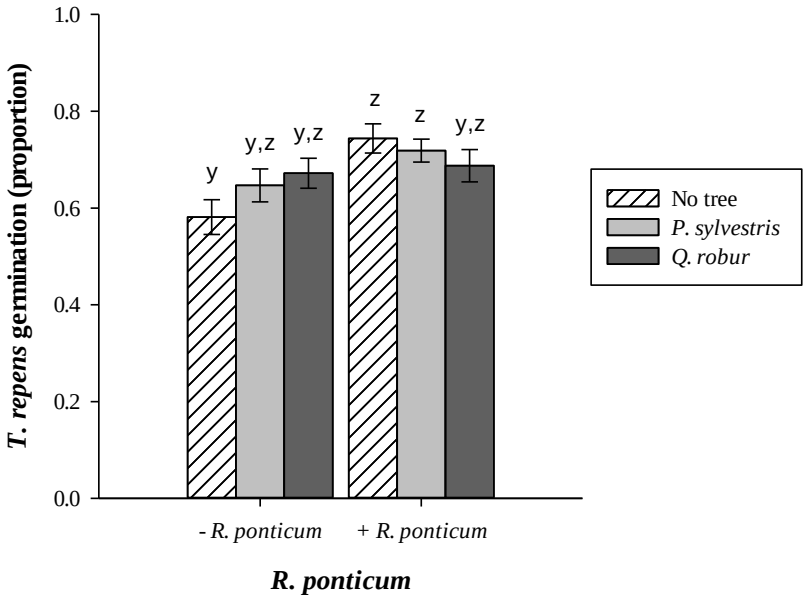


Figure 6. 13. Differences in mean germination of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions, with and without leachates from soil which had supported *P. sylvestris* or *Q. robur* (+/- 1 SE) (n= 16). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

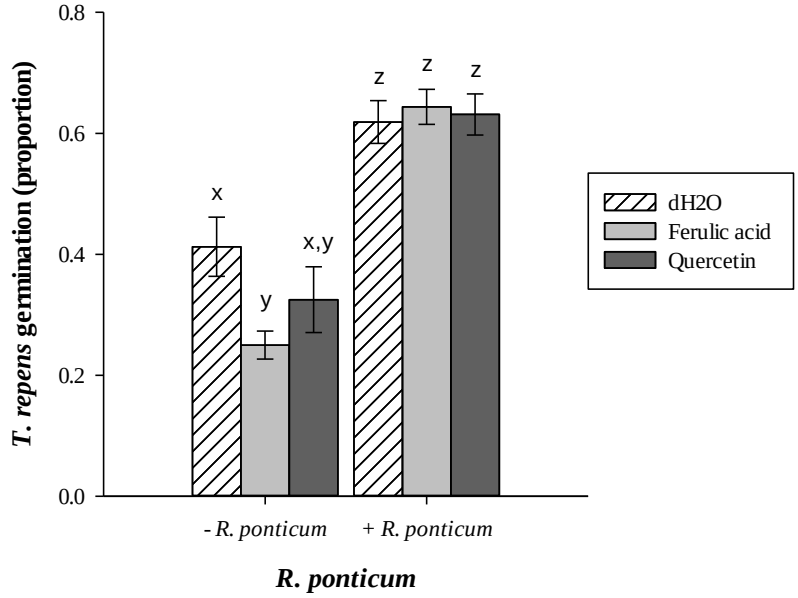


Figure 6. 14. Differences in mean germination of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions, with and without the addition of ferulic acid or quercetin (+/- 1 SE) (n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

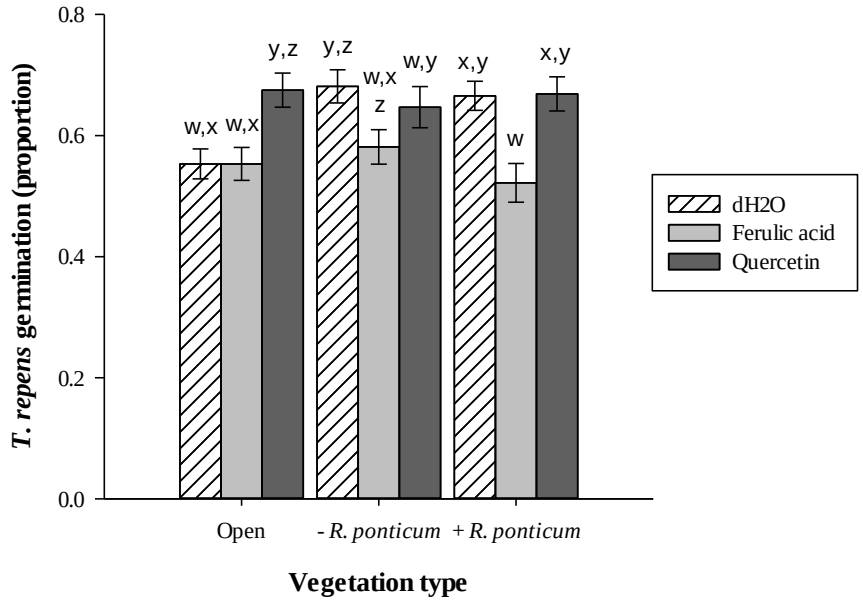


Figure 6. 15. Differences in mean germination of *T. repens* in aqueous leachates from open grassland, uninvaded woodland and woodland which had been invaded by *R. ponticum*, with and without the addition of ferulic acid or quercetin (+/- 1 SE) (n= 16). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

ii. Root elongation

Leachates from soil which had supported *P. sylvestris* or *Q. robur* under controlled conditions had no effect on the root elongation of *L. perenne* (*P. sylvestris* and *Q. robur* main effects $F_{1,60}$ = 0.76, P = 0.386 and $F_{1,60}$ = 0.52, P = 0.472 from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively) or *T. repens* (*P. sylvestris* and *Q. robur* main effects $F_{1,60}$ = 2.53, P = 0.117 and $F_{1,60}$ = 3.38, P = 0.071 from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively), and neither *P. sylvestris* or *Q. robur* leachates enhanced the effect of *R. ponticum* on either *L. perenne* (*R. ponticum* * *P. sylvestris* and *R. ponticum* * *Q. robur* interactions $F_{1,60}$ = 1.07, P = 0.306 and $F_{1,60}$ = 0.68, P = 0.414 from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively) or *T. repens* (*R. ponticum* * *P. sylvestris* and *R. ponticum* * *Q. robur* interactions $F_{1,60}$ = 0.01, P = 0.934 and $F_{1,60}$ = 0.11, P = 0.741 from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively).

Addition of known allelopathic compounds did not enhance the effect of leachates from soil which had supported *R. ponticum* on the root elongation of either *L. perenne* (*R. ponticum* * Allelopathic compound and Vegetation type * Allelopathic

compound interactions $F_{2,42} = 0.71$, $P = 0.500$ and $F_{4,135} = 1.18$, $P = 0.324$ from Model 4.8 and Model 4.9 analysis for lab soil and field soil respectively) or *T. repens* (*R. ponticum* * Allelopathic compound and Vegetation type * Allelopathic compound interactions $F_{2,42} = 0.98$, $P = 0.383$ and $F_{4,135} = 0.60$, $P = 0.663$ from Model 4.8 and Model 4.9 analysis for lab soil and field soil respectively).

iii. Leaf appearance

Leachates from soil which had supported *P. sylvestris* or *Q. robur* under controlled conditions had no effect on the leaf appearance of *L. perenne* (*P. sylvestris* and *Q. robur* main effects $F_{1,60} = 0.001$, $P = 0.977$ and $F_{1,60} = 0.07$, $P = 0.789$ from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively) or *T. repens* (*P. sylvestris* and *Q. robur* main effects $F_{1,60} = 0.23$, $P = 0.635$ and $F_{1,60} = 0.29$, $P = 0.591$ from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively), and neither *P. sylvestris* or *Q. robur* leachates enhanced the effect of *R. ponticum* on either *L. perenne* (*R. ponticum* * *P. sylvestris* and *R. ponticum* * *Q. robur* interactions $F_{1,60} = 2.25$, $P = 0.138$ and $F_{1,60} = 0.36$, $P = 0.549$ from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively) or *T. repens* (*R. ponticum* * *P. sylvestris* and *R. ponticum* * *Q. robur* interactions $F_{1,60} = 1.09$, $P = 0.302$ and $F_{1,60} = 0.04$, $P = 0.842$ from Model 4.7a and 4.7b analysis for *P. sylvestris* and *Q. robur* respectively).

Addition of known allelopathic compounds did not enhance the effect of leachates from field soil which had supported *R. ponticum* on the leaf appearance of either species (Vegetation type * Allelopathic compound interactions $F_{4,135} = 0.63$, $P = 0.639$ and $F_{4,135} = 0.88$, $P = 0.480$ Model 4.9 analysis for *L. perenne* and *T. repens* respectively), and they did not enhance the effect of leachates from soil which had supported *R. ponticum* under controlled conditions on *L. perenne* (*R. ponticum* * Allelopathic compound $F_{2,42} = 1.01$, $P = 0.374$ from Model 4.8 analysis). However, the combination of quercetin with soil which had supported *R. ponticum* under controlled conditions was found to have a greater inhibitory effect on leaf appearance of *T. repens*, than on their own (*R. ponticum* * Allelopathic compound interaction $F_{2,42} = 3.47$, $P = 0.040$ from Model 4.8 analysis) (Figure 6. 16).

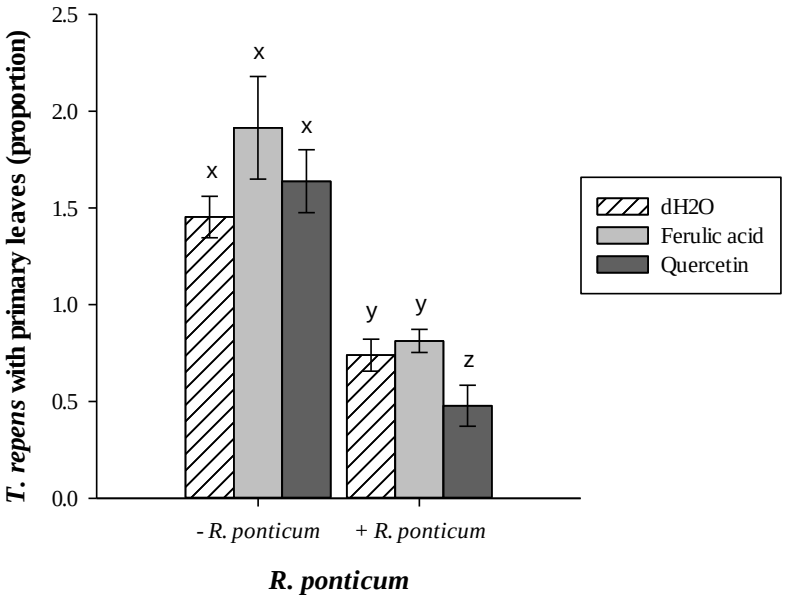


Figure 6. 16. Differences in mean leaf appearance of *T. repens* in aqueous leachates from soil which had supported *R. ponticum* under controlled conditions, with and without the addition of ferulic acid or quercetin (+/- 1 SE) (n= 8). Letters show which treatments Tukey's post-hoc analysis identified as being different from each other (P <0.050).

6.4 Discussion

Although reduced light availability was found to enhance the allelopathic effect of *R. ponticum* on the leaf appearance of *T. repens*, the combination of allelopathy and light stress could not explain the inhibition of growth of the native species beneath *R. ponticum* previously reported in the field (Cross, 1981; Eşen & Zedaker, 2004). Nutrient stress also appears to enhance the allelopathic effect of the rhododendron on the germination of *T. repens*, although even then, neither species was completely inhibited. Compounds produced by *Q. robur* and *P. sylvestris* were shown not to increase the allelopathic effect of *R. ponticum*. However, the reduced light availability and acidic conditions found in woodland in the New Forest did increase the accumulation of allelopathic compounds in the soil.

Previous studies have indicated that the combination of environmental stresses and allelopathic compounds likely play an important role in the inhibition of growth of the native species beneath *R. ponticum*, and light availability is known to be extremely important in affecting the inhibitory effect of allelopathic compounds (Von Elert and Jüttner, 1996). This study did show that leachates from soil which had supported *R. ponticum* had a greater inhibitory effect on the leaf appearance of *T. repens* under light limited growth conditions. This suggests that the combination of allelopathic compounds and the reduced light availability is important in the impact of *R. ponticum*, and *Rhododendron* species have been shown to produce compounds, including ferulic acid and quercetin, which interfere with photosynthesis (Moreland & Novitzky, 1987; Mersie & Singh, 1993), so it was expected that these allelochemicals would have a much greater inhibitory effect under light limited growth conditions (Von Elert & Jüttner, 1996).

However, the reduced light availability beneath stands of *R. ponticum* did not enhance the inhibitory effect of ferulic acid or quercetin on the germination or growth of either *L. perenne* or *T. repens*, and the inhibitory effect of ferulic acid on root elongation of *T. repens* was actually reduced in shaded conditions, supporting previous work by Lobón *et al*. (2002) which found that exudates from *Cistus ladanifer*, which have been shown to contain ferulic acid (Chaves *et al*., 2001), had the greatest inhibitory effect on both germination and seedling development under

long photoperiods (Lobón *et al*., 2002). This study also demonstrated that the reduced light conditions found beneath *R. ponticum* had no effect on the allelopathic effect of *R. ponticum* on the germination or root elongation of either species, or the leaf appearance of *L. perenne*, suggesting that other factors must be involved in the inhibition of growth of the native species beneath *R. ponticum* in the New Forest.

Previous work has shown that nutrient stress can also be extremely important in exacerbating the effect of allelopathic compounds (Kong. *et al*., 2002). This study demonstrated that addition of nutrients to the soil leachates removed the inhibitory effect of *R. ponticum* on the germination of *T. repens*, although addition of nutrients did not reduce the inhibitory effect when seedlings were grown directly in soil which had supported *R. ponticum*. However, even on its own, addition of nutrients to soil had no effect on the germination or growth of either of the species tested (Appendix VII), suggesting that the nutrients might be binding to the soil particles, making them unavailable for uptake by the plants and therefore not having any effect (Barber, 1995). Addition of nutrients were also found to have no effect on the inhibitory effect of *R. ponticum* on the germination of *L. perenne*, or on the root elongation or leaf appearance of either species, suggesting that other factors must be involved in the impact of *R. ponticum*.

Previous studies have shown that many of the tree species commonly found in the New Forest produce their own allelopathic compounds, and a number of compounds have been identified in the litter and soil from *Q. robur*, including ferulic acid, *p*-coumaric acid, vanillic acid, benzoic acid and cinnamic acid (Kuiters & Sarink, 1986), and *P. sylvestris*, including ferulic acid, *p*-coumaric acid and quercetin (Oleszek *et al*., 2002; Suominen *et al*., 2003). However, leachates from *P. sylvestris* and *Q. robur* were found to have no effect on the germination, root elongation or leaf appearance of either species. Eleven allelochemicals were identified in the exudate of *Cistus ladanifer*, which were shown to have a more negative effect on both germination and seedling growth of *Rumex crispus* (curly dock) when acting conjointly, than when acting alone (Chaves *et al*., 2001), and the inhibitory effect of ferulic acid (Einhellig, 1996) and aqueous extracts of *Sorghum bicolor*, *Helianthus annuus* and *Morus alba* (Khaliq *et al*., 2012) were all shown to be increased by low levels of herbicides, demonstrating that combinations of allelopathic compounds can have a much greater effect together than individually (Einhellig, 1996).

This study demonstrated that the combination of soil leachates from woodland which had been invaded by *R. ponticum* with ferulic acid had a greater inhibitory effect on the germination of *T. repens*, than individually, and the combination of soil leachates with quercetin had a greater inhibitory effect on the leaf appearance of *T. repens*. This suggests that the allelopathic compounds produced by *Q. robur* or *P. sylvestris* might be enhancing the inhibitory effect of allelopathic compounds produced by *R. ponticum* in the New Forest. However, the allelopathic effect of leachates from soil which had supported *R. ponticum* on the germination or growth of *L. perenne* and *T. repens* was not increased by leachates from either *Q. robur* or *P. sylvestris*, and leachates from *Q. robur* were found to reduce the effect of *R. ponticum* on the germination of *T. repens*. *Q. robur* has been shown to produce tannins (Salminen *et al*., 2004), which can form stable complexes with allelochemicals in the soil (Halvorson *et al*., 2009). These can differ in their allelopathic effect from the original compounds, or can make them unavailable for uptake by the target plant (Kang *et al*., 2002). Tannins can also reduce the availability of nitrogen in the soil, either by increasing microbial activity, or through the formation of tannin-protein complexes (Halvorson *et al*., 2009), and this has been shown to reduce the sensitivity of plants to some allelopathic compounds (Leflaive & Ten-Hage, 2009). Many other species found in the New Forest have also been shown to produce tannins, including *Betula pendula*, *P. sylvestris* (Suominen *et al*., 2003) and *Fagus sylvatica* (Bussotti *et al*., 1998), so although it was not shown in this study, it is likely that these species do play an important role in determining the inhibitory effect of compounds produced by *R. ponticum*, and further work is needed to show if this is the case.

MacDougall and Turkington (2005) showed that removing the dominant perennial grasses to simulate fire suppression in invaded oak savanna resulted in a shift in dominance from perennial grasses to perennial forbs, and suggested that rather than changes in environmental conditions being due to the arrival of invasive species, the conditions were already present and provided the ideal conditions for invasive species to become established (MacDougall & Turkington, 2005). Seedlings of *R. ponticum* have difficulty invading areas where there is already continuous ground cover from native plants (Cross, 1981), and it was suggested that the reduced light availability and the acidic pH conditions found in the woodland in the New Forest could already have been were more limiting for native species than they were for *R.*

ponticum. Diversity and ground cover in uninvaded woodland was found to be lower than in open grassland at many of the sites in the New Forest (personal observation). However, it is likely that there are some native species that are adapted to these conditions, and many species can tolerate a wide range of environmental conditions (Sterry, 2006), so pre-existing stresses cannot explain the complete inhibition of growth. However, light availability and soil pH can be extremely important in affecting the synthesis of allelopathic compounds, as well as their accumulation in the soil, and this study suggested that these conditions play another role in the success of *R. ponticum*.

Although light availability generally increases the synthesis and accumulation of allelopathic compounds (Dudt & Shure, 1994; Kato-Noguchi, 1999; Karageorgou *et al*., 2002), the concentration of allelopathic compounds in *Zingiber officinale* (Ghasemzadeh *et al*., 2010) and *Prymnesium parvum* (Granéli & Salomon, 2010) were found to be higher in the shade, and this study demonstrated that soil which had supported *R. ponticum* growing in reduced light conditions found in uninvaded woodland had a greater inhibitory effect on the germination of both *L. perenne* and *T. repens*. Although on its own pH had no effect, there was an interaction between light availability and soil pH, with acidic conditions increasing the accumulation of allelopathic compounds in the soil in the reduced light availability found in native woodland, and reducing the accumulation of allelopathic compounds in the soil in full sun. Previous studies have shown pH to be extremely important in affecting the sorption of allelochemicals, and generally, soil with a low pH exhibits a less inhibitory effect as phenolic acids bind to the negatively charged organic matter and clay particles (Whitehead *et al*., 1981; Dalton *et al*., 1989). However, pH has also been found to affect the synthesis and accumulation of allelopathic compounds in plant tissues, with the concentration of tannins and phenolic acids increasing with decreasing pH (Northup *et al*., 1995), and many phenolic acids have also been shown to be more stable in acidic conditions than in alkaline conditions (Gallet *et al*., 1999; Friedman & Jürgens, 2000). This suggests that as well as providing the ideal conditions for *R. ponticum* to invade, the reduced light availability and acidic pH conditions in woodland in the New Forest might also increase the synthesis and accumulation of allelopathic compounds in the soil beneath *R. ponticum* allowing them to have a greater effect on the native species, and allowing them to become so successful.

However, this study also found that light availability and pH had an effect on the accumulation of compounds in soil which had not supported *R. ponticum* (Appendix VIII). Many environmental conditions, including temperature and water availability, are directly correlated with light availability (Matlack, 1993), and soil pH has been shown to directly affect nutrient availability (Haynes & Swift, 1986). All these factors can have a direct effect on microbial populations (Killham, 1994). This change in microbial activity can affect the decomposition of organic matter, which can increase the concentration of any allelopathic compounds which are only released into the soil by decomposition (Singh *et al*., 2001). Many microbes also produce their own allelopathic compounds, which have been shown to inhibit the growth of several annual and perennial species (Barazani & Friedman, 2001; Singh *et al*., 2001). Therefore it is possible that in the reduced light availability and acidic conditions found in woodland in the New Forest, an increase in microbial activity might be responsible for the allelopathic effect on the native species, preventing their growth and providing the ideal conditions for *R. ponticum*, allowing it to become such an invasive species. Further work is therefore necessary to determine whether environmental stresses, such as light availability and soil pH, are increasing the synthesis and accumulation of allelopathic compounds by *R. ponticum*, or whether the increase in allelopathic activity is due to the microorganisms already present in the soil.

Chapter 7

General discussion

7.1 Key findings

This thesis has examined the influence of key environmental factors, including light availability and allelopathic compounds, on the growth of the native species beneath *R. ponticum* in the New Forest. The findings of this study will be discussed, not only in terms of their relevance to future research into the mechanisms involved in the impact of invasive plants, but also for successfully controlling their spread. *R. ponticum* is known to have an inhibitory effect on the native species (Dehnen-Schmutz *et al*., 2004), and environmental conditions were found to be altered beneath stands of *R. ponticum* compared to open grassland in the New Forest. However, there has been insufficient work investigating whether these differences in environmental conditions are enough to explain the impact on the native species, and the influence of these factors on the inhibition of growth. One of the main findings of this study was that although *R. ponticum* does produce allelopathic compounds, as well as reduce light availability to levels which should be sufficient to inhibit the native vegetation (Clinton & Vose, 1996), no single change in environmental conditions was enough to explain the inhibition of growth of the native species in the New Forest. This suggests that the impact of *R. ponticum* is not due to just one factor, but rather due to a complex combination of abiotic and biotic factors, such as competition for pollinators, or changes in herbivore activity, which can not only affect the susceptibility of plants to abiotic stresses (Nabity *et al*., 2009), but can affect the synthesis of allelopathic compounds (Thelen *et al*., 2005) (Figure 7. 1). This study also demonstrated that in the New Forest, light availability and soil pH were already altered from conditions in open grassland before the arrival of *R. ponticum*, which not only provided suitable sites for the rhododendron to establish (Cross, 1981), but enhanced its allelopathic effect on the native species.

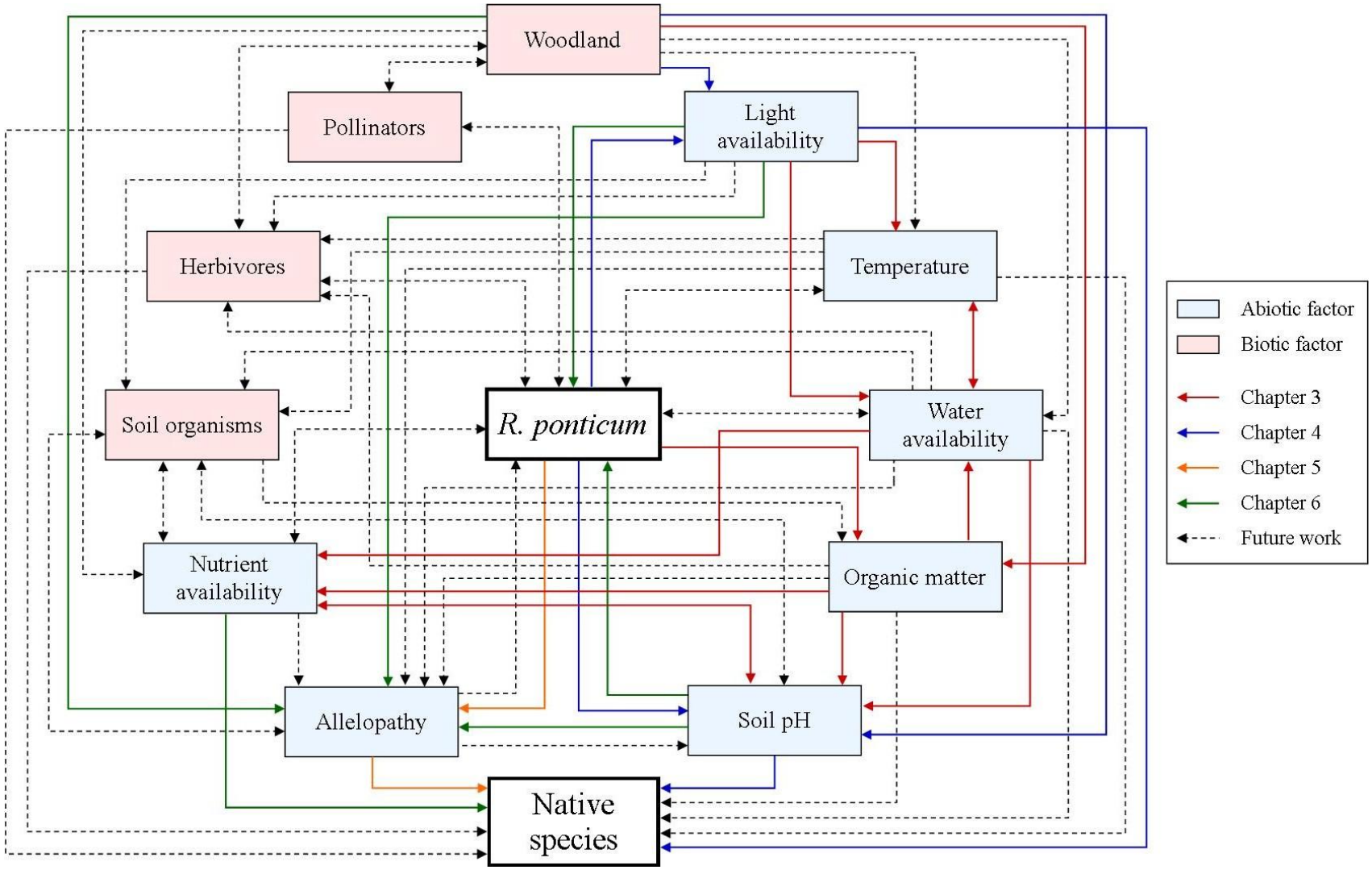


Figure 7. 1. Effect of *R. ponticum* on various biotic and abiotic factors, and the interactions between them.

7.2 Summary of key findings

Chapter 3:

- Reduced light availability beneath *R. ponticum* explained many of the differences in environmental conditions, and likely plays an important role in the inhibition of growth.
- Soil beneath *R. ponticum* was more acidic than that in open grassland, which could help to explain the impact of *R. ponticum* on the native species.
- Although temperature, water availability and organic matter were different beneath *R. ponticum* compared to open grassland, the degree of difference between many of the environmental conditions was not great enough to have a significant inhibitory effect on the native species.

Chapter 4

- Although light availability and soil pH were both different beneath *R. ponticum* compared to open grassland, neither of them could explain the inhibition of growth of the native species, either individually or in combination.
- Although soil beneath *R. ponticum* was more acidic than that in open grassland, this difference was due to the woodland in which the rhododendron was growing, and not due to the presence of the rhododendron.
- Light availability and soil pH in uninvaded woodland were already different from open grassland before the arrival of the rhododendron, and although they were not limiting for the native species, they could have provided the ideal conditions allowing *R. ponticum* to establish.

Chapter 5

- Although *R. ponticum* was found to have an allelopathic effect on the native species, allelopathy alone was not sufficient to explain the inhibition of growth of the native species beneath *R. ponticum* in the New Forest.

- On their own, leachates and root exudates from *R. ponticum* had no inhibitory effect on the species tested, suggesting that these compounds either build up over time, are transformed in the soil, or are interacting with other allelopathic compounds which were already present.

Chapter 6

- Although light and nutrient stress both enhanced the allelopathic effect of *R. ponticum*, it was still not enough to explain the inhibition of growth of the native species beneath *R. ponticum* in the New Forest, pointing to other factors being involved.
- The reduced light availability and acidic conditions found in woodland in the New Forest increased the accumulation of allelopathic compounds in the soil beneath *R. ponticum*.
- Pre-existing conditions and the presence of other species play an important role in the success of *R. ponticum* and the impact that it has on the native vegetation.

7.3 Influence of environmental conditions

Despite the fact that environmental factors do not act independently, and there are often interactions between them (Stark & Firestone, 1995; Yan *et al*., 1996; Ste-Marie & Paré, 1999), a number of workers investigating the effects of invasive plants on the native species have suggested that it is a single environmental factor, such as light limitation or allelopathy, that is responsible for the inhibition of native plant growth (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003). Reduced light availability is one of the most commonly implicated factors (Levine *et al*., 2003; Reinhart *et al*., 2006), and this study demonstrated that the low light conditions found beneath *R. ponticum* in the New Forest did have an inhibitory effect on the growth of *T. repens*. However, they had no inhibitory effect on the germination or growth of *L. perenne*, and although root elongation and leaf appearance of *T. repens* were reduced, light availability alone could only explain 75% of the inhibition. There was a strong correlation between light availability and many of the other abiotic conditions (Matlack, 1993; Fdz-Polanco *et al*., 1994; Stark & Firestone, 1995; Yan *et*

al., 1996), and temperature, water availability, organic matter and soil pH were also found to be different beneath *R. ponticum* compared to open grassland. Although the differences in these other environmental conditions were not sufficient to explain the inhibition of growth of the native species on their own, multiple environmental stresses have been shown to have a much greater effect on the native species together, rather than individually (Mittler, 2006). Therefore it is probable that the impact of *R. ponticum* on the native species is due to a combination of factors (Mittler, 2006), including water availability, the thickness of the leaf litter (Everham *et al*., 1996) and competition for pollinators (Chittka & Schürkens, 2001; Bjerknes *et al*., 2007), which were not sufficient to explain the impact on their own (Figure 7. 1).

This study found that leachates and root exudates from *R. ponticum* had no inhibitory effect on either of the species tested, and aqueous leachates from *R. ponticum* were shown to increase the germination of *L. perenne* and *T. repens*. Many ericaceous species have been found to produce allelopathic compounds which at low concentrations can stimulate growth (Mersie & Singh, 1993; Fries *et al*., 1997), and as well as increasing root elongation and leaf appearance of *T. repens* seedlings, *R. ponticum* was also shown to reduce the inhibitory effects of both soil leachates from uninvaded woodland and nutrients on the root elongation of *L. perenne* and *T. repens*, and of ferulic acid on the germination of *T. repens*. However, soil which had supported *R. ponticum* was still shown to have an allelopathic effect on the germination of *L. perenne* and germination and leaf appearance of *T. repens*. This suggests that although *R. ponticum* is releasing compounds which can stimulate growth at low concentrations, over time the concentration of these compounds build up in the soil, although this study did not identify the exact allelochemicals involved. However, allelopathy could only explain up to 60% of the inhibition, and several other species commonly found in the New Forest, including *Anemone nemorosa* (wood anemone), *Rumex crispus* and *Rumex sanguineus*, are capable of tolerating similar allelopathic compounds (Donath & Eckstein, 2008; Baltzinger *et al*., 2012).

Previous work has shown that the interaction between allelochemicals and environmental conditions is very important for plant growth. Many biotic and abiotic factors can have a significant effect on the susceptibility of plants to allelopathic compounds (Singh *et al*., 2001), and this study found that light and nutrient stress both enhanced the allelopathic effect of *R. ponticum* in the New Forest. However,

even then this could not explain the inhibition of growth, suggesting that it is a complex combination of biotic and abiotic stresses that is responsible for the impact of invasive species, which is not observed under laboratory conditions (Figure 7. 1).

7.4 Importance of pre-existing conditions

Recent work has suggested that the dominance of invasive species could have more to do with their ability to tolerate adverse conditions which were limiting for the native species (MacDougall & Turkington, 2005), and in the New Forest, several of the conditions were already different in uninvaded woodland compared to open grassland before the arrival of the rhododendron, due to other species present. Although it is likely that there are some native species that are adapted to these conditions, and many species can tolerate a wide range of environmental conditions (Sterry, 2006), diversity and ground cover in uninvaded woodland was found to be lower than in open grassland at many of the sites in the New Forest (personal observation), providing suitable sites for the rhododendron to establish. Light availability was already lower in the uninvaded woodland, and although rhododendrons were shown to have an effect on soil pH under controlled conditions, the acidic conditions beneath *R. ponticum* in the New Forest were found to be due more to woodland type, rather than the presence of the rhododendron. Although this study did not find a difference in nitrate availability between open grassland and woodland invaded by *R. ponticum*, sandy acidic soils, like those typically found in the New Forest (Tubbs, 2001; West, 2010), are generally low in nutrients (Raven *et al*., 2005), suggesting that nitrate availability was also already low in the New Forest before the arrival of the rhododendron (Powers, 1980). Due to the interactions between light availability and other environmental conditions (Matlack, 1993) it is also probable that other conditions were already different in uninvaded woodland compared to open grassland, although further work is needed to identify which of these factors were being altered by the rhododendron. As seeds of *R. ponticum* are at risk from desiccation, they are limited to shaded sites where there is more moisture, and they have difficulty becoming established in areas where there is already continuous ground cover from native plants (Cross, 1981; Stephenson *et al*., 2006). The mycorrhizal associations that develop within the roots of *R. ponticum* also appear to be limited to soils which are either high in organic matter or sandy, where

nutrient availability is low (Mitchell & Gibson, 2006). Although rhododendron can be found growing in a range of habitats (Dehnen-Schmutz *et al*., 2004), it is woodland that is most affected, with deciduous woodland providing the ideal conditions for invasion (Dehnen-Schmutz *et al*., 2004; Harris *et al*., 2011). Therefore, it is likely that pre-existing stresses are important in all the areas invaded by *R. ponticum*, not just the New Forest, and that these conditions are providing the ideal environment for the establishment of *R. ponticum*, which has not only evolved a higher growth rate in low light conditions (Erfmeier & Bruelheide, 2005), but the mycorrhizal associations enable it to tolerate low nutrient conditions (Mitchell & Gibson, 2006). This is not unique to *R. ponticum*, and many other invasive species have also been shown to be able to tolerate conditions which are not suitable for the native species. *Lepidium latifolium* (broadleaved pepperweed) has been found to tolerate extensive flooding (Chen *et al*., 2002), *Bromus diandrus* (great brome) was shown to tolerate increased salinity (Kolb & Alpert, 2003), and *Kalmia angustifolia* and *Kalmia latifolia* are able to tolerate low nutrient conditions (Inderjit & Mallik, 2002; Eppard *et al*., 2005).

7.5 Importance of other species

Although pre-existing environmental stresses were not sufficient to explain the inhibition of growth of the native species, the impact of invasive species was, in part, due to other species that were already present, and that the invasive species were exacerbating the already demanding conditions. Previous studies have shown that although *Acacia longifolia* (Sidney golden wattle) and *Tamarix ramosissima* (saltcedar) reduce water availability in invaded areas (Sala *et al*., 1996; Rascher *et al*., 2011), they are both better adapted than the native species to tolerate areas which are already water limited (Cleverly *et al*., 1997; Morais & Freitas, 2012), and although *Kalmia angustifolia* and *Kalmia latifolia* affect nutrient cycling (Bradley *et al*., 1997; Chastain Jr. *et al*., 2006), as well as producing allelopathic compounds that interfere with nutrient uptake (Yamasaki *et al*., 1998; Bradley *et al*., 2000; Eppard *et al*., 2005), both species are often limited to areas where nutrient availability is already low (Inderjit & Mallik, 2002; Eppard *et al*., 2005). This study demonstrated that *R. ponticum* reduced light availability by approximately 92%. Although previous studies have shown that this can be sufficient to inhibit plant growth (Clinton &

Vose, 1996; Lei *et al*., 2006), a number of species are capable of survival and growth under very low light conditions (Metcalfe, 1996; Hastwell & Facelli, 2003; McLaren & McDonald, 2003). Many of the species commonly found in uninvaded woodland in the New Forest also have some degree of shade tolerance (Sterry, 2006) so might be able to tolerate these low light conditions. However, *R. ponticum* is restricted to darker conditions where there is more moisture (Cross, 1981), and in the New Forest, the rhododendron is commonly found growing in woodland areas where average light availability was already reduced by over 50%. Although this is likely to be due to the woodland providing ideal conditions for *R. ponticum* to become established (Cross, 1981), these conditions might also be helping the rhododendron to outcompete the native species. As well as invasive populations of *R. ponticum* having evolved faster growth rates, particularly at low light conditions (Erfmeier & Bruelheide, 2005), the shade cast by the rhododendron combined with the shade created by the woodland species meant that in the New Forest, average light availability was 97% lower beneath stands of *R. ponticum* than in open grassland.

Various biotic and abiotic factors can influence the build-up of allelopathic compounds (Singh *et al*., 2001), and this study demonstrated that the reduced light availability and the acidic conditions in woodland in the New Forest increased the accumulation of allelopathic compounds in the soil beneath *R. ponticum*. This enhanced the inhibitory effect, and allowed it to have a greater impact on the native species. Differences in environmental conditions also have an effect on the activity of microorganisms (Killham, 1994). This will not only affect the transformation, and therefore activity, of allelochemicals in the soil (Singh *et al*., 2001), but many microorganisms have also been shown to produce their own allelopathic compounds (Barazani & Friedman, 2001; Singh *et al*., 2001). Although this was not investigated in this study, chapter 6 suggested that the conditions found in woodland in the New Forest are affecting the soil microorganisms which are having their own allelopathic effect on the native species, and further work is therefore necessary to determine the role of microorganisms in the inhibition of growth beneath *R. ponticum* in the New Forest.

Finally, many of the tree species commonly found in woodland in the New Forest have been shown to produce their own allelopathic compounds (Kuiters & Sarink, 1986). Despite combinations of compounds often having a greater effect together

than individually (Blum, 1996), this study found that leachates from *P. sylvestris* or *Q. robur* did not enhance the allelopathic potential of *R. ponticum* on the two species tested, and leachates from *Q. robur* reduced the effect of the rhododendron on the germination of *T. repens*. This was thought to be due to tannins produced by *Q. robur*, which can form stable complexes with allelochemicals in the soil (Halvorson *et al*., 2009), affecting their activity and uptake (Kang *et al*., 2002), and other tree species commonly found in the New Forest have also been shown to produce tannins (Bussotti *et al*., 1998; Suominen *et al*., 2003). However, many of the same species produce other allelopathic compounds, including ferulic acid and quercetin (Kuiters & Sarink, 1986; Laitinen *et al*., 2000), which were shown to enhance the allelopathic effect of *R. ponticum*.

7.6 Making predictions about invasive species

Plants have evolved different survival strategies, allowing them to occupy different habitats, and determining the composition of the community. Grime's Triangular *C-S-R* theory used traits of the adult stage of the life cycle to characterise plants based on their survival strategy (Grime, 1977; Booth *et al*., 2010) (Figure 7. 2). Competitors (*C*) are classed as those species that are able to out compete other plants by most efficiently tapping into available resources, through a combination of favourable characteristics, including rapid growth rate, high productivity, and high capacity for phenotypic plasticity. Stress tolerators (*S*) are those that live in areas of high intensity stress, such as deep shade or nutrient deficient soils, and generally have slow growth rates, long lived leaves, high rates of nutrient retention, and low phenotypic plasticity. Ruderals (*R*) are plant species that thrive in areas of high intensity disturbance, and are generally fast-growing, produce large amounts of seeds and rapidly complete their life cycles (Grime, 1977).

The findings of this study support predications about plant communities based on Grime's *C-S-R* theory, which tend to position invasive species at the extreme corners of the Grime's *C-S-R* triangle (Grime, 1977; Booth *et al*., 2010) and suggest that the success of invasive species is due to their high competitive ability or their ability to tolerate stressful conditions (MacDougall & Turkington, 2005). However, this theory is a considerable simplification of reality, as it implies that in high-disturbance or

stressful habitats, competition will be low. In nature there will usually be a wide range of strategies within a species, and survival is an outcome of the many traits and interactions that contribute to its success (Wilson & Lee, 2000). This study, as well as previous work, has indicated that *R. ponticum* has adapted to use all of these strategies, and suggested that the invasiveness of some species is due to this ability to take advantage of different conditions under different situations. It also suggested that all stages of the life cycle are extremely important, not just the adult stage which is the basis of Grimes' *C-S-R* triangle theory, and these all need to be taken into account when making predictions about plant communities. In order to become established, invasive species are often reliant on gaps in the vegetation, either as a result of disturbance (favouring the *R*-strategists), or due to high intensity stress (favouring the *S*-strategists). Studies have shown that many invasive species, including *Lonicera japonica* (Japanese honeysuckle), *Melaleuca quinquenervia* and *R. ponticum*, are, at least in part, dependant on disturbance, either natural (such as windfall or flooding) or due to human activity (such as forestry or agriculture), which results in gaps in the vegetation, providing suitable sites for the establishment and spread of the invasive species (Cross, 1981; Lozon & MacIsaac, 1997; Turner *et al*., 1997; Larson, 2002). However, once they have become established, selection will generally favour the *C*-strategists, which are able to out-compete the native species for essential resources such as nutrients and water (D'Antonio & Mahall, 1991). Again, *R. ponticum* is no exception, and its high growth rate allows it to out-compete the native species for light, water and nutrients (Erfmeier & Bruelheide, 2004). However, these species might still take advantage of further disturbance or already stressful conditions in order to invade new areas (Figure 7. 2). This not only makes it difficult to make predictions about them, as the impacts on native biodiversity and ecosystems are often species and context specific (Stout, 2011), but it also makes controlling them extremely challenging.

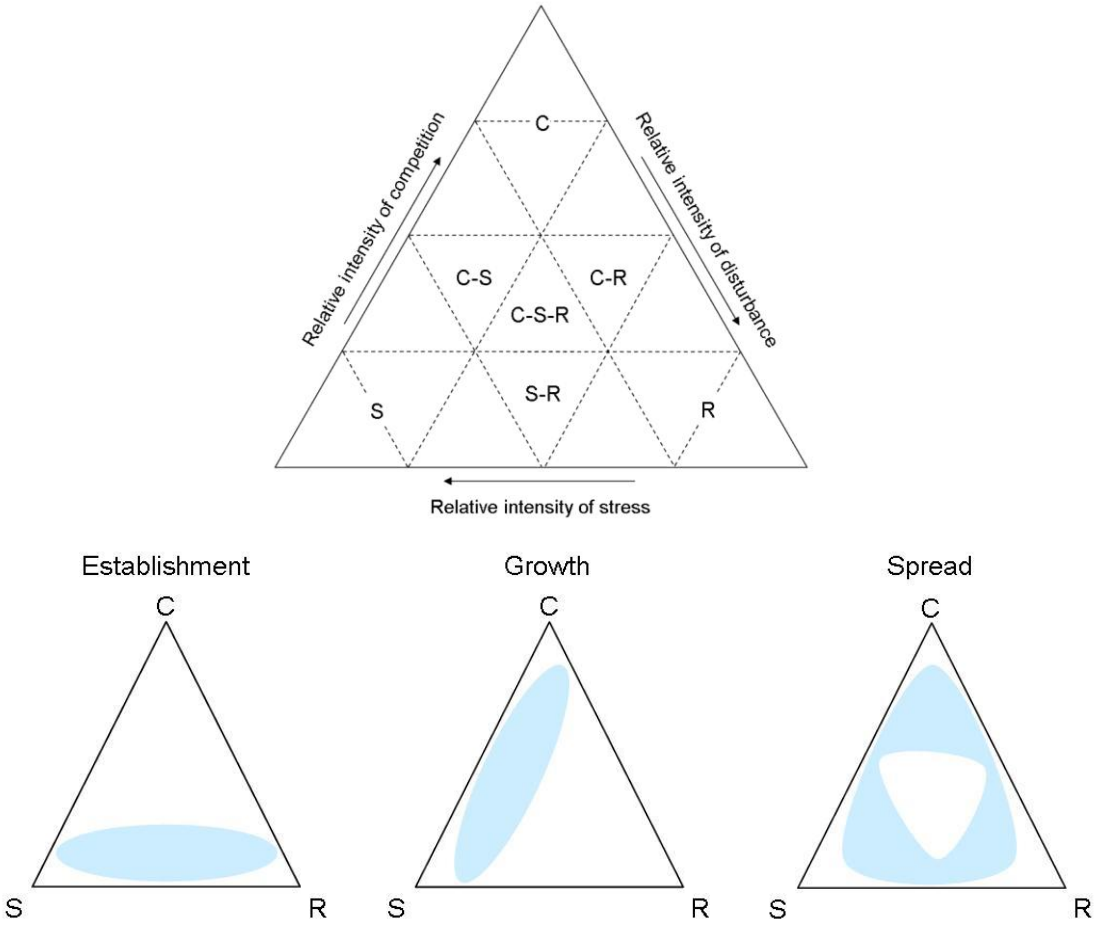


Figure 7. 2. Grime's C-S-R (Competitor- Stress tolerator- Ruderal) triangle (reproduced from Booth *et al*., 2010), and the predicted placement of *R. ponticum* along the three axis at different stages of its life cycle.

7.7 Limitations of this study and future work

The overall aim of this study was to investigate the influence of key environmental factors, including light availability and allelopathic compounds, on the growth of the native species beneath *R. ponticum* in the New Forest. As field research often involves too many interactive factors to be able to assess the influence of any one environmental factor on plant growth (Short, 1987), mesocosm experiments were conducted using passive greenhouse apparatus. Although this can have an effect on light transmittance, temperature, gas exchange and humidity (Debevec & MacLean, Jr., 1993; Kennedy, 1995a), they could still be used to provide an experimental ecosystem with close to natural conditions, but in which environmental factors could be realistically manipulated (Debevec & MacLean, Jr., 1993; Kennedy, 1995b). However, despite numerous workers suggesting that it is a single environmental

factor that is responsible for the inhibition of native plant growth beneath invasive species (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003), this study suggests that the inhibition of growth is not due to just one factor, but rather to a combination of biotic and abiotic factors (Figure 7. 1). This means that although manipulating environmental conditions in glasshouse mesocosm experiments can be useful for establishing the influence of various factors on the impact of invasive species, they can not be used exclusively when investigating the effects of invasive species on the growth and survival of the natives, as glasshouse experiments do not take into account the interactions between environmental conditions that are occurring under natural conditions (Singh *et al*., 2001; Mittler, 2006).

L. perenne and *T. repens* were chosen as the two study species as they were easy to use, and both have been used in previous studies, so a great deal was already known about their optimum conditions for growth. Although they are both grassland species and generally not found growing in woodland where *R. ponticum* had invaded, they are both shade tolerant and both will tolerate acidic conditions (Turkington & Burdon, 1983; Hannaway *et al*., 1999), so should be able to establish beneath the rhododendron. They have also been used as the bioassay species in a number of studies investigating allelopathy (Carballeira & Cuervo, 1980; Yang *et al*., 2007), and as both are commonly found in the south of England, including the New Forest (Sterry, 2006; personal observation), they are ecologically relevant, so could be used to demonstrate allelopathy, and not just phytotoxicity (Romeo & Weidenhamer, 1998). However, this study found that the two species varied significantly in their response to environmental stresses and allelopathic compounds. Although this demonstrated that some species were capable of tolerating the conditions found beneath *R. ponticum* in the New Forest, and pointed to a combination of factors being responsible for the inhibition, further work is necessary to test the influence of these conditions on a wider range of species. It would also be interesting to test several woodland species which are more likely to be found growing in the areas of the New Forest most affected by the rhododendron. The two study species that were used in this investigation have been found to tolerate a wide range of environmental stresses (Turkington & Burdon, 1983; Hannaway *et al*., 1999), and although the native woodland species should be better adapted to environmental stresses, particularly low light conditions, it is possible that they are more susceptible and that a single environmental stress is sufficient to account for the inhibition of growth.

This study focused on the influence of light availability, soil pH and allelopathy on the native species, which, based on previous work and a fundamental knowledge of plant growth, were the three environmental conditions that were most likely to be responsible for the inhibition of growth beneath *R. ponticum* in the New Forest (Raven *et al*., 2005). However, although these factors were all shown to contribute to the impact of *R. ponticum*, they were not enough to explain the inhibition of growth of the native species. Future work is necessary to test the effect of other conditions, such as water availability and organic matter, which on their own were expected to increase the growth of the native species, but in combination with other environmental stresses could be having an inhibitory effect (Singh *et al*., 2001; Raven *et al*., 2005) (Figure 7. 1). Although further glasshouse experiments would allow these conditions to be monitored more accurately, it would be interesting to carry out field studies to test the influence of these factors under more realistic conditions. The addition of activated carbon to soil beneath *R. ponticum* could be used to determine whether the rhododendron is having an allelopathic effect in the field (Nilsson, 1994; Ridenour & Callaway, 2001). Manipulating various environmental conditions in the field, such as light and nutrient availability, could also be used to test the combined effects of these factors under natural conditions (Clinton & Vose, 1996; Reinhart *et al*., 2006), although the interactions between the different environmental factors (Mittler, 2006), such as the effect of light intensity on water availability (Matlack, 1993), would need to be taken into account.

Although *R. ponticum* was shown to have an allelopathic effect on the native species, and previous work has shown that species in the rhododendron genus produce a variety of compounds, including quercetin, ferulic acid, *p*-coumaric acid, vanillic acid and scopoletin (Cao *et al*., 2002; Chou *et al*., 2010), there has been insufficient work to determine whether *R. ponticum* is producing the same compounds. Preliminary trials did identify several compounds, including grayanotoxin 1 and grayanotoxin 3, as well as several flavonoids (Appendix IV), but it is not known whether it is these that are responsible for the allelopathic effect, and it is likely that other compounds are also being produced (Cao *et al*., 2002; Chou *et al*., 2010). Future studies could fractionate the crude extracts using high performance liquid chromatography (HPLC) by collecting a fraction every fifteen seconds (Millar & Haynes, 1998; Anjum & Bajwa, 2010). Bioassays could then be conducted on these

samples to determine where the active compounds were eluting, and then liquid chromatography-mass spectrometry (LC-MS) could be used to identify the compounds responsible for the inhibitory effect (Millar & Haynes, 1998; Inderjit & Nilsen, 2003). However, bioassays testing the effect of these compounds would either need to be conducted in the field, or glasshouse conditions would need to be manipulated to replicate natural conditions, as it is likely that it is the environmental stresses present in the field that are enhancing the allelopathic effect sufficiently to have a significant effect on the native species (Singh *et al*., 2001; Inderjit & Mallik, 2002) (Figure 7. 1). Once the active compounds have been identified, HPLC could be used to calculate the concentrations present in the sample to determine whether their synthesis and accumulation in the soil are affected by environmental conditions, or whether the increased allelopathic effect of *R. ponticum* grown in low light and acidic conditions observed in this study was due to other factors in the soil, such as microorganisms. Soil from uninvaded woodland could also be analysed to determine whether soil organisms or other tree species are producing sufficient concentrations of allelopathic compounds to inhibit the growth of other native species on their own, or whether it is the combined effect of these compounds with *R. ponticum* allelochemicals that is responsible for the impact (Blum, 1996) (Figure 7. 1).

Numerous studies have investigated the effect of invasive species on abiotic conditions and the impact these changes have on the native plants (D'Antonio & Vitousek, 1992; Gordon, 1998; Levine *et al*., 2003), but there has been far less research into the impact of biotic factors. Many invasive plants have been shown to have an effect on herbivores, including mammals, birds and insects (Cross, 1981; Rodriguez, 2006; Tripathi *et al*., 2011). Cappuccino & Carpenter (2005) showed that invasive plants suffered less leaf herbivory than non-invasive plants, and as well as often being less palatable to herbivores (Rotherham, 2001; Caño *et al*., 2009), a number of invasive species, including *Lonicera maackii* and *Triadica sebifera* (tallow tree), produce phytochemicals which have anti-herbivore properties (Cipollini *et al*., 2008; Wang *et al*., 2012). Several invasive plants, including *Hydrilla verticillata* (Hydrilla), *Tamarix ramosissima*, and *R. ponticum*, have also been shown to create suitable habitats, providing shelter and protection from predators (Cross, 1981; Zavaleta *et al*., 2001; Rodriguez, 2006; Malo *et al*., 2013). Although studies have shown that changes in herbivore activity can affect the synthesis of allelopathic compounds (Thelen *et al*., 2005), few studies have

investigated the direct effect this change in herbivore activity has on the native plants. The increase in the number of herbivores and the unpalatability of the invasive species could result in an increase in grazing on the native plants (Malo *et al*., 2013), future work is necessary to examine the role this plays in the inhibition of growth beneath invasive species.

Although it was not investigated in this study, recent work investigating allelopathy has focused on the possibility of using these compounds as natural herbicides (Macías *et al*., 2001; Bhadoria, 2011). However, biotic and abiotic factors can influence the susceptibility of plants to allelochemicals, as well as their transformation, and therefore activity, in the soil (Singh *et al*., 2001), and this study supports previous work which shows that the inhibitory effect is often due to the combination of these compounds with other environmental stresses (Nilsson, 1994; Michelsen *et al*., 1995; Clinton & Vose, 1996). The intensity of competition between crops and weeds for space, light, moisture and nutrients will all differ under various field conditions, creating different situations for establishment and growth, thereby influencing the mechanism of allelopathic activity (Bhadoria, 2011). This means that many of the allelopathic compounds produced by invasive species, such as *Empetrum hermaphroditum*, *Kalmia latifolia* and *R. ponticum*, will not be feasible alternatives to synthetic herbicides as their effects are dependent on other environmental conditions (Nilsson, 1994; Eppard *et al*., 2005). It also means that future studies into the use of these allelochemicals as potential natural herbicides need to take into account agronomic practices, such as nutrient application and irrigation, and therefore, along with laboratory experiments, field experiments are essential in order to study the interaction between the allelochemicals and various biotic and abiotic factors (Bhadoria, 2011).

Every year, billions of pounds are spent across the world trying to control the spread of invasive species (Pimentel *et al*., 2001; Shaw & Tanner, 2008), using a combination of manual, mechanical and herbicidal methods (Turner *et al*., 1997; Eşen & Zedaker, 2004). Understanding the ecological mechanisms responsible for their inhibitory effect on the native species is crucial if we are to successfully manage the spread of invasive species (Levine *et al*., 2003). It not only helps in predicting which invaders are likely to have the greatest impact, and which sites are most at risk, but is also essential from a restoration standpoint, in knowing which

processes must be overcome if native species are to re-establish in invaded ecosystems (Levine *et al*., 2003). However, this study has demonstrated that the inhibition is due to a complex combination of factors, and no one factor alone can explain the impact (Figure 7. 1). It has also shown that environmental conditions are often limiting for the native species before the arrival of the invasive species, and therefore the role of the other species present needs to be taken into account. The ability of invasive species to use various survival strategies means that disturbance created by clearance would just provide further gaps in the vegetation. This would quickly result in the re-establishment of the invasive species (Lozon & MacIsaac, 1997), which are better suited to tolerate these conditions and are able to out-compete the native species (MacDougall & Turkington, 2005). This indicates that continuous management is needed to prevent the return of these species, and therefore, biological control might be the only effective method of control, as it can provide permanent weed suppression in a single release without further interference or cost (Shaw & Tanner, 2008). However, despite numerous studies, the release of *Aphalara itadori* into the UK in 2010 and *Stenopelmus rufinasus* (Azolla weevil) in 2012 were the only times a biological control approach has been used in Europe against an invasive plant (Shaw *et al*., 2011; CABI, 2013), and future work needs to focus on biological control as this might be the only way of successfully controlling the spread of these invasive species.

Appendices

Appendix I

Repeat measure analysis on light intensity.

Tests of Within-Subjects Effects

Measure: Light intensity

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Month	Sphericity Assumed	16.474	3	5.491	70.043	.000
	Greenhouse-Geisser	16.474	2.545	6.473	70.043	.000
	Huynh-Feldt	16.474	2.873	5.735	70.043	.000
	Lower-bound	16.474	1.000	16.474	70.043	.000
Month * Site	Sphericity Assumed	1.148	6	.191	2.441	.027
	Greenhouse-Geisser	1.148	5.090	.226	2.441	.036
	Huynh-Feldt	1.148	5.745	.200	2.441	.030
	Lower-bound	1.148	2.000	.574	2.441	.096
Month * Area	Sphericity Assumed	11.987	3	3.996	50.968	.000
	Greenhouse-Geisser	11.987	2.545	4.710	50.968	.000
	Huynh-Feldt	11.987	2.873	4.173	50.968	.000
	Lower-bound	11.987	1.000	11.987	50.968	.000
Month * Site * Area	Sphericity Assumed	.497	3	.166	2.111	.101
	Greenhouse-Geisser	.497	2.545	.195	2.111	.112
	Huynh-Feldt	.497	2.873	.173	2.111	.104
	Lower-bound	.497	1.000	.497	2.111	.152
Error(Month)	Sphericity Assumed	12.936	165	.078		
	Greenhouse-Geisser	12.936	139.963	.092		
	Huynh-Feldt	12.936	157.998	.082		
	Lower-bound	12.936	55.000	.235		

Tests of Between-Subjects Effects

Measure: Light intensity

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	133.475	1	133.475	4751.591	.000
Site	.464	2	.232	8.262	.001
Area	8.047	1	8.047	286.466	.000
Site * Area	.149	1	.149	5.310	.025
Error	1.545	55	.028		

Repeat measure analysis on air temperature.

Tests of Within-Subjects Effects

Measure: Air temperature

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Month	Sphericity Assumed	.790	3	.263	310.208	.000
	Greenhouse-Geisser	.790	2.039	.388	310.208	.000
	Huynh-Feldt	.790	2.230	.354	310.208	.000
	Lower-bound	.790	1.000	.790	310.208	.000
Month * Site	Sphericity Assumed	.436	3	.145	171.197	.000
	Greenhouse-Geisser	.436	2.039	.214	171.197	.000
	Huynh-Feldt	.436	2.230	.196	171.197	.000
	Lower-bound	.436	1.000	.436	171.197	.000
Month * Area	Sphericity Assumed	.081	3	.027	31.902	.000
	Greenhouse-Geisser	.081	2.039	.040	31.902	.000
	Huynh-Feldt	.081	2.230	.036	31.902	.000
	Lower-bound	.081	1.000	.081	31.902	.000
Month * Site * Area	Sphericity Assumed	.006	3	.002	2.508	.061
	Greenhouse-Geisser	.006	2.039	.003	2.508	.085
	Huynh-Feldt	.006	2.230	.003	2.508	.079
	Lower-bound	.006	1.000	.006	2.508	.119
Error(Month)	Sphericity Assumed	.143	168	.001		
	Greenhouse-Geisser	.143	114.166	.001		
	Huynh-Feldt	.143	124.866	.001		
	Lower-bound	.143	56.000	.003		

Tests of Between-Subjects Effects

Measure: Air temperature

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	73.267	1	73.267	289016.166	.000
Site	.057	1	.057	224.357	.000
Area	.013	1	.013	49.426	.000
Site * Area	.007	1	.007	25.949	.000
Error	.014	56	.000		

Repeat measure analysis on soil temperature.

Tests of Within-Subjects Effects

Measure: Soil temperature

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Month	Sphericity Assumed	1.354	3	.451	2084.921	.000
	Greenhouse-Geisser	1.354	1.834	.738	2084.921	.000
	Huynh-Feldt	1.354	1.994	.679	2084.921	.000
	Lower-bound	1.354	1.000	1.354	2084.921	.000
Month * Site	Sphericity Assumed	.051	3	.017	78.136	.000
	Greenhouse-Geisser	.051	1.834	.028	78.136	.000
	Huynh-Feldt	.051	1.994	.025	78.136	.000
	Lower-bound	.051	1.000	.051	78.136	.000
Month * Area	Sphericity Assumed	.050	3	.017	77.003	.000
	Greenhouse-Geisser	.050	1.834	.027	77.003	.000
	Huynh-Feldt	.050	1.994	.025	77.003	.000
	Lower-bound	.050	1.000	.050	77.003	.000
Month * Site * Area	Sphericity Assumed	.005	3	.002	7.847	.000
	Greenhouse-Geisser	.005	1.834	.003	7.847	.001
	Huynh-Feldt	.005	1.994	.003	7.847	.001
	Lower-bound	.005	1.000	.005	7.847	.007
Error(Month)	Sphericity Assumed	.036	168	.000		
	Greenhouse-Geisser	.036	102.696	.000		
	Huynh-Feldt	.036	111.689	.000		
	Lower-bound	.036	56.000	.001		

Tests of Between-Subjects Effects

Measure: Soil temperature

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	63.873	1	63.873	94650.545	.000
Site	.001	1	.001	.973	.328
Area	.057	1	.057	85.054	.000
Site * Area	.001	1	.001	.849	.361
Error	.038	56	.001		

Repeat measure analysis on rainfall.

Tests of Within-Subjects Effects

Measure: Rainfall

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Month	Sphericity Assumed	3.455	3	1.152	73.456	.000
	Greenhouse-Geisser	3.455	1.401	2.465	73.456	.000
	Huynh-Feldt	3.455	1.811	1.908	73.456	.000
	Lower-bound	3.455	1.000	3.455	73.456	.000
Month * Site	Sphericity Assumed	.395	3	.132	8.407	.000
	Greenhouse-Geisser	.395	1.401	.282	8.407	.005
	Huynh-Feldt	.395	1.811	.218	8.407	.002
	Lower-bound	.395	1.000	.395	8.407	.011
Month * Area	Sphericity Assumed	.075	3	.025	1.584	.206
	Greenhouse-Geisser	.075	1.401	.053	1.584	.228
	Huynh-Feldt	.075	1.811	.041	1.584	.224
	Lower-bound	.075	1.000	.075	1.584	.227
Month * Site * Area	Sphericity Assumed	.072	3	.024	1.526	.221
	Greenhouse-Geisser	.072	1.401	.051	1.526	.238
	Huynh-Feldt	.072	1.811	.040	1.526	.236
	Lower-bound	.072	1.000	.072	1.526	.236
Error(Month)	Sphericity Assumed	.706	45	.016		
	Greenhouse-Geisser	.706	21.022	.034		
	Huynh-Feldt	.706	27.166	.026		
	Lower-bound	.706	15.000	.047		

Tests of Between-Subjects Effects

Measure: Rainfall

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	20.822	1	20.822	845.444	.000
Site	.119	1	.119	4.830	.044
Area	.320	1	.320	12.999	.003
Site * Area	.147	1	.147	5.968	.027
Error	.369	15	.025		

Appendix II

Principal Component Analysis, ranking the different components according to the degree by which they explain the variances in measure correlations.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.313	47.324	47.324	3.313	47.324	47.324	3.305	47.214	47.214
2	1.814	25.917	73.241	1.814	25.917	73.241	1.822	26.026	73.241
3	.695	9.924	83.165						
4	.556	7.940	91.105						
5	.334	4.774	95.879						
6	.169	2.417	98.296						
7	.119	1.704	100.000						

Extraction Method: Principal Component Analysis.

Appendix III

Air temperature measured using EL-USB-2 data loggers, in open grassland and beneath *R. ponticum* at Exbury Gardens and Minstead Lodge.

To take into account differences between sites due to daily cycling, air temperature was measured at two plots in each area using EL-USB-2 data loggers (Lascar Electronics Ltd, Wiltshire, UK). Measurements were taken at 12.00 every day, between the 7th March and the 23rd September 2009 to allow comparisons to be made between sites. The two-factor nested ANOVA showed that air temperature was lower beneath the *R. ponticum* canopy (Vegetation type main effect $F_{1,1598} = 314.99$, $P < 0.001$), although this difference was only seen at Exbury Gardens (Site * Vegetation type interaction $F_{1,1598} = 238.71$, $P < 0.001$). Air temperature was also found to differ between sites (Site main effect $F_{1,1598} = 510.20$, $P < 0.001$), with higher temperatures observed at Exbury Gardens. Average air temperature under *R. ponticum* was found to be 15.9°C, compared to 21.1°C in open grassland, a difference of 1.1°C (Figure I).

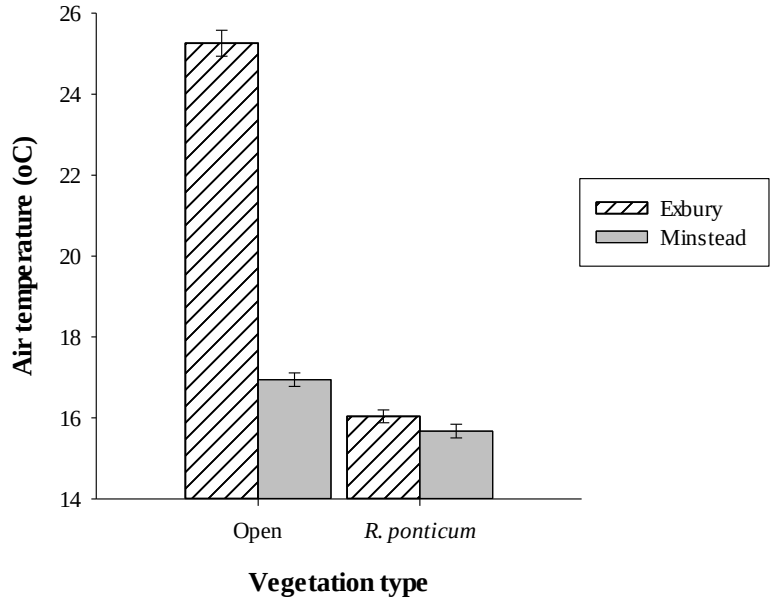


Figure I. Differences in air temperature (°C) between open grassland and beneath *R. ponticum*, at Exbury Gardens and Minstead Lodge in the New Forest (+/- 1 SE) (n= 402).

Appendix IV

Preliminary chemical analysis

Preliminary analysis of the compounds present in the leaves of *R. ponticum* was conducted at Kew Gardens, London in April 2009 (unpublished data, 2009). Fresh leaves were finely chopped and then 2 g of leaf material were placed in a beaker containing 1 ml methanol. The sample was left for 24 hours, and then analysed using liquid chromatography-mass spectrometry (LC-MS).

Mass Spectrometry did identify grayantotoxin I and grayantotoxin III in the leaves, but only at very low concentrations, and much lower than that found in nectar (Stevenson, P., 2009. pers. comm., 11 June). It also identified several compounds containing simple chromophores, as well as a number of fairly common flavonoids, but nothing particularly distinguishing (Stevenson, P., 2009. pers. comm., 11 June). It is possible that running the samples at a lower absorbance, and analyzing plant material and soil samples collected from the New Forest, might have identified further compounds, and future work had also aimed to isolate the active fractions using high performance liquid chromatography (HPLC). However, due to time constraints this was not possible.

Appendix V

Effect of different concentrations of known allelopathic compounds on the germination and growth of the two test species

To test the effect of different concentrations of known allelopathic compounds on the germination and growth of the two test species, 485 mg of ferulic acid or 755 mg of quercetin was dissolved in 500 ml distilled water to make up 5 mM concentration solutions. Serial dilutions were made to give 400 ml of 5 mM, 1 mM and 0.5 mM concentration solutions. These concentrations have been used in previous studies (Klein & Blum, 1990; Devi & Prasad, 1992; Yan *et al.*, 2010). Beakers were also left with 100% distilled water to use as a control. Twenty seeds of *L. perenne* or *T. repens* were grown in Petri dishes containing 15 ml of one of the solutions (see 5.2.5), with five Petri dishes for each sample. The pH of each sample was measured to ensure that any differences in growth were not due to pH (Tilman & Olff, 1991). Germination, root elongation and leaf appearance were then measured (see 2.6).

The one-factor ANOVA showed that ferulic acid had no effect on the germination of *L. perenne* (Concentration main effect $F_{3,16} = 0.43$, $P = 0.735$). However, it was found to have an inhibitory effect on the germination of *T. repens* (Concentration main effect $F_{3,16} = 10.20$, $P = 0.001$), but only at 5 mM concentration ($P = 0.002$). Ferulic acid was also shown to have an inhibitory effect on the root elongation of *L. perenne* (Concentration main effect $F_{3,16} = 5.82$, $P = 0.007$), but only at 5 mM concentration ($P = 0.018$). However, it had no effect on the root elongation of *T. repens* (Concentration main effect $F_{3,16} = 1.77$, $P = 0.194$), or on the leaf appearance of either of the species tested (Concentration main effect $F_{3,16} = 0.62$, $P = 0.613$ and $F_{3,16} = 1.68$, $P = 0.211$ for *L. perenne* and *T. repens* respectively).

The one-factor ANOVA showed that quercetin had an inhibitory effect on the germination of *T. repens* (Concentration main effect $F_{3,16} = 5.03$, $P = 0.012$), but only at 5 mM concentration ($P = 0.013$), although there was no difference between the 0.5 mM and the 5 mM concentration solutions ($P = 0.123$). However, it was found to have no effect on the germination of *L. perenne* (Concentration main effect $F_{3,16} = 1.49$, $P = 0.255$). Quercetin was also shown to have no effect on the root elongation (Concentration main effect $F_{3,16} = 0.92$, $P = 0.456$ and $F_{3,16} = 2.50$, $P =$

0.096 for *L. perenne* and *T. repens* respectively) or leaf appearance (Concentration main effect $F_{3,16} = 0.74$, $P = 0.545$ and $F_{3,16} = 1.73$, $P = 0.202$ for *L. perenne* and *T. repens* respectively) of either of the species tested.

Appendix VI

Effect of different concentrations of nutrients on the germination and growth of the two test species

To test the effect of different concentrations of nutrients on the germination and growth of the two test species, Miracle-Gro all purpose concentrated liquid plant food (Morrisons, Hampshire, UK) was added to beakers containing 200 ml distilled water, to make up 0.1%, 0.3%, and 0.5% concentration solutions (Ross *et al*., 2009). Beakers were also left with 100% distilled water to use as a control. Twenty seeds of *L. perenne* or *T. repens* were grown in Petri dishes containing 15 ml of one of the nutrient solutions (see 5.2.5), with ten Petri dishes for each sample. The pH of each sample was measured to ensure that any differences in growth were not due to pH (Tilman & Olff, 1991). Although pH was found to decrease as the nutrient concentration increased, in chapter 4 it was demonstrated that these differences were too small to have a significant effect on the species tested. Germination, root elongation and leaf appearance were then measured (see 2.6).

The one-factor ANOVA showed that addition of nutrients had no effect on the germination of *L. perenne* (Concentration main effect $F_{3,36} = 0.58$, $P = 0.634$). However, nutrients were found to have an effect on the germination of *T. repens* (Concentration main effect $F_{3,36} = 4.59$, $P = 0.008$), but the only difference was between the 0% and 0.5% nutrient solutions ($P = 0.004$), with germination slower when nutrients were added. Addition of nutrients had no effect on the root elongation of *L. perenne* (Concentration main effect $F_{3,36} = 0.37$, $P = 0.775$) or *T. repens* (Concentration main effect $F_{3,36} = 0.23$, $P = 0.876$), or on the leaf appearance of *L. perenne* (Concentration main effect $F_{3,36} = 0.37$, $P = 0.772$). However, nutrients were found to have an effect on the leaf appearance of *T. repens* (Concentration main effect $F_{3,36} = 6.37$, $P = 0.001$), with leaf appearance faster in the 0.1% and the 0.5% nutrient solutions compared to the 0% control ($P < 0.009$).

Appendix VII

Effect of adding nutrients to soil without *R. ponticum*.

The two-factor fully cross-factored ANOVA showed that addition of 0.3% NPK fertilizer (Miracle-Gro all purpose concentrated liquid plant food) to soil which had not supported *R. ponticum* had no effect on the germination of *L. perenne* (Nutrient main effect $F_{1,28} = 4.04$, $P = 0.054$) or *T. repens* (Nutrient main effect $F_{1,28} = 0.73$, $P = 0.401$). Addition of nutrients to soil which had supported *R. ponticum* were also found to have no effect on the root elongation of *L. perenne* (Nutrient main effect $F_{1,28} = 0.58$, $P = 0.454$) or *T. repens* (Nutrient main effect $F_{1,28} = 2.73$, $P = 0.110$), or on the leaf appearance of *L. perenne* (Nutrient main effect $F_{1,28} = 3.93$, $P = 0.057$). However, nutrients were shown to have an effect on the leaf appearance of *T. repens* (Nutrient main effect $F_{1,28} = 5.14$, $P = 0.031$), although this appeared to be due to the interaction between *R. ponticum* and nutrients (*R. ponticum* * Nutrients interaction $F_{1,28} = 2.76$, $P = 0.108$), and on its own, addition of nutrients had no effect ($P = 0.486$).

Appendix VIII

Effect of light availability (PAR) and pH conditions on the allelopathic potential of soil which had not supported *R. ponticum*.

As well as testing whether environmental conditions enhance the allelopathic potential of *R. ponticum*, the effect of different light availability (PAR) and pH conditions were also tested on soil which had not supported *R. ponticum* (see 6.2.3).

The two-factor fully cross-factored ANOVA showed that soil adjusted to pH 3.66 had a greater allelopathic effect on the germination of *T. repens* (pH main effect $F_{1,28}$ = 28.51, P <0.001). Soil kept under reduced light conditions was also shown to have a greater allelopathic effect on the germination of *T. repens* (PAR main effect $F_{1,28}$ = 11.33, P = 0.002), although there was also an interaction between light availability and pH (PAR * pH interaction $F_{1,28}$ = 6.51, P = 0.017), and this effect was only observed in the soil which had been adjusted to pH 3.66. Different light and pH conditions did not enhance the allelopathic activity of the soil on the germination of *L. perenne*, either on their own (Main effect $F_{1,28}$ = 1.39, P = 0.248 and $F_{1,28}$ = 2.19, P = 0.150 for light availability and soil pH respectively) or in combination (PAR * pH interaction $F_{1,28}$ = 1.92, P = 0.177).

Soil kept under reduced light conditions was found to have a greater allelopathic effect on the root elongation of *T. repens* (PAR main effect $F_{1,28}$ = 6.75, P = 0.015), although it had no effect on the germination of *L. perenne* (PAR main effect $F_{1,28}$ = 0.47, P = 0.500). pH was also shown not to enhance the allelopathic activity of the soil on the root elongation of the two species, either on its own (pH main effect $F_{1,28}$ = 0.61, P = 0.440 and $F_{1,28}$ = 3.65, P = 0.066 for *L. perenne* and *T. repens* respectively) or in combination with the reduced light availability (PAR * pH interactions $F_{1,28}$ = 0.04, P = 0.854 and $F_{1,28}$ = 2.02, P = 0.167 for *L. perenne* and *T. repens* respectively).

Soil adjusted to pH 3.66 was found to have a greater allelopathic effect on the leaf appearance of *T. repens* compared to soil adjusted to pH 4.62 (pH main effect $F_{1,28}$ = 8.95, P = 0.006), although pH had no effect on the allelopathic activity of the soil on leaf appearance of *L. perenne* (PAR main effect $F_{1,28}$ = 3.61, P = 0.068). Light

availability was also shown not to enhance the allelopathic activity of the soil on the leaf appearance of the two species (pH main effect $F_{1,28} = 1.71$, $P = 0.202$ and $F_{1,28} = 0.12$, $P = 0.730$ for *L. perenne* and *T. repens* respectively), and there was no interaction between light availability and soil pH for either of the species tested (PAR * pH interactions $F_{1,28} = 0.60$, $P = 0.446$ and $F_{1,28} = 2.60$, $P = 0.118$ for *L. perenne* and *T. repens* respectively).

References

Abdelgaleil, S.A.M. and Hashinaga, F., 2007. Allelopathic potential of two sesquiterpene lactones from *Magnolia grandiflora* L. *Biochemical Systematics and Ecology*, 35 (11), pp.737-742.

Aerts, R., Boot, R.G. and van der Aart, P.J., 1991. The relation between above and belowground biomass allocation patterns and competitive ability. *Oecologia*, 87 (4), pp.551-559.

Akhalkatsi, M., Abdaladze, O., Nakhutsrishvili, G. and Smith, W.K., 2006. Facilitation of seedlings microsite by *Rhododendron caucasicum* extends the *Betula litwinowii* alpine treeline, Caucasus Mountains, Republic of Georgia. *Arctic, Antarctic, and Alpine Research*, 38 (4), pp.481-488.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A. and Quarmby, C., 1974. *Chemical analysis of ecological materials*. Oxford: Blackwell Scientific Publications.

Allison, S.D. and Vitousek, P.M., 1998. Rapid nutrient cycling in leaf litter from invasive plants in Hawaii. *Oecologia*, 141 (4), pp.612-619.

Almeida, R., Gonçalves, S. and Romano, A., 2005. In vitro micropropagation of endangered *Rhododendron ponticum* L. subsp. baeticum (Boissier and Reuter) Handel-Mazzetti. *Biodiversity and Conservation*, 14 (5), pp.1059-1069.

Andersen, U.V. and Calov, B., 1996. Control of freshwater and riparian vegetation long-term effects of sheep grazing on giant hogweed (*Heracleum mantegazzianum*). *Hydrobiologia*, 340 (1-3), pp.277-284.

Anjum, T. and Bajwa, R., 2005. Importance of germination indices in interpretation of allelochemical effects. *International Journal of Agriculture and Biology*, 7 (3), pp.1560-8530.

Anjum, T. and Bajwa, R., 2010. Isolation of bioactive allelochemicals from sunflower (variety Suncross-42) through fractionation-guided bioassays. *Natural Product Research*, 24 (18), pp.1783-1788.

Angiras, N.N., Singh, S.D. and Singh, C.M., 1988. Allelopathic effects of weeds on germination and seedling growth of maize and soybean. *Indian Journal of Weed Science*, 20 (2), pp.82-87.

Arrieta, S. and Suárez, F., 2005. Spatial patterns of seedling emergence and survival as a critical phase in holly (*Ilex aquifolium* L.) woodland recruitment in Central Spain. *Forest Ecology and Management*, 205 (1-3), pp.267-282.

Arunachalam, A., Maithani, K., Pandey, H.N. and Tripathi, R.S., 1998. Leaf litter decomposition and nutrient mineralization patterns in regrowing stands of a humid subtropical forest after tree cutting. *Forest Ecology and Management*, 109 (1-3), pp.151-161.

Ashrafi, Z.Y., Mashhadi, H.R., Sadeghi, S. and Alizade, H.M., 2008. Allelopathic effects of sunflower (*Helianthus annuus*) on germination and growth of wild barley (*Hordeum spontaneum*). *Journal of Agricultural Science and Technology*, 4 (1), pp.219-229.

Aulakh, M.S., Wassmann, R., Bueno, C., Kreuzwieser, J. and Rennenberg, H., 2001. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. *Plant Biology*, 3 (2), pp.139-148.

Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. and Vivanco, J.M., 2003. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science*, 301 (5638), pp.1377-1380.

Ballester, A., Albo, J.M. and Vieitez, E., 1977. The allelopathic potential of *Erica scoparia* L. *Oecologia*, 30 (1), pp.55-61.

Ballester, A., Vieitez, A.M. and Vieitez, E., 1979. The allelopathic potential of *Erica australis* L. and *E. arborea* L. *Botanical Gazette*, 140 (4), pp.433-436.

Ballester, A., Vieitez, A.M. and Vieitez, E., 1982. Allelopathic potential of *Erica vagans, Calluna vulgaris*, and *Daboecia cantabrica*. *Journal of Chemical Ecology*, 8 (5), pp.851-857.

Baltzinger, M., Archaux, F. and Dumas, Y., 2012. Tree litter and forest understorey vegetation: A conceptual framework to understand the effects of tree litter on a perennial geophyte, *Anemone nemorosa*. *Annals of Botany*, 109 (6), pp.1175-1184.

Bao, G.H., Wang, L.Q., Cheng, K.F., Feng, Y.H., Li, X.Y. and Qin, G.W., 2003. Diterpenoid and phenolic glycosides from the roots of *Rhododendron molle*. *Planta Medica*, 69 (05), pp.434-439.

Barazani, O. and Friedman, J., 2001. Allelopathic bacteria and their impact on higher plants. *Critical Reviews in Microbiology*, 27 (1), pp.41-55.

Barber, S.A, 1995. *Soil nutrient bioavailability: A mechanistic approach*. 2nd ed. New York: John Wiley and Sons, Inc.

Bartlett, R. and James, B., 1980. Studying dried, stored soil samples - some pitfalls. *Soil Science Society of America Journal*, 44 (4), pp.721-724.

Basile, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montesano, D., Castaldo Cobianchi, R., Vuotto, M.L. and Ferrara, L., 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. *Fitoterapia*, 71, S110-S116.

Battaglia, L.L., Foré, S.A. and Sharitz, R.R., 2000. Seedling emergence, survival and size in relation to light and water availability in two bottomland hardwood species. *Journal of Ecology*, 88 (6), pp.1041-1050.

Baziramakenga, R., Leroux, G.D., Simard, R.R. and Nadeau, P., 1997. Allelopathic effects of phenolic acids on nucleic acid and protein levels in soybean seedlings. *Canadian Journal of Botany*, 75 (3), pp.445-450.

Beckage, B., Clark, J.S., Clinton, B.D. and Haines, B. L., 2000. A long-term study of tree seedling recruitment in southern Appalachian forests: The effects of canopy gaps and shrub understories. *Canadian Journal of Forest Research*, 30 (10), pp.1617-1631.

Bedford, B.L., Walbridge, M.R. and Aldous, A., 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology*, 80 (7), pp.2151-2169.

Beggs, C.J., Kuhn, K., Böcker, R. and Wellmann, E., 1987. Phytochrome-induced flavonoid biosynthesis in mustard (*Sinapis alba* L.) cotyledons: Enzymic control and differential regulation of anthocyanin and quercetin formation. *Planta*, 172 (1), pp.121-126.

Berg, G. and Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68 (1), pp.1-13.

Bertin, N. and Staudt, M., 1996. Effect of water stress on monoterpene emissions from young potted holm oak (*Quercus ilex* L.) trees. *Oecologia*, 107 (4), pp.456-462.

Bertin, C., Yang, X. and Weston, L.A., 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil*, 256 (1), pp.67-83.

Bhadoria, P.B.S., 2011. Allelopathy: A natural way towards weed management. *American Journal of Experimental Agriculture*, 1 (1), pp.7-20.

Bialy, Z., Oleszek, W., Lewis, J. and Fenwick, G.R., 1990. Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. *Plant and Soil*, 129 (2), pp.277-281.

Bjerke, J.W., Lerfall, K. and Elvebakk, A., 2002. Effects of ultraviolet radiation and PAR on the content of usnic and divaricatic acids in two arctic-alpine lichens. *Photochemical and Photobiological Sciences*, 1 (9), pp.678-685.

Bjerknes, A.L., Totland, O., Hegland, S.J. and Nielsen, A., 2007. Do alien plant invasions really affect pollination success in native plant species? *Biological Conservation*, 138 (1-2), pp.1-12.

Blum, U., 1996. Allelopathic interactions involving phenolic acids. *Journal of Nematology*, 28 (3), pp.259-267.

Blum, U., Dalton, B.R. and Shann, J.R., 1985. Effects of various mixtures of ferulic acid and some of its microbial metabolic products on cucumber leaf expansion and dry matter in nutrient culture. *Journal of Chemical Ecology*, 11 (5), pp.619-641.

Blum, U. and Rebbeck, J., 1989. Inhibition and recovery of cucumber roots given multiple treatments of ferulic acid in nutrient culture. *Journal of Chemical Ecology*, 15 (3), pp.917-928.

Boettcher, S.E. and Kalisz, P.J., 1991. Single-tree influence on earthworms in forest soils in eastern Kentucky. *Soil Science Society of America Journal*, 55 (3), pp.862-865.

Bollen, J., Van de Sompel, H., Hagberg, A. and Chute, R., 2009. A principal component analysis of 39 scientific impact measures. *PLoS ONE*, 4 (6): e6022.

Bolte, A. and Villanueva, I., 2006. Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.). *European Journal of Forest Research*, 125 (1), pp.15-26.

Booth, B.D., Murphy, S.D. and Swanton, C.J., 2010. *Invasive plant ecology in natural and agricultural systems*. 2nd ed. Oxfordshire: CAB International.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. and Prati, D., 2005. Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144 (1), pp.1-11.

Bossuyt, B., Cosyns, E. and Hoffmann, M., 2007. The role of soil seed banks in the restoration of dry acidic dune grassland after burning of *Ulex europaeus* scrub. *Applied Vegetation Science*, 10 (1), pp.131-138.

Bradley, R.L., Titus, B.D. and Fyles, J.W., 1997. Nitrogen acquisition and competitive ability of *Kalmia angustifolia* L., paper birch (*Betula papyrifera* Marsh) and black spruce (*Picea mariana* (Mill.) B.S.P.) seedlings grown on different humus forms. *Plant and Soil*, 195 (2), pp.209-220.

Bradley, R.L., Titus, B.D. and Preston, C.P., 2000. Changes to mineral N cycling and microbial communities in black spruce humus after additions of $(NH_4)_2SO_4$ and condensed tannins extracted from *Kalmia angustifolia* and balsam fir. *Soil Biology and Biochemistry*, 32 (8-9), pp.1227-1240.

Braithwaite, R.W., Lonsdale, W.M. and Estbergs, J.A., 1989. Alien vegetation and native biota in tropical Australia: The impact of *Mimosa pigra*. *Biological Conservation*. 48 (3), pp.189-210.

Bramley, H., Turner, D.W., Tyerman, S.D. and Turner, N.C., 2007. Water flow in the roots of crop species: The influence of root structure, aquaporin activity, and waterlogging. *Advances in Agronomy*, 96 (1), pp.133-196.

Brasier, C.M., Beales, P.A., Kirk, S.A., Denman, S. and Rose, J., 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research*, 109 (8), pp.853-859.

Bush, L.P., Wilkinson, H.H. and Schardl, C.L., 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. *Plant Physiology*, 114 (1), pp.1-7.

Bussotti, F., Gravano, E., Grossoni, P. and Tani, C., 1998. Occurrence of tannins in leaves of beech trees (*Fagus sylvatica*) along an ecological gradient, detected by histochemical and ultrastructural analyses. *New Phytologist*, 138 (3), pp.469-479.

Caamal-Maldonado, J.A., Jiménez-Osornio, J.J., Torres-Barragán, A. and Anaya, A.L., 2001. The use of allelopathic legume cover and mulch species for weed control in cropping systems. *Agronomy Journal*, 93 (1), pp.27-36.

CABI, 2012. *Current invasive species projects*. [online]. Available at: <http://www.cabi.org>. [Accessed 25 January 2012].

CABI, 2013. *Progress with weed biocontrol projects*. [online]. Available at: <http://www.cabi.org>. [Accessed 05 November 2013].

Cahill, J.F.,Jr. and Casper, B.B., 2000. Investigating the relationship between neighbor root biomass and belowground competition: Field evidence for symmetric competition belowground. *Oikos*, 90 (2), pp.311-320.

Callaway, R.M. and Aschehoug, E.T., 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science*, 290 (5491), pp.521-523.

Callaway, R.M. and Ridenour, W.M., 2004. Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, 2 (8), pp.436-443.

Cambardella, C.A., Moorman, T.B., Parkin, T.B., Karlen, D.L., Novak, J.M., Turco, R.F. and Konopka, A.E., 1994. Field-scale variability of soil properties in central Iowa soils. *Soil Science Society of America Journal*, 58 (5), pp.1501-1511.

Cannon, J.P., Allen, E.B., Allen, M.F., Dudley, L.M. and Jurinak, J.J., 1995. The effects of oxalates produced by *Salsola tragus* on the phosphorus nutrition of *Stipa pulchra*. *Oecologia*, 102, pp.265-272.

Caño, L., Escarré, J., Vrieling, K. and Sans, F.X., 2009. Palatability to a generalist herbivore, defence and growth of invasive and native *Senecio* species: Testing the evolution of increased competitive ability hypothesis. *Oecologia*, 159 (1), pp.95-106.

Cao, Y., Lou, C., Fang, Y. and Ye, J., 2002. Determination of active ingredients of *Rhododendron dauricum* L. by capillary electrophoresis with electrochemical detection. *Journal of Chromatography A*, 943 (1), pp.153-157.

Cappuccino, N. and Carpenter, D., 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. *Biology Letters*, 1 (4), pp.435-438.

Carballeira, A., 1980. Phenolic inhibitors in *Erica australis* L. and in associated soil. *Journal of Chemical Ecology*, 6 (5), pp.593-596.

Carballeira, A. and Cuervo, A., 1980. Seasonal variation in allelopathic potential of soils from *Erica australis* L. heathland. *Acta Oecologia*, 1 (4), pp.345-353.

Catovsky, S. and Bazzaz, F.A., 2000. The role of resource interactions and seedling regeneration in maintaining a positive feedback in hemlock stands. *Journal of Ecology*, 88 (1), pp.100-112.

Cavieres, L.A., Badano, E.I., Sierra-Almeida, A. and Molina-Montenegro, M.A., 2007. Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the high Andes of Central Chile. *Arctic, Antarctic, and Alpine Research*, 39 (2), pp.229-236.

Chase, W.R., Nair, M.G. and Putnam, A.R., 1991. 2,2′-OXO-1,1′-azobenzene: Selective toxicity of rye (*Secale cereale* L.) allelochemicals to weed and crop species: II. *Journal of Chemical Ecology*, 17 (1), pp.9-19.

Chastain Jr., R.A., Currie, W.S. and Townsend, P.A., 2006. Carbon sequestration and nutrient cycling implications of the evergreen understory layer in Appalachian forests. *Forest Ecology and Management*, 231 (1), pp.63-77.

Chaves, N., Sosa, T., Alias, J.C. and Escudero, J.C., 2001. Identification and effects of interaction phytotoxic compounds from exudate of *Cistus ladanifer* leaves. *Journal of Chemical Ecology*, 27 (3), pp.611-621.

Chen, H., Qualls, R.G. and Miller, G.C., 2002. Adaptive responses of *Lepidium latifolium* to soil flooding: Biomass allocation, adventitious rooting, aerenchyma formation and ethylene production. *Environmental and Experimental Botany*, 48 (2), pp.119-128.

Cheng, X., Pend, R., Chen, J., Luo, Y., Zhang, Q. An, S., Chen, J. and Li, B., 2007. CH_4 and N_2O emissions from *Spartina alterniflora* and *Phragmites australis* in experimental mesocosms. *Chemosphere*, 68 (3), pp.420-427.

Chittka, L. and Schürkens, S., 2001. Successful invasion of a floral market. *Nature*, 411 (1), pp.653-654.

Chou, S.C., Huang, C.H., Hsu, T.W., Wu, C.C. and Chou, C.H., 2010. Allelopathic potential of *Rhododendron formosanum* Hemsl in Taiwan. *Allelopathy Journal*, 25 (1), pp.73-92.

Chou, S.C., Krishna, V. and Chou, C.H., 2009. Hydrophobic metabolites from *Rhododendron formosanum* and their allelopathic activities. *Natural Product Communications*, 4 (9), pp.1189-1192.

Cipollini, D., Stevenson, R., Enright, S., Eyles, A. and Bonello, P., 2008. Phenolic metabolites in leaves of the invasive shrub, *Lonicera maackii*, and their potential phytotoxic and anti-herbivore effects. *Journal of Chemical Ecology*, 34 (2), pp.144-152.

Cleverly, J.R., Smith, S.D., Sala, A. and Devitt, D.A., 1997. Invasive capacity of *Tamarix ramosissima* in a Mojave Desert floodplain: The role of drought. *Oecologia*, 111 (1), pp.12-18.

Cline, J.F., Uresk, D.W. and Rickard, W.H., 1977. Comparison of water used by a sagebrush-bunchgrass community and a cheatgrass community. *Journal of Range Management*, 30 (3), pp.199-201.

Clinton, B.D., 1995. Temporal variation in photosynthetically active radiation (PAR) in mesic southern Appalachian hardwood forest with and without *Rhododendron* understories. *Proceedings, 10th Central Hardwood Forest Conference*, pp.534-540.

Clinton, B.D., 2003. Light, temperature, and soil moisture responses to elevation, evergreen understory, and small canopy gaps in the Southern Appalachians. *Forest Ecology and Management*, 186 (1-3), pp.243-255.

Clinton, B.D. and Vose, J.M., 1996. Effects of *Rhododendron maximum* L. on *Acer rubrum* L. seedling establishment. *Castanea*, 61 (1), pp.38-45.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. and MacIsaac, H.J., 2004. Is invasion success explained by the enemy release hypothesis?. *Ecology Letters*, 7 (8), pp.721-733.

Coley, P.D., Bryant, J.P. and Chaplin III, F.S., 1985. Resource availability and plant antiherbivore defense. *Science*, 230 (4728), pp.895-899.

Coxon, P., Hannon, G. and Foss, P., 1994. Climatic deterioration and the end of the Gortian Interglacial in sediments from Derrynadivva and Burren Townland, near Castlebar, County Mayo, Ireland. *Journal of Quaternary Science*, 9 (1), pp.33-46.

Cronin, G. and Lodge, D.M., 2003. Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia*, 137 (1), pp.32-41.

Cross, J.R., 1975. Botanical flora of the British Isles: *Rhododendron ponticum* L. *Journal of Ecology*, 63 (1), pp.345-364.

Cross, J.R., 1981. The establishment of *Rhododendron ponticum* in the Killarney oakwoods, S.W. Ireland. *The Journal of Ecology*, 69 (3), pp.807-824.

Cruz-Ortega, R., Ayala-Cordero, G. and Anaya, A. L., 2002. Allelochemical stress produced by the aqueous leachate of *Callicarpa acuminata*: Effects on roots of bean, maize, and tomato. *Physiologia Plantarum*, 116 (1), pp.20-27.

Cullen, J., 2005. *Hardy Rhododendron species. A guide to identification*. Edinburgh: Timber Press, Inc.

Ćurković-Perica, M. and Ježić, M., 2010. Detrimental effect of quercetin on phytoplasma-infected *Catharanthus roseus* (L.) G. Don shoots grown in vitro. *Acta Botanica Croatica*, 69 (2), pp.155-162.

Dalton, B.R., Blum, U. and Weed, S.B., 1989. Differential sorption of exogenously applied ferulic, p-coumaric, p-hydroxybenzoic, and vanillic acids in soil. *Soil Science Society of America Journal*, 53 (3), pp.757-762.

Daniel, O., Meier, M.S., Schlatter, J. and Frischknecht, P., 1999. Selected phenolic compounds in cultivated plants: Ecologic functions, health implications, and modulation by pesticides. *Environmental Health Perspectives*, 107 (Suppl 1), pp.109-114.

D'Antonio, C.M. and Mahall, B.E., 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. *American Journal of Botany*, 78 (7), pp.885-894.

D'Antonio, C.M. and Vitousek, P.M., 1992, Biological invasions by exotic grasses, the grass fire cycle, and global change. *Annual Review of Ecology, Evolution and Systematics*, 23 (1), pp.63-87.

DAISIE, 2012. *European Invasive Alien Species Gateway*. [online]. Available at: <http://www.europe-aliens.org>. [Accessed 20 January 2012].

Day, W., Legg, B.J., French, B.K., Johnston, A.E., Lawlor, D.W. and Jeffers, W.D., 1978. A drought experiment using mobile shelters: The effect of drought on barley yield, water use and nutrient uptake. *Journal of Agricultural Science*, 91 (3), pp.599-623.

Debevec, E.M. and MacLean Jr., S.F., 1993. Design of greenhouses for the manipulation of temperature in tundra plant communities. *Arctic and Alpine Research*, 25 (1), pp.56-62.

De Bruxelles, S., 2010. *War declared on invasive plants that cost Britain £2.7bn a year*. *Times Online*, [online] 24 Feb. Available at: <http://www.times online.co.uk/tol/news/environment/article7038322.ece> [Accessed 23 September 2010].

Defra, 2013. *The New Forest - Special Area of Conservation* [online] Available at: <http://jncc.defra.gov.uk/protectedsites/sacselection/sac.asp?EUCode =UK0012557> [Accessed 09 January 2013].

Dehnen-Schmutz, K., Perrings, C. and Williamson, M., 2004. Controlling *Rhododendron ponticum* in the British Isles: An economic analysis. *Journal of Experimental Management*, 70 (4), pp.323-332.

Delgado, J.D., Arroyo, N.L., Arévalo, J.R. and Fernández-Palacios, J.M., 2007. Edge effects of roads on temperature, light, canopy cover, and canopy height in laurel and pine forests (Tenerife, Canary Islands). *Landscape and Urban Planning*, 81 (4), pp.328-340.

Del Moral, R. and Cates, R.G., 1971. Allelopathic potential of the dominant vegetation of Western Washington. *Ecology*, 52 (6), pp.1030-1037.

Devi, S.R. and Prasad, M.N.V., 1992. Effect of ferulic acid on growth and hydrolytic enzyme activities of germinating maize seeds. *Journal of Chemical Ecology*, 18 (11), pp.1981-1990.

Dieterman, L.J., Lin, C.Y., Rohrbaugh, L.M. and Wender, S.H., 1964. Accumulation of ayapin and scopolin in sunflower plants treated with 2, 4-dichlorophenoxyacetic acid. *Archives of Biochemistry and Biophysics*, 106 (1), pp.275-279.

Dietzsch, A.C., Stanley, D.A. and Stout, J.C., 2011. Relative abundance of an invasive alien plant affects native pollination processes. *Oecologia*, 167 (2), pp.469-479.

Discover Life, 2013. *Map of Rhododendron*. [online]. Available at: <http://www.discoverlife.org>. [Accessed 05 November 2013].

Donath, T.W. and Eckstein, R.L., 2008. Grass and oak litter exert different effects on seedling emergence of herbaceous perennials from grasslands and woodlands. *Journal of Ecology*, 96 (2), pp.272-280.

Doncaster, C.P. and Davey, A.J.H., 2007. Analysis of varience and covariance. How to choose and construct models for the life sciences. New York: Cambridge University Press.

Donnelly, M.J., Green, D.M. and Walters, L.J., 2008. Allelopathic effects of fruits of the Brazilian pepper *Schinus terebinthifolius* on growth, leaf production and biomass of seedlings of the red mangrove *Rhizophora mangle* and the black mangrove *Avicennia germinans*. *Journal of Experimental Marine Biology and Ecology*, 357 (2), pp.149-156.

Dudt, J.F. and Shure, D.J., 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, 75 (1), pp.86-98.

Dunbar, K.R. and Facelli, J.M., 1999. The impact of a novel invasive species, *Orbea variegata* (African carrion flower), on the chenopod shrublands of South Australia. *Journal of Arid Environments*, 41 (1), pp.37-48.

Dukes, J.S. and Mooney, H.A., 2004. Disruption of ecosystem processes in western North America by invasive species. *Revista Chilena de Historia Natural*, 77 (3), pp.411-437.

Duryea, M.L., English, R.J. and Hermansen, L.A., 1999. A comparison of landscape mulches: Chemical, allelopathic, and decomposition properties. *Journal of Arboriculture*, 25 (2), pp.88-96.

Ehrenfeld, J.G., Koutev, P. and Huang, W. 2001. Changes in soil functions following invasions of exotic understorey plants in deciduous forests. *Ecological Applications*, 11 (5), pp.1287-1300.

Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*, 6 (6), pp.503-523.

Einhellig, F.A., 1987. Interactions among allelochemicals and other stress factors of the plant environment. *American Chemical Society Symposium Series*, 330, pp.343-357.

Einhellig, F.A., 1994. Mechanism of action of allelochemicals in allelopathy. *American Chemical Society Symposium Series*, 582, pp. 96-116.

Einhellig, F.A., 1996. Interactions involving allelopathy in cropping systems. *Agronomy Journal*, 88 (6), pp.886-893.

Einhellig, F.A. and Eckrich, P.C., 1984. Interactions of temperature and ferulic acid stress on grain sorghum and soybeans. *Journal of Chemical Ecology*, 10 (1), pp.161-170.

Einhellig, F.A., Rice, E.L., Risser, P.G. and Wender, S.H., 1970. Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin, and chlorogenic acids in tobacco, sunflower, and pigweed. *Bulletin of the Torrey Botanical Club*, 97 (1), pp.22-33.

Elmore, C.D., 1980. Inhibition of turnip (*Brassica rapa*) seed germination by velvetleaf (*Abutilon theophrasti*) seed. *Weed Science*, 28 (6), pp.658-660.

English-Loeb, G., Stout, M.J. and Duffey, S.S., 1997. Drought stress in tomatoes: Changes in plant chemistry and potential nonlinear consequences for insect herbivores. *Oikos*, 79 (3), pp.456-468.

Eppard, H.R., Horton, J.L., Nilsen, E.T., Galusky, P. and Clinton, B.D., 2005. Investigating the allelopathic potential of *Kalmia latifolia* L. (Ericaceae). *Southeastern Naturalist*, 4 (3), pp.383-392.

Erfmeier, A. and Bruelheide, H., 2004. Comparison of native and invasive *Rhododendron ponticum* populations: Growth, reproduction and morphology under field conditions. *Flora*, 199 (2), pp.120-133.

Erfmeier, A. and Bruelheide, H., 2005. Invasive and native *Rhododendron ponticum* populations: Is there evidence of genotypic differences in germination and growth? *Ecography*, 28 (4), pp.417-428.

Erfmeier, A. and Bruelheide, H., 2010. Invasibility or invasiveness? Effects of habitat, genotype, and their interaction on invasive *Rhododendron ponticum* populations. *Biological Invasions*, 12 (3), pp.657-676.

Erfmeier, A. and Bruelheide, H., 2011. Maintenance of high genetic diversity during invasion of *Rhododendron ponticum*. *International Journal of Plant Sciences*, 172 (6), pp.795-806.

Ericsson, T., 1995. Growth and shoot: root ratio of seedlings in relation to nutrient availability. *Plant and Soil*, 168-169 (1), pp.205-214.

Eriksen, F.I. and Whitney, A.S., 1981. Effects of light intensity on growth of some tropical forage species. I. Interaction of light intensity and nitrogen fertilization on six forage grasses. *Agronomy Journal*, 73 (3), pp.427-433.

Ertürk, Ö., Karakaş, F.P., Pehlivan, D. and Nas, N., 2009. The antibacterial and antifungal effects of *Rhododendron* derived mad honey and extracts of four *Rhododendron* species. *Turkish Journal of Biology*, 33 (2), pp.151-158.

Eşen, D. and Zedaker, S.M., 2004. Control of rhododendron (*Rhododendron ponticum* and *R. flavum*) in the eastern beech (*Fagus orientalis*) forests of Turkey. *New Forests*, 27 (1), pp.69-79.

Everham, E.M., Myster, R.W. and VanDeGenachte, E., 1996. Effects of light, moisture, temperature, and litter on the regeneration of five tree species in the tropical montane wet forest of Puerto Rico. *American Journal of Botany*, 83 (8), pp.1063-1068.

Farley, R.A. and Fitter, A.H., 1999. Temporal and spatial variation in soil resources in a deciduous woodland. *Journal of Ecology*, 87 (4), pp.688-696.

Fdz-Polanco, F., Villaverde, S. and Garcia, P.A., 1994. Temperature effect on nitrifying bacteria activity in biofilters: Activation and free ammonia inhibition. *Water Science and Technology*, 30 (11), pp.121-130.

Filella, I. and Peñuelas, J., 1999. Altitudinal differences in U.V. absorbance, U.V. reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. *Plant Ecology*, 145 (1), pp.157-165.

Fiorentino, A., D'Abrosca, B., Pacifico, S., Izzo, A., Letizia, M., Esposito, A. and Monaco, P., 2008. Potential allelopathic effects of stilbenoids and flavonoids from leaves of *Carex distachya* desf. *Biochemical Systematics and Ecology*, 36 (9), pp.691-698.

Fistarol, G., Legrand, C. and Graneli, E., 2005. Allelopathic effect on a nutrient-limited phytoplankton species. *Aquatic Microbial Ecology*, 41 (2), pp.153-161.

Fleischbein, K., Wilcke, W., Goller, R., Boy, J., Valarezo, C., Zech, W. and Knoblich, K., 2005. Rainfall interception in a lower montane forest in Ecuador: Effects of canopy properties. *Hydrological Processes*, 19 (7), pp.1355-1371.

Frank, A.B., Power, J.F. and Willis, W.O., 1973. Effect of temperature and plant water stress on photosynthesis, diffusion resistance, and leaf water potential in spring wheat. *Agronomy journal*, 65 (5), pp. 777-780.

Friebe, A., Roth, U., Kück, P., Schnabl, H. and Schulz, M., 1997. Effects of 2,4-dihydroxy-1,4-benzoxazin-3-ones on the activity of plasma membrane H^+-ATPase. *Phytochemistry*, 44 (6), pp.979-983.

Friedman, J. and Waller, G.R., 1983. Seeds as allelopathic agents. *Journal of Chemical Ecology*, 9 (8), pp.1107-1117.

Friedman, M. and Jürgens, H.S., 2000. Effect of pH on the stability of plant phenolic compounds. *Journal of Agricultural and Food Chemistry*, 48 (6), pp.2101-2110.

Fries, L.L., Pacovsky, R.S., Safir, G.R. and Siqueira, J.O., 1997. Plant growth and arbuscular mycorrhizal fungal colonization affected by exogenously applied phenolic compounds. *Journal of Chemical Ecology*, 23 (7), pp.1755-1767.

Fry, W.E. and Goodwin, S.B., 1997. Resurgence of the Irish potato famine fungus. *BioScience*, 47 (6), pp.363-371.

Fujii, Y., Shibuya, T., Nakatani, K., Itani, T., Hiradate, S. and Parvez, M.M., 2004. Assessment method for allelopathic effect from leaf litter leachates. *Weed Biology and Management*, 4 (1), pp.19-23.

Gallet, C., 1994. Allelopathic potential in bilberry-spruce forests: Influence of phenolic compounds on spruce seedlings. *Journal of Chemical Ecology*, 20 (5), pp.1009-1024.

Gallet, C., Nilsson, M.C. and Zackrisson, O., 1999. Phenolic metabolites of ecological significance in *Empetrum hermaphroditum* leaves and associated humus. *Plant and Soil*, 210 (1), pp.1-9.

Gerhardt, K.E., Lampi, M.A. and Greenberg, B.M., 2008. The effects of far-red light on plant growth and flavonoid accumulation in *Brassica napus* in the presence of ultraviolet B radiation. *Photochemistry and Photobiology*, 84 (6), pp.1445-454.

Ghasemzadeh, A., Jaafar, H.Z., Rahmat, A., Wahab, P.E.M. and Halim, M.R.A., 2010. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). *International Journal of Molecular Sciences*, 11 (10), pp.3885-3897.

Gholz, H.L., Hawk, G.M., Campbell, A. and Cromack, Jr, K., 1985. Early vegetation recovery and element cycles on a clear-cut watershed in western Oregon. *Canadian Journal of Forest Research*, 15 (2), pp.400-409.

Gifford, R.M., 1995. Whole plant respiration and photosynthesis of wheat under increased CO_2 concentration and temperature: Long-term vs. short-term distinctions for modelling. *Global Change Biology*, 1 (6), pp.385-396.

Gliessman, S.R. and Muller, C.H., 1978. The allelopathic mechanisms of dominance in bracken (*Pteridium aquilinum*) in Southern California. *Journal of Chemical Ecology*, 4 (3), pp.337-362.

Glinwood, R., Pettersson, J., Ahmed, E., Ninkovic, V., Birkett, M. and Pickett, J., 2003. Change in acceptability of barley plants to aphids after exposure to allelochemicals from couch-grass (*Elytrigia repens*). *Journal of Chemical Ecology*, 29 (2), pp.261-274.

Global Invasive Species Database, 2013. *Global Invasive Species Database* [online] Available at: <http://www.issg.org/database/welcome/> [Accessed 31 October 2013].

Goldberg, D.E., 1985. Effects of soil pH, competition, and seed predation on the distributions of two tree species. *Ecology*, 66 (2), pp.503-511.

Gorchov, D.L. and Trisel, D.E., 2003. Competitive effects of the invasive shrub, *Lonicera maackii* (Rupr.) Herder (Caprifoliaceae), on the growth and survival of native tree seedlings. *Plant Ecology*, 166 (1), pp.13-24.

Gordon, D.R., 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. *Ecological Applications*, 8 (4), pp.975-989.

Gordon, J.C., 1969. Effect of shade on photosynthesis and dry weight distribution in yellow birch (*Betula alleghaniensis* Britton) seedlings. *Ecology*, 50 (5), pp.924-927.

Gould, K.S. and Lister, C., 2006. Flavonoid functions in plants. In: Andersen, Ø.M. and Markham, K.R., eds. 2006. *Flavonoids: Chemistry, biochemistry and applications*. Florida: CRC Press. Ch.8.

Gould, A.M.A. and Gorchov, D.L., 2000. Effects of the exotic invasive shrub *Lonicera maackii* on the survival and fecundity of three species of native annuals. *American Midland Naturalist*, 144 (1), pp.36-50.

Granéli, E. and Johansson, N., 2003. Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N-or P-deficient conditions. *Harmful Algae*, 2 (2), pp.135-145.

Granéli, E. and Salomon, P.S., 2010. Factors influencing allelopathy and toxicity in *Prymnesium parvum*. *Journal of the American Water Resources Association*, 46 (1), pp.108-120.

Gratzer, G., Rai, P.B., Darabant, A., Chhetri, P.B., Eckmüllner, O. and Glatzel, G., 2004. Leaf characteristics and growth response to light of understory *Rhododendron hodgsonii* in the Bhutan Himalayas. *Ekologia*, 23 (3), pp.283-297.

Gray, D.E., Pallardy, S.G., Garrett, H.E. and Rottinghaus, G.E., 2003a. Acute drought stress and plant age effects on alkamide and phenolic acid content in purple coneflower roots. *Planta Medica*, 69 (1), pp.50-55.

Gray, D.E., Pallardy, S.G., Garrett, H.E. and Rottinghaus, G.E., 2003b. Effect of acute drought stress and time of harvest on phytochemistry and dry weight of St. John's wort leaves and flowers. *Planta Medica*, 69 (11), pp.1024-1030.

Grayston, S.J., Vaughan, D. and Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, 5 (1), pp.29-56.

Grime, J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, 111 (982), pp.1169-1194.

Grubb, P.J., Lee, W.G., Kollmann, J. and Wilson, J.B., 1996. Interaction of irradiance and soil nutrient supply on growth of seedlings of ten European tall-shrub species and *Fagus sylvatica*. *Journal of Ecology*, 84 (6), pp.827-840.

Hadwiger, L.A., 1972. Increased levels of pisatin and phenylalanine ammonia lyase activity in *Pisum sativum* treated with antihistaminic, antiviral, antimalarial, tranquilizing, or other drugs. *Biochemical and Biophysical Research Communications*, 46 (1), pp.71-79.

Hagedorn, F., Steiner, K.G. and Sekayange, L., 1997. Effect of rainfall pattern on nitrogen mineralization and leaching in a green manure experiment in South Rwanda. *Plant and Soil*, 195 (2), pp. 365-375.

Haling, R.E., Simpson, R.J., Delhaize, E., Hocking, P.J. and Richardson, A.E., 2010. Effect of lime on root growth, morphology and the rhizosheath of cereal seedlings growing in an acid soil. *Plant and Soil*, 327 (1-2), pp.199-212.

Halvorson, J.J., Gonzalez, J.M., Hagerman, A.E. and Smith, J.L., 2009. Sorption of tannin and related phenolic compounds and effects on soluble-N in soil. *Soil Biology and Biochemistry*, 41 (9), pp.2002-2010.

Hannaway, D., Fransen, S., Cropper, J., Teel, M., Chaney, M., Griggs, T., Halse, R., Hart, J., Cheeke, P., Hansen, D., Klinger, R. and Lane, W., 1999. *Perennial ryegrass (Lolium perenne L.)* [online] Available at: <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/17827/pnw503.pdf?sequence=1> [Accessed 11 January 2010].

Harborne, J.B. and Williams, C.A., 1971. Leaf survey of flavonoids and simple phenols in the genus *Rhododendron*. *Phytochemistry*, 10 (11), pp.2727-2744.

Hardy, R., Wright, P., Gribbin, J. and Kington, J., 1982. *The weather book*. London: Michael Joseph Limited.

Harris, C.M., Stanford, H.L., Edwards, C., Travis, J.M.J. and Park, K.J., 2011. Integrating demographic data and a mechanistic dispersal model to predict invasion spread of *Rhododendron ponticum* in different habitats. *Ecological Informatics*, 6 (3), pp.187-195.

Hastwell, G.T. and Facelli, J.M., 2003. Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *Journal of Ecology*, 91 (6), pp.941-950.

Havaux, M., 1992. Stress tolerance of photosystem II in vivo: Antagonistic effects of water, heat, and photoinhibition stresses. *Plant Physiology*. 100 (1), pp.424-432.

Haynes, R.J. and Swift, R.S., 1986. Effects of soil acidification and subsequent leaching on levels of extractable nutrients in a soil. *Plant and Soil*, 95 (3), pp.327-336.

Hebeisen, T., Lüscher, A. and Nösberger, J., 1997. Effects of elevated atmospheric CO2 and nitrogen fertilisation on yield of *Trifolium repens* and *Lolium perenne*. *Acta Oecologica*, 18 (3), pp.277-284.

Hobbie, S.E., 1992. Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution*, 7 (10), pp.336-339.

Hoffman, W.A. and Jackson, R.B., 2000. Vegetation-climate feedbacks in the conversion of tropical savanna to grassland. *Journal of Climate*, 13 (9), pp.1593-1602.

Hudson, B.D, 1994. Soil organic matter and available water capacity. *Journal of Soil and Water Conservation*, 49 (2), pp.189-194.

Hunter, M.D., Adl, S., Pringle, C.M. and Coleman, D.C., 2003. Relative effects of macroinvertebrates and habitat on the chemistry of litter during decomposition. *Pedobiologia*, 47 (2), pp.101-115.

Hussain, S., Siddiqui, S.U., Khalid, S., Jamal, A., Qayyum, A and Ahmad, Z., 2007. Allelopathic potential of senna (*Cassia angusifolia vahl.*) on germination and seedling characters of some major cereal crops and their associated grassy weeds. *Pakistan Journal of Botany*, 39 (4), pp.1145-1153.

Ibrahima, A., Joffre, R. and Gillon, D., 1995. Changes in litter during the initial leaching phase: An experiment on the leaf litter of Mediterranean species. *Soil Biology and Biochemistry*, 27 (7), pp.931-939.

Inderjit and Dakshini, K.M.M., 1994. Allelopathic effect of *Pluchea lanceolata* (Asteraceae) on characteristics of four soils and tomato and mustard growth. *American Journal of Botany*, 81 (7), pp.799-804.

Inderjit and Del Moral, R., 1997. Is separating resource competition from allelopathy realistic? *The Botanical Review*, 63 (3), pp.221-230.

Inderjit and Duke, S.O., 2003. Ecophysiological aspects of allelopathy. *Planta*, 217 (4), pp.529-539.

Inderjit and Mallik, A.U., 2002. Can *Kalmia angustifolia* interference to black spruce (*Picea mariana*) be explained by allelopathy? *Forest Ecology and Management*, 160 (1-3), pp.75-84.

Inderjit and Nilsen, E.T., 2003. Bioassays and field studies for allelopathy in terrestrial plants: Progress and problems. *Critical Reviews in Plant Sciences*, 22 (3-4), pp.221-238.

Isfahan, M.N. and Shariati, M., 2007. The effect of some allelochemicals on seed germination of *Coronilla varia* L. seeds. *American-Eurasian Journal of Agricultural and Environmental Science*, 2 (5), pp.534-538.

Jaakola, L., Määttä-Riihinen, K., Kärenlampi, S. and Hohtola, A., 2004. Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta*, 218 (5), pp.721-728.

Jalal, M.A.F. and Read, D.J., 1983. The organic acid composition of *Calluna* heathland soil with special reference to phyto- and fungitoxicity. II. Monthly quantitative determination of the organic acid content of *Calluna* and spruce dominated soils. *Plant and Soil*, 70 (2), pp.273-286.

Jan, S., Parween, T. and Siddiqi, T.O., 2012. Enhancement in furanocoumarin content and phenylalanine ammonia lyase activity in developing seedlings of *Psoralea corylifolia* L. in response to gamma irradiation of seeds. *Radiation and Environmental Biophysics*, 51 (3), 341-347.

Jeangros, B. and Nösberger, J., 2006. Effects of an established sward of *Lolium perenne* L. on the growth and development of *Rumex obtusifolius* L. seedlings. *Grass and Forage Science*, 45 (1), pp.1-7.

Jefferson, L.V. and Pennacchio, M., 2003. Allelopathic effects of foliage extracts from four *Chenopodiaceae* species on seed germination. *Journal of Arid Environments*, 55 (2), pp.275-285.

Jose, S. and Gillespie, A.R., 1998. Allelopathy in black walnut (*Juglans nigra* L.) alley cropping. I. Spatio-temporal variation in soil juglone in a black walnut–corn (*Zea* mays *L*.) alley cropping system in the midwestern USA. *Plant and Soil*, 203 (2), pp.191-197.

Judd, S. and Rotherham, I.D., 1992. The phytophagous insect fauna of *Rhododendron ponticum* L. in Britain. *Entomologist*, 111 (3), pp.134-150.

Kang, K.H., Dec, J., Park, H. and Bollag, J.M., 2002. Transformation of the fungicide cyprodinil by a laccase of *Trametes villosa* in the presence of phenolic mediators and humic acid. *Water research*, 36 (19), pp.4907-4915.

Karageorgou, P., Levizou, E. and Manetas, Y., 2002. The influence of drought, shade and availability of mineral nutrients on exudate phenolics of *Dittrichia viscosa*. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 197 (4), pp.285-289.

Karlsson, P.S. and Nordell, K.O., 1996. Effects of soil temperature on the nitrogen economy and growth of mountain birch seedlings near its presumed low temperature distribution limit. *Ecoscience*, 3 (2), pp.183-189.

Kato-Noguchi, H., 1999. Effect of light-irradiation on allelopathic potential of germinating maize. *Phytochemistry*, 52 (6), pp.1023-1027.

Kato-Noguchi, H., Ino, T., Sata, N. and Yamamura, S., 2002. Isolation and identification of a potent allelopathic substance in rice root exudates. *Physiologia Plantarum*, 115 (3), pp.401-405.

Kato, S. and Komiyama, A., 2002. Spatial and seasonal heterogeneity in understory light conditions caused by differential leaf flushing of deciduous overstory trees. *Ecological Research*, 17 (6), pp.687-693.

Keane, R.M. and Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, 17 (4), pp.164-170.

Kemertelidze, E.P., Shalashvili, K.G., Korsantiya, B.M., Nizharadze, N.O. and Chipashvili, N.S., 2007. Therapeutic effect of phenolic compounds isolated from *Rhododendron ungernii* leaves. *Pharmaceutical Chemistry Journal*, 41 (1), pp.10-13.

Kennedy, A.D., 1995a. Temperature effects of passive greenhouse apparatus in high-latitude climate change experiments. *Functional Ecology*, 9 (2), pp.340-350.

Kennedy, A.D., 1995b. Simulated climate change: Are passive greenhouses a valid microcosm for testing the biological effects of environmental perturbations?. *Global Change Biology*, 1 (1), pp.29-42.

Khaliq, A., Matloob, A., Tanweer, A. and Khan, M.B., 2012. Naturally occurring phytotoxins in allelopathic plants help reduce herbicide dose in wheat. *Natural Product Research*, 26 (12), pp.1156-1160.

Khan, M.A., Hussain, N., Abid, M. and Imran, T., 2004. Screening of wheat (*Triticum aestivum* L.) cultivars for saline conditions under irrigated arid environment. *Journal of Research Science*, 15 (4), pp.471-477.

Kikvidze, Z., Pugnaire, F.I., Brooker, R.W., Choler, P., Lortie, C.J., Michalet, R. and Callaway, R.M., 2005. Linking patterns and processes in alpine plant communities: A global study. *Ecology*, 86 (6), pp.1395-1400.

Killham, K., 1994. *Soil ecology*. Cambridge: Cambridge University Press.

Klein, K. and Blum, U., 1990. Inhibition of cucumber leaf expansion by ferulic acid in split-root experiments. *Journal of Chemical Ecology*, 16 (2), pp.455-463.

Klocke, J.A., Mei-Ying, H., Shin-Foon, C. and Kubo, I., 1991. Grayanoid diterpene insect antifeedants and insecticides from *Rhododendron molle*. *Phytochemistry*, 30 (6), pp.1797-1800.

Knox, G.W., 1989. Water use and average growth index of five species of container grown woody landscape plants. *Journal of Environmental Horticulture*, 7 (4), pp.136-139.

Koca, I. and Koca, A.F., 2007. Poisoning by mad honey: A brief review. *Food and Chemical Toxicology*, 45 (8), pp.1315-1318.

Koeppe, D.E., Rohrbaugh, L.M., Rice, E.L. and Wender, S.H., 1970. The effect of age and chilling temperature on the concentration of scopolin and caffeoylquinic acids in tobacco. *Physiologia Plantarum*, 23 (2), pp.258-266.

Kohli, R.K., Batish, D.R. and Singh, H.P., 1998. Eucalypt oils for the control of parthenium (*Parthenium hysterophorus* L.). *Crop Protection*, 17 (2), pp.119-122.

Kohli, R.K. and Singh, D., 1991. Allelopathic impact of volatile components from eucalyptus on crop plants. *Biologia Plantarum*, 33 (6), pp.475-483.

Kolářová, P., Bezděčková, L. and Procházková, Z., 2010. Effect of gibberellic acid and ethephon on the germination of European beech dormant and chilled beechnuts. *Journal of Forest Science*, 56 (9), pp.389-396.

Kolb, A. and Alpert, P., 2003. Effects of nitrogen and salinity on growth and competition between a native grass and an invasive congener. *Biological Invasions*, 5 (3), pp.229-238.

Komissarenko, N.F., Levashova, I.G. and Shnyakina, G.P., 1973. Phenolic compounds of *Rhododendron dahuricum*. *Chemistry of Natural Compounds*, 9 (5), pp.629.

Kong, C., Hu, F. and Xu, X., 2002. Allelopathic potential and chemical constituents of volatiles from *Ageratum conyzoides* under stress. *Journal of Chemical Ecology*, 28 (6), pp.1173-1182.

Kraus, T.E.C., Zasoski, R.J., Dahlgren, R.A., Horwath, W.R. and Preston, C.M., 2004. Carbon and nitrogen dynamics in a forest soil amended with purified tannins from different plant species. *Soil Biology and Biochemistry*, 36 (2), pp.309-321.

Kuiters, A.T. and Sarink, H.M., 1986. Leaching of phenolic compounds from leaf and needle litter of several deciduous and coniferous trees. *Soil Biology and Biochemistry*, 18 (5), pp.475-480.

Laine, K.M. and Henttonen, H., 1987. Phenolics/nitrogen ratios in the blueberry *Vaccinium myrtillus* in relation to temperature and microtine density in Finnish Lapland. *Oikos*, 50 (3), pp.389-395.

Laitinen, M. L., Julkunen-Tiitto, R. and Rousi, M., 2000. Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology*, 26 (7), pp.1609-1622.

Larson, D.L., 2002. Native weeds and exotic plants: Relationships to disturbance in mixed-grass prairie. *Plant Ecology*, 169 (2), pp.317-333.

Lawrence, J.G., Colwell, A. and Sexton, O.J., 1991. The ecological impact of allelopathy in *Ailanthus altissima* (Simaroubaceae). *American Journal of Botany*, 78 (7), pp.948-958.

Leege, L.M. and Murphy, P.G., 2001. Ecological effects of the non-native *Pinus nigra* on sand dune communities. *Canadian Journal of Botany*, 79 (4), pp.429-437.

Leflaive, J. and Ten-Hage, L., 2009. Allelopathic interactions in benthic biofilms: Effects of abiotic conditions on production of and sensitivity to allelochemicals. *Journal of the North American Benthological Society*, 28 (2), pp.273-282.

Lei, T.T., Nilsen, E.T. and Semones, S.W., 2006. Light environment under *Rhododendron maximum* thickets and estimated carbon gain of regenerating forest tree seedlings. *Plant Ecology*, 184 (1), pp.143-156.

Lei, T.T., Semones, S.W., Walker, J.F., Clinton, B.D. and Nilsen, E.T., 2002. Effects of *Rhododendron maximum* thickets on tree seed dispersal, seedling morphology, and survivorship. *International Journal of Plant Sciences*, 163 (6), pp.991–1000.

Leishman, M.R. and Westoby, M., 1994. The role of large seed size in shaded conditions: Experimental evidence. *Functional Ecology*, 8 (2), pp.205-214.

Levine, J.M., Vilá, M., D'Antonio, C.M., Dukes, J.S., Grigulis, K. and Lavorel, S., 2003. Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London B*, 270 (1517), pp.775-781.

Li, Y., Liu, Y.B. and Yu, S.S., 2013. Grayanoids from the Ericaceae family: Structures, biological activities and mechanism of action. *Phytochemistry Reviews*, 12 (2), pp.305-325.

Lichtenthaler, H.K., Buschmann, C., Döll, M., Fietz, H.J., Bach, T., Kozel, U., Meier, D. and Rahmsdorf, U., 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research*, 2 (2), pp.115-141.

Lipscomb, M.V. and Nilsen, E.T., 1990. Environmental and physiological factors influencing the natural distribution of evergreen and deciduous ericaceous shrubs on northeast and southwest facing slopes of the Southern Appalachian Mountains: II. Water relations. *American Journal of Botany*, 77 (4), pp.517-526.

Liu, X., Chen, Q., Wang, Z., Xie, L. and Xu, Z., 2008. Allelopathic effects of essential oil from *Eucalyptus grandis* × *E. urophylla* on pathogenic fungi and pest insects. *Frontiers of Forestry in China*, 3 (2), pp.232-236.

Lobón, N.C., Gallego, J.C., Díaz, T.S. and García, J.C., 2002. Allelopathic potential of *Cistus ladanifer* chemicals in response to variations of light and temperature. *Chemoecology*, 12 (3), pp.139-145.

Lodhi, M.A.K. and Killingbeck, K.T., 1982. Effects of pine-produced chemicals on selected understory species in a *Pinus ponderosa* community. *Journal of Chemical Ecology*, 8 (1), pp.275-283.

Löf, M., 2000. Establishment and growth in seedlings of *Fagus sylvatica* and *Quercus robur*: Influence of interference from herbaceous vegetation. *Canadian Journal of Forest Research*, 30 (6), pp.855-864.

Lozon, J.D. and MacIsaac, H.J., 1997. Biological invasions: Are they dependent on disturbance? *Environmental Reviews*, 5 (2), pp.131-144.

Luthria, D.L., Mukhopadhyay, S. and Krizek, D.T., 2006. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar U.V. radiation. *Journal of Food Composition and Analysis*, 19 (8), pp.771-777.

Lyu, S.W. and Blum, U., 1990. Effects of ferulic acid, an allelopathic compound, on net P, K, and water uptake by cucumber seedlings in a split-root system. *Journal of Chemical Ecology*, 16 (8), pp.2429-2439.

MacDougall, A.S. and Turkington, R., 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, 86 (1), pp.42-55.

Macías, F.A., Molinillo, J.M., Galindo, J.C., Varela, R.M., Simonet, A.M. and Castellano, D., 2001. The use of allelopathic studies in the search for natural herbicides. *Journal of Crop Production*, 4 (2), pp.237-255.

Macías, F.A., Simonet, A.M. and Esteban, M.D., 1994. Potential allelopathic lupane triterpenes from bioactive fractions of *melilotus messanensis*. *Phytochemistry*, 36 (6), pp.1369-1379.

Maithani, K., Arunachalam, A., Tripathi, R.S. and Pandey, H.N., 1998. Influence of leaf litter quality on N mineralization in soils of subtropical humid forest regrowths. *Biology and Fertility of Soils*, 27 (1), pp.44-50.

Mallik, U. and Pellissier, F., 2000. Effects of *Vaccinium myrtillus* on spruce regeneration: Testing the notion of coevolutionary significance of allelopathy. *Journal of Chemical Ecology*, 26 (9), pp.2197-2209.

Malo, A.F., Godsall, B., Prebble, C., Grange, Z., McCandless, S., Taylor, A. and Coulson, T., 2013. Positive effects of an invasive shrub on aggregation and abundance of a native small rodent. *Behavioral Ecology*, 24 (3), pp.759-767.

Mark, U. and Tevini, M., 1996. Combination effects of UV-B radiation and temperature on sunflower (*Helianthus annuus* L., cv. Polstar) and maize (*Zea mays* L., cv. Zenit 2000) seedlings. *Journal of Plant Physiology*, 148 (1-2), pp.49-56.

Matlack, G.R., 1993. Microenvironment variation within and among forest edge sites in the eastern United States. *Biological Conservation*, 66 (3), pp.185-194.

McLaren, K.P. and McDonald, M.A., 2003. The effects of moisture and shade on seed germination and seedling survival in a tropical dry forest in Jamaica. *Forest Ecology and Management*, 183 (1-3), pp.61-75.

Mersie, W. and Singh, M., 1993. Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. *Journal of Chemical Ecology*, 19 (7), pp.1293-1301.

Metcalfe, D.J., 1996. Germination of small-seeded tropical rain forest plants exposed to different spectral compositions. *Canadian Journal of Botany*, 74 (4), pp.516-520.

Michelsen, A., Schmidt, I.K., Jonasson, S., Dighton, J., Jones, H.E. and Callaghan, T.V., 1995. Inhibition of growth, and effects on nutrient uptake of arctic graminoids by leaf extracts-allelopathy or resource competition between plants and microbes?. *Oecologia*, 103 (4), pp.407-418.

Millar, J.G. and Haynes, K.F. eds., 1998. *Methods in chemical ecology volume 1: Chemical methods*. Massachusetts: Kluwer Academic Publishers.

Milne, R.I. and Abbott, R.J., 2000. Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. in the British Isles. *Molecular Ecology*, 9 (5), pp.541-556.

Mitchell, D.T. and Gibson, B.R., 2006. Ericoid mycorrhizal association: Ability to adapt to a broad range of habitats. *Mycologist*, 20 (1), pp.2-9.

Mitchell, R.J., Marrs, R.H., Le Duc, M.G. and Auld, M.H.D., 1997. A study of succession on lowland heaths in Dorset, southern England: Changes in vegetation and soil chemical properties. *Journal of Applied Ecology*, 34 (6), pp.1426-1444.

Mittler, R., 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, 11 (1), pp.15-19.

Modgil, D. and Kapil, M., 1990. Allelopathic activity of *Pinus roxburghii* Sarg. and *Rhododendron arboreum* Sm. leaves. *Indian Forester*, 116 (6), pp.512-514.

Monk, C.D., McGinty, D.T. and Day, F.P.Jr., 1985. The ecological importance of *Kalmia latifolia* and *Rhododendron maximum* in the deciduous forest of the southern Applalachians. *Bulletin of the Torrey Botanical Club*, 112 (2), pp.187-193.

Morais, M.C. and Freitas, H., 2012. The acclimation potential of *Acacia longifolia* to water stress: Implications for invasiveness. *Plant Science*, 196 (1), pp.77-84.

Moreland, D.E. and Novitzky, W.P., 1987. Interference by luteolin, quercetin, and taxifolin with chloroplast-mediated electron transport and phosphorylation. *Plant and Soil*, 98 (1), pp.145-159.

Morelli, G. and Ruberti, I., 2000. Shade avoidance responses. Driving auxin along lateral routes. *Plant Physiology*, 122 (3), pp.621-626.

Muller, C.H., 1966. The role of chemical inhibition (allelopathy) in vegetational composition. *Bulletin of the Torrey Botanical Club*, 93 (5), pp.332-351.

Muscolo, A. and Sidari, M., 2006. Seasonal fluctuations in soil phenolics of a coniferous forest: Effects on seed germination of different coniferous species. *Plant and Soil*, 284 (1), pp.305-318.

Nabity, P.D., Zavala, J.A. and DeLucia, E.H., 2009. Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. *Annals of Botany*, 103 (4), pp.655-663.

National Biodiversity Network's Gateway, 2013. *Grid map for Rhododendron ponticum L. [Rhododendron]*. [online] Available at: <https://data.nbn.org.uk/Taxa/NBNSYS0000003888/Grid_Map> [Accessed 18 November 2013].

New Forest Life, 2007. *The New Forest LIFE projects*. [online] Available at: <http://www.newforestlife.org.uk/index.htm> [Accessed 16 January 2013].

Nilsen, E.T. and Horton, J., 2002. *Rhododendron maximum* in the USA: Similarities to *Rhododendron ponticum* in Britain and ecological mechanisms for community effects. *International Rhododendron Conference*. Royal Botanic Gardens, Edinburgh 17-19 May 2002.

Nilsen, E.T., Clinton, B.D., Lei, T.T., Miller, O.K., Semones, S.W. and Walker, J.F., 2001. Does *Rhododendron maximum* L. (Ericaceae) reduce the availability of resources above and belowground for canopy tree seedlings? *American Midland Naturalist*, 145 (2), pp.325-343.

Nilsen, E.T., Walker, J.F., Miller, O.K., Semones, S.W., Lei, T.T. and Clinton. B.D., 1999. Inhibition of seedling survival under *Rhododendron maximum* (Ericaceae): Could allelopathy be a cause? *American Journal of Botany*, 86 (11), pp.1597-1605.

Nilsson, M.C., 1994. Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. *Oecologia*, 98 (1), pp.1-7.

Nilsson, M.C., Gallet, C. and Wallstedt, A., 1998. Temporal variability of phenolics and Batatasin-III in *Empetrum hermaphroditum* leaves over an eight-year period: Interpretations of ecological function. *Oikos*, 81 (1), pp.6-16.

Nilsson, M.C., Zackrisson, O., Sterner, O. and Wallstedt, A., 2000. Characterisation of the differential interference effects of two boreal dwarf shrub species. *Oecologia*, 123 (1), pp.122-128.

Nilsson, U., Gemmel, P., Löf, M. and Welander, T., 1996. Germination and early growth of sown *Quercus robur* L. in relation to soil preparation, sowing depths and prevention against predation. *New Forests*, 12 (1), pp.69-86.

Nisar, M., Ali, S. and Qaisar, M., 2011. Preliminary phytochemical screening of flowers, leaves, bark, stem and roots of *Rhododendron arboreum*. *Middle-East Journal of Scientific Research*, 10 (4), pp.472-476.

Nishida, N., Tamotsu, S., Nagata, N., Saito, S. and Sakai, A., 2005. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of Chemical Ecology*, 31 (15), pp.1187-1203.

Northup, R.R., Dahlgren, R.A. and Yu, Z., 1995. Intraspecific variation of conifer phenolic concentration on a marine terrace soil acidity gradient: A new interpretation. *Plant and Soil*, 171 (2), pp.255-262.

Nye, P.H., 1981. Changes of pH across the rhizosphere induced by roots. *Plant and Soil*, 61 (1-2), pp.7-26.

NZflora, 2013. *Flora of New Zealand: Rhododendron ponticum L*. [online] Available at: <http://www.nzflora.info/factsheet/Weed/Rhododendron_ponticum.html> [Accessed 08 November 2013].

Odén, P.C., Brandtberg, P.O., Andersson, R., Gref, R., Zackrisson, O. and Nilsson, M.C., 1992. Isolation and characterization of a germination inhibitor from leaves of *Empetrum hermaphroditum* hagerup. *Scandinavian Journal of Forest Research*, 7 (1), pp.497-502.

Oleskog, G. and Sahlén, K., 2000. Effects of seedbed substrate on moisture conditions and germination of Scots pine (*Pinus sylvestris*) seeds in a mixed conifer stand. *New Forests*, 20 (2), pp.119-133.

Oleszek, W., Stochmal, A., Karolewski, P., Simonet, A.M., Macias, F.A. and Tava, A., 2002. Flavonoids from *Pinus sylvestris* needles and their variation in trees of different origin grown for nearly a century at the same area. *Biochemical Systematics and Ecology*, 30 (11), pp.1011-1022.

Ostman, N.L. and Weaver, G.T., 1982. Autumnal nutrient transfers by retranslocation, leaching, and litter fall in a chestnut oak forest in southern Illinois. *Canadian Journal of Forest Research*, 12 (1), pp.40-51.

Özhan, H., Akdemir, R., Yazici, M., Gündüz, H., Duran, S. and Uyan, C., 2004. Cardiac emergencies caused by honey ingestion: A single centre experience. *Emergency Medicine Journal*, 21 (6), pp.742-744.

Parvez, M.M., Tomita-Yokotani, K., Fujii, Y., Konishi, T. and Iwashina, T., 2004. Effects of quercetin and its seven derivatives on the growth of *Arabidopsis thaliana* and *Neurospora crassa*. *Biochemical Systematics and Ecology*, 32 (7), pp.631-635.

Patterson, D.T., 1981. Effects of allelopathic chemicals on growth and physiological responses of soybean (*Glycine max*). *Weed Science*, 29 (1), pp.53-59.

Pearson, V. and Read, D.J., 1973. The biology of mycorrhiza in the *Ericaceae*. *New Phytol*ogist, 72 (2), pp.371-379.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. and Tsomondo, T., 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment*, 84 (1), pp.1-20.

Pimentel, D., Zuniga, R. and Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52 (3), pp.273-288.

Ponder, F. and Tadros, S.H., 1985. Juglone concentration in soil beneath black walnut interplanted with nitrogen-fixing species. *Journal of Chemical Ecology*, 11 (7), pp.937-942.

Poorter, H. and Nagel, O., 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: A quantitative review. *Australian Journal of Plant Physiology*, 27 (12), pp.1191-1191.

Powers, R.F., 1980. Mineralizable soil nitrogen as an index of nitrogen availability to forest trees. *Soil Science Society of America Journal*, 44 (6), pp.1314-1320.

Pramanik, M.H.R., Nagai, M., Asao, T. and Matsui, Y., 2000. Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (*Cucumis sativus*) in hydroponic culture. *Journal of Chemical Ecology*, 26 (8), pp.1953-1967.

Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M., and Lowe, A.J., 2008. Adaptive evolution in invasive species. *Trends in Plant Science*, 13 (6), pp.288-294.

Puritch, G.S., 1973. Effect of water stress on photosynthesis, respiration, and transpiration of four Abies species. *Canadian Journal of Forest Research*, 3 (2), pp.293-298.

Pyšek, P. and Richardson, D.M., 2010. Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources*, 35 (1), pp.25-55.

Qasem, J.R. and Foy, C.L., 2001. Weed allelopathy, its ecological impacts and future prospects: A review. In: Kohli, R.K., Singh, H.P. and Batish, D.R., eds. 2001. *Allelopathy in Agroecosystems*. New York: Food Products Press. Ch.2.

Rascher, K.G., Große-Stoltenberg, A., Máguas, C. and Werner, C., 2011. Understory invasion by *Acacia longifolia* alters the water balance and carbon gain of a Mediterranean pine forest. *Ecosystems*, 14 (6), pp.904-919.

Raven, P.H., Evert, R.F. and Eichhorn, S.E., 2005. *Biology of plants*. 7th ed. New York: W.H Freeman and Company Publishers.

Rayamajhi, M.B., Van, T.K., Center, T.D., Goolsby, J.A., Pratt, P.D. and Racelis, A., 2002. Biological attributes of the canopy-held *Melaleuca quinquenervia* seeds in Australia and Florida. *Journal of Aquatic Plant Management*, 40 (2), pp.87-91.

Razavi, S.M., 2011. Plant coumarins as allelopathic agents. *International Journal of Biological Chemistry*, 5 (1), pp.86-90.

Read, D.J., 1996. The structure and function of the ericoid mycorrhizal root. *Annals of Botany*, 77 (4), pp.365-374.

Read, D.J. and Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems - A journey towards relevance?. *New Phytologist*, 157 (3), pp.475-492.

Reigosa, M.J., Souto, X.C. and González, L., 1999. Effect of phenolic compounds on the germination of six weeds species. *Plant Growth Regulation*, 28 (2), pp.83-88.

Reinhart, K.O., Gurnee, J., Tirado, R. and Callaway, R.M., 2006. Invasion through quantitative effects: Intense shade drives native decline and invasive success. *Ecological Applications*, 16 (5), pp.1821-1831.

Rice, E.L., 1974. *Physiological ecology: Allelopathy*. New York: Academic Press, Inc.

Rice, E.L., 1992. Allelopathic effects on nitrogen cycling. In: S.J.H. Rizvi and V. Rizvi, eds. 1992. *Allelopathy: Basic and applied aspects*. London: Chapman and Hall, pp.31-58.

Rice, E.L. and Pancholy, S.K., 1974. Inhibition of nitrification by climax ecosystems. III. Inhibitors other than tannins. *American Journal of Botany*, 61 (10), pp.1095-1103.

Ridenour, W.M. and Callaway, R.M., 2001. The relative importance of allelopathy in interference: The effects of an invasive weed on a native bunchgrass. *Oecologia*, 126 (3), pp.444-450.

Rizvi, S.J.H., Mishra, G.P. and Rizvi, V., 1989. Allelopathic effects of nicotine on maize I. Its possible importance in crop rotation. *Plant and Soil*, 116 (2), pp.289-291.

Rodriguez, L.F., 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions*, 8 (4), pp.927-939.

Rolot, J.L. and Seutin, H., 1999. Soilless production of potato minitubers using a hydroponic technique. *Potato Research*, 42 (3-4), pp.457-469.

Romeo, J.T. and Weidenhamer, J.D., 1998. Bioassays for allelopathy in terrestrial plants. In: Haynes, K.F. and Millar, J.G., eds. 1998. *Methods in Chemical Ecology volume 2: Bioassay methods.* Massachusetts: Kluwer Academic Publishers. Ch.4.

Ross, K.C., Colquhoun, J.B. and Mallory-Smith, C.A., 2009. Small broomrape (*Orobanche minor*) germination and early development in response to plant species. *Weed Science*, 52 (2), pp.260-266.

Rotherham, I.D., 2001. Rhododendron gone wild: Conservation implications of *Rhododendron ponticum* in Britain. *Biologist*, 48 (1), pp.7-11.

Rotherham, I.D. and Read, D.J., 1988. Aspects of the ecology of *Rhododendron ponticum* with reference to its competitive and invasive properties. *Aspects of Applied Biology*, 16 (1), pp.327-335.

Royal Horticultural Society, 2006. *Encyclopedia of plants and flowers*. 4th ed. London: Dorling Kindersley Ltd.

Sack, L. and Grubb, P.J., 2002. The combined impacts of deep shade and drought on the growth and biomass allocation of shade-tolerant woody seedlings. *Oecologia*, 131 (2), pp.175-185.

Sala, A., Smith, S.D. and Devitt, D.A., 1996. Water use by *Tamarix ramosissima* and associated phreatophytes in a Mojave Desert floodplain. *Ecological Applications*, 6 (3), pp.888-898.

Salminen, J.P., Roslin, T., Karonen, M., Sinkkonen, J., Pihlaja, K. and Pulkkinen, P., 2004. Seasonal variation in the content of hydrolyzable tannins, flavonoid glycosides, and proanthocyanidins in oak leaves. *Journal of Chemical Ecology*, 30 (9), pp.1693-1711.

Santisteban, J.I., Mediavilla, R., López-Pamo, E., Dabrio, C.J., Ruiz Zapata, M.B., Gil García, M.J., Castaño, S. and Martínez-Alfaro, P.E., 2004. Loss on ignition: A qualitative or quantitative method for organic matter and carbonate mineral content in sediments? *Journal of Paleolimnology*, 32 (3), pp.287-299.

Sarah, S., Hussain, F., Ehsan, M. and Burni, T., 2011. Allelopathic potential of *Polypogon monspeliensis* L. against two cultivars of wheat. *African Journal of Biotechnology*, 10 (85), pp.19723-19728.

Sariyildiz, T. and Küçük, M., 2009. Influence of slope position, stand type and rhododendron (*Rhododendron ponticum*) on litter decomposition rates of oriental beech (*Fagus orientalis*) and spruce (*Picea orientalis*). *European Journal of Forest Research*, 128 (4), pp.351-360.

Saverimuttu, T. and Westoby, M., 1996. Seedling longevity under deep shade in relation to seed size. *Journal of Ecology*, 84 (5), pp.681-689.

Schmidt, S.K., 1988. Degradation of juglone by soil bacteria. *Journal of Chemical Ecology*, 14 (7), pp.1561-1571.

Schulz, M., Kussmann, P., Knop, M., Kriegs, B., Gresens, F., Eichert, T., Ulbrich, A., Marx, F., Fabricius, H., Goldbach, H. and Noga, G., 2007. Allelopathic monoterpenes interfere with *Arabidopsis thaliana* cuticular waxes and enhance transpiration. *Plant Signaling and Behavior*, 2 (4), pp.231-239.

Seabloom, E.W., Harpole, W.S., Reichman, O.J. and Tilman, D., 2003. Invasion, competitive dominance, and resource use by exotic and native California grassland species. *Proceedings of the National Academy of Sciences of the United States of America*, 100 (23), pp.13384-13389.

Sharma, N., Sharma, U.K., Gupta, A.P. and Sinha, A.K., 2010. Simultaneous determination of epicatechin, syringic acid, quercetin-3-*O*-galactoside and quercitrin in the leaves of *Rhododendron* species by using a validated HPTLC method. *Journal of Food Composition and Analysis*, 23 (3), pp.214-219.

Shaw, D. and Tanner, R., 2008. Weed like to see less of them. *The Journal of the Institute of Biology*, 55 (4), pp.208-214.

Shaw, R.H., Tanner, R., Djeddour, D. and Cortat, G., 2011. Classical biological control of *Fallopia japonica* in the United Kingdom - lessons for Europe. *Weed Research*, 51 (6), pp.552-558.

Short, F.T., 1987. Effects of sediment nutrients on seagrasses: Literature review and mesocosm experiment. *Aquatic Botany*, 27 (1), pp. 41-57.

Shrestha, P., Vaidya, R. and Sherpa, K., 2009. Mad honey poisoning: A rare case report of seven cases. *Nepal Medical College Journal*, 11 (3), pp.212-213.

Singh, H.P., Batish, D.R. and Kohli, R.K., 2001. Allelopathy in agroecosystems: An overview. In: Kohli, R.K., Singh, H.P. and Batish, D.R., eds. 2001. *Allelopathy in Agroecosystems*. New York: Food Products Press. Ch.1.

Sisodia, S. and Siddiqui, M.B., 2009. Allelopathic potential of rhizosphere soil of *Croton bonplandianum* on growth and establishment of some crop and weed plants. *African Journal of Agricultural Research*, 4 (5), pp.461-467.

Smith, S.D., Huxman, T.E., Zitzer, S.F., Charlet, T.N., Housman, D.C., Coleman, J.S., Fenstermaker, L.K., Seemann, J.R. and Nowak, R.S., 2000. Elevated CO2 increases productivity and invasive species success in an arid ecosystem. *Nature*, 408 (6808), pp.79-82.

Souto, X.C., Gonzalez, L., and Reigosa, M.J., 1994. Comparative analysis of allelopathic effects produced by four forestry species during decomposition process in their soils in Galecia (NW Spain). *Journal of Chemical Ecology*, 20 (11), pp.3005-3015.

Stark, J.M. and Firestone, M.K., 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, 61 (1), pp.218-221.

Ste-Marie, C. and Paré, D., 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. *Soil Biology and Biochemistry*, 31 (11), pp.1579-1589.

Stephenson, C.M., MacKenzie, M.L., Edwards, C. and Travis, J.M.J., 2006. Modelling establishment probabilities of an exotic plant, *Rhododendron ponticum*, invading a heterogeneous, woodland landscape using logistic regression with spatial autocorrelation. *Ecological Modelling*, 193 (3), pp.747-758.

Sterry, P., 2006. *Complete British wild flowers*. London: Harper Collins Publishers Ltd.

Stock, W.D., Wienand, K.T. and Baker, A.C., 1995. Impacts of invading N2-fixing *Acacia* species on patterns of nutrient cycling in two Cape ecosystems: Evidence from soil incubation studies and 15 N natural abundance values. *Oecologia*, 101 (3), pp.375-382.

Stout, J.C., 2007a. Pollination of invasive *Rhododendron ponticum* (Ericaceae) in Ireland. *Apidologie*, 38 (2), pp.198-206.

Stout, J.C., 2007b. Reproductive biology of the invasive exotic shrub, *Rhododendron ponticum* L. (Ericaceae). *Botanical Journal of the Linnean Society*, 155 (3), pp.373-381.

Stout, J.C., Parnell, J A.N., Arroyo, J. and Crowe, T.P., 2006. Pollination ecology and seed production of *Rhododendron ponticum* in native and exotic ranges. *Biodiversity and Conservation*, 15 (2), pp.755-777.

Stout, J.C., 2011. Plant invasions: Their threats in an Irish context. *Biology and Environment: Proceedings of the Royal Irish Academy*, 111B: 135 - 141.

Stratton, L.C. and Goldstein, G., 2001. Carbon uptake, growth and resource-use efficiency in one invasive and six native Hawaiian dry forest tree species. *Tree Physiology*, 21 (18), pp.1327-1334.

Streck, N.A., Weiss, A., Xue, Q. and Baenziger, P.S., 2003. Incorporating a chronology response into the prediction of leaf appearance rate in winter wheat. *Annals of Botany*, 92 (2), pp.181-190.

Subbarao, G.V., Ishikawa, T., Ito, O., Nakahara, K., Wang, H.Y. and Berry, W.L., 2006. A bioluminescence assay to detect nitrification inhibitors released from plant roots: A case study with *Brachiaria humidicola*. *Plant and Soil*, 288 (1-2), pp.101-112.

Sütlüpmar, N., Mat, A. and Satganoglu, Y., 1993. Poisoning by toxic honey in Turkey. *Archives of toxicology*, 67 (2), pp.148-150.

Sutton, C.A. and Wilkinson, D.M., 2007. The effects of *Rhododendron* on testate amoebae communities in woodland soils in North West England. *Acta Protozoologica*, 46 (4), pp.333-338.

Suominen, K., Kitunen, V. and Smolander, A., 2003. Characteristics of dissolved organic matter and phenolic compounds in forest soils under silver birch (*Betula pendula*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). *European Journal of Soil Science*, 54 (2), pp.287-293.

Swaroop, A., Prakash Gupta, A. and Kumar Sinha, A., 2005. Simultaneous determination of quercetin, rutin and coumaric acid in flowers of *Rhododendron arboreum* by HPTLC. *Chromatographia*, 62 (11-12), pp.649-652.

Szabó, B., Tyihák, E., Szabó, G. and Botz, L., 2003. Mycotoxin and drought stress induced change of alkaloid content of *Papaver somniferum* plantlets. *Acta Botanica Hungarica*, 45 (3), pp.409-417.

Szakiel, A. and Mroczek, A., 2007. Distribution of triterpene acids and their derivatives in organs of cowberry (*Vaccinium vits-idaea*, L.) plant. *Acta Biochimica Polonica*, 54 (4), pp.733-740.

Takahashi, L., Sert, M.A., Kelmer-Bracht, A.M., Bracht, A. and Ishii-Iwamoto, E.L., 1998. Effects of rutin and quercetin on mitochondrial metabolism and on ATP levels in germinating tissues of *Glycine max*. *Plant Physiology and Biochemistry*, 36 (7), pp.495-501.

Tang, C. and Yu, Q., 1999. Impact of chemical composition of legume residues and initial soil pH on pH change of a soil after residue incorporation. *Plant and Soil*, 215 (1), pp.29-38.

Tang, C.S., Cai, W.F., Kohl, K. and Nishimoto, R.K., 1995. Plant stress and allelopathy. *American Chemical Society Symposium Series*, 582, pp.142-157.

Teasdale, J.R., 1993. Interaction of light, soil moisture, and temperature with weed suppression by hairy vetch residue. *Weed Science*, 41 (1), pp.46-51.

Tegelberg, R., Julkunen-Tiitto, R. and Aphalo, P.J., 2004. Red : far-red light ratio and UV-B radiation: Their effects on leaf phenolics and growth of silver birch seedlings. *Plant, Cell and Environment*, 27 (8), pp.1005-1013.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M.C., Schwager, M. and Jeltsch, F., 2004. Animal species diversity driven by habitat heterogeneity/diversity: The importance of keystone structures. *Journal of Biogeography*, 31 (1), pp.79–92.

Thelen, G.C., Vivanco, J.M., Newingham, B., Good, W., Bais, H.P., Landres, P., Caesar, A. and Callaway, R.M., 2005. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecology Letters*, 8 (2), pp.209-217.

Thiele, J. and Otte, A., 2007. Impact of *Heracleum mantegazzianum* on invaded vegetation and human activities. In: Pyšek, P., Cock, M.J.W., Nentwing, W. and Ravn, H., eds. 2007. *Ecology and management of giant hogweed (Heracleum mantegazziannum)*. Oxfordshire: CABI publishing. Ch.9.

Thomas, R.G., 1981. Flower initiation in relation to cool season growth of four lines of white clover. *New Zealand Journal of Agricultural Research*, 24 (1), pp.37-41.

Tilman, D. and Olff, H., 1991. An experimental study of the effects of pH and nitrogen on grassland vegetation. *Acta Oecologica*, 12, pp.427-442.

Tiwari, O.N. and Chauhan, U.K., 2006. *Rhododendron* conservation in Sikkim Himalaya. *Current Science*, 90 (4), pp.532-541.

Tow, P.G. and Lazenby, A., 2001. *Competition and succession in pastures*. New York, CABI publishing.

Tripathi, A.K., Bhakuni, R.S., Upadhyay, S. and Gaur, R., 2011. Insect feeding deterrent and growth inhibitory activities of scopoletin isolated from *Artemisia annua* against *Spilarctia obliqua* (Lepidoptera: Noctuidae). *Insect Science*, 18 (2), pp.189-194.

Tubbs, C.R., 2001. *The New Forest: History, ecology and conservation*. 2nd ed. Lyndhurst: New Forest Ninth Centenary Trust.

Turk, M.A. and Tawaha, A.M., 2003. Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). *Crop Protection*, 22 (4), pp.673-677.

Turkington, R. and Burdon, J.J., 1983. The biology of Canadian weeds: *Trifolium repens* L. *Canadian Journal of Plant Science*, 63 (1), pp.243-266.

Turner, C.E., Center, T.D., Burrows, D.W. and Buckingham, G.R., 1997. Ecology and management of *Melaleuca quinquenervia*, an invader of wetlands in Florida, U.S.A. *Wetlands Ecology and Management*, 5 (3), pp.165-178.

Turner, J.A. and Rice, E.L., 1975. Microbial decomposition of ferulic acid in soil. *Journal of Chemical Ecology*, 1 (1), pp.41-58.

U.S Department of Agriculture, 2004. *Soil Survey Laboratory Methods Manual*. [online] Available at: <ftp://ftp-fc.sc.egov.usda.gov/NSSC/Lab_Methods_Manual/SSIR42_2004_view.pdf> [Accessed 23 February 2009].

Van Miegroet, H. and Cole, D.W., 1984. The impact of nitrification on soil acidification and cation leaching in a red alder ecosystem. *Journal of Environmental Quality*, 13 (4), pp.586-590.

Veteli, T.O., Kuokkanen, K., Julkunen-Tiiti, R., Roininen, H. and Tahvanainen, J., 2002. Effects of elevated CO_2 and temperature on plant growth and herbivore defensive chemistry. *Global Change Biology*, 8 (12), pp.1240–1252.

Vitousek, P.M. and Walker, L.R., 1989. Biological invasion by *Myrica faya* in Hawaii: Plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs*, 59 (3), pp.247-266.

Von Elert, E. and Jüttner, F., 1996. Factors influencing the allelopathic activity of the planktonic cyanobacterium *Trichormus doliolum*. *Phycologia*, 35 (6S), pp.68-73.

Wang, Y., Siemann, E., Wheeler, G.S., Zhu, L., Gu, X. and Ding, J., 2012. Genetic variation in anti−herbivore chemical defences in an invasive plant. *Journal of Ecology*, 100 (4), pp.894-904.

Wardle, D.A., Nicholson, K.S., Ahmed, M. and Rahman, A., 1994. Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L. *Plant and Soil*, 163 (2), pp.287-297.

Webber, J.F., 2007. Status of *Phytophthora ramorum* and *P. kernoviae* in Europe. *Proceedings of the Sudden Oak Death Third Science Symposium*, pp.5-9.

Webster, I.T. and Day, C.R.B., 1993. The impacts of shade on evaporation rates and temperatures in stock watering troughs. *Australian Journal of Agricultural Research*, 44 (2), pp.287-298.

Went, F.W., 1953. The effect of temperature on plant growth. *Annual Review of Plant Physiology*, 4 (1), pp.347-362.

West, I.M., 2010. *Geology of the New Forest National Park. Internet field guide*. [online] Available at: <http://www.soton.ac.uk/~imw/New-Forest-Geology-Guide.htm> [Accessed 15 April 2013].

Whitehead, D.C., Dibb, H. and Hartley, R.D., 1981. Extractant pH and the release of phenolic compounds from soils, plant roots and leaf litter. *Soil Biology and Biochemistry*, 13 (5), pp.343-348.

Wikimedia, 2013. *New Forest National Park UK location map*. [online] Available at: <http://commons.wikimedia.org/wiki/File:New_Forest_National_Park_UK_location_map.svg> [Accessed 05 November 2013].

Wilson, B.R., Moffat, A.J. and Nortcliff, S., 1997. The nature of three ancient woodland soils in southern England. *Journal of Biogeography*, 24 (5), pp.633-646.

Wilson, J.B. and Lee, W.G., 2000. C-S-R triangle theory: Community level predictions, tests, evaluation of criticisms, and relation to other theories. *Oikos*, 91 (1), pp.77-96.

Wilson, R.E. and Rice, E.L., 1968. Allelopathy as expressed by *Helianthus annuus* and its role in old-field succession. *Bulletin of the Torrey Botanical Club*, 95 (5), pp.432-448.

Wink, M., 1988. Plant breeding: Importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics*, 75 (2), pp.225-233.

Wink, M., Schmeller, T. and Latz-Brüning, B., 1998. Modes of action of allelochemical alkaloids: Interaction with neuroreceptors, DNA, and other molecular targets. *Journal of Chemical Ecology*, 24 (11), pp.1881-1937.

Wium-Andersen, S., Anthoni, U. and Houen, G., 1983. Elemental sulphur, a possible allelopathic compound from *Ceratophyllum demersum*. *Phytochemistry*, 22 (11), pp.2613.

Woods, K.D., 1993. Effects of invasion by *Lonicera tatarica* L. on herbs and tree seedlings in four New England forests. *American Midland Naturalist*, 130 (1), pp.62-74.

Wuensch, K.L., 2004. *Principal components analysis*. [online] Available at: <http://core.ecu.edu/psyc/wuenschk/MV/FA/PCA.doc> [Accessed 28 October 2011].

Wurzburger, N. and Hendrick, R.L., 2007. Rhododendron thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. *Pedobiologia*, 50 (6), pp.563-576.

Xuan, T.D., Tawata, S., Khanh, T.D. and Chung, I.M., 2005. Decomposition of allelopathic plants in soil. *Journal of Agronomy and Crop Science*, 191 (3), pp.162-171.

Yamamoto, Y., 2009. Movement of allelopathic compound coumarin from plant residue of sweet vernalgrass (*Anthoxanthum odoratum* L.) to soil. *Grassland Science*, 55 (1), pp.36-40.

Yamasaki, S.H., Fyles, J.W., Egger, K.N. and Titus, B.D., 1998. The effects of *Kalmia angustifolia* on the growth, nutrition, and ecto-mycorrhizal symbiont community of black spruce. *Forest Ecology and Management*, 105 (1-3), pp.197-207.

Yan, F., Schubert, S. and Mengel, K., 1996. Soil pH increase due to biological decarboxylation of organic anions. *Soil Biology and Biochemistry*, 28 (4-5), pp.617-624.

Yan, J., Bi, H.H., Liu, Y.Z., Zhang, M., Zhou, Z.Y. and Tan, J.W., 2010. Phenolic compounds from *Merremia umbellata* subsp. *orientalis* and their allelopathic effects on Arabidopsis seed germination. *Molecules*, 15 (11), pp.8241-8250.

Yang, R.Y., Mei, L.X., Tang, J.J. and Chen, X., 2007. Allelopathic effects of invasive *Solidago canadensis* L. on germination and growth of native Chinese plant species. *Allelopathy Journal*, 19 (1), pp.241-248.

Zavaleta, E.S., Hobbs, R.J. and Mooney, H.A., 2001. Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution*, 16 (8), pp.454-459.

Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Mooney, H.A. and Field, C.B., 2003. Additive effects of simulated climate changes, elevated CO_2, and nitrogen deposition on grassland diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 100 (13), pp.7650-7654.

Zhang, C. and Fu, S., 2010. Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. *Allelopathy Journal*, 26 (1), pp.91-100.

Zhao, X., Zheng, G., Niu, X., Li, W., Wang, F. and Li, S., 2009. Terpenes from *Eupatorium adenophorum* and their allelopathic effects on *Arabidopsis* seeds germination. *Journal of Agricultural and Food Chemistry*, 57 (2), pp.478-482.

Zheng, W.L., Tian, D.L., Quan, H., Lan, X.Z. and Zhang, Y.M., 2009. Allelopathic effects of aqueous extracts from several species of plants on *Rhodiola fastigiata* in Tibet Plateau. *Journal of Northeast Forestry University*, 37 (1), pp.32-34.

Zhu, H. and Mallik, A.U., 1994. Interactions between kalmia and black spruce: Isolation and identification of allelopathic compounds. *Journal of Chemical Ecology*, 20 (2), pp.407-421.