The effect of drought stress on the green spruce aphid

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Declaration of Originality

I declare that all the work presented in this thesis has not previously been submitted for a university degree and is entirely my own work.

Copyright Declaration

Abstract

The green spruce aphid, *Elatobium abietinum* (Walker) (Hemiptera: Aphididae), is the most important defoliating pest of Sitka spruce, *Picea sitchensis* (Bong.) Carr., in the U.K. Currently, populations of this aphid are limited by freezing periods in the winter, while interactions between climate and other factors regulate population dynamics.

Climate change in the U.K. is predicted to result in: (1) warmer winters, improving overwinter survival by aphid populations, and (2) an increase in hot and dry summers, likely to place Sitka spruce under drought stress. These could promote aphid densities and increased damage to the trees, resulting in losses to plant growth and productivity.

Few studies have been conducted on the effect of drought stress on arboreal herbivores. This project sought to explore the effects of different intensities of spring-summer drought stress on *E. abietinum* on Sitka spruce. Populations and their effects on their host plant, in terms of needle retention and impact on tree growth, were observed in a semi-field nursery setting. The performance of individual aphids was also observed under controlled conditions at intervals following bud-burst in spring, and again in autumn. Finally, a study was conducted on the consumption rates of specialist and generalist Coccinellid predators feeding on aphids reared under differing drought intensities.

Elatobium abietinum exhibited an overall positive response to moderate intermittent drought stress, while severe stress was typically detrimental. When considered with aphid size, Coccinellid predator consumption rates reflected these findings. Changes to damage levels on Sitka spruce can therefore be expected under drought stress; increases are likely under moderate intermittent stress, though the nature of changes under severe stress levels remain unclear.

The results revealed complex interactions between drought stress, *E. abietinum* and Sitka spruce. Given the potential impact of the aphid, it is important to understand the possible responses under climate change.

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“*Alice*: Would you tell me, please, which way I ought to go from here?
Cheshire cat: That depends a great deal on where you want to get to.
A: I don’t much care where...
C: Then it doesn’t matter which way you go.
A: ...so long as I get somewhere.
C: Oh, you’re sure to do that, if you only walk long enough.”
- *Lewis Carroll, ‘Alice in Wonderland’*

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Overview

Sitka spruce, *Picea sitchensis* (Bong.) Carr. plays a dominant role in British commercial forestry (Samuel *et al.*, 2007). Green spruce aphid, *Elatobium abietinum* (Walker), is the most important defoliator of Sitka spruce in the U.K. (Evans *et al.*, 2002), causing damage not only to commercial plantations, but also to nursery stock, Christmas trees and ornamental plantings. It is anholocyclic, feeding on spruce as adults and nymphs throughout the year (Fisher & Dixon, 1986).

Currently, *E. abietinum* populations are limited primarily by freezing periods during the winter. The interaction between the climate and other factors, particularly natural enemies, causes a cyclical pattern in the aphid's population dynamics, in which high, seriously defoliating populations of the aphid tend to re-occur every three to six years (Evans *et al.*, 2002). Climate change is predicted to result in warmer winters (Murphy *et al.*, 2009). An increase in winter temperatures would allow improved overwintering rates (Evans *et al.*, 2002; Day *et al.*, 2010), which might lead to an increase in the frequency and intensity of aphid attack and greater damage.

A further prediction is that, due to climate change, there will be a greater frequency of hot and dry summers in the U.K. (Murphy *et al.*, 2009). It has been suggested that such conditions, which would cause drought stress in Sitka spruce, could promote higher aphid densities and an increase in damage (Straw *et al.*, 2005), though a greater impact in these circumstances has not been proven. Experimental studies on other aphid species have exhibited a decrease in aphid abundance, survival and fecundity on continuously drought-stressed plants (Kennedy *et al.*, 1958; McVean & Dixon, 2001; Hale *et al.*, 2003). Conversely, on plants subjected to intermittent, or ‘pulsed’, water stress, other aphid species appeared to benefit and showed positive effects from the treatment (Huberty & Denno, 2004; Mody *et al.*, 2009). Overall, however, few studies have been conducted to assess the impact of drought stress on arboreal herbivorous insects (Koricheva *et al.*, 1998).

The green spruce aphid

Elatobium abietinum is most commonly found in the apterous form (see Figure 1.1). These are usually 1-2mm in body length, and pale green with two darker green longitudinal stripes (Blackman & Eastop, 1994).

Figure 1.1: Apterous *Elatobium abietinum*

Source: Forestry Commission Picture Library Image, ©2010.

This aphid is widely distributed throughout north-western Europe, with the British Isles, France, Germany, Austria, Switzerland and Denmark comprising the largest continuous area of its distribution (Carter & Halldórsson, 1998). Worldwide, this aphid is also found in the Americas, Tasmania and New Zealand (Figure 1.2).

In the United Kingdom, *E. abietinum* is anholocyclic, reproducing parthenogenetically as apterous viviparae throughout the year (Fisher & Dixon (1986), Figure 1.3). The populations peak in late spring-early summer (typically late May), accompanied by the production of migrant alate females (Fisher & Dixon, 1986; Carter & Halldórsson, 1998). Alate production is thought to be induced by an increase in photoperiod, with host

Figure 1.2: Known global distribution of *Elatobium abietinum*
Modified from Day *et al.* (1998).

quality and crowding modifying the response (Fisher & Dixon, 1986). After the peak, populations decline rapidly in response to decreases in the nutritional quality of the phloem sap (Day & Kidd, 1998). A small population of aphids persists through the summer months, which increases again in number in the autumn, when plant nutritional quality improves following the cessation of growth and onset of dormancy (Evans *et al.*, 2002).

The size of the spring population peak correlates closely with the number of aphids overwintering, which in turn depends on two things: (1) the number of aphids present in the preceding autumn, and (2) the winter survival rates (Evans *et al.*, 2002). Currently, *E. abietinum* populations are restricted by low winter temperatures. Extended periods below 7 °C reduce aphid survival through starvation and torpor, and temperatures below -7 °C, particularly sudden frosts, kill most individuals, preventing damaging populations the following spring (Powell & Parry, 1976).

Elatobium abietinum also exhibits cyclical population dynamics. In Britain, high population numbers occur approximately every three to five years (Fisher, 1987; Day & Kidd, 1998; Evans *et al.*, 2002). These cycles are thought to be the result of the effects of climate and density-related processes, including a delayed density-dependent impact

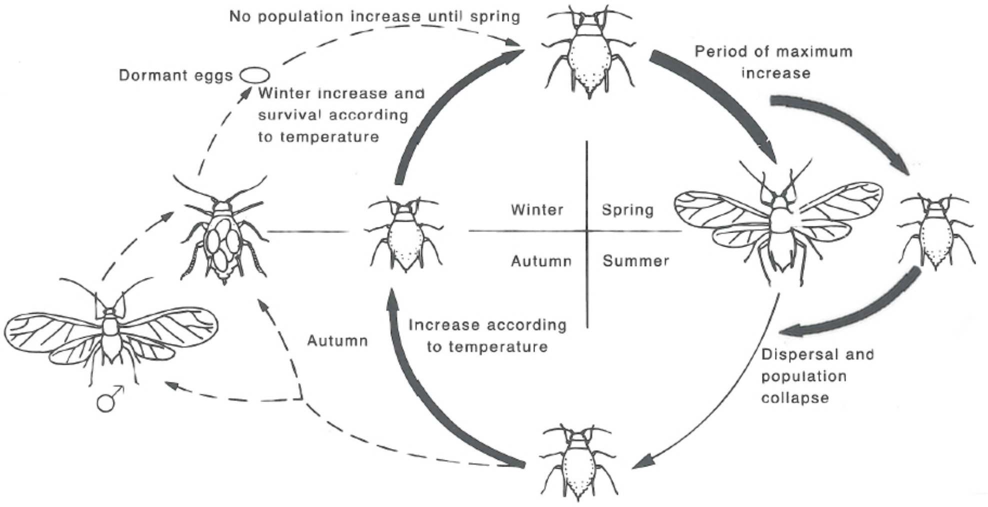

Figure 1.3: Life cycle of *Elatobium abietinum*

Continuous arrowed lines denote the anholocycle, which is the most common cycle in Europe. Broken lines denote the forms found in the complete holocycle. Thickness of the lines indicates how populous the colonies are in that stage. A mixture of successful overwintering forms is characteristic in paracyclic populations. Modified from Carter & Halldórsson (1998).

of predators and parasites (Day & Kidd, 1998), which help maintain low levels of aphids in years following an outbreak (Day *et al.*, 2010).

Recent modelling of alate populations in the United Kingdom concluded that the population maximum can be predicted from knowledge of relatively few parameters. These were chill bouts (winter temperature), thermal sun (spring temperature), and density dependence (interannual negative feedback), suggesting that, ultimately, *E. abietinum* populations are influenced by similar climatic and endogenous factors applying to both aerial populations and apterous populations in the forest (Day *et al.*, 2010).

As suggested by its common name, the green spruce aphid feeds on various species of spruce (*Picea*). In Britain, *E. abietinum* is a serious defoliating pest of commercial Sitka spruce and, to a lesser extent, Norway spruce (*P. abies* (L.) Karst) plantations (Nichols, 1987; Evans *et al.*, 2002). The aphids feed exclusively on one-year old and older needles (Carter, 1977; Evans *et al.*, 2002), and on the current season's needles from the autumn onwards (Straw *et al.*, 1998a). The feeding habit of *E. abietinum* led Carter & Halldórsson (1998) to describe the species as an "oddity", as it is unable to feed on nutrient-rich, actively growing foliage, preferring the older needle resource instead.

Research has shown that this is due to high concentrations of terpene compounds in the epicuticular wax, which defend the current year's needles (Jackson & Dixon, 1996).

Salivary secretions at feeding sites cause the development of chlorotic, yellow bands on the needle tissue, which eventually extend over the entire needle (Fisher, 1987; Carter, 1989). This damage causes needles to brown and die, leading to premature abscission and defoliation (Nichols, 1987; Evans *et al.*, 2002). Hence the marked, visible symptom of infestation is browning of the canopy followed by needle loss. A study of the probing behaviour of *E. abietinum* has shown that penetration of the stylets was not only frequently accompanied by a heavy deposition of saliva, but also increased divergence and branching of the salivary sheath, factors associated with impenetrability of the needle endodermis (Parry, 1971). This may go some way to explain the heavy damage sustained by Sitka spruce under *E. abietinum* feeding. *Elatobium abietinum* reared on chlorotic needles are heavier than those reared on green needles and infestation alters the amino acid balance of the needles, though not the total concentration (Fisher, 1987). Extensive defoliation reduces shoot growth and dry matter production (Carter, 1977; Straw *et al.*, 2005). Although infestations rarely kill mature trees, severe infestations and defoliation of young trees, in exceptional circumstances, can cause tree death (Straw *et al.*, 1998a; Straw *et al.*, 2005).

The damage caused by *E. abietinum* to spruce has been observed to be species-specific (Theobald, 1914). A comparative study by Nichols (1987) on twenty different species of spruce showed that North American spruce species exhibited greater susceptibility to *E. abietinum*, which performed better on these species and in particular on Sitka spruce. Furthermore, though there was great variation, Asian spruce species were the least favoured, whereas performance on European spruce species was intermediate (Nichols, 1987).

Sitka spruce

Sitka spruce is the most widely used conifer for afforestation and replanting in Great Britain. It accounts for 36% of the forest estate and 61% of all conifer species planted (Samuel *et al.*, 2007). In the decade following 1981, Sitka spruce accounted for 65% of the total planted area and 71% of all conifers (Samuel *et al.*, 2007), and the species continues to play a dominant role in British commercial forestry (Straw *et al.*, 1998b; Gardiner *et al.*, 2011).

Sitka spruce was introduced to Great Britain from North America in 1831 (Samuel *et al.*, 2007). A predominantly northern species, the natural range extends through a narrow, 3000km belt running along the Pacific coast, from Alaska to California. The range is dependent on the presence of abundant moisture during the growing season; for example, on the east side of Vancouver Island, which is a rain shadow with low annual rainfall and frequent severe summer droughts, Sitka spruce is restricted to stream sides, tidal areas and beaches (Samuel *et al.*, 2007).

Sitka spruce has been planted extensively in regions with a mild and oceanic climate, such as the northern and western parts of Britain, and in particular Scotland (Straw *et al.*, 2005; Green & Ray, 2009). Although these are the areas where Sitka spruce is the most productive conifer species, these climates are also favourable for *E. abietinum* (Straw *et al.*, 2005). Maritime areas are characterised by mild winters and wet, relatively cool summers, which allow the aphids to persist on the spruce as anholocyclic, parthenogenetic populations throughout the year (Straw *et al.*, 2006).

Sitka spruce requires a very mild and wet climate for optimal growth, and is very intolerant of drought. It is not recommended for planting in areas with a mean annual rainfall of less than 700mm (Jarvis & Mullins, 1987). This basic requirement gives rise to potentially severe effects of hot and dry summers on this species.

Elatobium abietinum on Sitka spruce

Several factors have been shown to affect both *E. abietinum* population dynamics and individual performance on Sitka spruce, and the effects of the aphid on its host. These include both endogenous and extrinsic elements, often applicable to both apterous and alate forms, and which can vary between regions.

Abiotic effects

Soil nutrients

Sitka spruce is often planted on poor upland soils that require application of artificial fertiliser to achieve satisfactory tree growth (Chandler & Dale, 1990), and this can also affect aphid population size. Although larger populations of *E. abietinum* have been observed under high nutrient conditions, a greater number of needles were lost per aphid in a low nutrient treatment resulting in similar overall defoliation rates between the two conditions (Straw & Green, 2001). Nutrient conditions also affected the growth response of the host Sitka spruce. Infestations caused greater reductions in growth, and were related to both aphid density and defoliation under low nutrient conditions (Straw & Green, 2001). Conversely, where nutrients are not a limited resource, such growth reductions were smaller and related to neither aphid density nor defoliation (Straw & Green, 2001).

Shade

In older forests with understory spruce seedlings, shading can play an important role in the population sizes of *E. abietinum*. Cumulative aphid densities can be three to four times higher on shaded plants when compared to completely unshaded plants (Bertin *et al.*, 2010). Furthermore, the effects of shade and aphid infestation interact. Under shade, seedling biomass is reduced with aphid infestation causing additional reductions, such that, under infestation, lead extension growth can be reduced by up to 17% in shade but is only reduced by up to 3% in full light (Bertin *et al.*, 2010).

Temperature

Halldórsson *et al.* (2001) conducted a study on *E. abietinum* aphid populations from various north European countries, including Iceland and Britain. They found that while aphid populations from different countries had different growth rates and pre-natal periods, fecundity was similar and faster growth rates were not detrimental in terms of reduced adult weight. An important observation of this study was in the effects of winter temperature and high summer temperature. Aphids from different countries showed no difference in frost tolerance or in mortality at potentially lethal temperatures, indicating random mortality, while high temperatures appeared to select for better adapted genotypes of aphid (Halldórsson *et al.*, 2001).

Season

The distribution of the aphid population on a host tree is affected by season. In September through to November, the highest aphid densities are usually found on three and four year old foliage on branches low in the canopy. Over the winter period, and ensuing spring, the population shifts upwards and outwards such that by June the highest densities are located near current and one year old branches nearer the top of the tree (Straw *et al.*, 2006). There is, however, wide variation not only between sites but also between trees (Straw *et al.*, 2006), so much so that density counts must be obtained at regular intervals throughout the canopy during periods of aphid abundance, in order to be able to establish realistic population size estimates.

Differences in aphid density have been suggested to reflect changes in tree vigour, rather than induced changes in host quality (Straw *et al.*, 2005). This corresponds with the findings of Williams *et al.* (2005), who found that high spring populations of *E. abietinum* did not induce defensive mechanisms in Sitka spruce, though an improvement in nutritional quality of the host for the autumn generation was observed.

Biotic effects

The importance of spruce species

Elatobium abietinum is a specialist across the *Picea* genus. The weight of immature aphids is significantly affected by the *Picea* host, such that final weight is significantly lower on least favoured plants (Nichols, 1987). In addition to such effects of host plant species, Sitka spruce has what could almost be described as an ‘over-reaction’ to *E. abietinum*, showing different damage to that caused by the same aphid on Norway spruce. Sitka spruce has been found to be both more heavily attacked and more susceptible to damage than Norway spruce (Parry, 1974a; Nichols, 1987; Carter & Nichols, 1988). Chlorotic banding and needle death occur at a slower rate on Norway spruce than on Sitka spruce, for example (Dumbleton, 1932). The difference in damage has been attributed to differences in probing behaviour by Parry (1971). Stylet penetration in Sitka spruce was associated with increased multiple branching of the salivary sheath and a greater salivary deposition than was observed on Norway spruce (Parry, 1971).

The importance of natural enemies

Natural enemies are also thought to affect the population dynamics of *E. abietinum*. Their effects are of great interest as they offer the only means of control in established forests, where chemical control is often not viable both environmentally and economically (Timms, 2004). It is thought that, in the U.K., the presence of natural enemies regulates the spring peak size of *E. abietinum*, moderates the rate of decline, and that they also serve a role in suppressing a second autumn peak (Hussey, 1952; Crute & Day, 1990). This is supported by the findings of Austarå *et al.* (1998), who found that in natural enemy-poor Iceland the main population peak is observed in the autumn, whereas such peaks are rare in other areas where the aphid is anholocyclic.

Elatobium abietinum is preyed upon by a number of generalist and specialist natural enemies, though there are some contradictions as to the importance of different types. While studies by Parry (1992), Leather & Owuor (1996) and Leather & Kidd (1998) suggest that coccinellids are the main predators, Crute & Day (1990) found that hemerobiids and syrphids were more important. The value of syrphids, however, may be limited by their biology; whereas coccinellids are aphidophagous as adults and larvae and can be present within the canopy in greater numbers, syrphid adults are nectivorous and must forage in a floral resource (Leather & Kidd, 1998; Timms, 2004).

The importance of hymenopterous parasitoids and entomopathogenic fungi has not yet been fully investigated (Austarå *et al.*, 1998; Nielsen *et al.*, 2001), and although birds have been observed to consume a large number of aphids, they are not thought to have a significant impact on aphid populations (Bejer-Peterson, 1962).

The natural enemy fauna, while affected by prey abundance, often exhibit regular patterns of seasonal abundance. Modelling of hemerobiid and syrphid predators of *E. abietinum* suggests that these predators have little effect on the spring population peak, but are capable of reducing the size of the autumn peak (Crute & Day, 1990). A more in-depth study by Timms (2004) found that most control by natural enemies was by few, specific aphidophagous predators, with generalists only having a small effect. Furthermore, it was suggested that overwintering predators contributed to the control of *E. abietinum* populations early in the season, with larvae mediating the spring peak and facilitating the population crash. Finally, an autumn peak was prevented or at least modulated by the new generation of aphidophagous adults (Timms, 2004).

The effects of infestation on plant growth

Elatobium abietinum infestation of Sitka spruce rarely causes tree mortality (Carter, 1977; Straw *et al.*, 2000). A current year's needles are protected from aphid attack until the autumn, conferred by secondary compounds present in the needle wax (Jackson & Dixon, 1996), and it is this which permits the trees to recover (Straw *et al.*, 1998b). The main effect of *E. abietinum* on Sitka spruce is on growth and needle loss, which are reduced and increased respectively. In addition to the more short-term and immediate effects in terms of growth reduction, Sitka spruce may also take several years following attack to recover and return to normal growth rates (Straw, 1995).

Elatobium abietinum density has been found to correlate with needle loss (Straw *et al.*, 2005), though at higher densities a lower relative impact of individual aphids has been observed (Day & McClean, 1991; Straw *et al.*, 1998b). Infested trees lose a higher proportion of older needles (Straw *et al.*, 1998b). Photosynthetic potential of host trees is compromised by needle loss, leading to reductions in growth, which has been shown to strongly correlate with defoliation levels (Seaby & Mowat, 1993; Straw *et al.*, 2011).

An immediate effect of infestation on height and lead shoot length has been frequently observed in Sitka spruce, with a reduction of height increment between 6 - 30% (Warrington & Whittaker, 1990; Seaby & Mowat, 1993; Thomas & Miller, 1994; Straw *et al.*, 1998a; Straw *et al.*, 2000; Straw & Green, 2001). Greater reductions of 40 - 62% have also been found in 5 - 6 year old trees (Carter, 1977; Carter & Nichols, 1988). Other aphid species, such as *Cinara* spp., have also been found to reduce leader growth (Inouye & Yamaguchi, 1955; Johnson, 1965). *Cinara* individuals, however, are larger than *E. abietinum*, live and feed on the stems of the trees rather than the needles, and do not cause defoliation. This suggests a more direct effect on growth by *Cinara*, plausibly explained by their large extraction levels of phloem sap causing reductions in shoot water potential and nutrient availability (Straw *et al.*, 2000). *Elatobium abietinum*, on the other hand, may cause a physiologically specific influence on leader shoot growth (Straw *et al.*, 2000).

Stem diameter increment shows a delayed response to aphid infestation. Reduction levels vary, with Straw *et al.* (2000) finding a reduction of 12% in the year following *E. abietinum* infestation. Thomas & Miller (1994) also observed reductions comparable to those in height. Reductions in needle size have also been observed in years following infestation, coupled with reduced dry biomass (Straw *et al.*, 2000). The reductions in diameter and volume increments have been found to be affected particularly by changes to the photosynthetic production of a current year's needles, whereas needle size changes are plausibly attributable to a direct influence of the aphid on needle development and as such is likely related to reductions in height growth (Straw *et al.*, 2000). Similarly, bud development has been found to be affected by a similar mechanism (Straw *et al.*, 2000; Straw *et al.*, 2005).

Climate change

Climate change in the U.K. is predicted to result in warmer winters and hot, dry summers (Murphy *et al.*, 2009). Forests in north-western Britain are likely to experience a milder, moister climate, whereas those in southern and eastern Britain are likely to experience a greater severity and frequency of dry spells during the summer months (West & Morison, 2009).

These projected changes, and climate change in general, are likely to cause a wide range of effects on trees and their pests, both directly and indirectly. Examples of such effects include, *inter alia*:

- Altered growing conditions;
- Modified rates of development and growth, as well a wood production;
- Altered frequency and types of abiotic disturbances;
- Changes in the severity, timing, seasonality and types of invertebrate and vertebrate pests and diseases;

- Changes to species composition of forest communities; and
- Shifts in climatic limitations to species survival (West & Morison, 2009).

The projections suggest that, particularly for southern England, climatic limitations to species survival may shift towards factors such as tolerance of summer drought, rather than the current tolerance of frost and cold hardiness. Regardless, the balance between insects pests, their hosts and their natural enemies can be expected to alter under changed climate (Straw *et al.*, 2005; Green & Ray, 2009; Broadmeadow *et al.*, 2009a), rendering predictions on insect damage to forests difficult to make.

In addition to this, the composition of forest flora and fauna may be affected; new areas may become suitable for existing pests or for the introduction of new species, and changes to such ranges could influence the natural control of pest species (Timms, 2004; Broadmeadow *et al.*, 2009a). These threats may become of greater importance through the direct effects of climate change on tree function and the modifications of the interactions between the pests and their host.

Forests and climate change

Global carbon emissions have already caused recent changes in the climate of the U.K., and climate modelling predicts continuing change (Murphy *et al.*, 2009; West & Morison, 2009). Forests are widely believed to have a potential role in the abatement of climate change by acting as carbon sinks, however their management and sustainability is likely to be influenced by changes to their environment. Over 20% of land surface is covered by closed forests, with an estimated two-hundred times as much carbon contained as is released annually from fossil fuels (Kimmins, 1996). Forest ecosystems are liable to be affected by temperature increases and changes to atmospheric gas concentrations associated with climate change (Jarvis, 1994; Jarvis *et al.*, 2009), with changes to phenology likely (Broadmeadow *et al.*, 2009b).

Determining the impact of climate change is also further complicated by the interactive effects of many predicted changes. The importance of such interactions has repeatedly been shown (Warrington & Whittaker, 1990; Lukac *et al.*, 2010; Albert *et al.*, 2011). For example, tree growth rates and productivity may increase in response to climatic warming, rising atmospheric CO_2 levels and lengthened growing seasons combined. Evidence for such an increase in growth increment is limited in the U.K. (Broadmeadow *et al.*, 2009b), and care must be taken to consider the impact of improved forest management strategies and nitrogen deposition when considering the evidence. Any increases in plant growth are dependent on there being sufficient soil moisture, as growth increments are reduced under drought conditions. Severe drought in very dry summers has been documented to cause damage to tree stands, particularly where the species are not well suited to site conditions. Sitka spruce thrives on deep, moist and well-drained soils. Dry summers have been shown to cause abiotic damage to stands on sites in eastern Scotland with shallow free-draining soils, where affected trees suffered from stem cracking (Broadmeadow *et al.*, 2009b). Under drought conditions, the xylem of Sitka spruce collapses, and this results in the appearance of stem lesions and cracks in the cambium (Green & Ray, 2009).

Forestry is a long-term investment and decisions in the industry necessarily involve long time frames. This adds considerable complexity to forest management, as it becomes important to not only consider present conditions but also future conditions, management strategy and sustainability. Climate change widens the range of issues that need to be taken into account and adds uncertainty into the decision-making process, further complicating the situation.

The impact of increasing temperatures

One of the main predictions of climate change is an increase in temperatures. Such a change would have far-reaching consequences, as it would not only affect plant growth and development, but also that of insect pests and their natural enemies, while also lengthening growing seasons. Furthermore, responses to such changes are likely to be complex and varied by species.

Increases in ambient temperature will have direct effects plant growth by altering photosynthetic rates and efficiency. Sitka spruce, for example, has an optimum temperature for growth of approximately 18 °C, which is above current projections if other variables do not change (Jarvis *et al.*, 2009). An increase in air temperature, then, would initially appear to benefit the growth of young Sitka spruce plantations. Such increases, however, also cause increases in local water vapour pressure deficit (VPD) (Jarvis, 1994). Conifers, and in particular Sitka spruce, are typically very sensitive to ambient VPD, with stomatal closure resulting from the higher levels of VPD associated with increased temperature (Jarvis, 1994). Stomatal closure results in a suppression of photosynthesis by limiting CO_2 availability, which could present the potential for reduced growth rates.

The trend in responses is not always so clear cut. Optimal Rubisco enzyme activity is observed at 25 °C, for example, though it has been shown to acclimate to temperature (Lukac *et al.*, 2010). Centritto *et al.* (2011) also observed no acclimation of photosynthesis to increased temperatures, though light and dark respiration did. Although increased stomatal closure under elevated VPD was observed by Jarvis (1994) in Sitka spruce, photorespiration, or leaf gas exchange, have been supported by Lukac *et al.* (2010) and Albert *et al.* (2011) in other forest tree species. Changes to plant chemistry are also possible, with Hu *et al.* (2013) finding increased foliar metabolite levels in *Quercus* species under elevated temperatures.

As well as such direct effects on plant growth and productivity, indirect effects of temperature on plants may also be observed under altered climate. For example, nutrient uptake and availability is likely to be affected by increased temperatures in complex ways (Lukac *et al.*, 2010). Such impacts on tree physiology are likely to complicate not only a forest's response to climate change and drought, but also the interaction between a tree and its pest species.

Temperature also has a major influence on insect success, and therefore an increase under climate change is likely to be one of the most significant variables in changes to growth, survival and development rates, and abundance of insect herbivores. It is also likely to result in changes to the range of insect herbivores and their natural enemies. Furthermore, an increase in severity and frequency of attacks is likely at any given latitude, along with increases in diversity (Bale *et al.*, 2002).

The effect of temperature is likely to depend on the extent of temperature increase; too much of an increase, for example, may result in sublethal effects. For example, Chiu *et al.* (2012) observed that though slight temperature increases of 1.2 °C did not significantly affect development and generation time of *Myzus varians* Davidson, warming of 3.7 °C resulted in no aphids reaching adulthood. Within a suitable range of temperatures, insect development time has been shown to decrease with increasing temperature (Kuo *et al.*, 2006b; Hazell *et al.*, 2010; Musolin *et al.*, 2010), while temperatures higher than the range suppress nymph development or kill them outright (Davis *et al.*, 2006; Kuo *et al.*, 2006a). It has been suggested that this may be due to endosymbionts being killed off (Ohtaka & Ishikawa, 1991).

In the case of *E. abietinum*, temperatures are currently below the optimal range for this species for the majority of the year; a rise in temperature should lead to an increase in fecundity and development rates in the aphids, through direct effects on physiological processes (Evans *et al.*, 2002). These would in turn lead to shorter generation times which could bring about higher population densities (Day & Crute, 1990). Such effects would be most pronounced in spring and autumn, when poor host nutritional quality is not a limiting factor to development.

Temperature is a major factor affecting population dynamics (Powell & Parry, 1976; Crute & Day, 1990), and rather than impacts on individual aphid performance it is likely that the greatest effects will be seen in this aspect. This can be subdivided into several components: population development, migration time, and overwinter survival.

Warmer springs would result in insect herbivore populations being able to start developing sooner, while prolonged growth seasons for the plants would also increase the amount of time during which herbivorous insects could cause damage. Warmer weather can be expected to increase accumulated day-degrees of aphids, and in *E. abietinum* it has been predicted that such an effect would result in increased population growth and faster recruitment rates (Day & Crute, 1990). Such a response, however, is not guaranteed. Adler *et al.* (2007), for example, observed no effect of long-term warming on the densities of *Obtusicauda cowenni* Hunter, an aphid on sagebrush, in a field study. Rather, they observed a tendency towards reduced aphid abundance where predators were excluded.

Migration time may well be advanced under increased ambient temperature. In the case of aphids, including *E. abietinum*, it has been found that increases as small as 1 °C could cause such an effect (Zhou *et al.*, 1995). Parry (1977) found that peak alate *E. abietinum* numbers correlated with aphid density, itself related to temperature. Uninfested trees may therefore be colonised earlier in the year if the production of alate morphs is advanced, resulting in a longer period during which the trees may be damaged. Furthermore, timing of infestation has an effect on Sitka spruce growth, with greater reductions observed earlier in the season (Straw *et al.*, 2000; Straw *et al.*, 2005).

Increased temperatures during the winter would improve overwinter survival rates, and an increase in temperature over the winter months, as predicted by Murphy *et al.* (2009), would greatly affect *E. abietinum.* At present, winter temperatures are close to freezing (Murphy *et al.*, 2009) with frequent occurrences of frosts, and these limit the number of years with severe defoliation to about one in every three to six years (Evans *et al.*, 2002). Climate change is expected to decrease the frequency and occurrence of frosts, and decrease accumulated day-degrees below freezing by up to 40% by 2020, and by 60 - 70% by 2050 (Evans *et al.*, 2002). A consequence of this is that a greater proportion of overwintering aphids are likely to survive, leading to an increase in the severity of defoliation and frequency of outbreaks (Evans *et al.*, 2002; Straw, 1995; Day *et al.*, 2010).

Natural enemies of herbivorous insects can be expected to respond to increased temperatures in a similar manner. Predators and parasitoids are important in reducing *E. abietinum* numbers, and changes in temperature, as well as aphid availability are likely to affect their population dynamics as well as that of the aphids. Climate change may affect the range and distribution of aphid natural enemies, and this could alter aphid performance (Awmack *et al.*, 1997). Furthermore, a wide range of generalist predators and parasites occur on spruce, and *E. abietinum* is vulnerable to these throughout the year (Evans *et al.*, 2002). An increase in the number of natural enemies, in response to the increase in aphid numbers predicted to occur as a result of climate change and warming, could be expected to impact on the size of an aphid population (Straw *et al.*, 2009). On the other hand, other insect species, such as the winter moth *Operophtera brumata* L., may be able to escape natural enemy control more frequently under a warmer climate, through a reduction in the time when they are vulnerable to attack by predators and parasites. Reduced mortality from natural enemies may be a consequence of a faster development time in response to warmer temperatures (Evans *et al.*, 2002).

Development times of natural enemies can be expected to change under elevated temperatures. The time spent in larval instars by *Aphidecta obliterata* (L.), for example, is reduced at 20 °C when compared with that spent at 15 °C. Pupal duration showed a similar response (Timms & Leather, 2008).

Feeding rates are also affected by temperature. *Aphidecta obliterata* consumption of *E. abietinum* was influenced by temperature, with a larger number of prey items consumed at 20 °C compared with 15 °C (Timms & Leather, 2008). Vucic-Pestic *et al.* (2011) observed increases in metabolism and decreases in the handling time of prey by three Carabid beetle predators. Specifically, mobile prey items (flightless *Drosophila hydei* Sturt.) suffered an increased rate of attack, whereas a mostly resident prey item (*Alphitobius diaperinus* (Panzer) larvae) did not show altered attack rates. Despite these findings, Vucic-Pestic *et al.* (2011) found that warmer temperatures decreased the energetic efficiencies of the predators, which could lead to predator starvation despite abundant prey resources.

Population dynamics of natural enemies are expected to be altered under increased temperatures. Using simulated models to predict changes to the dynamics of *Sitobion avenae* (Fab.) and the coccinellid *Coccinella septempunctata* L., Skirvin *et al.* (1997) observed that the predators would be most effective at limiting aphid populations at temperatures 2 °C higher than current summer temperatures, whereas under a 1 °C increase aphids would be favoured. It should be noted that these temperature increases correspond with those predicted for temperate forest areas (Kirschbaum *et al.*, 1996).

A further effect may be seen with increased winter temperatures. These are likely to not only improve overwinter survival rates of the natural enemies, but may also mean that they can remain active during the winter period. This could result in improved insect pest suppression, reducing the pest spring peak; which is often associated with the number of individuals surviving the winter.

The alteration of host-pest synchrony

A well-known relationship exists between temperature and plant phenological stage, and as such increases in temperature will cause direct effects to plant phenology. Changes in leafing, flushing, or bud burst dates provide clear evidence of the impact of climate warming. Oak leafing, for example, currently occurs some three weeks earlier than it did in the 1950s (Broadmeadow *et al.*, 2009b). In a study on the first flowering dates for

405 plant species, Amano *et al.* (2010) found an estimated community-level advancement of 2 - 13 days earlier in the last 25 years compared to any other 25-year period since 1760. Their index was closely correlated with February-April mean temperatures, with every 1 °C increase in temperature corresponding to the first flowering date being brought forward by five days.

Gunderson *et al.* (2012) found evidence that climate warming alone could extend the plant growing season at both ends in deciduous forests, despite complications to stand-level impacts due to variation in other environmental factors. They observed that increases in temperature of 2 °C brought forward bud-burst while chlorophyll was retained longer and leaf abscission delayed in the autumn, resulting in prolonged growing seasons.

Such an extension in the growing season in Sitka spruce, as determined by the onset of bud burst until autumn dormancy, may result in a prolonged period in which the trees are vulnerable to attack or provide a better resource for their pest species. This could increase the amount of damage sustained in a season, and have implications for forest growth and development.

Spruce nutritional suitability for *E. abietinum* is highest just before budburst in spring, and once the flush has been completed the total nitrogen content and amino-acid balance of the phloem sap decline (Fisher, 1987). This causes a drop in spruce aphid development and fecundity. It is expected that changes to Sitka spruce phenology will cause the plant to remain suitable for rapid *E. abietinum* development for a relatively longer period in warm springs, while also becoming a more suitable host earlier. This would facilitate an increase in average population size, with populations increasing earlier and more rapidly. Furthermore, if the growing season is prolonged, the period of time during which *E. abietinum* densities can cause damage is prolonged. Autumn peaks of *E. abietinum* are associated with the onset of spruce dormancy, however Evans *et al.* (2002) speculate that warmer spring may only advance this by a few days. This may further lead to a longer period of time in which the spruce host will have to endure high aphid densities.

Phenology is determined primarily by the interaction between photoperiod and temperature, though the two factors are not required to work in tandem to elicit an effect (Bale *et al.*, 2002). Changes to phenology can also be expected to affect synchronicity between a plant host and its pest insects, affecting different pest species in differing ways depending on their life-history strategies. For example, on Sitka spruce, larval survival of the winter moth, *Operophtera brumata*, is greatly affected by the synchronicity of egg hatch and bud-burst (Straw, 1995; Evans *et al.*, 2002). Conversely, *E. abietinum* persists anholocyclically on Sitka spruce in the U.K., and changes to bud-burst timing are not likely to affect populations beyond what has already been described above.

Changes to phenology under climate change are also likely to affect the interaction between pests and their natural enemies. A decoupling of synchronicity between insects hosts and their parasitoids may come about in several ways.

One such cause would be where either the pest or natural enemy species uses a climate-related cue such as temperature to trigger emergence or development, while the other uses a cue such as day-length or some other non-climate-associated cue (Tanaka *et al.*, 1987; Walther, 2010). Evans *et al.* (2013), for example, showed that a phenological mismatch between the cereal leaf beetle, *Oulema melanopus* (L.), and a parasitoid wasp, *Tetrastichus julis* (Walker), in warm springs reduces the rate of parasitism and therefore weakened biological control of the pest species. Whereas the beetle pest responded to accumulated degree-days, the parasitoid wasp varied little in this regard (therefore probably responding to a non-climatic cue for development). Alternatively, should different responses to the same climatic cue be exhibited by species, asynchronicity could be expected to ensue (Visser & Holleman, 2001).

The interaction between insects and their natural enemies may also be destabilised further by inter-year variation, with an exacerbating effect of climate change. Climate change models predict an increase in the incidence of extreme events (Meehl & Tebaldi, 2004), and should a frequency threshold be exceeded it is predicted that pest populations may be released from natural enemy pressure through extinction of the latter's population (Godfray *et al.*, 1994). This suggests that the persistence of host-natural enemy meta-populations will be affected by phenological asynchrony through changes to rates of colonisation and extinction (Jeffs & Lewis, 2013).

It should also be noted that there is a possibility for increased synchrony between insect pest and natural enemies, which would increase predation pressure on the pest population (Jeffs & Lewis, 2013). While this may suppress the pest population to a greater extent, it could cause local extinction of the pest host population and therefore destabilise the pest-natural enemy meta-population balance, leading to the collapse of both species (Van Nouhuys & Lei, 2004).

The effects of changes to atmospheric gases

Climate change is often associated with changes in atmospheric conditions. Typically, increased levels of atmospheric gases are predicted, with a considerable rise in CO_2 predicted in this century (Evans *et al.*, 2002). CO_2 levels had increased from 280ppm prior to the industrial revolution to 390ppm in 2011 (Abrams, 2011). The potential effects of such changes on both trees and their insect herbivores have been explored in a variety of studies.

In general, CO_2 increases tend to improve plant growth, as its uptake and fixation are primarily used by plants for photosynthesis. Therefore, increasing levels of this particular gas stimulate tree growth and physiology until the point of saturation (Karnosky, 2003; Korner, 2003; Lukac *et al.*, 2010; Norby *et al.*, 2010). In contrast, increased O_3 has a detrimental effect which counteracts that of CO_2 (Isebrands *et al.*, 2001; Karnosky *et al.*, 2007). Elevated CO_2 levels have also been shown to affect leaf senescence (Warren *et al.*, 2011).

Changes to Sitka spruce growth rates have been observed under altered atmospheric gas levels, though results vary. Townend (1993) observed an increase of up to 10% in the growth rates of Sitka spruce seedlings under CO_2 levels of 600ppm. On the other hand, Warrington & Whittaker (1990) observed little effect of elevated SO_2 levels alone on plant growth, though a significant reduction in growth was observed when *E. abietinum* were also present.

One effect of altered atmospheric conditions may be changes to the properties of leaves or needles, and this in turn could have implications for the performance of their insect pests. Eamus *et al.* (1990), for example, observed increases in CO_2 assimilation rates, chlorophyll content, stomatal conductance and daily transpiration rates in Norway spruce under exposure to O_3. Furthermore, exposure to this pollutant increased needle wettability (Barnes *et al.*, 1990). Given that *E. abietinum* tends not to be washed off during rainfall (Hussey, 1952; Straw, 1995), believed to be due to the small surface area of spruce needles, changes to such properties may have effects on aphid survival.

Insect responses to altered atmospheric conditions appear to vary by feeding guild. It is generally accepted that increased CO_2 would lead to declines in food quality for leaf-chewing insects, as such increases cause reductions in leaf nitrogen concentrations while those of secondary compounds may increase (Evans *et al.*, 2002). Watt *et al.* (1996) suggested that the impact on leaf-feeding insect performance is closely related to the impact on plant nitrogen under altered CO_2 levels, reducing growth and survival where those levels are elevated. The potential impacts on phloem-feeding insects such as aphids, however, show less clear results (Docherty *et al.*, 1997; Watt *et al.*, 1998). Aphids showed varying responses, with some studies suggesting a positive impact on tree aphids (Docherty *et al.*, 1997) and others finding that there would be no beneficial effect (Awmack *et al.*, 1997).

Warrington & Whittaker (1990) observed an increase in *E. abietinum* numbers under increased SO_2 levels, suggesting that increased levels of this particular pollutant may increase the potential impact of the aphid as a pest. Kidd (1991) also speculated that increases of these pollutants would lead to increased population densities of *Cinara pinea* (Mord.), the result of whose study concur with those of Port & Thompson (1980) and Heliövaara & Väisänen (1990). Mean relative growth rates of *E. abietinum* have also been found to increase under exposure to SO_2 and NO_2 (McNeill & Whittaker, 1990), with the addition of NO_2 further enhancing the response (Whittaker, 2001). Studies on several other coniferous aphids, including *Cinara pilicornis* (Hartig), *C. pini* (L.) and *Schizolachnus pineti* (Fab.) have showed further evidence of this trend (Watt *et al.*, 1998). Despite this, evidence exists that suggests that such changes to growth rates are not reflected in the population densities observed (Awmack *et al.*, 2004; Mondor *et al.*, 2010).

Changes to atmospheric conditions could not only affect the behaviour of phytophagous insect, but may also do so for their natural enemies. For example, Awmack *et al.* (1997) observed a decreased response to aphid alarm pheromones by *Aulacorthum solani* (Kalt.) under elevated CO_2 levels comparable with those predicted for the end of century. Gate *et al.* (1995), however, observed that the proportion of hosts parasitised and searching efficiency of parasitoids was reduced by 10% under O_3 fumigation, and that under elevated

O_3 and NO_2 levels they were less able to distinguish between different host densities. Changes in atmospheric gases, therefore, have the potential to modulate the searching behaviour of natural enemies, potentially by interfering with olfactory responses, while simultaneously improving the hunting success rates of the natural enemies by suppressing prey alarm responses. Both mechanisms would result in effects of the natural enemies on prey density, though the two may effectively cancel each other out.

Drought

The greatest threat to British forestry is likely to be that of an increased frequency and severity of drought. The reasons for this are two-fold. Firstly, serious impacts on drought-sensitive species in established plantations are likely to be widespread, particularly in southern and eastern Britain. Secondly, though most current forestry tree species will remain suitable across much of the U.K., it may be necessary to introduce new, drought-tolerant species in particularly drought-prone areas (Broadmeadow *et al.*, 2009a). The effect of drought stress is also likely to be complicated by the fact that it interacts with other factors, such as increases to temperature and atmospheric gas levels. While many established conifer plantations will reach maturity before serious impacts become apparent, appropriate modification of species choice in reforestation and restocking must be considered imminently, which will present current forest managers with a challenge.

Drought and trees

Water stress is an important factor for tree health, and the predicted increase in the frequency of drought events in the U.K. will have implications for forest health in various areas of Britain (Green & Ray, 2009). The effects of drought on trees have been widely studied, and stem from effects on physiological condition leading to changes to physical condition, growth, and plant chemistry. Tolerance and resistance, both to the original drought stress and also to pests and pathogens, can also be compromised.

The most extreme effect of water stress to trees is mortality. This often comes about as a result of severe symptoms of direct damage. Several tree mortality events have recently been linked to drought (Allen *et al.*, 2010; Anderegg *et al.*, 2013). A drought event in Scotland in 2003, for example, led to 14 - 20% mortality of Sitka spruce at some sites (Green *et al.*, 2008), while many surviving trees showed varying degrees of damage to physical condition (Green & Ray, 2009).

The mechanisms behind tree death as a response to drought remain largely unknown (Anderegg *et al.*, 2013), and it is likely that a combination of mechanisms come into play to cause mortality. One mechanism is hydraulic deterioration. In a study on the hydraulic performance of trembling aspen, *Populus tremuloides* Michx., under drought stress, Anderegg *et al.* (2013) observed that hydraulic damage not only persisted but increased in dying trees over multiple years, with limited indications of recovery. An increased vulnerability to xylem cavitation mediated the damage.

Cavitation rates are affected by plant species as well as water availability. Scots pine, *Pinus sylvestris* (L.), is a drought-tolerant species, and, as would be expected, cavitation rates are lower in this species when compared with the drought-susceptible Sitka spruce (Jackson *et al.*, 1995). Despite this, Jackson *et al.* (1995) did not observe an increase in the cavitation rates of droughted Sitka spruce, though decreases in sap flow rate and water potential were nonetheless observed. Scots pine, however, did show an increase in cavitation rates under drought.

Beyond mortality, several other symptoms of direct damage on trees can be observed. Foliage wilting or browning (Green & Ray, 2009) and premature abscission (Warren *et al.*, 2011) are among these. Furthermore, drought and elevated CO_2 have been shown to have an additive interactive effect on leaf senescence and abscission (Warren *et al.*, 2011), with increased responses when both stressors were applied. Warren *et al.* (2011) found that elevated CO_2 reduced sap flow rate by 28%, rising to reductions of 45% during drought, in a study on *Liquidambar styraciflua* L. sweetgum trees. Additionally, canopy conductance was also reduced. The authors speculated that, under moderate drought conditions, elevated CO_2 levels had the capacity to reduce leaf water usage, but that in acute drought situations stomatal closure may increase and offset any potential benefits. Studies such as this highlight the often important interactive effects that drought has with other elements of climate change.

Crown condition is also directly affected by drought, and crown dieback is considered a symptom of drought stress (Green & Ray, 2009). Crown dieback is associated with depletions of carbon reserves in trees. In holm oak, *Quercus ilex* L., Galiano *et al.* (2012) observed complex spatial patterns in tree-level response to drought, which was affected by soil depth, individual tree characteristics and stem number. Additionally, reductions of carbon reserves of up to 60% were found under drought, leading the authors to suggest that under repeated limited water availability a progressive depletion would result in reduced forest resilience and increased incidences of crown dieback.

While drought can cause whole-tree mortality in the long term, or also in the short term during acute instances, it can have immediate effects on bud mortality. Bud survival is critical to sustain tree growth across seasons, but has been found to be reduced under drought stress in trees (Barigah *et al.*, 2013). This has severe implications on a tree's potential for growth and timber production, though it is not the only factor affecting it. The symptoms outlined above all contribute to impacts on both above- and below-ground growth, through reduction of photosynthetic and transpiration area (Ryan, 2011).

Drought is known to impair cell division and expansion in trees (Hsiao, 1973). These occur at lower water stress thresholds than those for photosynthetic inhibition (Hsiao *et al.*, 1976). Cell differentiation is also affected by drought stress, supported by the findings of Gruber *et al.* (2010). In this study on Scots pine, they found a strong influence of drought on the cell differentiation process, causing changes to dynamics and duration of radial widths and wood formation of earlywood cells. Such effects can lead to reduced total stem wood growth (Ge *et al.*, 2011).

The effects of drought on growth are mediated by stress-tolerance and provenance of tree stock, and these are often related to differences in biomass allocation. Scots pine seedlings, for example, with a drier central-Asian provenance survived longer under drought than those from mesic European and coastal sources in a study by Cregg & Zhang (2001). Asian seedlings were not only smaller, but allocated a greater biomass to roots than seedlings from European sources. Reductions in lead shoot extension (Nzokou & Cregg, 2010; Arend *et al.*, 2011) and stem diameter (Arend *et al.*, 2011; Sánchez-Salguero *et al.*, 2012) have been found to result from drought stress, while root growth was typically promoted (Arend *et al.*, 2011). Such findings are intuitively logical, as increased root biomass may allow for a marginally increased water uptake and therefore the potential for increased survival. This is not always the case, however, as a study by

Olesinski *et al.* (2011) found that monthly fine root production was reduced by low soil water content, though it was increased in periods of recovery, in balsam fir (*Abies balsamea* L. Mill.). These changes did not affect leaf biomass production, therefore suggesting a balance between root biomass and foliage supported by fine root dynamics over multiple seasons.

As well as their function in water uptake, roots also play an important role in resource storage. In fact, it has been shown that under water stress growth can be switched to reserve storage. Galvez *et al.* (2011) showed that, in aspen, seedlings grown under severe drought stress showed a significant increase, of two orders of magnitude, in sugar and starch content. Furthermore, when compared with non-droughted seedlings, there was a higher starch content relative to sugar content. The same droughted seedlings, however, showed decreases in gas exchange and water-relative parameters which did not mirror the observations made on roots.

Different aspects of plant life-history can be affected in differing ways by limited water availability. A study by Moser *et al.* (2010) showed that cocoa plants, *Theobroma cocao* L., showed no reductions in lead biomass, stem and branch wood production or fine root biomass under fairly severe drought stress. Despite this, production of cocoa bean was reduced, resulting in a decreased bean yield at the end of the season. Changes to phenological timings also respond to drought. Misson *et al.* (2011) found that spring rainfall exclusion caused greater and sustained suppression of leaf water potential during key development phases in holm oak. This led to a reduced shoot lengthening phase, which gave a reduction of functionally mature leaves, and reduced the number of instances of female fruit maturation.

Impacts of drought stress on physiological processes and photosynthesis have been alluded to in the above paragraphs. Declines in water potential under drought conditions are well-established fact (Cregg & Zhang, 2001; Ditmarová *et al.*, 2009; Vaz *et al.*, 2010), but it should be noted that physiological processes in plants respond at different water potential levels (Hsiao, 1973; Ditmarová *et al.*, 2009). This highlights the importance of drought severity, as it can influence a tree's response.

The decreases in water potential are typically accompanied by a suppression of photosynthesis-related measures; stomatal conductance, photosynthetic rate and efficiency, transpiration and respiration rates have all repeatedly shown to be reduced (Ditmarová *et al.*, 2009; Guo *et al.*, 2010; Ibáñez *et al.*, 2010; Liu *et al.*, 2010; Vaz *et al.*, 2010; Albert *et al.*, 2011; Centritto *et al.*, 2011; Crous *et al.*, 2011). The down-regulation of photosynthesis is not only due to decreased leaf water potentials and increased stomatal closure. Chlorophyll concentration has been shown to be reduced under drought stress by both Ditmarová *et al.* (2009) and Guo *et al.* (2010) in Norway spruce and poplar respectively, while enzymes necessary to the process, such as Rubisco (responsible for CO_2 fixation), may also have their activity curtailed (Vaz *et al.*, 2010; Albert *et al.*, 2011). Rates of photosynthetic electron transportation are also suppressed under limited water availability (Vaz *et al.*, 2010; Albert *et al.*, 2011). Many of these factors are further mediated by other aspects such as atmospheric gas concentrations and temperature, which are predicted to alter under climate change, further complicating plant responses. As such, it will be important for future management to understand these mechanisms and interactions in greater depth than is currently available.

A final aspect to be addressed is the effect that drought stress may have on host tree resistance to insect herbivores under drought stress. The Growth-Differentiation Balance (GCB) hypothesis of plant defence states that a trade-off exists between plant growth and defence for two reasons: (1) physiological restrictions of secondary metabolism and structural reinforcement; and (2) secondary metabolism requires a diversion of resources from the new leaf area production (Herms & Mattson, 1992). Both biotic and abiotic factors, such as those changes predicted under climate change, can affect the balance between growth and defence in plants.

Secondary metabolites have been shown to be affected under drought stress. Major (1990), for example, observed an increase in monoterpene levels in Sitka spruce under drought stress. In oaks (*Quercus* spp.), Hu *et al.* (2013) also observed changes to foliar metabolites. Anti-oxidant levels, including those of γ-glutamylcysteine and total glutathione and proline levels, were increased with drought. No changes were observed to foliar ascorbate, glutathione disulfide and dehydroascrobic acid levels, though levels of all chemicals did differ between three oak species. These findings led the authors to conclude that the stress response of plants was species-dependent, as well as stress-dependent. Given the changes to terpene compound levels shown by Major (1990), it is plausible that the same may be true for secondary defence compounds. On the other hand, the GCB hypothesis predictions may lead one to infer that a plant may favour survival rather than defence against insects. This is supported by Straw (1995) and Green & Ray (2009), who propose that drought stress may render trees more susceptible to pest and pathogen damage. The findings of Gutbrodt *et al.* (2012) provide evidence of this. They observed an effect of drought stress on constitutive herbivore resistance in apple plants, *Malus domestica* Borkh., but not induced resistance, with the response modulated by drought intensity. While leaf glucose concentrations increased with increasing drought intensity, phenolic compounds decreased instead. This led to *Spodoptera littoralis* Bois. showing a preference for these plants.

The Plant Water Stress hypotheses

The plant stress hypothesis (PSH) was originally conceived by White (1969), based on observations of the outbreak dynamics of psyllids on water-stressed Eucalyptus trees in Australia. The White (1969) PSH asserted that during prolonged periods of water deficit changes in the plant physiology, and more specifically in available nitrogen, was the cause of the insect outbreaks. It was suggested that the increase in available plant nitrogen promoted population outbreaks through improved growth and reproduction (White, 1969).

Since its inception, this original version of the PSH has been challenged. A greater understanding of plant physiological changes, including those of allelochemicals, turgor pressure and water content, and its varying effects on different feeding guilds of insects, has led to modifications to the original prediction. Larsson (1989), for example, refined the hypothesis and predicted that different feeding guilds of insects would respond differently to plant water stress, as they would experience the changes in plant nutrition, allelochemistry and growth differently. Within this context, phloem and cambium feeders could be expected to respond more positively than chewing insects and gall-formers to water stressed plants (Larsson, 1989).

A second hypothesis was developed by Price (1991). The Plant Vigor Hypothesis (PVH) proposed that herbivores should prefer vigorously growing, healthy plants, which would provide insects better food quality and faster growth. The hypothesis predicts that, for galling insects, higher shoot growth rates should favour larval performance when compared with that on slower growing shoots (Price, 1991). Plants growing in environments providing abundant water, mineral nutrients and access to appropriate levels of sunlight should grow more vigorously than those plants that are deficient in any those resources, making such plants superior in nutritional quality (Price, 1991). Furthermore, Price (1991) suggested that the two hypotheses should not be seen as strict alternatives, but as ends to a spectrum accounting for the diversity of herbivorous insect responses to plants.

Huberty & Denno (2004) developed the Pulsed Water Stress Hypothesis (PWSH) to explain the discrepancy between observed outbreaks of herbivorous insects on water stressed plants, and the negative effects often detected in experiments where plants were continuously stressed. They proposed that bouts of intermittent stress would allow recovery of turgor pressure, thus allowing phloem-feeding insects, such as aphids, to take advantage of stress-induced increases in nitrogen, which would have been inaccessible during continuous stress (Huberty & Denno, 2004). These findings explain previous results obtained in several studies, such as that conducted by Major (1990) on *E. abietinum.* This hypothesis was further refined by Mody *et al.* (2009), who observed that the magnitude and intensity of the stress bouts could enhance or reduce phloem-feeding insect performance on intermittently stressed plants.

The plant stress hypotheses are relevant to the predictions for hot, dry summers and drought conditions made under the climate change model, as the nature of these conditions, and specifically the amount and frequency of any summer rainfall, is likely to affect the response of *E. abietinum* populations to stressed trees. Prolonged periods without rainfall are likely to produce conditions of continuous stress, which have been shown by various studies, such as that of Kennedy *et al.* (1958), to negatively affect aphid populations. Occasional rainfall would, however, produce intermittent stress conditions, which are suggested to improve aphid performance (Major, 1990; Huberty & Denno, 2004; Mody *et al.*, 2009). Furthermore, the frequency and quantity of rainfall would further affect aphid performance, as predicted by Mody *et al.* (2009).

Drought, insects and aphids

Studies of the effects of drought on arboreal herbivores are somewhat limited, with even fewer such studies assessing the impact of drought stress on arboreal aphids (Koricheva *et al.*, 1998). Most studies on trees are conducted on potted plants, as are many on agricultural and potted plants, to limit the logistic complexities. Overall, stressed plants showed no significant effect on insect growth, fecundity, survival or colonization density (Koricheva *et al.*, 1998). Despite this, prolonged or severe drought is speculated to trigger an increased frequency and severity of forest insect outbreaks (Straw, 1995; Green & Ray, 2009; Jactel *et al.*, 2012) and may potentially facilitate growth loss and mortality when interacting with hydraulic failure and other changes to plant physiology (McDowell *et al.*, 2008). It is therefore important to understand the impact of drought stress on insect performance as well as their damage to trees (Jactel *et al.*, 2012).

In regards to drought stress, the nature of the herbivorous insect response, both in terms of magnitude and direction, has repeatedly been shown to be affected by feeding guild. As a general rule, boring (which also includes mining) and sucking insects showed improved performance on drought stressed plants, whereas gall-makers and chewers were negatively affected (Larsson & Björkman, 1993; Koricheva *et al.*, 1998; Björkman & Larsson, 1999). Such generalisations, however, must be made with caution, as even within feeding guilds species-specific responses have been shown to drought stress.

Larval weight of a leaf-mining lepidopteran was found to be reduced under drought stress by Björkman & Larsson (1999), however they suggested that the feeding preference of *Epinotia tedella* (Cl.), which feeds on older needles, may render the species particularly responsive to drought. A species-specific response is supported by the findings of Staley *et al.* (2006), who observed an increase in abundance of one leaf-mining species, while three others remained unaffected. Wood boring beetles also show a similar, complex response. *Hylotrupes bajulus* L. beetle larvae on Scots pine, for example, were unaffected by drought stress even though a significant impact on wood development and anatomy was observed (Heijari *et al.*, 2010). *Tomicus destruens* Woll. larval survival was, however, reduced on maritime pine (*Pinus pinaster* Ait.) in a study by Branco *et al.* (2010), in contrast with the overall findings for the feeding guild stipulated by Koricheva *et al.*

(1998).

In the case of chewing insects, Björkman & Larsson (1999) observed that the sawfly, *Gilpinia hercyniae* (Htg.), met the predictions of the PSH; namely, that no response was shown to drought stress by this species. These findings mirror those of Larsson & Björkman (1993), in work on the same species. These studies were, however, conducted on mature trees, and the authors speculated that fundamental differences between seedlings and mature trees may mediate the response to drought stress on the two age classes of host. These findings are, however, in contrast to those of Gutbrodt *et al.* (2011). In this study, the performance and feeding preferences of *S. littoralis* and *Pieris brassicae* (L.) on a Brassicaceous host were monitored under drought stress. *Pieris brassicae* performed better on drought-stressed plants, though a feeding preference for well-watered plants was shown. In contrast, *S. littoralis* preferred severely stressed plants. Drought was found to reduce the level of secondary defence compounds in the host plant leaves, with the lowest levels shown under the severest level of drought treatment, which may go some way to explaining the performance results (Gutbrodt *et al.*, 2011).

It should be noted, however, that the Gutbrodt *et al.* (2011) study was conducted on a non-woody host plant, and it is plausible that the physiological responses of such plants may differ to their woody counterparts. In a study on apple trees conducted by Mody *et al.* (2009), *S. littoralis* showed a non-monotonic preference and performance response to drought stress. Specifically, apple trees under the highest level of stress were most attractive and suitable for the caterpillar herbivores, while low-stress plants were the least and control plants were intermediately preferred. This contrasted with the predictions of the PSH, while supporting the PWSH in the importance of intermittent stress. Mody *et al.* (2009) used their findings to modify the PWSH by illustrating the importance of intensity even under intermittent stress.

Despite an apparent overall improvement of performance of sucking insects under drought conditions (Koricheva *et al.*, 1998), aphids show a varied and inconsistent response. Negative effects have been shown several times. Kennedy *et al.* (1958), for example, observed a reduction in nymph deposition by *Aphid fabae* Scop., despite improvements in phloem sap. McVean & Dixon (2001) observed similar responses in *Acyrthosiphon pisum* (Harr.), with fewer aphids observed on continuously stressed plants when compared to a well-watered control.

Positive effects of drought stress on aphids have also been recorded. Major (1990) observed an increase in *E. abietinum* population size on intermittently droughted Sitka spruce, while Tariq *et al.* (2010) observed improvements to fecundity and intrinsic rates of increase under moderate drought stress in both *Myzus persicae* Sulz. and *Brevicoryne brassicae* (L.). The latter study, however, also showed comparatively reduced performance on severely drought stressed plants, emphasising the importance of stress intensity on the response of aphids. Khan *et al.* (2010) also observed larger *M. persicae* populations on stressed cabbages.

Other studies have instead observed no effect of droughted host plants on aphid performance. Simpson *et al.* (2012) concluded that *M. persicae* population growth and survival rates were not affected by drought on cabbage plants, and as such their findings did not support the plant stress hypotheses. A similar result was found in regard to *B. brassicae* by Khan *et al.* (2010). In a study on *E. abietinum* and Sitka spruce, Warrington & Whittaker (1990) observed a small but non-significant increase in mean aphid numbers under drought stress and concluded that, despite having a significant impact on plant growth, drought had little effect on aphid numbers. In a study conducted on *Rhopalosiphum padi* L., Aslam *et al.* (2013) found no effect of drought on total aphid population size. Rather, what was significantly affected was the population demography, with a greater number of adults on droughted plants.

Host plant species can also contribute to the nature of aphid responses to drought stress. A good example of this is provided in work conducted by Hale *et al.* (2003) on *R. padi*. In this study, the intrinsic rate of increase of the aphids was reduced on three grass species, while remaining unaffected on a fourth. Plant-herbivore interactions were also found to be affected by genotype in a field experiment conducted on soybean, *Glycine max* Merr., by Grinnan *et al.* (2013).

All the evidence thus far presented indicates a highly complex and often species-specific response of insect herbivores to drought stress, mediated by drought intensity and frequency as well as host species. An added layer of complexity might also be expected in the interaction between insect pests and their natural enemies under drought stress.

Despite the number of studies conducted on the interaction of pests and their natural enemies under altered temperature or atmospheric gas concentrations, few have been conducted under altered water availability. One such study, conducted by Aslam *et al.* (2013), indicated that parasitisation of *R. padi* by *Aphidius ervi* Haliday was reduced on drought-stressed plants. They further observed that phsyiological changes in aphids did not appear to affect parasitoid preferences, therefore suggesting that the changes to attack and parasitisation rates were due to drought-induced changes to aphid demographics.

Introduction to the project

Although research has been conducted on the effect of drought stress on aphids, most of these studies have been on aphids associated with agricultural crops (e.g. Kennedy *et al.* (1958); McVean & Dixon (2001); Hale *et al.* (2003)). Very few have been conducted on trees and arboreal aphids (Koricheva *et al.*, 1998). For these aphids the situation is unclear, and given the enormous potential impact of *E. abietinum* research is needed to assess the likely response of this aphid to climate change, and especially the interaction with the effects of drought on the host tree.

The purpose of the research herein presented was to gain a clearer understanding of the effects of drought stress on *E. abietinum.* The main objectives were:

- To test the hypothesis that drought-stress in spring improves host-plant quality for *E. abietinum* and leads to more rapid population development;
- To determine whether host tolerance, in terms of needle retention and impact of infestation on tree growth, varies with drought stress;
- To test the hypothesis that spring-summer drought advances the onset of dormancy in spruce and enables aphid populations to increase earlier in the autumn;
- To test the hypothesis that drought stress improves host-plant quality for *E. abietinum* in the autumn; and
- To investigate the potential effects of drought on the tritrophic interactions of Sitka spruce, *E. abietinum* and its natural enemies.

In order to meet these objectives a two-year semi-nursery field trial was established at Silwood Park, Ascot (U.K.) in which aphid density, needle loss and plant growth were monitored. An investigation on individual aphid performance parameters was conducted under controlled conditions, staggered through time following budburst and in the autumn. Additionally, an experiment on the consumption rate of a specialist and generalist coccinellid predator was also conducted. The effects of five different drought treatments, encompassing different drought intensities and frequencies, was explored throughout the work, with the results herein presented and discussed.

Chapter 2

A non-damaging method for creating drought stress and monitoring water levels in potted Sitka spruce

Introduction

Stress, or resource limitation, is known to affect both plants and the interactions of the plant with phytophagous insects (Herms & Mattson, 1992; Koricheva *et al.*, 1998). Drought is an important abiotic stress, with extensive implications on plant growth, functioning and productivity. Drought events have been observed increasingly frequently around the globe (Mishra & Singh, 2010), and furthermore, climate change in the U.K. is predicted to result in an increase in the number of incidences of summer drought (Murphy *et al.*, 2009). Consequently, great interest has been taken by a variety of disciplines on the effects of drought.

Drought can be broadly classified into five categories: (i) agricultural, (ii) hydrological, (iii) meteorological, (iv) socio-economic, and (v) ground water drought (Mishra & Singh, 2010). Although all relate to shortages in water supply and precipitation, hydrological and socio-economic drought are associated with a failure of water resources and management systems to meet pre-existing water demands. In contrast, meteorological, agricultural and ground water drought are defined as a lack of precipitation over a region or declining moisture or water levels.

A range of drought indices have been derived, particularly in order to study agricultural droughts, and many of these can be applied to not only agricultural crops but also forest systems. Several indices are associated with precipitation including, but not limited to, the standardised precipitation index (SPI), Palmer drought severity index (PDSI), crop moisture index (CMI), effective precipitation (EP) and surface water supply index (SWSI) (Mishra & Singh, 2010). Indices based directly on soil moisture have also been developed, including the soil moisture deficit (SDMI) and evapotranspiration deficit (ETDI) indices (Narasimhan & Srinivasan, 2005). Furthermore, a soil moisture index (SMI), based on observed water content and known field capacity, was also recently developed by Hunt *et al.* (2009).

Experimental studies on the effects of drought are often carried out on potted plants, be they trees or agricultural crops, as the water regimes can be varied or manipulated to achieve different levels and patterns of drought.

It is crucial to have a reasonable watering regime in any manipulative study of drought effects. All methods require the retention of water from certain treatments, but there is no standard or set protocol across studies. There are two aspects to be considered in any study: timing of watering and quantity of water provided.

Timing of watering

Timing of water application is frequently based on plant condition, usually through leaf wilting or drooping (Kennedy *et al.*, 1958; Mody *et al.*, 2009; Gutbrodt *et al.*, 2011; Gutbrodt *et al.*, 2012). While this may be useful for agricultural crop plants, which show such systems readily and rapidly, the same cannot be said for coniferous plants with needles, which often do not. Another means is to withhold water for periods of time comparable with those observed under field conditions (Nakai & Kisanuki, 2011), watering only after such a length of time has transpired. Following the same rationale, a selection of reasoned intervals under which plants would experience stress have also been used (Cregg & Zhang, 2001; Simpson *et al.*, 2012). A similar, though perhaps more extreme method, is complete retention of water after some starting date for the duration of the experiment, such as in Warrington & Whittaker (1990). A more time-intensive method often used is the application of water whenever some lower bound is reached, be it determined by moisture meter (Heijari *et al.*, 2010; Aslam *et al.*, 2013) or pot weight (Ditmarová *et al.*, 2009).

Quantity of water provided

One method of establishing the amount of water provided is to determine treatments as percentages of a pot's field capacity. Khan *et al.* (2011) employed this means in their study, while Heijari *et al.* (2010) used it in terms of the amount of water in the soil pore space. Similarly, Aslam *et al.* (2013) provided enough water to maintain determined levels of soil water content.

A second commonly used method for establishing drought treatments is to base the amount of water provided on that received by a well-watered control, whereby different levels of drought receive a different percentage of the control treatment. Several studies, such as those by Mody *et al.* (2009); Gutbrodt *et al.* (2011); Gutbrodt *et al.* (2012), have utilised this technique.

Though these two methods appear to be the most commonly used means of determining the quantity of water to be provided as experimental treatments, a variety of other determinants have been used. The provision of water to meet certain reasoned target rates (Nzokou & Cregg, 2010), or based on rainfall reduction predictions (Aslam *et al.*, 2013), can be used to reflect potential conditions in the field, when used judiciously.

Returning pots back to a fully watered state cyclically (Cregg & Zhang, 2001), as well as the more extreme option of not watering during the entirety of the experimental period (Warrington & Whittaker, 1990) have also been used in studies to establish drought stress.

In order to determine or monitor soil moisture levels, a variety of techniques have been developed. Fawcett & Collis-George (1967), for example, devised a method using Whatman No.42 filter paper to determine moisture characteristics of soil. Soil water retention curves can also be used to calculate soil water potential and corresponding water content (Cockfield & Potter, 1986; Liu *et al.*, 2010). More recently, studies have taken advantage of advanced technology to determine soil moisture content. Profile probes and moisture meters can be used to monitor soil water content (Simpson *et al.*, 2012; Aslam *et al.*, 2013). Water potentials of growing mediums can be established using psychrometers (Hale *et al.*, 2003) or dew point potentiometers (Liu *et al.*, 2010), while leaf water potential can be assessed using a pressure bomb or chamber (Cockfield & Potter, 1986; Major, 1990; Marchin *et al.*, 2010; Crous *et al.*, 2011). Other parameters of plants affected by drought which can also be monitored include stomatal closure and gas exchange measurements (Vaz *et al.*, 2010; Albert *et al.*, 2011), while tree crown condition has also been used in mature forests (Galiano *et al.*, 2012). Other studies, such as those conducted by Ditmarová *et al.* (2009) and Branco *et al.* (2010), devised systems using pot weight to monitor moisture content of the growing medium, adding water when some lower bound in weight was reached.

Several of the experiments presented as part of this thesis required that the study trees be carried over across years. The number of tree replicates used at any one time also had the potential to be large, particularly in instances where several studies were being conducted simultaneously (as was the case through 2010 and 2011). As such, not only was a suitable watering regime required, it was also necessary to develop a reliable, fast and non-damaging method to monitor the water levels in the experimental tree pots. The method needed to be consistently applicable across experiments and trials, as without this, comparisons could not be drawn. It was decided that a system based on a combination of pot weight and soil moisture sensors would be the optimal solution.

Aims and Objectives

1. Develop a watering regime and a reliable, non-damaging method to create and monitor water levels in potted Sitka spruce (*Picea sitchensis* (Bong.) Carr.).

2. The method must be able to be applied in a field, laboratory (CT) room and greenhouse setting.

3. Due to the number of potted saplings to be checked on a regular basis, the method must require as little time as possible per pot while still maintaining reliability.

Materials and Methods

Drought treatments

Five drought treatments were decided upon, to reflect different intensities and fluctuation frequencies of drought stress:

1. Field capacity (**FC**) - Plants maintained at field capacity;
2. Moderate continuous stress (**MS**) - Plants maintained at 60% of field capacity;
3. Continuous severe stress (**CS**) - Plants maintained at 20% of field capacity;
4. Intermittent severe stress (**IS1**) - Plants subjected to fluctuating stress, whereby plots were allowed to dry out to 20% field capacity, and were then watered back up to field capacity;
5. Intermittent moderate stress (**IS2**) - Plants subjected to fluctuating stress, whereby plots were allowed to dry out to 30% field capacity, and were then watered back up 70% of field capacity.

For two-year-old Sitka spruce pots

In 2010, forty 3L pots containing a standard growing medium of 2:1:1 peat, bark and perlite with 20g controlled release granular fertiliser mixed in (Osmacote® Plus: 16%N + 8%P + 11%K + 2%MgO; Scotts Ltd, U.K.) were obtained from Alice Holt Forest Research Station, Surrey (U.K.). The amount of potting mixture used when potting each Sitka spruce was standardised, and the amount of mixture in each of the forty pots reflected the amount used when potting the trees.

The filled pots were placed in a warm, dry, but well-ventilated greenhouse in order to dry out the growing medium, which was regularly disturbed to ensure even drying. While the growing medium was still completely dry, each pot was weighed and a moisture reading obtained. The soil moisture was measured as percentage volume (% volume) using an SM200 Soil Moisture Sensor and an HH2 Meter (Delta T Devices, Cambridge). These values were designated as 0% of field capacity.

The pots were then brought up to saturation point and left to drain freely for half an hour to ensure they were not waterlogged. Each pot was then re-weighed and a further moisture reading was also taken. These values were designated as field capacity. Both weight and % volume readings were taken daily thereafter until they approached their starting weight, to provide reference points for the readings at different percentages of field capacity.

Each of the 250 experimental tree pots was then weighed at field capacity and the mean weight calculated. The difference between the mean weight of the treeless pots at field capacity and the mean weight of the experimental tree pots at field capacity was assumed to equate to the weight of the tree. Knowing the weight of the tree allowed the weight of the tree pots at various percentages of field capacity to be established (Table 2.1). This could then be corroborated by the moisture readings taken on the treeless study pots, in order to compensate for the effect of tree growth on the pot weight.

For re-potted three-year-old Sitka spruce pots

In 2011, forty 7L pots containing the standard growing medium were filled with a standardised amount of mixture, reflecting the amount of mixture already in the pots and the added amount used when re-potting the trees. The filled pots were placed in a warm, dry, but well-ventilated greenhouse in order to dry out the growing medium, which was regularly disturbed to ensure even drying. While the growing medium was completely dry, each pot was weighed and a moisture reading taken. These values were designated as 0% of field capacity.

The pots were then brought up to saturation point and left to drain freely for half an hour to ensure they were not waterlogged. Each pot was then re-weighed and a moisture reading taken. These values were designated as field capacity. Both weight and % volume readings were taken daily thereafter until they approached their starting weight, to provide reference points for the readings at different percentages of field capacity.

After re-potting, each of the surviving experimental tree pots was then weighed at field capacity and the mean weight calculated for each drought treatment. The difference between the mean weight of the treeless pots at field capacity and the mean weight of the experimental tree pots at field capacity was assumed to equate to the weight of the tree. The weight of the tree pots at various percentages of field capacity were then established and corroborated by the moisture readings taken on the treeless study pots. This allowed for compensation of the changes in weight throughout the season due to tree growth.

Statistical analysis

Comparisons between readings taken on 3L pots and 7L pots were compared using linear mixed effects models. Pot capacity was modelled as a fixed effect, while pot number was modelled as a random effect (groups: pot = 40; n = 40, estimated d.f. for each parameter = 1).

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

The % volume and weight decreases of tree-less pots following watering are shown in Figures 2.1 and 2.2 for 3L and 7L pots, respectively.

The model intercept for pot weight significantly differed between the two pot sizes (${\chi^2}_1 = 6479.1$, $P < 0.001$); as would be expected, 7L pots were heavier ($t = 71.51$, $P < 0.001$). Furthermore, 7L pots were found to dry out faster, reflected by a steeper weight loss slope ($t = 80.49$, $P < 0.001$). There was, however, no significant difference in the % volume decreases through time between the two pot sizes (${\chi^2}_1 = 0.196$, $P > 0.05$).

The mean % volume and weight values calculated for the different levels required for the drought treatments are shown in Table 2.1. Figure 2.3 shows the relationship between the two measures for 3L (Figure 2.3(A)) and 7L (Figure 2.3(B)) pots, respectively.

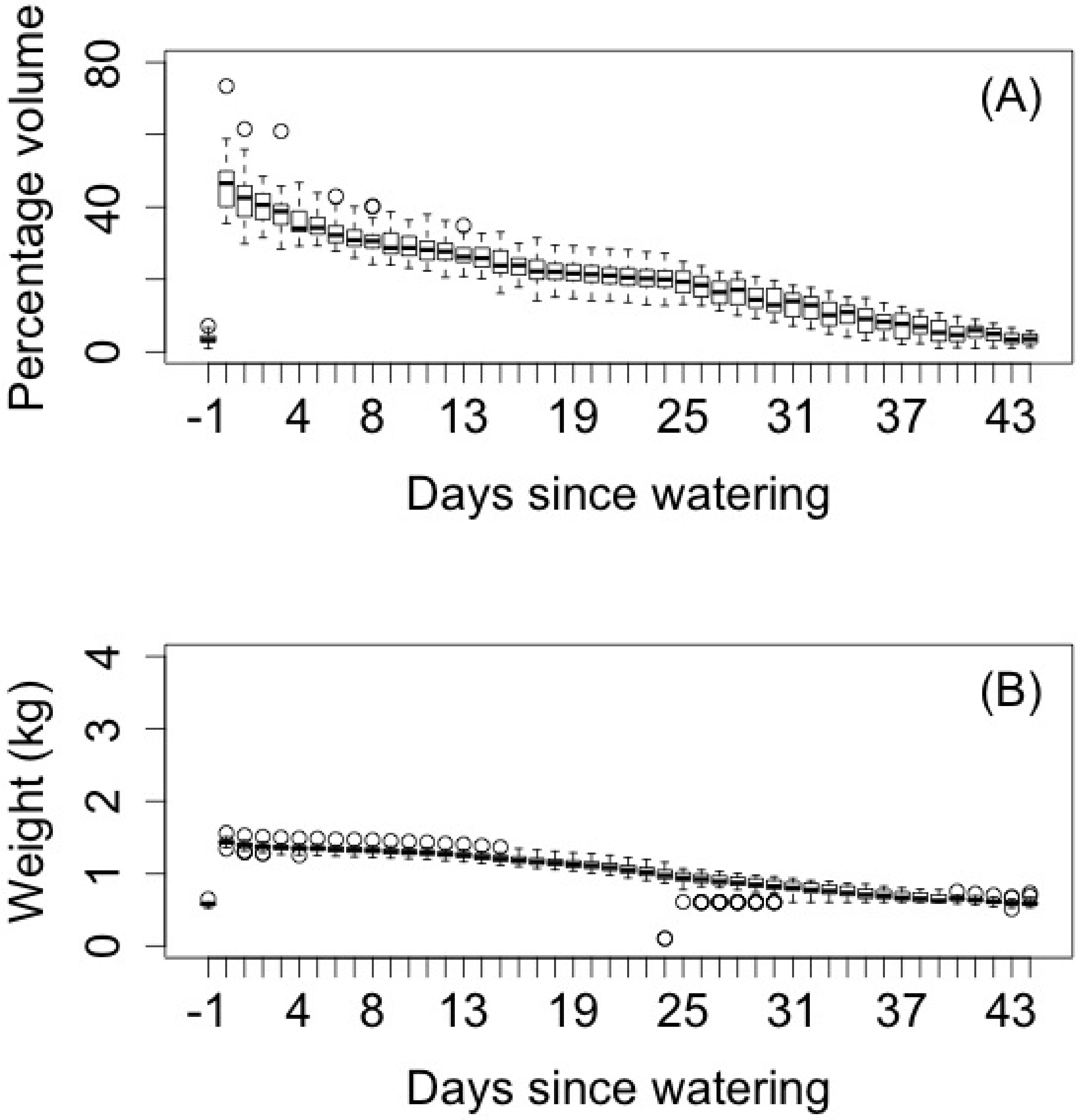

Figure 2.1: Weight and percentage volume following watering in tree-less 3L pots.

(A) Percentage volume through time since watering; (B) Weight through time since watering. Where: day -1 indicates pre-watering readings (defined as 0% of field capacity) and day 0 indicates day of watering to saturation (defined as field capacity.)

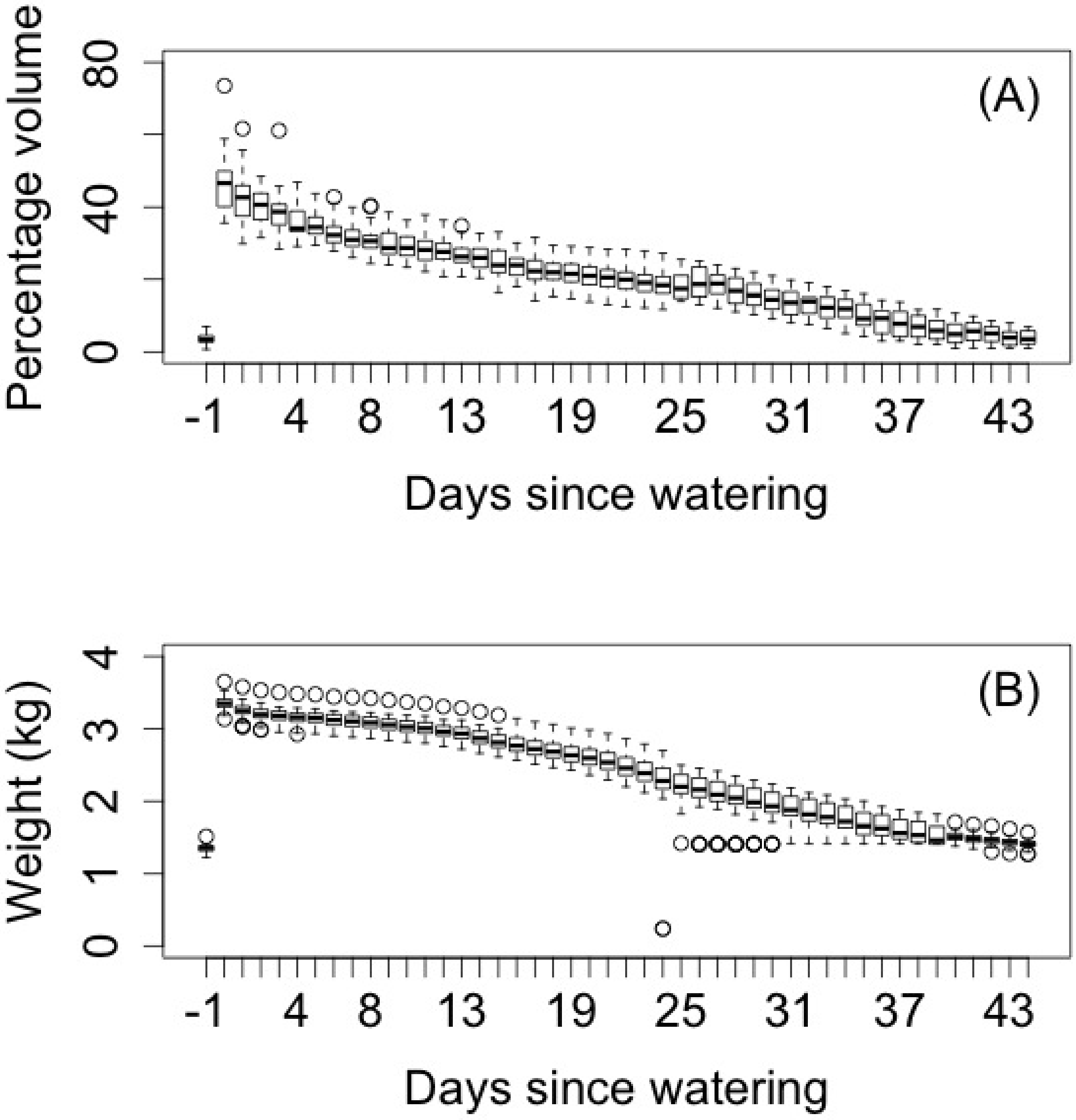

Figure 2.2: Weight and percentage volume following watering in tree-less 7L pots. (A) Percentage volume through time since watering; (B) Weight through time since watering. Where: day -1 indicates pre-watering readings (defined as 0% of field capacity) and day 0 indicates day of watering to saturation (defined as field capacity.)

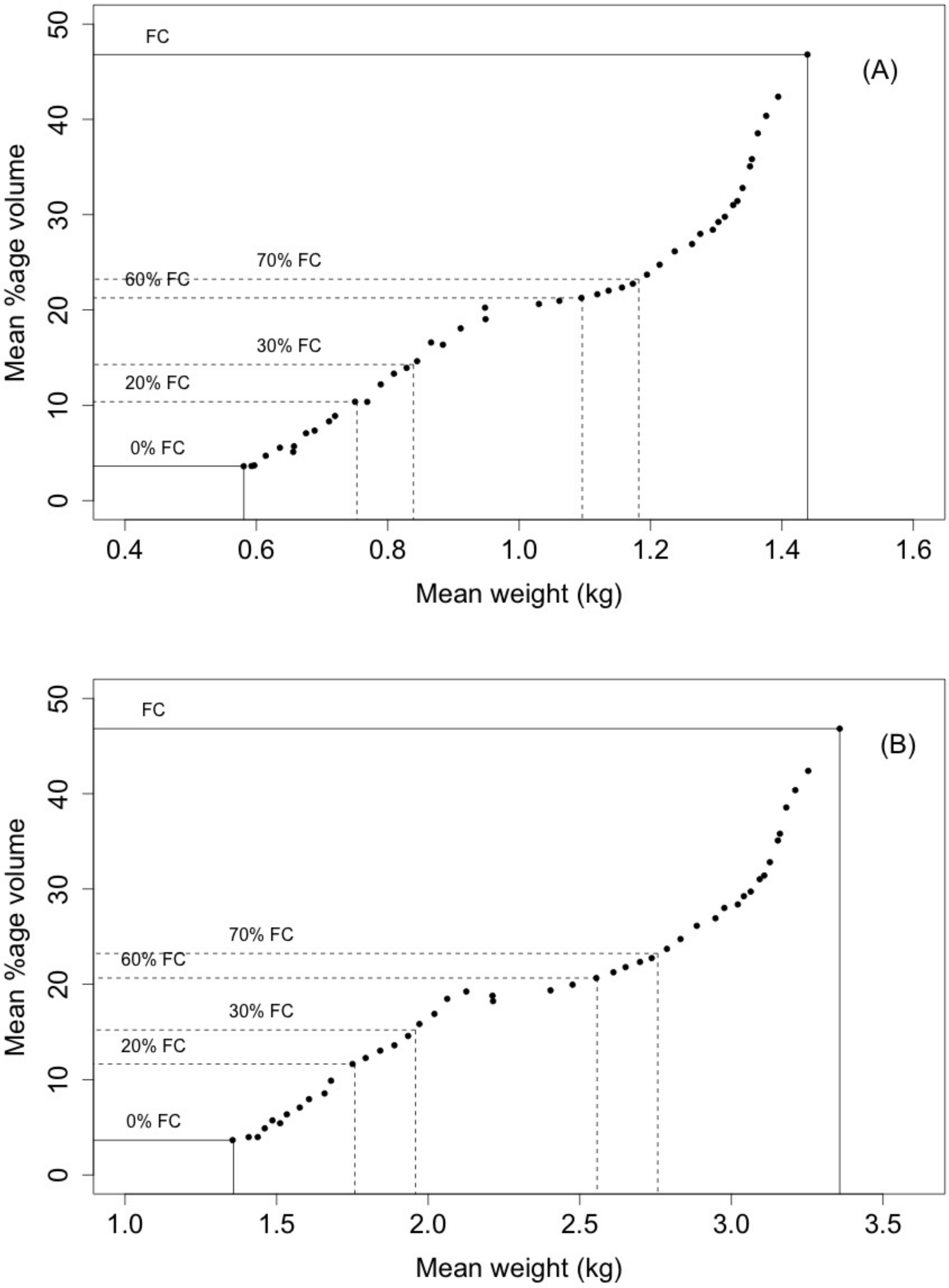

Figure 2.3: Relationship between pot weight and percentage volume in 3L (A) and 7L (B) pots.

FC = field capacity.

Table 2.1:

Weight and percentage volume of tree-less pots at given levels relative to field capacity. FC = field capacity.

	3L pots		**7L pots**	
Soil moisture content	**$\bar{x}$ weight (kg)**	**$\bar{x}$ % volume**	**$\bar{x}$ weight (kg)**	**$\bar{x}$ % volume**
FC	1.439	46.803	3.357	46.828
70% FC	1.182	23.229	2.757	23.235
60% FC	1.096	21.265	2.557	20.660
30% FC	0.839	14.274	1.958	15.207
20% FC	0.753	10.370	1.758	11.638
0% FC	0.581	3.608	1.358	3.653

Discussion

One drawback of many drought indices is that they do not directly use soil water levels as part of the index, which can sometimes make it difficult to relate them to water stress experienced by plants (Hunt *et al.*, 2009). Hunt *et al.* (2009)'s proposed soil moisture index (SMI), based on observed water content and known field capacity, overcomes this limitation, by being logically related to water stress. Although the use of pot weight and % volume may not make use of any drought indices, it should provide a reasonable means of creating, maintaining and monitoring the drought stress experienced by potted plants.

Fawcett & Collis-George (1967) stated that their filter-paper method for determining soil moisture characteristics could only be used under special circumstances, with a set of specific conditions which had to be met for successful use. The same can be said for the system herein presented, as calibration would be critical to the success of the system, in terms of the use of the pot weights to determine water content. This is due to the fact that, given the length of the experiments, plant growth is to be expected, which in turn is likely to be affected by the drought treatment applied to the plant (see Chapter 4).

The 7L pots were found to dry out faster, which may seem counter-intuitive, due to the decreased surface area to volume ratio. This could, however, be explained by differences in the greenhouse conditions between 2010 and 2011, which was when the 3L and 7L pot measurements were taken, respectively. On the other hand, the % volume values at the determined percentages of field capacity were not found to differ significantly between the two pot sizes. As such, % volume can be thought of as not being affected by the pot evapotranspiration rates. Furthermore, use of % volume measurements provided the necessary consistency throughout the growing season and experimental periods, and allowed for changes in pot weight due to plant growth to be accounted for resulting in the consistent maintenance of the drought treatments.

The drought treatment levels were selected on the grounds of stressing the Sitka spruce saplings, but not the extent of inducing mortality in the short-term, during the experiments. This would allow for the saplings to be carried over across years. Furthermore, treatments were established to assess the response of *Elatobium abietinum* (Walker) to different types of drought stress - both continuous (proposed to affect insect herbivores by White (1969)) and intermittent (proposed by Huberty & Denno (2004)) drought stress, while also considering stress magnitude (as proposed by Mody *et al.* (2009)).

A final consideration must be given to the exclusion of rainwater from the pots. When outdoors, rainwater must be excluded from the experimental plants regardless of whether they are potted, as was the case for the Sitka spruce saplings used as part of this thesis, or whether they are planted directly into the soil. For the latter, rainwater exclusion is typically achieved through the use of 'roofs' positioned above the experimental plots (Björkman, 1998; Björkman & Larsson, 1999; McVean & Dixon, 2001; Staley *et al.*, 2006; Staley *et al.*, 2007).

As part of this thesis' work, one experiment was conducted solely outdoors in a semi-nursery environment (see Chapter 3), while for remaining experiments saplings were typically maintained outdoors and only brought into controlled conditions during the experiments themselves. In order to exclude rainwater from the pots, each sapling, while outdoors, was fixed with a well-sealed plastic skirt attached securely to the base of each tree using garden wire and insulating foam tape. In order to avoid moisture leaching into the pots from the ground, the pots were placed on raised pallets. This had the added advantage of air pruning the sapling roots in the pots.

Conclusions

1. Results indicate that, with calibration, using pot weight and % volume can provide a quick and reliable monitoring system to use as part of the experimental procedures.
2. Although some accuracy may be sacrificed in the case of pot weight, this should be compensated for due to the % volume being used in conjunction to determine how much, and how often, water is added to the pots.

Chapter 3

Drought stress effects on *Elatobium abietinum* in an outdoor nursery experiment

Introduction

Climate change in the U.K. is predicted to result in milder winters, as well as an increased frequency of hot and dry summers (Evans *et al.*, 2002; Murphy *et al.*, 2009; Day *et al.*, 2010). Changes to the climate and their effect on insect pest populations, particularly aphids, have long been recognised as a serious issue facing agriculture and forestry (Bale *et al.*, 2002). The green spruce aphid, *Elatobium abietinum* (Walker), is one species expected to respond to such changes, and to which such concerns are of particular relevance. It is the most serious defoliating pest on Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in Britain (Straw, 1995; Evans *et al.*, 2002). Infestations lead to the rapid appearance of yellow chlorotic bands on one-year-old and older needles as a result of feeding damage (Parry, 1971; Fisher, 1987). This leads to premature needle loss and can further cause reductions in growth (Straw *et al.*, 1998a; Straw *et al.*, 2000).

Populations of *E. abietinum* in Britain are anholocyclic, taking advantage of the maritime climate, and are currently limited by freezing periods in the winter (Day & Crute, 1990). As such, any increase in winter temperatures is likely to affect overwinter survival and lead to higher abundance in spring(Carter, 1989; Straw, 1995). Sitka spruce is particularly susceptible to damage by *E. abietinum* (Fisher, 1987; Nichols, 1987), and, owing to the tree species' preference for maritime climates (Samuel *et al.*, 2007), with relatively cool and wet summers, Sitka spruce is also likely to be severely affected by drought conditions. As such, attempts have long been made to model potential effects of drought on the suitability and growth of Sitka spruce (Jarvis & Mullins, 1987).

Very few studies have explored the effect of stress on population performance of arboreal aphids (Koricheva *et al.*, 1998), though some have been conducted on the effect on tree growth. Ditmarová *et al.* (2009) explored the drought response of four-year-old Norway spruce (*Picea abies* Karst.) seedlings, and observed that this included a number of physiological and biochemical changes occurring in parallel. These alterations enhanced the ability of plants to survive drought periods.

It has been suggested that current knowledge renders it unreasonable to expect that the general response of aphids to climate change may be predicted on the basis of any single plant factor or component (Pritchard *et al.*, 2007). Adler *et al.* (2007), for example, were unable to support the prediction that warming increased aphid abundance or population growth in an aphid-sagebrush interaction, based on a field study. Conversely, some studies have suggested that stress can cause a response in aphid population dynamics (Major, 1990; McVean & Dixon, 2001).

Several other factors are also known to affect population size of *E. abietinum* on Sitka spruce, such as nutrient and light availability. In a potted plant study, larger aphid populations developed on trees grown under high nutrient conditions, though greater needle loss occurred in low nutrient treatments, resulting in similar defoliation rates (Straw & Green, 2001). In another study on potted plants, *E. abietinum* population density was found to be significantly increased, between three- to four-fold, under shaded conditions (Bertin *et al.*, 2010).

The effects of infestation on tree and root growth are related to aphid density, reductions in which were exacerbated under low nutrient and light conditions (Bertin *et al.*, 2010; Straw & Green, 2001). Needle loss has been shown to be strongly correlated with aphid density, with a local influence of aphids and defoliation on shoot growth within the canopy (Straw *et al.*, 1998b). This in turn can be affected by aphid position in the canopy, which changes throughout a season (Straw *et al.*, 2006). Reductions in growth increments appear to be caused by *E. abietinum*'s effect on shoot extension during infestation (Straw *et al.*, 2000).

It has been suggested that, in field situations, aphid density is a reflection of changes in tree vigour rather than the result of changes to host quality, based on aphid density, defoliation rates and growth losses under high and low aphid populations (Straw *et al.*, 2005). In another potted plant study, however, Williams *et al.* (2005) observed that while defensive mechanisms in Sitka spruce are not triggered by high spring populations of *E. abietinum*, such populations improve host nutritional quality for autumn generations.

Though experimental work is conducted at various levels, the principles applied are consistent, regardless of whether the work is conducted on potted plants or in the field. Potted plants present an opportunity to assess effects of various factors on aphids in a semi-field, nursery or laboratory environment while avoiding the technical difficulties associated with using large trees. The technique has been used in many studies (e.g. Warrington & Whittaker (1990); Straw & Green (2001); Williams *et al.* (2005); Ditmarová *et al.* (2009); Bertin *et al.* (2010), *inter alia*). Whereas field trials assessing the effect of *E. abietinum* on Sitka spruce require uninfested tree treatments to be obtained by spraying experimental trees with insecticide, potted plants allow for experiments to start with insect-free material (Straw *et al.*, 1998a). Although this achieves the same kind of comparison, field experiments are dependent on high aphid populations occurring naturally. Furthermore, phytotoxic and growth promoting effects of insecticides must be considered before application. In studies on potted plants, defoliated trees are produced by artificial inoculation with aphids, but the trees are necessarily small and it is therefore often difficult to extrapolate or be confident that results can be applied to older trees in the field.

The study presented here was established to assess the impact of drought stress and *E. abietinum* infestation on Sitka spruce performance in a nursery environment, and to establish whether differing levels of drought stress affected aphid population size and dynamics.

Aims and Objectives

1. Determine whether spring drought stress improves host-plant quality for *E. abietinum*, leading to more rapid population development.
2. Investigate whether spring-summer drought advances the onset of dormancy in Sitka spruce, enabling increased aphid populations earlier in the autumn.
3. Test the hypothesis that a stronger response is shown during a second year of spring-summer drought stress.
4. Establish whether host tolerance, in terms of needle retention, varies with drought stress.

Materials and Methods

Drought treatments

Five drought levels were explored in this study:

1. Field capacity (**FC**) - Plants maintained at field capacity;
2. Moderate continuous stress (**MS**) - Plants maintained at 60% of field capacity;
3. Continuous severe stress (**CS**) - Plants maintained at 20% of field capacity;
4. Intermittent severe stress (**IS1**) - Plants subjected to fluctuating stress, whereby plots were allowed to dry out to 20% field capacity, and were then watered back up to field capacity;
5. Intermittent moderate stress (**IS2**) - Plants subjected to fluctuating stress, whereby plots were allowed to dry out to 30% field capacity, and were then watered back up 70% of field capacity.

These treatments were maintained using a combination of pot weight and soil moisture content, as established on pots in a greenhouse (see Chapter 2). The soil moisture was measured as percentage volume using an SM200 Soil Moisture Sensor and an HH2 Meter

(Delta T Devices, Cambridge). Pots were checked daily to ensure the correct application of the drought treatments.

Plant material

Two-year-old Sitka spruce saplings (vegetatively propagated, Ident. QSS 04 (0R18TE)) were obtained from the Forestry Commission Delamere Nursery, Cheshire (U.K.) during the winter in 2010. These were potted up in 3L pots, using a standard 2:1:1 peat, bark and perlite growing medium mixed at Alice Holt Forest Research Station, Surrey (U.K.). They were also supplied with 20g of Osmacote® Plus controlled release granular fertiliser (16%N + 8%P + 11%K + 2%MgO; Scotts Ltd, U.K.), which was mixed into the growing medium.

The same trees were utilised for the second year of the experiment. As such, in early March 2011, trees which had survived the winter were re-potted into 7L pots with the same growing medium as was used in the first year (mixed at Alice Holt Forest Research Station, Surrey, U.K.). They were then returned to their pallets, and left to re-establish for one week before restarting the drought treatments. During this time, they were all well-watered. Trees which had not survived were removed from the experiment (see Table 3.1).

Each pot was routinely and regularly weeded throughout the duration of the experiment. Each tree was also checked daily for the presence of *Cinara pilicornis*; when these aphids were found, they were immediately removed using a fine paintbrush. Furthermore, non-aphid treatment trees were also examined for *E. abietinum*, and where any were found these were also removed.

Experimental design

A total of 250 Sitka spruce trees were maintained outdoors on raised platforms, in a 5 x 5 Latin square (Figure 3.1). Each pallet was assigned ten trees, which were then numbered (Figure 3.2). Each tree was allocated an aphid treatment (with or without aphids) at random, such that five trees per pallet were infested and the other five were not. Trees were positioned such that there was no contact between individuals, to ensure that the

Table 3.1:

Number of trees surviving into the second year of the field trial. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. A+ = With aphids; A- = Without aphids.

Drought treatment	**Aphid presence**	**N. surviving**	**N. dead**
FC	*A+*	19	6
	A-	17	8
MS	*A+*	23	2
	A-	22	3
CS	*A+*	20	5
	A-	21	4
IS1	*A+*	15	10
	A-	21	4
IS2	*A+*	14	11
	A-	16	9

apterous aphids were unable to disperse between infested and uninfested trees. The trees were watered using an automatic irrigation system, which was monitored regularly by checking the weight and soil moisture content of the pots.

Each tree was fitted with a sealed plastic skirt to ensure that rainwater did not reach the growing medium. These skirts were attached securely to the base of each tree using garden wire and insulating foam tape, to create a water-proof seal that caused minimal damage to the plant. Strong duct tape was used to seal joins in the plastic. The skirts were regularly inspected to maintain seal and condition, as were the ties at the base of each tree to ensure that they were not restricting growth. Skirts were attached to coincide with the start of drought treatment in both years.

Stock cultures of *E. abietinum* were maintained and used for the inoculation of trees.

Figure 3.1: Field experiment set-up, showing 250 Sitka spruce on raised platforms in a 5 x 5 Latin square.

Figure 3.2: Pallet set-up in the field experiment. Ten Sitka spruce were randomly assigned to each pallet. Each pallet was assigned a drought treatment and was individually irrigated.

These were reared on cut branches in buckets of water in a CT room at 15 °C, with 70% RH and a 16:8 photoperiod. In 2010, the branches were initially sourced from Hafren Forest, Wales (U.K.), with further branches obtained from Alice Holt Forest Research Station, Surrey (U.K.). In 2011, all branches were sourced from Alice Holt.

Year 1

Trees were inoculated on the 21st April 2010 by tying three 3cm lengths of aphid-infested shoots from stock *E. abietinum* cultures to the designated trees using black cotton thread. Each shoot section had approximately thirty apterous aphids. One section was tied to the leader shoot, and two others to side shoots. The inoculation sections were removed after one week (27th April 2010), which was sufficient time for the aphids to move onto the experimental trees.

The drought treatments were maintained until mid-November 2010, after which the irrigation system was switched off and the plastic skirts removed. Trees were left to over-winter, though they were routinely checked.

Year 2

In 2011, trees were inoculated on the 28th March, and then again on the 28th April as the initial attempt did not succeed. Six 3cm lengths of aphid-infested shoots from the stock *E. abietinum* culture were attached to the designated trees with black cotton thread. In the initial attempt, each shoot section had approximately 35 apterous aphids, whereas in the second attempt there were only approximately 20 apterous aphids per section. Two sections were tied onto the leader shoot; one on the one-year-old section, and one on the two-year old section. The remaining four were tied onto side shoots; two on one-year-old sections, and two one two-year-old sections. Additionally, sections were only tied where there were needles for the aphids to migrate onto. The inoculation sections were removed after one week, to allow sufficient time for the aphids to move onto the experimental trees.

Drought treatments were terminated in mid-November 2011. The plastic skirts were removed and trees were again left to overwinter until January, when final plant growth measurements were taken (refer to Chapter 4).

Aphid counts and density

It was necessary to utilise a non-damaging method of data collection in order to be able to retain trees over the period of two years, and the protocol used was as described by Straw *et al.* (1998b). The number of *E. abietinum* visible through a 3cm window cut into a small plastic sheet were counted directly on the shoots in a number of set positions, as follows:

Year 1:

One count against the leading shoot on a one-year-old section;

Two counts on the underside of two side shoots on one-year-old sections.

Year 2:

One count against the leading shoot on a one-year-old section;

One count against a two-year-old section of leading shoot;

Two counts on the underside of two side shoots on one-year-old sections;

Two counts on the underside of two side shoots on two-year-old sections.

Counts were taken weekly on all aphid-treated inoculated plants between the 29th April and 05th October 2010, and between the 04th April and 25th October 2011.

Aphid density

Weekly aphid counts (E_S) were summed together for each tree and converted to estimates of aphids·100 needles^{-1} (EN) to allow for comparison between drought treatments, using the following equation:

$$EN = 100 \cdot \left(\frac{E_S}{(2 \cdot N_S)} \right) \tag{I}$$

where (N_S) was the needle count. When no aphids or needles were observed (i.e. where $E_S = 0$ or $N_S = 0$), it was assumed that no aphids were present and that $EN = 0$.

Needle counts and retention

Needle counts were taken using the same method as per the aphid counts, with the exception that a recording was made on each of the experimental trees. The number of needles defined by the edges of the 3cm windows and originating down one side of the shoot were counted from photographs taken of each location. This was such that the needles could be counted when time allowed and with greater accuracy.

Counts were taken weekly on all aphid-treated inoculated plants between the 29th April and 05th October 2010, and between the 04th April and 25th October 2011.

Percentage needle loss was then calculated based on readings taken on the first week and final week of counting for each year.

Statistical analysis

The effect of drought stress on EN data within each week were analysed using linear mixed effects models to take into account pseudoreplication. The data was cube-root transformed for normality. Drought was modelled as a fixed effect, while pallet was modelled as a random effect (groups: pallet = 5; estimated d.f. for each parameter = 4).

The effect of drought and aphid infestation on percentage needle loss, as well as their interaction, were also analysed using linear mixed effects models, though transformation of data was unnecessary. When assessing needle loss within the years, drought and aphid presence were modelled as fixed effects, while pallet was modelled as a random effect (groups: pallet = 5; estimated d.f. for each parameter: drought = 4, aphid presence = 1). When comparing the rate of one-year-old needle loss between years, drought, aphid presence and year were modelled as fixed effects, while pallet was modelled as a random effect (groups: pallet = 5; estimated d.f. for each parameter: drought = 8, aphid presence = 5, year = 1).

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

Aphid density in the first year of drought treatment (2010)

Counts of *E. abietinum* showed typical population dynamics with a spring peak under all drought treatments during the first year of drought stress application (Figure 3.3). Analyses conducted on each week are summarised in Table 3.2. Significant differences in EN on drought stressed trees were observed at weeks eight (${\chi^2}_4 = 18.386$, $P < 0.01$, Figure 3.4(A)), nine (${\chi^2}_4 = 22.098$, $P < 0.001$, Figure 3.4(B)), twelve (${\chi^2}_4 = 16.440$, $P < 0.01$, Figure 3.4(C)) and twenty-one (${\chi^2}_4 = 16.496$, $P < 0.01$, Figure 3.4(D)).

The highest point in the aphid peak was achieved under all drought treatments in week five (Figure 3.3), though there was no significant difference between the size of the peaks. A significantly higher EN was maintained at weeks eight and nine on trees under the IS2 drought treatment ($t = 3.41$ and 3.24 respectively, $P < 0.05$). At week twelve, EN under the MS drought treatment were higher than for the other treatments ($t = 2.16$, $P < 0.05$). While aphids on CS trees did not show significantly higher population densities during the spring peak, there was a second peak in the autumn in this treatment alone at week twenty-one ($t = 2.59$, $P < 0.05$).

Aphid density in the second year of drought treatment (2011)

During the second year of drought stress application, *E. abietinum* counts again showed typical population dynamics under all drought treatments (Figure 3.5). Analyses conducted on each week are summarised in Table 3.3. Significant differences in EN on drought stressed trees were observed at weeks five (${\chi^2}_4 = 32.572$, $P < 0.001$, Figure 3.6(A)), six (${\chi^2}_4 = 11.396$, $P < 0.05$, Figure 3.6(B)), nine (${\chi^2}_4 = 10.912$, $P < 0.05$, Figure 3.6(C)), ten (${\chi^2}_4 = 17.463$, $P < 0.01$, Figure 3.6(D)), eleven (${\chi^2}_4 = 21.148$, $P < 0.001$, Figure 3.6(E)), twelve (${\chi^2}_4 = 11.732$, $P < 0.05$, Figure 3.7(A)), thirteen (${\chi^2}_4 =$

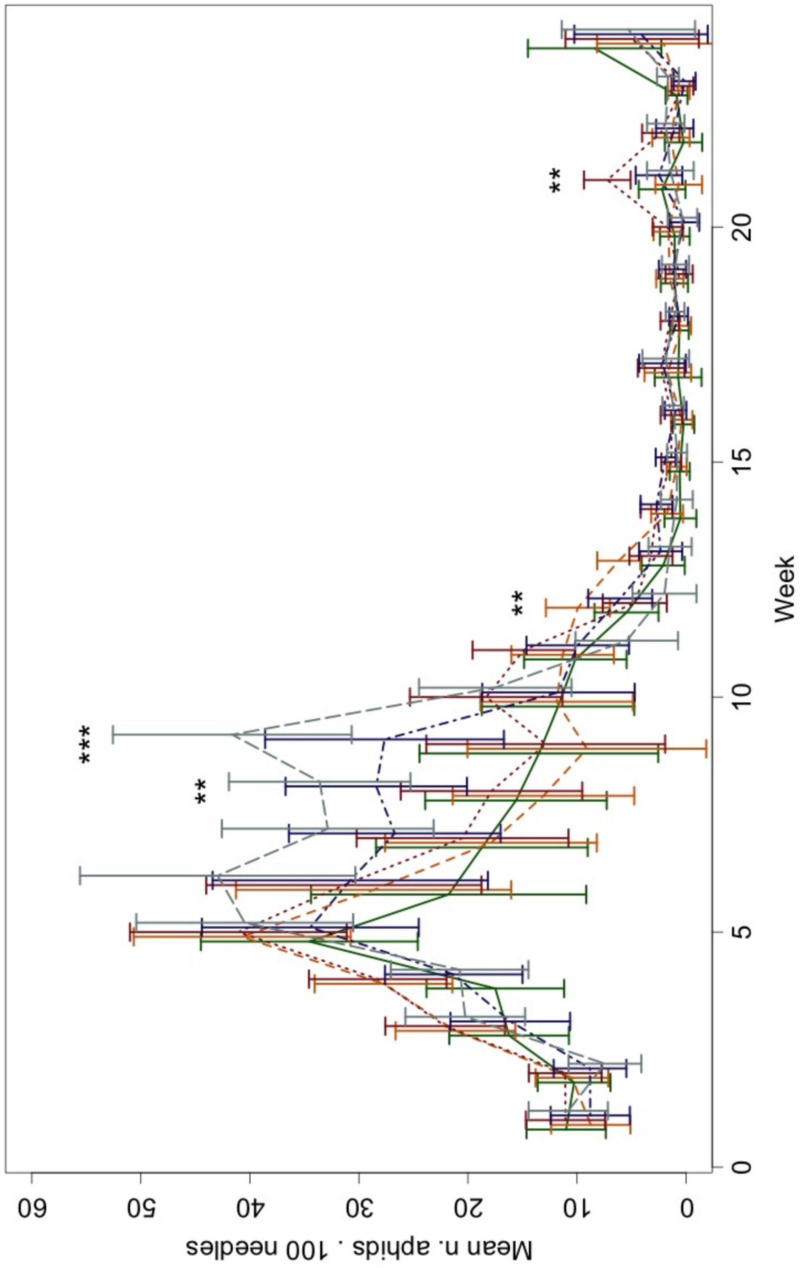

Figure 3.3: Mean number of aphids·100 needles in 2010. Bars indicate Least Significant Difference. Green = FC (field capacity); Orange = MS (60% field capacity); Red = CS (20% field capacity); Blue = IS1 (allowed to fluctuate from field capacity to 20% field capacity); Grey = IS2 (allowed to fluctuate from 70% to 30% field capacity). *** = P < 0.001; ** = P < 0.01; * = P < 0.05. Drought treatment means offset along x-axis at each weekly point, to allow for clear distinction between bar overlap.

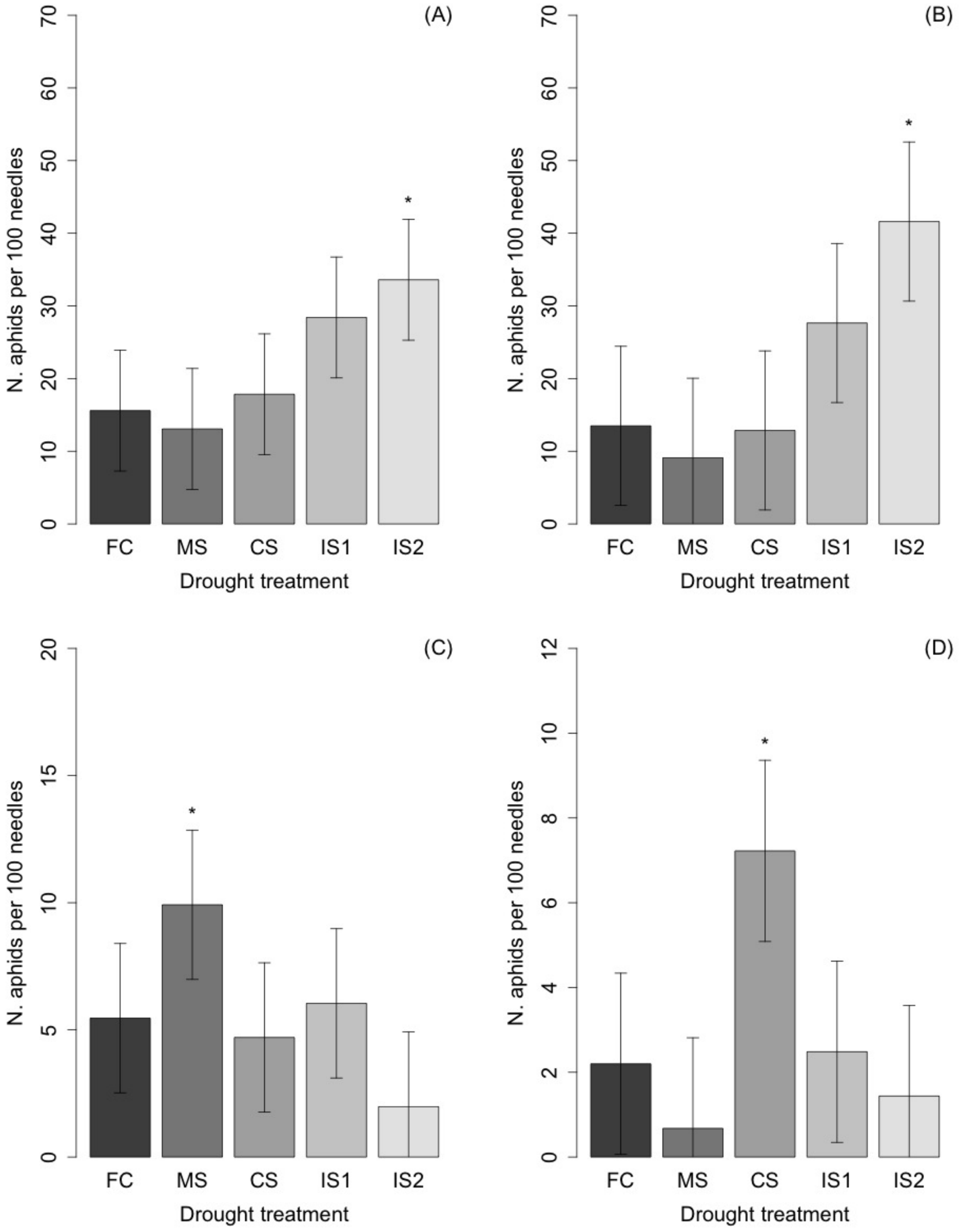

Figure 3.4: Mean number of aphids·100 needles in 2010, at weeks eight (A), nine (B), twelve (C) and twenty-one (D). Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = P < 0.001; ** = P < 0.01; * = P < 0.05.

18.136, $P < 0.01$, Figure 3.7(B)), fourteen (${\chi^2}_4 = 13.65$, $P < 0.01$, Figure 3.7(C)) and twenty-six (${\chi^2}_4 = 20.338$, $P < 0.001$, Figure 3.7(D)).

While the population was building in spring, higher EN was observed at week five under CS, IS1 and IS2 drought treatments ($t = 4.12$, $t = 3.12$ and $t = 3.88$ respectively, $P < 0.05$). Despite this, at week six, EN was reduced under the IS1 treatment ($t = 2.06$, $P < 0.05$). The spring peak was achieved in week nine for all drought treatments except IS2, under which the peak was observed in week ten (Figure 3.5). No difference in peak size was observed in week nine (under FC, MS, CS and IS1 drought treatments), but the results suggest a higher peak population under IS2 drought treatment as, during week ten, EN was higher only on IS2 trees ($t = 2.40$, $P < 0.05$.) Furthermore, though no differences were observed in comparison with the FC control in week nine, EN on CS and IS1 trees was lower than that observed on IS2 trees ($t = 2.76$ and $t = 2.99$ respectively, $P < 0.05$). In weeks eleven through to fourteen, EN under the IS2 drought treatment continued to be consistently higher than under the other drought treatments (Week 11: $t = 2.74$, $P < 0.05$; Week 12: $t = 2.99$, $P < 0.05$; Week 13: $t = 3.68$, $P < 0.05$; Week 14: $t = 3.39$, $P < 0.05$). As observed during 2010, only aphids on CS trees exhibited an autumn peak in numbers, at week twenty-six ($t = 3.53$, $P < 0.05$).

Needle loss

A summary of mean percentage needle loss values is presented in Table 3.4. In the first season of drought treatment, during 2010, drought stress was not found to significantly affect the percentage needle loss of Sitka spruce over twenty-four weeks (${\chi^2}_4 = 6.34$, $P > 0.05$, Figure 3.8). Infestation with *E. abietinum*, however, was found to have a significant impact (${\chi^2}_4 = 313.92$, $P < 0.001$), with a higher percentage loss on trees where aphids were present ($t = 7.65$, $P < 0.01$). There was no interaction between drought and aphid presence (${\chi^2}_4 = 4.13$, $P > 0.05$).

In 2011, during the second year of drought treatment, a similar overall response was observed (Figure 3.9A). Although drought did not significantly affect percentage needle loss (${\chi^2}_4 = 2.89$, $P > 0.05$), aphid presence caused a significant impact (${\chi^2}_1 = 268.87$, $P < 0.001$), with a higher percentage needle loss on trees where aphids were present (t =

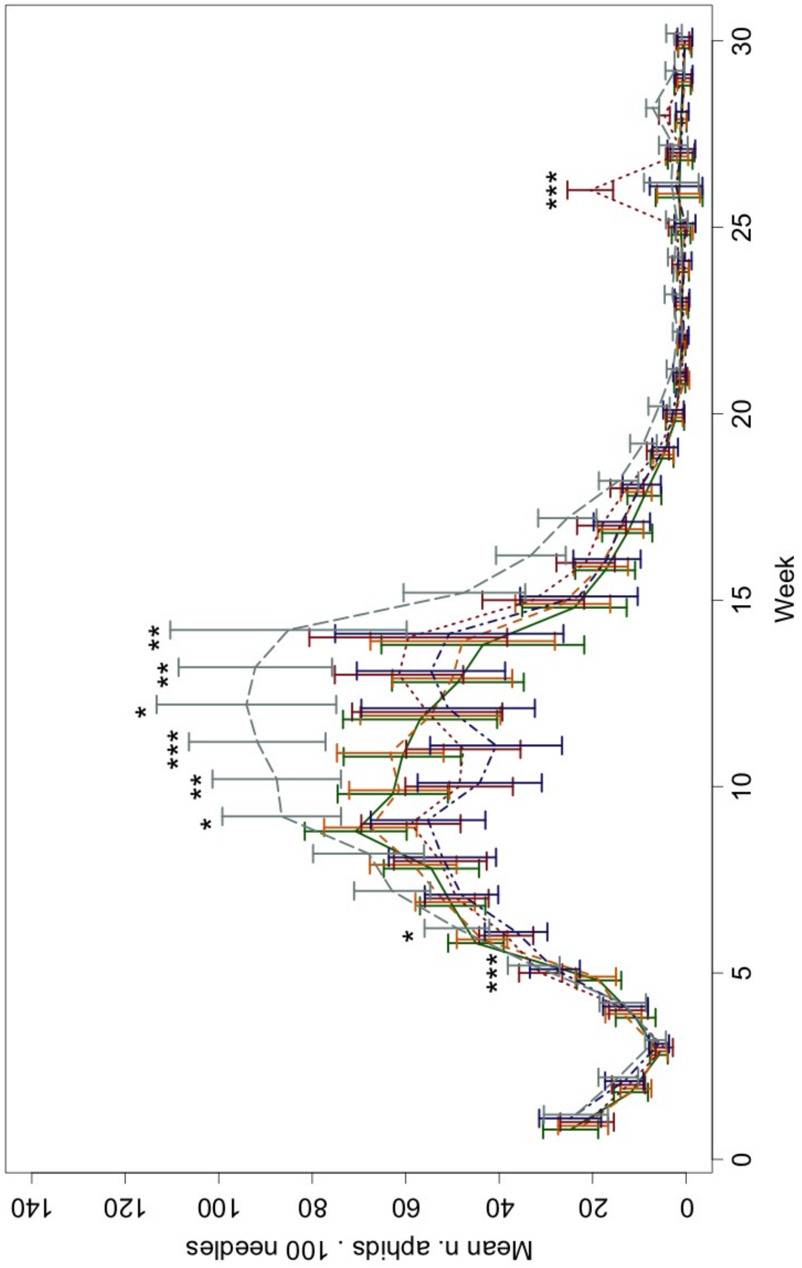

Figure 3.5: Mean number of aphids·100 needles in 2011. Bars indicate Least Significant Difference. Green = FC (field capacity); Orange = MS (60% field capacity); Red = CS (20% field capacity); Blue = IS1 (allowed to fluctuate from field capacity to 20% field capacity); Grey = IS2 (allowed to fluctuate from 70% to 30% field capacity). *** = P < 0.001; ** = P < 0.01; * = P < 0.05. Drought treatment means offset along x-axis at each weekly point, to allow for clear distinction between bar overlap.

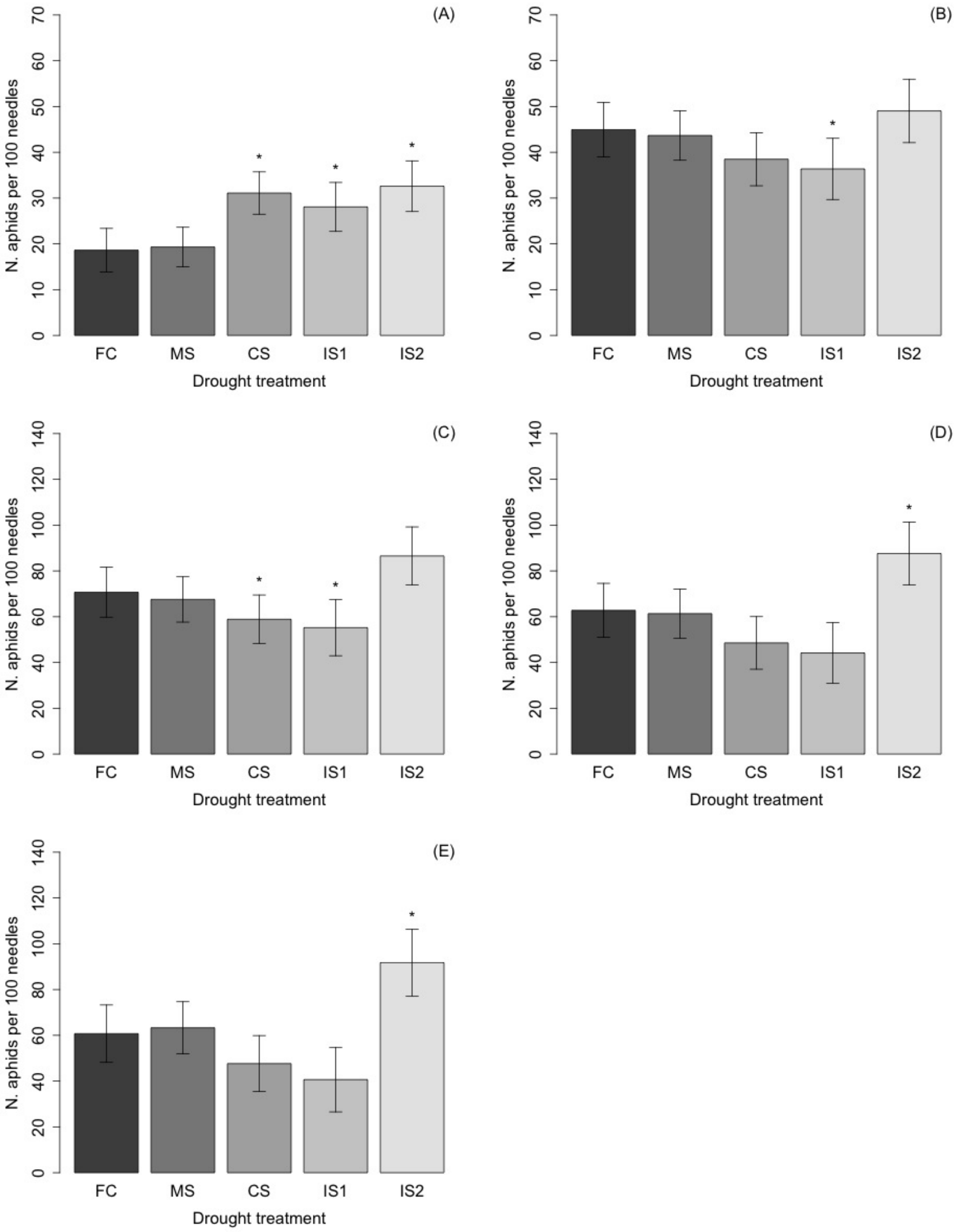

Figure 3.6: Mean number of aphids·100 needles in 2011, at weeks five (A), six (B), nine (C), ten (D) and eleven (E). Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

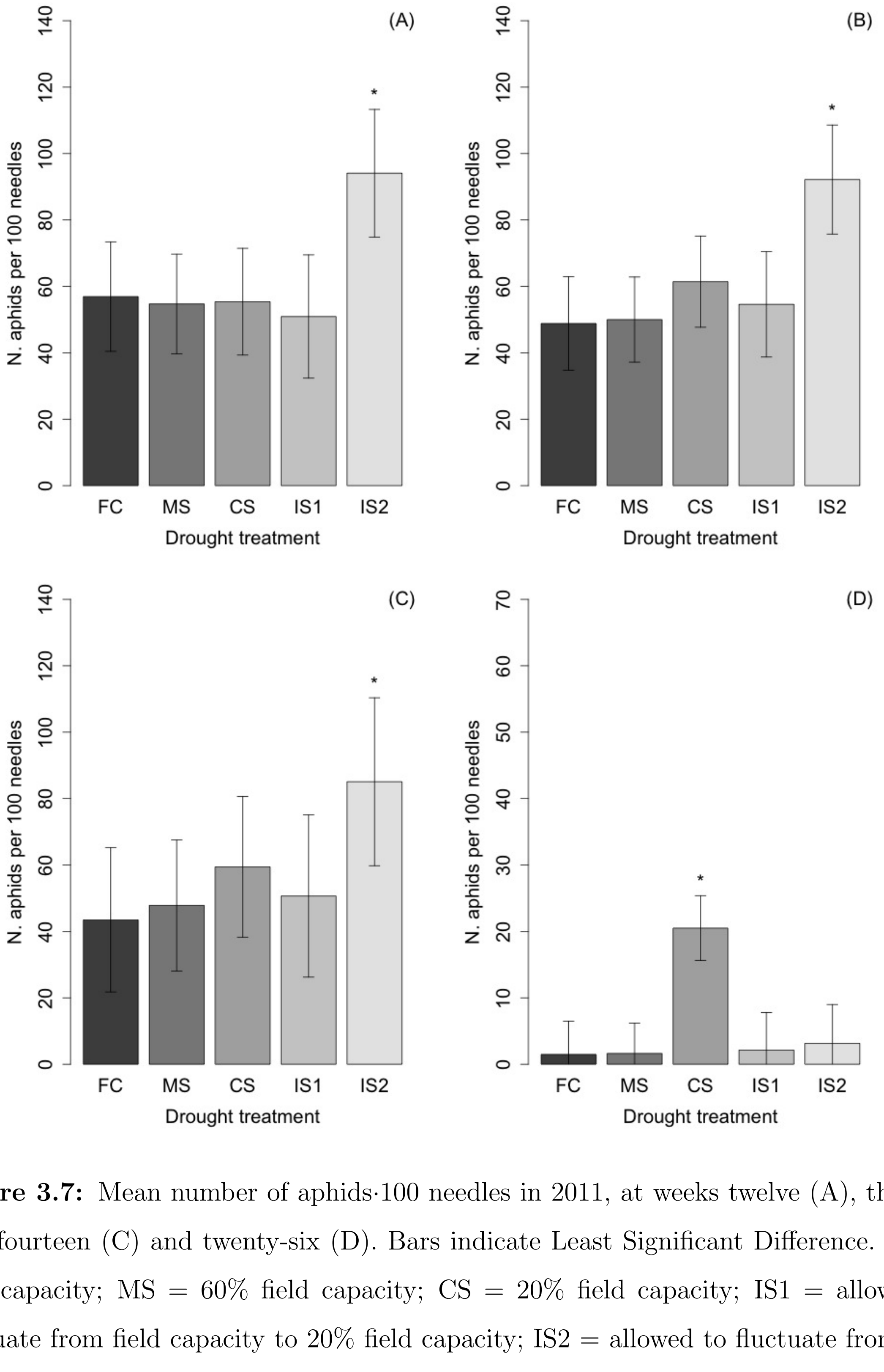

Figure 3.7: Mean number of aphids·100 needles in 2011, at weeks twelve (A), thirteen (B), fourteen (C) and twenty-six (D). Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

6.65, $P < 0.01$). There was also no interaction between drought and aphid presence ($\chi^2{}_4$ = 2.90, $P > 0.05$).

Similar patterns were observed in 2011 when both one-year-old needles (drought: $\chi^2{}_4$ = 6.07, $P > 0.05$; aphid presence: $\chi^2{}_1$ = 155.01, $P < 0.001$; Figure 3.9B) and two-year-old needles (drought: $\chi^2{}_4$ = 7.07, $P > 0.05$; aphid presence: $\chi^2{}_1$ = 5379.78, $P < 0.001$; 3.9C) were analysed separately. In both cases, a higher percentage needle loss was observed on aphid-infested trees (1-yr-old needles: t = 3.73, $P < 0.05$; 2-yr-old needles: t = 30.60, $P < 0.05$). Additionally, an interaction between drought treatment and aphid presence was observed for the percentage needle loss of one-year-old needles ($\chi^2{}_4$ = 11.27, $P < 0.05$).

Comparison of one-year-old needle percentage loss

While no three-way interaction was observed between year, drought treatment and aphid presence ($\chi^2{}_4$ = 9.47, $P > 0.05$), a two-way interaction was observed between drought treatment and aphid presence ($\chi^2{}_4$ = 13.80, $P < 0.01$). Furthermore, while there was no significant difference between the 2010 and 2011, both drought ($\chi^2{}_4$ = 11.13, $P < 0.05$) and aphid presence ($\chi^2{}_1$ = 464.83, $P < 0.001$) were found to have independent effects on needle loss.

Discussion

The effect of drought stress on Sitka spruce and on the population dynamics of *E. abietinum* is not yet well understood, and the interactions and effects of the aphid may be exacerbated or ameliorated by the effects of drier summers due to climate change. What information is available is not consistent, and while some literature suggests that higher aphid populations should be found on continuously or intermittently stressed plants, various studies have not observed this.

McVean & Dixon (2001), for example, observed that fewer *Acyrthosiphon pisum* (Harris) were found on drought stressed plants when compared with well watered controls. Conversely, Huberty & Denno (2004) suggested that intermittent stress levels may provide optimal conditions for aphids due to a periodic return to suitable turgor pressure for feeding, allowing aphids to access the improved quality sap. This explains the findings

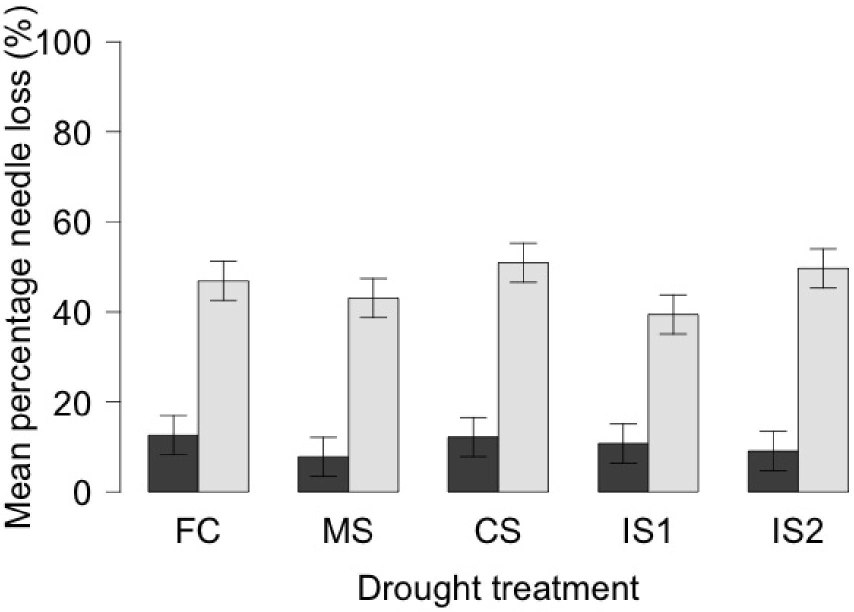

Figure 3.8: The effect of drought stress and *E. abietinum* infestation on percentage needle loss in Sitka spruce in a first year of drought treatment (2010). Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

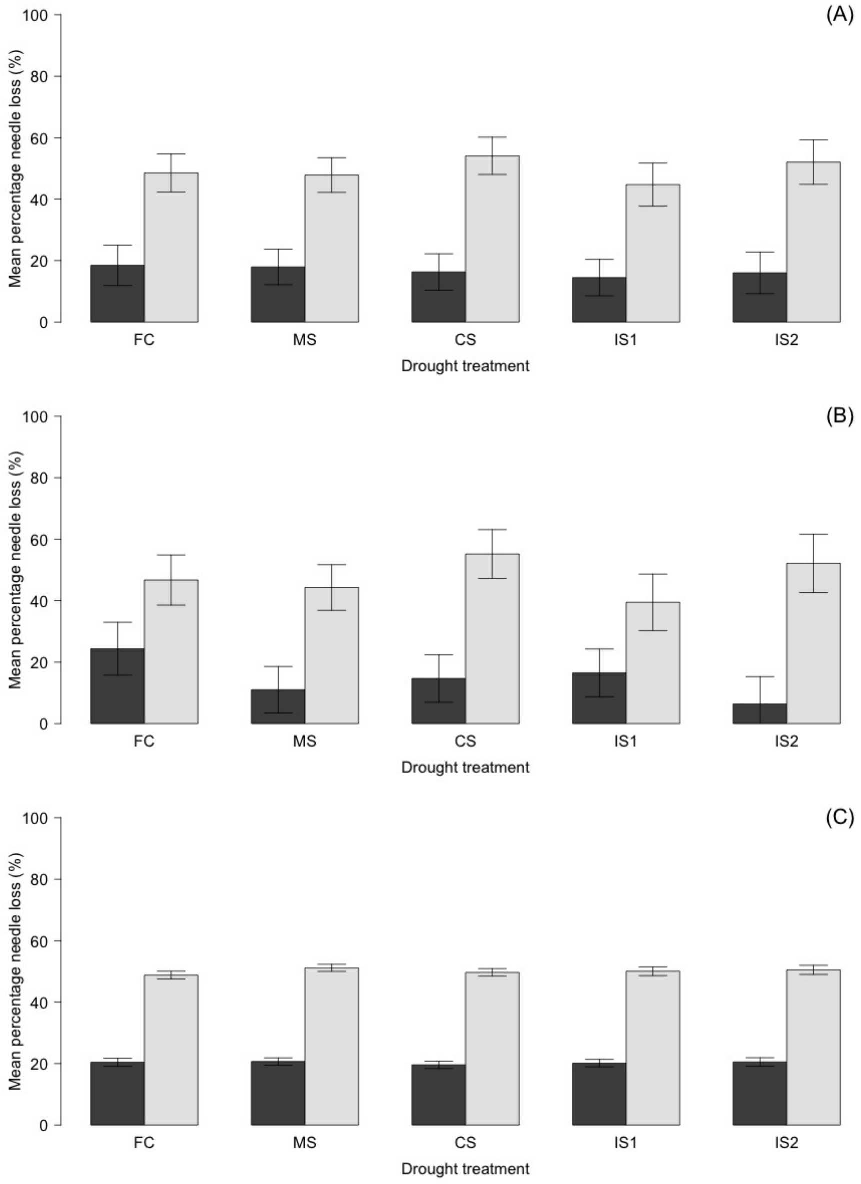

Figure 3.9: The effect of drought stress and *E. abietinum* infestation on percentage needle loss in Sitka spruce after a second year of drought treatment (2011)). Bars indicate Least Significant Difference. (A) Overall needle loss; (B) One-year-old needle loss; (C) Two-year-old needle loss. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

of Major (1990) who, in a study of drought effects on Sitka spruce and *E. abietinum*, observed that intermittently stressed plants supported the highest populations of aphid, followed by well watered controls, with continuously stressed plants supporting the lowest aphid population. Furthermore, the mean number of aphids produced per adult per day was also found to be higher on intermittently stressed plants, with aphids on continuously stressed plants producing the least. Warrington & Whittaker (1990) also observed an effect of drought stress on Sitka spruce, though little effect of drought was found on aphid numbers. Although both drought and aphid attack were found to affect spruce growth, the impact of the combination of the two was not found to be additive, and it was suggested that the effects of both independently of each other were so severe that insufficient plasticity was left in the trees to show further growth reductions.

During the first year of drought stress, in 2010, the results suggested that there was no difference in the spring peak population size, although the peak was observed one week later under moderate intermittent drought treatment (IS2). This implies that aphid performance parameters, such as aphid fecundity and nymph survival are likely to be similar approaching the spring peak. Trees under moderate intermittent stress, furthermore, maintained a significantly higher population number for a longer period after the peak when compared with well-watered control trees. This suggests that, on trees under this treatment, *E. abietinum* may have had reduced mortality or have been able to maintain higher performance than on trees submitted to the other drought treatments. This may reflect an improvement in host plant quality, and matches the findings of Major (1990) in relation to *E. abietinum*, and support Mody *et al.* (2009) overall.

In 2011, during the second year of drought stress, a similar pattern was observed. Namely, higher aphid densities were supported by trees under moderate intermittent drought over the course of several weeks. Again, this suggests improved host nutritional quality and aphid performance under moderate intermittent drought, corroborated by the findings of Major (1990) and supporting Mody *et al.* (2009)'s drought stress hypothesis. Further observations can also be made. The peak 'behaviour' of the aphids differed between the types of drought treatment; populations on well-watered control trees and trees subjected to moderate continuous stress had a single peak before declining, while those under the severe-type stresses (CS and IS1) showed a double peak effectively prolonging the period of high aphid density. While the findings overall support Huberty & Denno (2004)'s 'Pulsed Stress Hypothesis', whereby sap-feeding herbivorous insects benefit from intermittent stress, this does not explain the double peak observed under continuous severe stress. Rather, under this drought treatment, White (1969)'s hypothesis is supported; simply, stress improved host quality for herbivorous insects.

Björkman & Larsson (1999) found that there was no significant difference between the build-up of *Cinara costata* (Zett.) aphid densities on control and drought stressed Norway spruce. This led them to speculate that, where drought is severe, tree physiology may be pushed beyond the point where stress effects are positive and instead become negative, with the net results being such that no effect is caused (Björkman & Larsson, 1999). Spruce provenance and the interaction between stress treatment and genotype has been shown to have no significant effect on aphid population performance (Larsson & Björkman, 1993). Variation between individual trees can, however, be large enough such that no statistical difference may be observed, as was found by Larsson & Björkman (1993).

The findings of Warrington & Whittaker (1990), who observed that the greatest impact of drought was on tree growth rather than on the aphids, imply that water stress did render the host less acceptable to the aphids. Their findings, and those of this study, can be explained by the observations of Huberty & Denno (2004), who suggested that intermittent stress would allow aphids to take advantage of favourable nutritional conditions while overcoming turgor pressure reductions. Differences in aphid density are primarily a reflection of changes to general tree vigour (Straw *et al.*, 2005), and it may be that under moderate intermittent stress tree vigour is maintained for longer after the spring peak while the vigour of trees under other treatments drops more rapidly.

Ramírez & Verdugo (2009) conducted a study on the effects of drought on a poplar hybrid (*Populus* spp.) and its aphid, *Chaitophorus leucomelas* Koch. They found that while water availability affected both tolerance and resistance by the tree to the aphid, there was no trade-off between the two mechanisms. Drought stressed trees allocated a greater proportion of resources to tolerance of the aphids, whereas unstressed, well watered trees allocated more to resistance, leading Ramírez & Verdugo (2009) to suggest that tolerance may have a lesser cost than resistance and that water availability could modulate a shift to either strategy.

Aphids are well-adapted to deal with heterogeneity in host plant nutritional quality (Pritchard *et al.*, 2007). As such, the effects of drought stress on the population dynamics of *E. abietinum* may not be affected or detectable on a short scale, such as in a single growing season. Furthermore, *E. abietinum* feed on one year old and older needles, which may reduce the effects of drought in the short term - there may be enough plasticity in an aphid's ability to adapt and respond to stress to overcome such changes in host quality in the short term. Other insects have been shown to be able to adapt to or tolerate drought stress by physiological or physical means. Larval performance of the wood borer *Hylotrupes bajulus* (L.) was not affected by drought stress on Scots pine seedlings, despite changes in wood characteristics (Heijari *et al.*, 2010), and large body size in the seed beetle *Callosobruchus maculatus* (Fab.) correlated with enhanced tolerance to water stress (Yoder *et al.*, 2010).

During the first season of drought stress, the apparent maintenance of higher aphid densities on the moderate intermittent drought treatment suggested that larger differences between treatments might be observed following a second season of drought treatment. Indeed, not only was a stronger response exhibited under this drought treatment (showed by a comparatively longer number of weeks with significantly higher aphid densities), but a different nature in the response of populations under the remaining drought treatments was also observed. Bertin *et al.* (2010) observed that light levels affected *E. abietinum* population growth and that these effects accumulated over time. Drought may elicit a similar, cumulative response.

Another observation of the study presented here was that only aphids on trees under continuous severe drought stress exhibited a second, autumn peak. This was true in both years of the study. New needles are chemically defended from aphid attack during the spring and early summer (Jackson & Dixon, 1996). It is the retention of this current growth, combined with the peak aphid population's occurrence in spring when new needles are protected, that results in trees generally not being killed by *E. abietinum* attack. Previous studies have suggested that spring infestation improves the nutritional quality of Sitka spruce for autumn generations of *E. abietinum* (Williams *et al.*, 2005), and this may explain why the autumn peak was observed. As there was no such peak in other treatments, however, it is likely that some other effect interacted with the drought treatment under continuous stress. Furthermore, *E. abietinum* population dynamics, though strongly affected by weather and climate, are also moderated by internal processes (Day & Kidd, 1998), and these may influenced the population responses. It may also be that, under continuous severe stress, dormancy of Sitka spruce is brought forward, resulting in an improved host quality which the aphids are able to take advantage of.

Various physical factors of needle structure are known to affect *E. abietinum* probing and feeding behaviour on Sitka spruce (Parry, 1971; Nichols, 1987). It is possible that drought causes further changes to such behaviour, potentially compensating any negative, or positive effects, which may have been conveyed by changes in host quality, though this is speculative.

Percentage needles loss was not affected by drought during an initial season of stress. It was, however, greatly increased under aphid infestation. No interaction was found between the presence of aphids and drought. Following the second season of drought stress this held true both overall and for two-year-old needles, however, in the case of one-year-old needles, the nature of the response differed. While a higher percentage needle loss was always observed for all treatments under aphid infestation, an interaction between drought treatment and aphid presence was also found. In the absence of aphids, needle loss was higher for well-watered control trees, whereas under moderate intermittent drought stress it was reduced. The interaction of drought treatment and aphid presence was also present when comparing between the response of the one-year-old needles in the two years, highlighting the difference in responses to drought and aphid presence between the two years.

Previous studies have shown that defoliation level is associated with aphid density (Straw & Green, 2001; Williams *et al.*, 2005). As no difference was found in peak population size, this may explain why drought did not appear to affect needle loss. Furthermore, low *E. abietinum* densities have been shown to result in high defoliation rates (Straw *et al.*, 2005), and where nutrients are limited these effects are exacerbated (Straw & Green, 2001). Hopmans *et al.* (2008) and Eyles *et al.* (2011) indicate that drought stress and nutrient deficiency increase needle shedding in *Pinus radiata*, and that this is associated with infestation by the Monterey pine aphid, *Essigella californica* (Essig). Severe drought may reduce nutrient uptake by the tree roots, such that low densities would nevertheless cause extensive needle loss, though this is entirely speculative.

Aphids are able to rapidly respond to changes in host plant nutritional quality, enabling them to take advantage of improvements in quality under drought. This study has shown that the response of *E. abietinum* populations to drought stress is complex, and is affected by the level of drought intensities and frequency. It has also highlighted the importance of longer-term study, as responses differ between years suggesting a cumulative effect of drought stress. Needle loss was always higher on trees under aphid attack, but the importance of drought level on needle retention was only observed after repeated seasons under drought stress. This suggests that the effect of drought stress on Sitka spruce and on *E. abietinum* populations may be cumulative.

Conclusions

1. *Elatobium abietinum* population development was not affected by drought stress. In a second year of drought stress, however, a higher peak number of aphids was observed under moderate intermittent stress.

2. Trees subjected to moderate intermittent stress supported higher aphid densities for longer around the spring peak, suggesting an improved host quality under this type and level and drought.

3. Spring-summer drought may advance the onset of dormancy in Sitka spruce under continuous severe drought stress, as shown by the treatment causing a peak in *E. abietinum* density in the autumn.

4. Host tolerance, in terms of needle retention, is most greatly affected by aphid attack, where a greater proportion of needles are lost under aphid attack. However, after a second year of drought stress, an interaction between drought and aphid presence is observed - where no aphids are present, a higher rate of needle loss is observed on well-watered controls whereas under moderate intermittent stress more needles are retained.

5. The effects of drought stress are likely to be cumulative. Additionally, the intensity and frequency of drought bouts cause different responses in the aphid populations.

Summary Tables

Table 3.2:

Summary of weekly *E. abietinum* aphids·100 needles^{-1} (EN) on Sitka spruce under five different drought levels, during the first year of the field trial (2010). FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

	FC	MS	CS	IS1	IS2		
Week	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\chi^2{}_4$	**P**
1	10.99 ± 1.62	8.74 ± 1.61	11.05 ± 2.14	8.79 ± 1.63	10.81 ± 2.36	0.683	n.s.
2	10.27 ± 1.75	10.46 ± 1.70	11.08 ± 2.50	8.81 ± 1.51	7.44 ± 1.09	0.604	n.s.
3	16.23 ± 1.88	21.15 ± 3.00	22.09 ± 2.89	16.12 ± 2.34	20.25 ± 3.18	3.529	n.s.
4	17.49 ± 2.32	27.75 ± 3.02	28.28 ± 5.07	21.31 ± 2.25	20.77 ± 2.89	7.215	n.s.
5	34.55 ± 5.33	40.70 ± 6.13	41.06 ± 5.49	34.46 ± 4.31	40.47 ± 4.92	1.926	n.s.
6	21.78 ± 5.85	28.66 ± 4.60	31.38 ± 6.54	30.80 ± 9.80	42.96 ± 7.65	8.983	n.s.
7	18.73 ± 4.86	17.89 ± 3.56	20.50 ± 4.64	26.72 ± 7.05	32.86 ± 5.12	5.324	n.s.
8	15.60 ± 3.64	13.07 ± 2.20	17.84 ± 2.95	28.41 ± 7.36	33.60 ± 4.85	18.386	< 0.01
9	13.50 ± 3.15	9.10 ± 2.63	12.87 ± 2.87	27.65 ± 9.44	41.61 ± 7.93	22.098	< 0.001
10	11.74 ± 2.18	11.86 ± 3.47	18.33 ± 4.59	11.71 ± 2.57	17.50 ± 4.08	1.773	n.s.
11	10.16 ± 2.48	11.32 ± 2.28	14.87 ± 3.45	9.94 ± 1.76	5.43 ± 1.43	5.966	n.s.
12	5.46 ± 1.62	9.92 ± 2.86	4.70 ± 1.41	6.04 ± 1.29	1.98 ± 0.66	16.440	< 0.01
13	2.10 ± 0.57	6.17 ± 2.17	3.22 ± 0.88	2.34 ± 0.81	1.47 ± 0.84	7.183	n.s.
14	0.52 ± 0.26	1.74 ± 0.56	2.70 ± 1.09	2.71 ± 1.07	0.85 ± 0.50	5.181	n.s.
15	0.58 ± 0.30	0.87 ± 0.27	1.35 ± 0.48	1.87 ± 0.80	0.82 ± 0.39	2.456	n.s.
16	0.23 ± 0.13	0.40 ± 0.30	1.34 ± 0.76	0.96 ± 0.57	1.19 ± 0.47	3.293	n.s.
17	0.72 ± 0.51	1.67 ± 1.21	2.28 ± 1.35	2.16 ± 1.26	1.86 ± 0.97	0.832	n.s.
18	0.62 ± 0.38	0.40 ± 0.22	1.48 ± 0.64	0.68 ± 0.42	1.01 ± 0.45	2.836	n.s.
19	1.07 ± 0.48	1.49 ± 0.59	0.64 ± 0.31	1.25 ± 0.93	0.97 ± 0.66	1.949	n.s.
20	1.04 ± 0.76	1.62 ± 0.77	1.71 ± 1.03	0.15 ± 0.15	0.34 ± 0.23	4.998	n.s.
21	2.20 ± 0.84	0.68 ± 0.39	7.22 ± 1.93	2.48 ± 0.79	1.44 ± 0.52	16.496	< 0.01
22	0.23 ± 0.16	1.39 ± 0.93	2.32 ± 1.26	1.03 ± 0.87	1.86 ± 1.02	2.350	n.s.
23	0.87 ± 0.38	0.68 ± 0.49	0.33 ± 0.33	0.12 ± 0.12	1.66 ± 0.90	5.304	n.s.
24	8.38 ± 3.74	2.05 ± 1.19	4.94 ± 2.56	4.13 ± 2.16	5.29 ± 4.49	4.322	n.s.

Table 3.3:

Summary of weekly *E. abietinum* aphids·100 needles^{-1} (EN) on Sitka spruce under five different drought levels, during the second year of the field trial (2011). FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

	FC	MS	CS	IS1	IS2		
Week	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2_4	**P**
1	24.70 ± 3.26	22.03 ± 1.47	21.18 ± 2.38	24.81 ± 2.88	23.59 ± 4.11	0.848	n.s.
2	11.79 ± 1.64	10.73 ± 1.08	12.31 ± 1.50	13.22 ± 2.76	14.49 ± 2.04	2.355	n.s.
3	5.83 ± 1.03	5.70 ± 0.88	4.68 ± 1.05	5.79 ± 1.15	6.53 ± 1.46	1.366	n.s.
4	10.80 ± 2.40	13.39 ± 1.08	12.34 ± 1.49	12.96 ± 2.44	13.54 ± 2.28	3.623	n.s.
5	18.63 ± 2.79	19.31 ± 2.58	31.10 ± 2.27	28.07 ± 2.53	32.60 ± 3.00	32.572	< 0.001
6	44.97 ± 3.20	43.67 ± 2.52	38.49 ± 2.39	36.37 ± 3.10	49.04 ± 2.29	11.396	< 0.05
7	49.94 ± 3.85	51.55 ± 3.05	49.07 ± 3.15	48.06 ± 2.92	62.89 ± 4.96	7.999	n.s.
8	54.50 ± 3.98	58.38 ± 4.35	52.60 ± 4.76	52.13 ± 4.44	67.94 ± 4.80	7.357	n.s.
9	70.69 ± 7.91	67.55 ± 6.25	58.87 ± 5.00	55.21 ± 5.13	86.53 ± 6.90	10.912	< 0.05
10	62.77 ± 8.28	61.36 ± 6.35	48.56 ± 5.66	44.15 ± 5.71	87.60 ± 8.70	17.463	<0.01
11	60.73 ± 8.44	63.29 ± 6.84	47.66 ± 5.06	40.65 ± 6.07	91.76 ± 10.25	21.148	<0.001
12	56.92 ± 8.81	54.71 ± 6.97	55.38 ± 6.07	50.92 ± 7.90	94.07 ± 13.18	11.732	<0.05
13	48.82 ± 4.57	50.00 ± 5.29	61.42 ± 5.63	54.58 ± 7.04	92.18 ± 12.41	18.136	<0.01
14	43.49 ± 4.04	47.82 ± 3.68	59.44 ± 6.83	50.66 ± 6.55	85.08 ± 19.66	13.650	<0.01
15	23.89 ± 2.90	26.39 ± 2.56	32.70 ± 3.50	22.93 ± 2.12	47.44 ± 10.36	8.395	n.s.
16	17.30 ± 2.60	18.26 ± 2.18	21.50 ± 2.42	16.91 ± 2.13	33.20 ± 5.81	6.915	n.s.
17	12.60 ± 2.01	14.01 ± 1.94	18.09 ± 3.06	13.75 ± 2.27	25.40 ± 4.96	3.160	n.s.
18	8.91 ± 1.58	10.66 ± 1.95	12.66 ± 2.11	9.49 ± 1.40	14.43 ± 4.08	2.662	n.s.
19	5.12 ± 0.97	4.84 ± 1.23	6.03 ± 1.18	4.48 ± 1.15	9.13 ± 2.79	1.077	n.s.
20	2.38 ± 0.89	2.49 ± 0.78	2.23 ± 0.91	2.69 ± 0.82	5.78 ± 1.95	2.793	n.s.
21	1.36 ± 0.66	0.39 ± 0.27	0.87 ± 0.51	1.44 ± 0.59	2.78 ± 1.06	8.049	n.s.
22	0.99 ± 0.40	1.02 ± 0.43	0.61 ± 0.34	0.42 ± 0.29	1.83 ± 0.81	2.428	n.s.
23	0.95 ± 0.67	1.20 ± 0.51	0.78 ± 0.58	0.86 ± 0.59	2.94 ± 1.41	3.489	n.s.
24	0.64 ± 0.64	0.60 ± 0.35	1.73 ± 0.82	0.24 ± 0.24	2.42 ± 1.35	4.075	n.s.
25	1.06 ± 0.84	0.38 ± 0.38	1.79 ± 1.26	0.24 ± 0.24	1.96 ± 1.52	1.754	n.s.
26	1.48 ± 1.07	1.63 ± 1.37	20.50 ± 6.24	2.13 ± 1.89	3.15 ± 1.97	20.338	<0.001
27	1.25 ± 1.01	1.94 ± 1.38	0.77 ± 0.54	1.01 ± 0.69	2.73 ± 1.51	1.433	n.s.
28	1.12 ± 0.77	0.97 ± 0.57	4.64 ± 3.75	0.80 ± 0.57	7.13 ± 3.91	4.374	n.s.
29	0.75 ± 0.75	0.26 ± 0.26	0.52 ± 0.52	0.56 ± 0.56	2.39 ± 1.75	2.361	n.s.
30	0.26 ± 0.26	0.41 ± 0.28	0.71 ± 0.71	0.25 ± 0.25	2.59 ± 1.48	4.977	n.s.

Table 3.4:

Summary of weekly *E. abietinum* aphids·100 needles^{-1} (EN) on Sitka spruce under five different drought levels, during the second year of the field trial (2011). FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. A+ = With aphids; A- = Without aphids.

	2010		**2011**					
	1-year-old needles		Overall		1-year-old needles		2-year-old needles	
	A+	A-	A+	A-	A+	A-	A+	A-
Drought	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**
FC	46.9 ± 3.59	12.6 ± 2.68	48.6 ± 2.80	18.5 ± 2.75	46.7 ± 4.47	24.4 ± 6.17	48.8 ± 0.78	20.4 ± 0.50
MS	43.1 ± 3.34	7.8 ± 1.91	47.9 ± 2.31	17.9 ± 4.35	44.3 ± 3.74	11.0 ± 2.49	51.2 ± 0.65	20.7 ± 0.41
CS	50.9 ± 3.80	12.2 ± 2.48	54.1 ± 2.84	16.3 ± 1.89	55.2 ± 4.12	14.7 ± 3.06	49.7 ± 0.78	19.6 ± 0.41
IS1	39.5 ± 3.57	10.8 ± 2.54	44.7 ± 2.66	14.5 ± 1.98	39.5 ± 4.96	16.5 ± 5.05	50.1 ± 0.76	20.2 ± 0.49
IS2	49.6 ± 3.27	9.1 ± 4.11	52.1 ± 2.78	16.0 ± 5.90	52.2 ± 4.50	6.4 ± 2.01	50.5 ± 1.15	20.5 ± 0.38

Chapter 4

The effects of drought stress and *Elatobium abietinum* infestation on the growth of Sitka spruce

Introduction

Sitka spruce, *Picea sitchensis* (Bong.) Carr., is the predominant commercial conifer species used for afforestation and replanting in Great Britain (Straw *et al.*, 1998b; Gardiner *et al.*, 2011), accounting for 36% percent of the forest estate and 61% of all conifer species planted (Samuel *et al.*, 2007). More than half of the total sawn timber volume produced in Great Britain currently is Sitka spruce, a proportion which is expected to increase in the future (Halsall *et al.*, 2006).

Sitka spruce requires a mild, maritime climate for optimal growth, such as is found in the northern and western parts of Britain and, in particular, Scotland (Straw *et al.*, 2005; Green & Ray, 2009). These are characterised by mild winters and wet, relatively cool summers. Although these areas are where Sitka spruce is the most productive conifer species, such climactic conditions are also favourable for the green spruce aphid, *Elatobium abietinum* (Walker), a major defoliating pest of spruce, allowing populations to persist throughout the year anholocyclically and parthenogenetically (Straw *et al.*, 2005).

Elatobium abietinum feed on one-year-old and older needles, causing chlorosis and premature abscission (Fisher, 1987). Although high aphid densities can cause severe defoliation, potentially resulting in the almost complete loss of older needle age classes, the aphid rarely causes mortality in Sitka spruce (Carter, 1977; Straw *et al.*, 2000). Rather, the main effect observed of *E. abietinum* is a reduction in the growth increment of the host spruce (Carter, 1977). Due to the potential commercial implications of reduced timber yield that would correlate with growth reductions, several studies have been conducted to estimate such reductions. Studies have typically focused around small trees in pots or on estimated reductions following natural outbreaks (Carter, 1977; Warrington & Whittaker, 1990; Straw, 1995), however a series of experiments was conducted in Hafren Forest, Wales, to estimate the impact of natural and enhanced populations (Straw *et al.*, 1998b; Straw *et al.*, 2000; Straw *et al.*, 2002).

Elatobium abietinum density correlates with needle loss, with infested trees losing a higher proportion of their older needles (Straw *et al.*, 1998b). Lead shoot length, and therefore height, were also found to be reduced under aphid infestation (Straw *et al.*, 1998b; Straw *et al.*, 2000). Stem diameter increment and needle size, on the other hand, have been shown to have a delayed response to aphid infestations, though reductions are nonetheless observed and correlate with aphid density rather than needle loss (Straw *et al.*, 1998a; Straw *et al.*, 2000; Straw *et al.*, 2002). As well as the immediate effect of the aphids on growth, Sitka spruce may also take several years following attack to recover and return to normal growth rates (Straw, 1995).

Climate change in the U.K. is predicted to cause an increase in the frequency of summer drought (Murphy *et al.*, 2009). Drought can damage trees directly, with symptoms including foliage wilting and browning, crown dieback, stem splitting or cracking (Green & Ray, 2009; Galiano *et al.*, 2012), and bud death (Barigah *et al.*, 2013). Reductions to root biomass have also been observed (Moser *et al.*, 2010), while root electrolyte leakage (McKay & White, 1996) and xylem cavitation (Jackson *et al.*, 1995) have also been found in spruce. Drought has also been shown to change a plethora of physiological factors in plants, such as photosynthetic capacity and performance (Ditmarová *et al.*, 2009; Vaz *et al.*, 2010; Albert *et al.*, 2011), leaf respiration and gas exchange (Ibáñez *et al.*, 2010; Crous *et al.*, 2012; Crous *et al.*, 2011) and leaf water potential (Cregg & Zhang, 2001; Ditmarová *et al.*, 2009), *inter alia.* Drought has also been shown to affect plant growth. Radial growth is typically reduced (McLane *et al.*, 2011; Eilmann & Rigling, 2012; Sánchez-Salguero *et al.*, 2012), as are shoot height and stem diameter (Arend *et* *al.*, 2011). Reductions in leader extension and root biomass have been observed for Sitka spruce (Warrington & Whittaker, 1990; Straw & Green, 2001).

Sitka spruce is intolerant to drought, and is not recommended for planting in areas with a mean annual rainfall of less than 700mm (Jarvis & Mullins, 1987). Additionally, it is thought that drought stress may render spruce more susceptible to damage by *E. abietinum*, potentially due to induced changes in plant chemistry and consequent effects on aphid performance. Major (1990), for example, observed increased *E. abietinum* population size under intermittent drought stress. Increased aphid population sizes, and the potential for more frequent outbreak years, could lead to increased damage (Straw, 1995). Furthermore, though the aphids may not directly cause tree mortality drought is known to increase rates of tree death (Green & Ray, 2009; Ryan, 2011; Anderegg *et al.*, 2013), and additional effects on plant growth rates may also be observed.

Drought has been found to alter the interaction between the host plant and its herbivorous insect pests and pathogens, causing changes to inflicted damage (Jactel *et al.*, 2012). The interaction between multiple stressors and conditions have been shown to affect plant functioning (Straw & Green, 2001; Bansal *et al.*, 2013), and the importance of considering them across a range of durations, frequencies and intensities has been highlighted by Jactel *et al.* (2012) and Mitchell *et al.* (2013).

Aims and Objectives

1. Determine whether host tolerance, in terms of impact of aphid infestation on tree growth and lead shoot survival, varies with drought stress.
2. Determine whether the impact of aphid infestation on plant growth varies across multiple seasons under drought stress.

Materials and Methods

Five drought levels were explored in this study:

1. **FC** - Plants maintained at field capacity;

2. **MS** - Plants maintained at 60% of field capacity;
3. **CS** - Plants maintained at 20% of field capacity;
4. **IS1** - Plants subjected to fluctuating stress, whereby pots were allowed to dry out to 20% of field capacity, and were then watered back up to field capacity;
5. **IS2** - Plants subjected to fluctuating stress, whereby pots were allowed to dry out to 30% of field capacity, and were then watered up to 70% of field capacity.

These treatments were maintained using a combination of pot weight and soil moisture content, as established on pots in a greenhouse (see Chapter 2). The soil moisture was measured as percentage volume using an SM200 Soil Moisture Sensor and an HH2 Meter (Delta T Devices, Cambridge). Pots were checked daily to ensure the correct application of the drought treatments.

Plant material

Two-year-old Sitka spruce saplings (vegetatively propagated, Ident. QSS 04 (0R18TE)) were obtained from the Forestry Commission Delamere Nursery, Cheshire (U.K.) during the winter in 2010. These were potted up in 3L pots, using a standard 2:1:1 peat, bark and perlite growing medium mixed at Alice Holt Forest Research Station, Surrey (U.K.), supplied with 20g of Osmacote® Plus controlled release granular fertiliser (16%N + 8%P + 11%K + 2%MgO; Scotts Ltd, U.K.) mixed into the growing medium.

The same trees were utilised for the second year of the experiment. As such, in early March 2011, trees which had survived the winter were re-potted into 7L pots with the same growing medium as was used in the first year (mixed at Alice Holt Forest Research Station, Surrey, U.K.). They were then returned to their pallets, and left to re-establish for one week before restarting the drought treatments. During this time, they were all well-watered. Trees which had not survived were removed from the experiment (refer to Table 3.1 in Chapter 3).

Each pot was routinely and regularly weeded throughout the duration of the experiment. Each tree was also checked daily for the presence of *Cinara pilicornis*, which were immediately removed using a fine paintbrush if found. Furthermore, non-aphid treatment trees were also examined for *E. abietinum*, and where any were found these were also removed.

Experimental design

A total of 250 Sitka spruce trees were maintained outdoors on raised platforms, in a 5 x 5 Latin square. Each pallet was assigned ten trees, and each tree was allocated an aphid treatment (with or without aphids) at random such that five trees per pallet were infested and the other five were not. Trees were positioned in such a way that there was no contact between individuals, to ensure that the apterous aphids were unable to disperse between infested and uninfested trees. The trees were watered using an automatic irrigation system, and monitored regularly by checking the weight and soil moisture content of the pots.

Each tree was fitted with a sealed plastic skirt to ensure that rainwater did not reach the growing medium. These skirts were attached securely to the base of each tree using garden wire and insulating foam tape, to create a water-proof seal that caused minimal damage to the plant. Strong duct tape was used to seal joins in the plastic. The skirts were regularly inspected to maintain seal and condition, as were the ties at the base of each tree to ensure that they were not restricting growth. Skirts were attached to coincide with the start of drought treatment in both years.

Stock cultures of *E. abietinum* were maintained and used for the inoculation of trees. These were reared on cut branches in buckets of water in a CT room at 15 °C, with 70% RH and a 16:8 photoperiod. In 2010, the branches were initially sourced from Hafren Forest, Wales (U.K.), with further branches obtained from Alice Holt Forest Research Station, Surrey (U.K.). In 2011, all branches were sourced from Alice Holt.

Year 1

Trees were inoculated on the 21st April 2010 by tying three 3cm lengths of aphid-infested shoots from stock *E. abietinum* cultures to the designated trees using black cotton thread. Each shoot section had approximately thirty apterous aphids. One section was tied to the leader shoot, and two others to side shoots. The inoculation sections were removed one week later, which was sufficient time for the aphids to move onto the experimental trees.

The drought treatments were maintained until mid-November 2010, after which the irrigation system was switched off and the plastic skirts removed. Trees were left to overwinter, though they were routinely checked.

Year 2

In 2011, trees were inoculated on the 28th March, and then again on the 28th April as the initial attempt did not succeed. Six 3cm lengths of aphid-infested shoots from the stock *E. abietinum* culture were attached to the designated trees with black cotton thread. In the initial attempt, each shoot section had approximately 35 apterous aphids, whereas in the second attempt there were only approximately 20 apterous aphids per section. Two sections were tied onto the leader shoot; one on the one-year-old section, and one on the two-year old section. The remaining four were tied onto side shoots; two on one-year-old sections, and two one two-year-old sections. Additionally, sections were only tied where there were needles for the aphids to migrate onto. The inoculation sections were removed after one week, to allow sufficient time for the aphids to move onto the experimental trees.

Drought treatments were terminated in mid-November 2011, when the irrigation system was switched off and plastic skirts removed again.

Plant growth measurements

Initial measurements of Sitka spruce height, midpoint diameter and collar diameter were taken in April 2010. Measurements for growth at the end of the first year were recorded in January 2011, and those for the end of the second year in January 2012. Measurements were used to calculate annual growth increments and lead extension. Survival of the lead shoot at the end of the first drought season was also recorded in January 2011 (where this had occurred, the new lead shoot was noted for further height and lead shoot measurements).

Overwinter survival into the second year was determined in March 2011 during the re-potting of the saplings.

Statistical analysis

The effect of drought stress and aphid infestation on the plant growth measurements were analysed using linear mixed effects models to take into account pseudoreplication. Total midpoint and collar diameter data for the end of each year was log-transformed for normality, as was the midpoint data for the initial measurements. Lead extension survival and tree survival into the second year of the experiment were analysed with a binomial distribution. Drought and aphid presence were modelled as fixed effects, while pallet was modelled as a random effect (groups: pallet = 5; estimated d.f. for each parameter = 4).

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

Initial measurements

No significant differences were found in the height (drought: ${\chi^2}_4 = 2.39$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 2.93$, $P > 0.05$; Figure 4.1(A)), midpoint diameter (drought: ${\chi^2}_4 = 1.10$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 0.91$, $P > 0.05$; Figure 4.2(A)) and collar diameter (drought: ${\chi^2}_4 = 5.47$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 0.08$, $P > 0.05$; Figure 4.3(A)) of the Sitka spruce saplings at the start of the experiment.

Height and lead extension

No significant effects of drought treatment or aphid presence were found on the height of the Sitka spruce saplings at both the end of the first (drought: ${\chi^2}_4 = 1.07$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 0.29$, $P > 0.05$) and second (drought: ${\chi^2}_4 = 0.11$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 0.05$, $P > 0.05$) years of the experiment. Mean total heights are summarised in Table 4.3. The same was also true for lead shoot extension (Year 1 - drought: ${\chi^2}_4 = 0.97$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 0.66$, $P > 0.05$, Figure 4.1(B)); Year 2 - drought: ${\chi^2}_4 = 0.36$, $P > 0.05$; aphid presence: ${\chi^2}_1 = 0.11$, $P > 0.05$, Figure 4.1(C)). No significant interactions were observed between drought treatment and aphid attack for any of these measures, and as such were removed from the model.

Midpoint diameter growth

Midpoint diameter was found to be significantly affected in both years by drought treatment (year 1: ${\chi^2}_4 = 12.09$, $P < 0.05$; year 2: ${\chi^2}_4 = 11.87$, $P < 0.05$), though the treatments were not found to significantly differ from the well-watered control. An effect of aphid presence was also observed (year 1: ${\chi^2}_1 = 10.12$, $P < 0.01$; year 2: ${\chi^2}_1 = 5.30$, $P < 0.05$), whereby midpoint diameter was smaller for aphid-infested trees (year 1: $t = 3.18$, $P < 0.05$; year 2: $t = 2.30$, $P < 0.05$). Mean total midpoints diameters are summarised in Table 4.4.

The increment by which the midpoint diameter increased was not affected by drought in either of the two years (year 1: ${\chi^2}_4 = 8.74$, $P > 0.05$; year 2: ${\chi^2}_4 = 7.70$, $P > 0.05$, Figure 4.2(B) & (C)). Aphid attack was found to significantly affect the increment (${\chi^2}_1 = 5.46$, $P < 0.05$), reducing it where aphids were present ($t = 2.34$, $P < 0.05$), after the first year of drought treatment. No such effect was observed after the second year of drought treatment (${\chi^2}_1 = 1.91$, $P > 0.05$).

No significant interactions were observed between drought treatment and aphid attack for any of these measures, and as such were removed from the model.

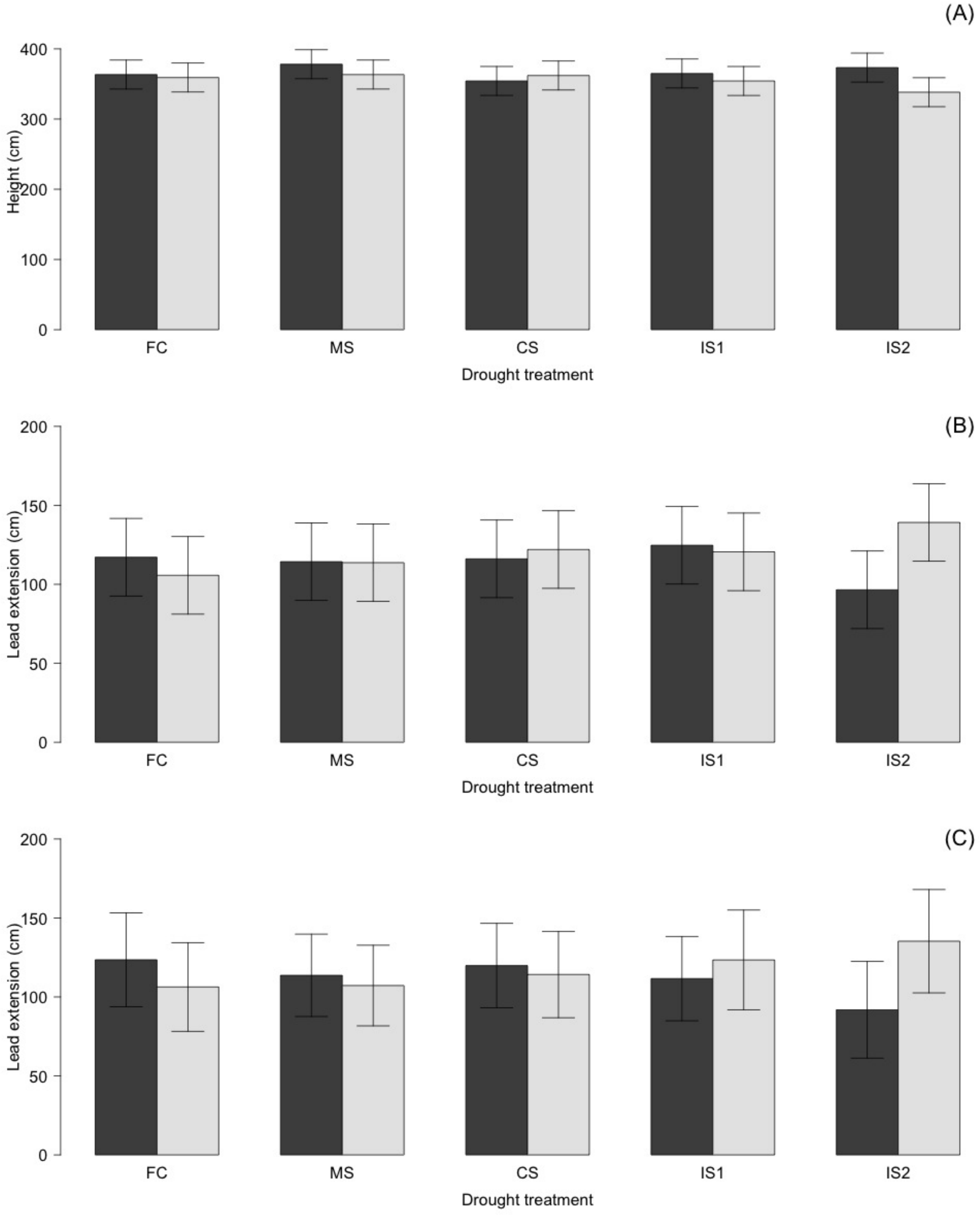

Figure 4.1: Effect of drought stress and *E. abietinum* infestation on height of Sitka spruce.

Bars indicate Least Significant Difference. (A) Initial height; (B) Lead shoot extension after one year of drought treatment; (C) Lead shoot extension after two years of drought treatment. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

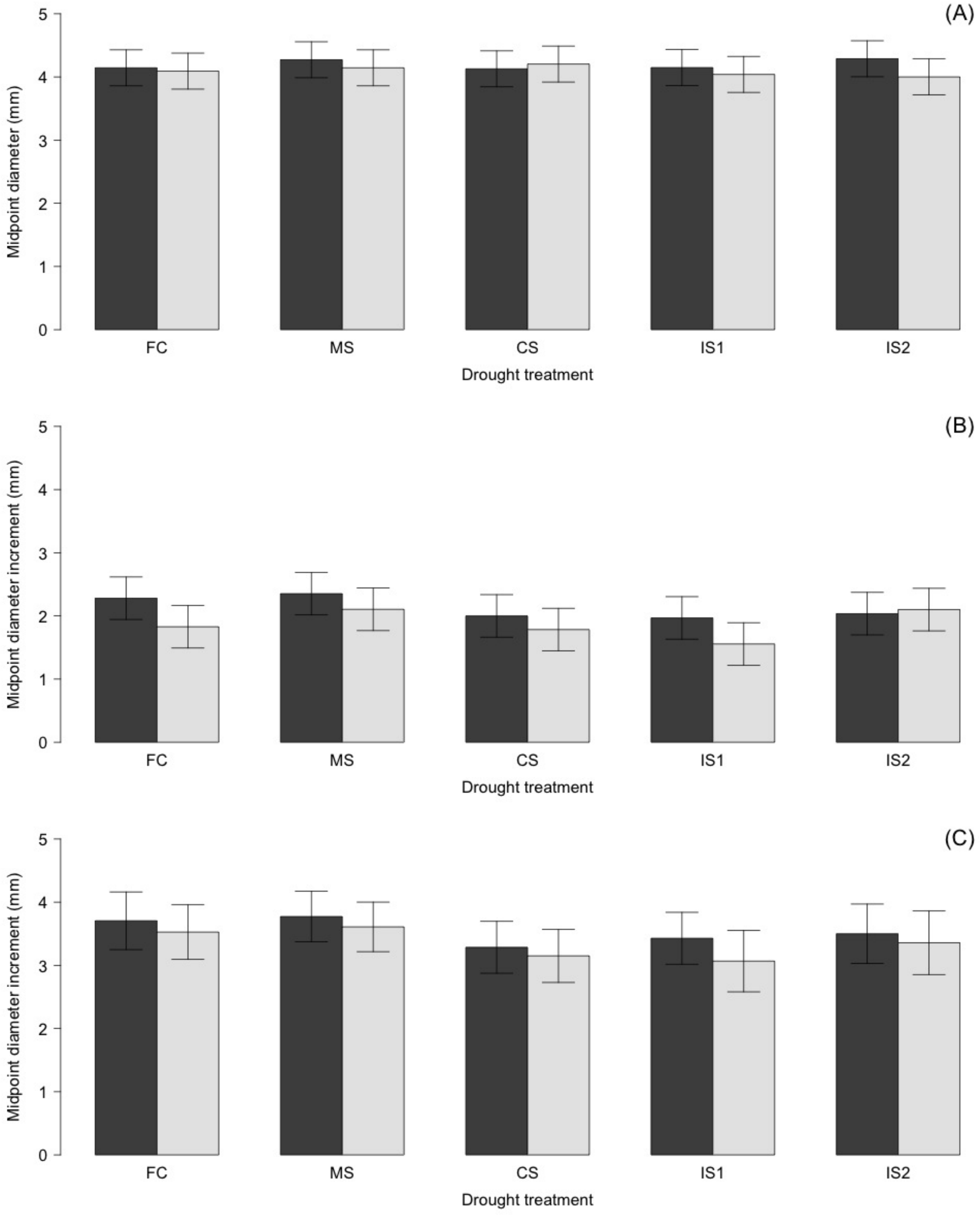

Figure 4.2: Effect of drought stress and *E. abietinum* infestation on midpoint diameter of Sitka spruce.

Bars indicate Least Significant Difference. (A) Initial midpoint diameter; (B) Midpoint diameter increment after one year of drought treatment; (C) Midpoint diameter increment after two years of drought treatment. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

Collar diameter growth

No significant effects of drought treatment or aphid presence were found on the collar diameter of the experimental trees at both the end of the first (drought: $\chi^2{}_4 = 2.40$, $P > 0.05$; aphid presence: $\chi^2{}_1 = 2.36$, $P > 0.05$) and second (drought: $\chi^2{}_4 = 4.08$, $P > 0.05$; aphid presence: $\chi^2{}_1 = 2.71$, $P > 0.05$) years of the trial. Mean total collar diameters are summarised in Table 4.5. The same was also true for the increment by which the collar diameter increased (Year 1 - drought: $\chi^2{}_4 = 4.89$, $P > 0.05$; aphid presence: $\chi^2{}_1 = 1.82$, $P > 0.05$; Figure 4.3(B)). Year 2 - drought: $\chi^2{}_4 = 5.76$, $P > 0.05$; aphid presence: $\chi^2{}_1 = 3.06$, $P > 0.05$; Figure 4.3(C)). Again, there were no significant interactions between drought treatment and aphid attack for any of these measures, and as such they were removed from the model.

Lead shoot survival, and survival into the second year of drought treatment

Survival of the lead shoot after the first season of drought treatment was not affected by either drought ($\chi^2{}_4 = 2.15$, $P > 0.05$) or aphid presence ($\chi^2{}_1 = 0.08$, $P > 0.05$; Figure 4.4). In contrast, tree overwinter survival was found to be affected by drought ($\chi^2{}_4 = 12.74$, $P < 0.05$, Figure 4.5), where a greater proportion survived into the second year under the MS drought treatment ($z = 2.21$, $P < 0.05$). Aphid presence was not found to affect this proportion, however ($\chi^2{}_1 = 0.82$, $P > 0.05$). There were no significant interactions between drought treatment and aphid attack.

Discussion

Many studies on the effect of *E. abietinum* on Sitka spruce growth have shown that the aphids cause reductions, and others have indicated reductions caused by drought. Warrington & Whittaker (1990) found that both drought and aphid attack had direct effects on Sitka spruce, but that the combined effects of both stressors was not additive. In contrast to these findings, the study herein presented found no effect of either drought or aphid presence on height and collar diameter across two years of spring-summer drought

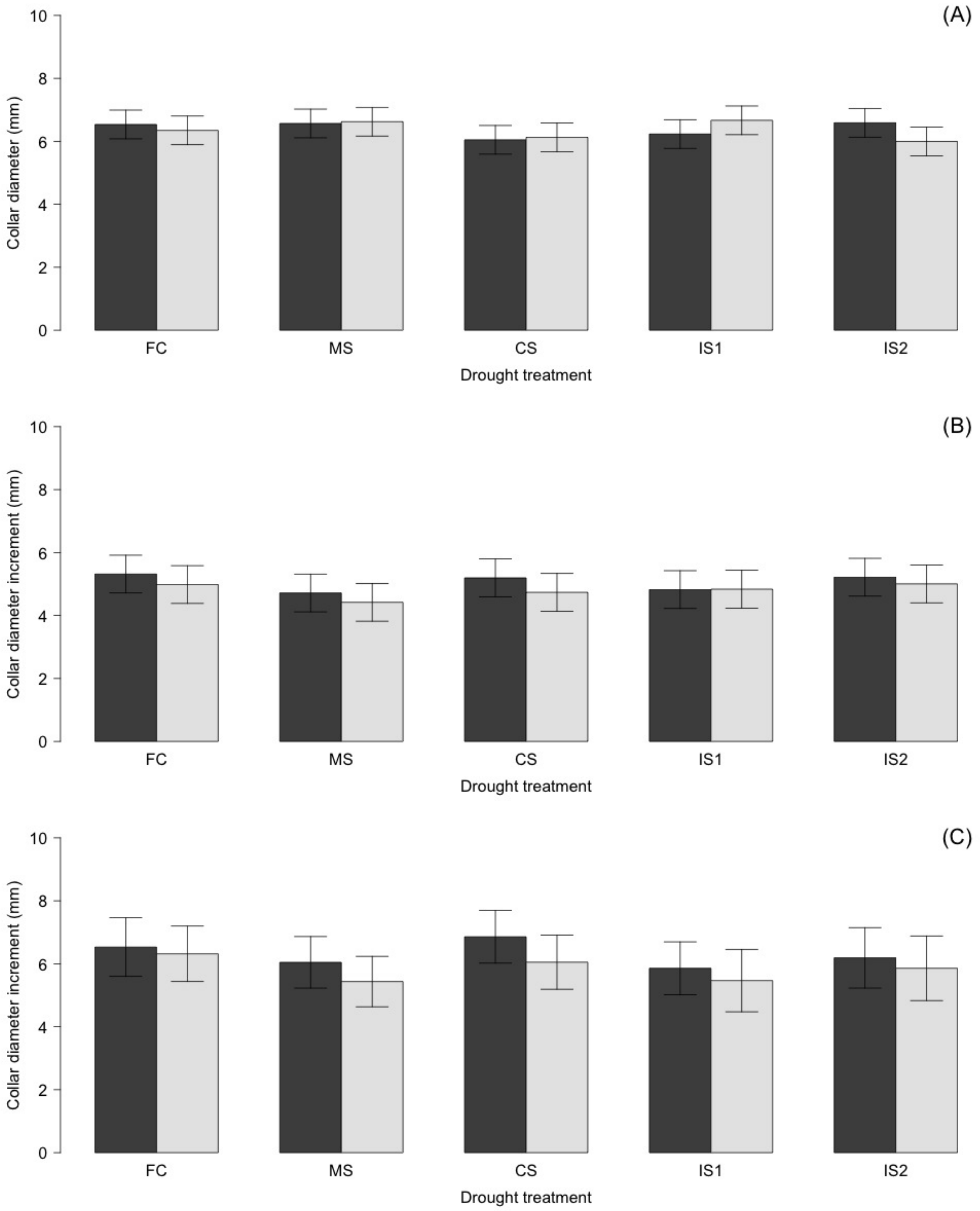

Figure 4.3: Effect of drought stress and *E. abietinum* infestation on collar diameter of Sitka spruce.

Bars indicate Least Significant Difference. (A) Initial midpoint diameter; (B) Collar diameter increment after one year of drought treatment; (C) Collar diameter increment after two years of drought treatment. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

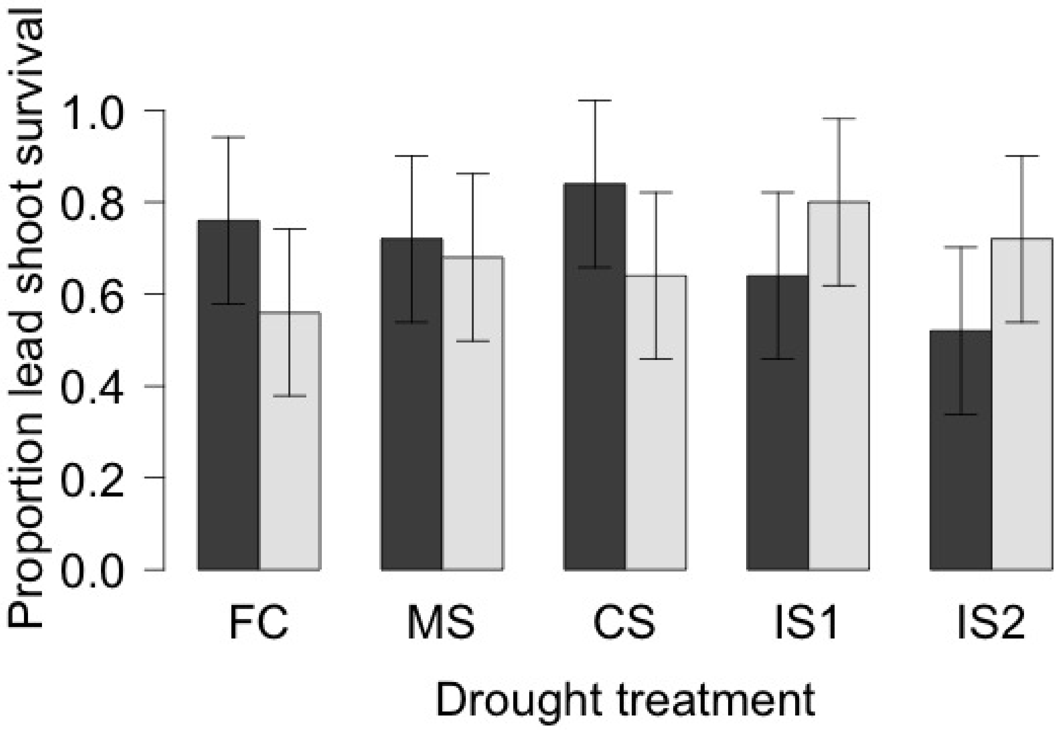

Figure 4.4: Effect of drought stress and *E. abietinum* infestation on Sitka spruce lead shoot survival after one year of drought treatment.

Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

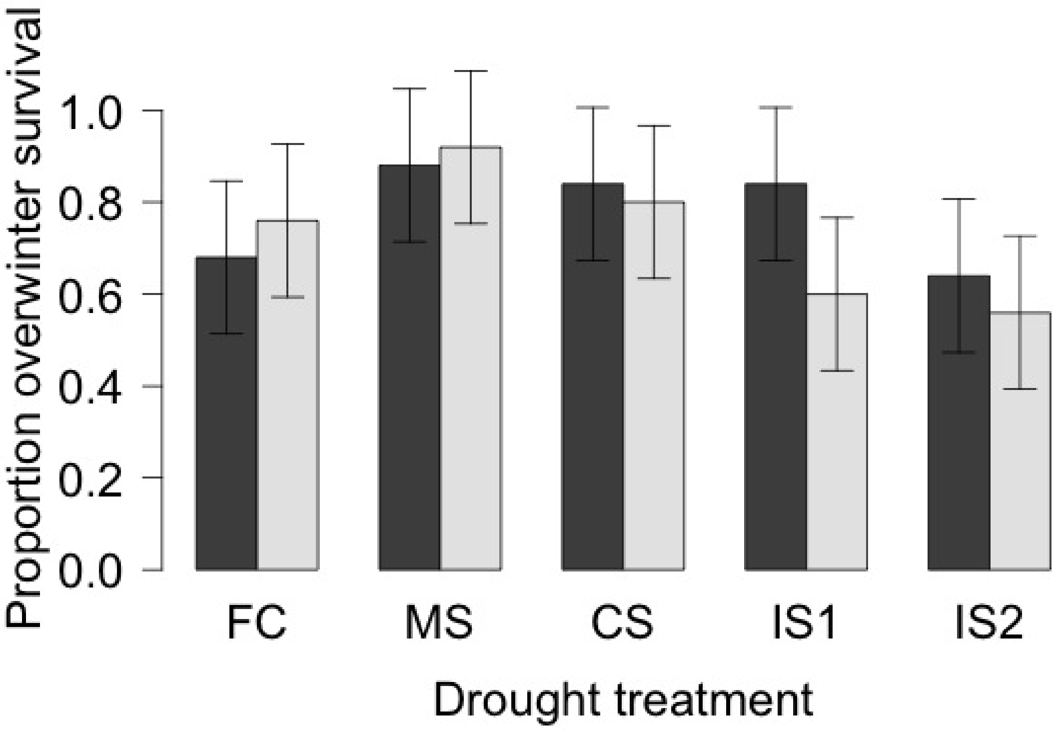

Figure 4.5: Effect of drought stress and *E. abietinum* infestation on Sitka spruce overwinter survival after one year of drought treatment.

Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Dark grey bars = without aphids; Light grey bars = with aphids.

stress. Effects of both were observed, however, on midpoint diameter.

Reductions of between 1.2% and 6.8% under aphid infestation were observed in the first year, except under CS drought treatment which saw an increase of 1.8% under aphid attack. In the second year, reductions of between 3.2% and 9.5% were observed for all treatments under aphid infestation. Although no interactions were observed between drought and aphid presence, drought was also observed to have an effect on midpoint diameter at the end of each year. Moderate drought resulted in a higher mean midpoint diameter, whereas severe stress typically reduced it - in the first year, a reduction was observed only under the severe continuous drought treatment, with an increase under intermittent drought, but in the second year both severe treatments showed a reduction. The percentage differences are summarised in Tables 4.1 and 4.2.

Table 4.1:

Mean percentage differences in midpoint diameter of Sitka spruce during the field trial between *E. abietinum* infestation treatments. Differences shown are in comparison to the 'without aphids' treatment. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

Drought treatment	**End of year 1**	**End of year 2**
FC	- 1.21%	- 5.51%
MS	- 3.04%	- 5.83%
CS	+ 1.67%	- 3.42%
IS1	- 2.65%	- 9.48%
IS2	- 6.76%	- 3.19%

The findings in this study do not replicate those repeatedly observed in previous literature on Sitka spruce. Certainly, as far as the effects of *E. abietinum* infestation are concerned, the results are atypical.

Aphid infestation has an immediate effect on height and lead shoot extension, which have always been found to be reduced in Sitka spruce under aphid attack. Studies typ-

Table 4.2:

Mean percentage differences in midpoint diameter of Sitka spruce during the field trial between different levels of drought treatment. Differences shown are in comparison to the well-watered control. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

Drought treatment	**End of year 1**	**End of year 2**
MS	+ 2.14%	+ 2.71%
CS	- 0.96%	- 10.50%
IS1	+ 1.68%	- 1.66%
IS2	+ 1.21%	+ 5.49%

ically find reductions in height increment of between 10 - 30% (Seaby & Mowat, 1993; Thomas & Miller, 1994; Straw *et al.*, 1998a), though greater reductions of 40 - 62% in 5-6 year old Sitka spruce have also been observed (Carter, 1977; Carter & Nichols, 1988). Warrington & Whittaker (1990) observed a height increment reduction of 13%, comparable to the findings of Straw *et al.* (2000) who found reductions of 6 - 23%. Furthermore, the increment reductions have been found to be affected for several years after infestation (Straw *et al.*, 1998a). The nutrient conditions of Sitka spruce have also been found to affect the height increment reductions, with greater reductions of between 15 - 44% in low nutrient conditions and 11 - 27% under hight nutrient conditions (Straw & Green, 2001).

Stem diameter increments, on the other hand, have been found to show a delayed response to aphid infestation (Day & McClean, 1991; Straw *et al.*, 1998a), though the increments are still reduced. Straw *et al.* (2000) observed reductions of 12% in the year following aphid infestation, while Thomas & Miller (1994) found reductions comparable to those in height. The findings of the study herein presented, however, indicate an immediate effect on midpoint diameter.

Reductions in growth are associated with defoliation rates and hence also to aphid density (Straw *et al.*, 2005). The work presented here indicates that needle loss was affected by aphid presence (refer to Chapter 3), and drought to some extent in the second year of drought application. Aphid density and population behaviour was also affected by drought treatment. Despite these findings, the correlation of needle loss and growth reductions, as found by Straw *et al.* (1998b); Straw *et al.* (2000); Straw *et al.* (2005), were not observed in this study.

Drought has been predicted to make spruce more susceptible to *E. abietinum* damage (Straw, 1995). Furthermore, drought not only causes direct damage to trees (Green & Ray, 2009), but has repeatedly been shown to affect growth. Warrington & Whittaker (1990) observed reductions of 31% when water was withheld from potted Sitka spruce saplings, while reductions in both shoot length and stem diameter increment were observed by Arend *et al.* (2011) in three oak (*Quercus*) species. Eilmann & Rigling (2012) found substantial reductions in the growth of four species of Pinaceaen conifers, as did Sánchez-Salguero *et al.* (2012) in four pine (*Pinus*) species and Guo *et al.* (2010) in three poplar (*Populus*) clones.

Warrington & Whittaker (1990) did not observe an additive effect of drought and aphid attack on the growth reductions in Sitka spruce. Both were, however, found to have had large impacts, the reduction was no greater under the presence of both stressors, and this lead the authors to suggest this was due to a limited plasticity in the response of Sitka spruce. Even so, reductions to growth were observed, whereas they were not in the study herein presented. Furthermore, while it is known that tree size affects the size of both growth (Straw *et al.*, 1998a) and drought tolerance (Way, 2011), reductions are still to be expected.

The lack of response to either drought stress or aphid presence in the Sitka spruce saplings used in this experiment suggests that there may have been a methodological problem. It may also reflect a problem with the drought treatment levels; perhaps they were not strong or different enough to elicit different responses, but this does not correspond with the findings of Chapter 3, where aphid densities (though not overall needle loss) were found to be affected differently depending on drought intensity and frequency.

Another potential explanation for the apparent lack of growth reductions may be that drought stress affects the tolerance and resistance to aphids. Resistance can be defined as the inherent ability of a host to prevent or reduce pest infestation and development (Dent, 1991; Gordh & Headrick, 2001), whereas tolerance may be defined as "the ability of a host plant to withstand injury due to pest activity" (Gordh & Headrick, 2001). The continuum hypothesis (Maschinski & Whitham, 1989) predicts that plant tolerance should be facilitated in resource-rich environments, but the results of the current study contradict this. Rather, they may support the defence-stress cost hypothesis proposed by Siemens *et al.* (2003), which predicts an increase in host plant defence costs under stressful growing conditions.

Ramírez & Verdugo (2009) observed that, under reduced water availability, tolerance and resistance of a *Populus* hybrid to the aphid *Chaitophorus leucomelas* Koch were affected without a trade-off between the two, as reflected by effects of drought and aphid attack on plant growth. Specifically, the study found a significant effect of water availability on both tolerance and resistance, with no cost of tolerance and a cost in growth of resistance under drought stress. They observed growth compensation to herbivore damage under drought. Furthermore, those poplar clones with higher resistance to aphids grew less when the aphids were not present, while tolerance in branch length for trees under drought stress was approximately twice that of well-watered control trees. Katjiua & Ward (2006) suggested that the degree to which tolerance and resistance are expressed is resource-dependant based on studies of the deciduous tree, *Terminalia sericea* Butch., supporting similar findings by Prittinen *et al.* (2003) on *Betula pendula* Roth. These studies go to provide evidence that trade-offs between tolerance and resistance are dependant on environmental conditions. Osier & Lindroth (2006) observed negative correlations between resource allocations to growth and resistance in *Populus tremuloides* Michz. under stressful conditions while yet others have found the opposite to be true (Koricheva, 2002; Stevens *et al.*, 2007), which suggests that plant responses to multiple stressors may not only be complex, but also affected by biotic factors such as plant age and type.

It is possible that, under simultaneous *E. abietinum* attack and drought stress, a general plant response may be elicited in Sitka spruce saplings and result in an increased tolerance, while resistance to the aphid may bring about reduced growth in the absence of the pests, similarly to the findings of Ramírez & Verdugo (2009). If growth under aphid attack and drought stress was increased, while growth was reduced under drought in the absence of aphids, it may be possible that any differences in response cancelled each other out. Further investigation into such a relationship, as well as the potential underlying mechanisms, would be essential in untangling the nature of the response.

Conclusions

1. Height and collar diameter growth in Sitka spruce were not found to be affected by drought or *E. abietinum* infestation. However, an effect of both was found on midpoint diameter.

2. The results of this study did not replicate those repeatedly observed in previous literature on Sitka spruce, which typically uncovered reductions in Sitka spruce growth increments as a response to aphid attack.

3. The findings suggest that there may be a potential trade-off between tolerance and resistance to *E. abietinum* under drought stress, or that there were methodological problems with the experiment.

Summary tables

Table 4.3:

Summary of Sitka spruce height during the field trial under drought treatment and *E. abietinum* infestation. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. A+ = With aphids; A- = Without aphids.

Drought treatment	**Aphid presence**	**Initial height (cm)**	**At end of year 1 (cm)**	**At end of year 2 (cm)**
		$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**
FC	A-	363.36 ± 9.98	480.52 ± 14.13	608.53 ± 27.85
	A+	359.12 ± 7.97	464.80 ± 13.51	569.26 ± 28.02
MS	A-	378.10 ± 16.82	492.44 ± 12.10	598.05 ± 18.10
	A+	363.36 ± 9.99	477.08 ± 14.07	577.09 ± 22.61
CS	A-	354.28 ± 10.02	470.40 ± 16.26	594.00 ± 31.85
	A+	362.00 ± 8.00	484.00 ± 12.87	588.00 ± 27.18
IS1	A-	364.88 ± 11.18	489.60 ± 16.20	588.10 ± 25.25
	A+	354.24 ± 8.80	474.80 ± 11.99	592.20 ± 31.34
IS2	A-	373.32 ± 10.43	469.84 ± 16.66	555.19 ± 33.23
	A+	338.24 ± 9.12	477.40 ± 16.20	612.36 ± 41.70

Table 4.4:

Summary of Sitka spruce midpoint during the field trial under drought treatment and *E. abietinum* infestation. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. A+ = With aphids; A- = Without aphids.

Drought treatment	**Aphid presence**	**Initial midpoint diameter (mm)**	**At end of year 1 (mm)**	**At end of year 2 (mm)**
		$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**
FC	A-	4.14 ± 0.14	6.42 ± 0.22	10.16 ± 0.52
	A+	4.09 ± 0.11	5.92 ± 0.20	9.60 ± 0.49
MS	A-	4.27 ± 0.15	6.62 ± 0.25	10.46 ± 0.52
	A+	4.14 ± 0.12	6.25 ± 0.14	9.85 ± 0.27
CS	A-	4.13 ± 0.14	6.13 ± 0.12	9.35 ± 0.18
	A+	4.20 ± 0.11	5.99 ± 0.15	9.03 ± 0.33
IS1	A-	4.15 ± 0.17	6.12 ± 0.17	9.49 ± 0.32
	A+	4.04 ± 0.15	5.60 ± 0.15	8.59 ± 0.36
IS2	A-	4.29 ± 0.18	6.32 ± 0.18	9.72 ± 0.37
	A+	4.00 ± 0.16	6.10 ± 0.16	9.41 ± 0.31

Table 4.5:

Summary of Sitka spruce collar during the field trial under drought treatment and *E. abietinum* infestation. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. A+ = With aphids; A- = Without aphids.

Drought treatment	**Aphid presence**	**Initial collar diameter (mm)**	**At end of year 1 (mm)**	**At end of year 2 (mm)**
		$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**
FC	A-	6.54 ± 0.22	11.85 ± 0.41	18.04 ± 0.88
	A+	6.35 ± 0.25	11.33 ± 0.39	17.77 ± 0.92
MS	A-	6.57 ± 0.19	11.28 ± 0.23	17.45 ± 0.64
	A+	6.62 ± 0.24	11.04 ± 0.23	16.31 ± 0.39
CS	A-	6.05 ± 0.20	11.24 ± 0.29	18.09 ± 0.79
	A+	6.13 ± 0.18	10.86 ± 0.25	16.93 ± 0.66
IS1	A-	6.23 ± 0.25	11.06 ± 0.30	16.46 ± 0.46
	A+	6.67 ± 0.26	11.50 ± 0.34	16.69 ± 0.78
IS2	A-	6.59 ± 0.27	11.80 ± 0.33	17.77 ± 0.63
	A+	6.00 ± 0.26	11.00 ± 0.35	16.63 ± 0.66

Chapter 5

Aphid performance on drought-stressed Sitka spruce under controlled laboratory conditions

Introduction

The green spruce aphid, *Elatobium abietinum* (Walker), is the most serious defoliating pest of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) in Britain, and populations of this aphid are predicted to show a strong response to climate change (Straw, 1995). The frequency of summer drought is expected to increase as a result of climate change in the U.K. (Murphy *et al.*, 2009), which in turn would be expected to affect the quality of Sitka spruce as a host plant for *E. abietinum.* It is often difficult to make a direct assessment of plant nutritional quality and chemical defences, however performance parameters can be used as indicative, albeit indirect, measures of host quality (Wyatt & White, 1977; Leather & Dixon, 1984; Dixon, 1987). Individual aphid performance parameters may also provide useful indicators of population potential when it is neither practical nor possible to monitor population development directly.

A variety of measures for aphid performance have been devised. These include measures such as mean relative growth rate (mRGR) (Radford, 1967), adult weight, fecundity, intrinsic rate of increase (r_m), generation time and longevity (Awmack & Leather, 2002; Awmack & Leather, 2007). Some performance measures are more accurate predictors of aphid fitness than others, but all have been used extensively in studies on both arboreal and agricultural aphids species (e.g. Kennedy *et al.* (1958); Fisher (1987); Warrington & Whittaker (1990); McVean & Dixon (2001); Williams *et al.* (2005); Mody *et al.* (2009)).

Several studies have been conducted on the effect of drought stress on aphid performance in a laboratory environment using potted plants. Many of these studies have been conducted on crop plants and their aphids, with very few conducted on trees and arboreal aphids (Koricheva *et al.*, 1998). Although the effects of drought on plants has been well-documented, the indirect effect on aphids and other insect herbivores is not clearly understood, with different studies pointing to different trends. Kennedy *et al.* (1958) and McVean & Dixon (2001) observed negative effects under continuously stressed plants. Warrington & Whittaker (1990) observed a small but non-significant increase in mean aphid numbers on plants under drought treatments, and determined that, despite having an impact on plant growth, drought alone had relatively little effect on the aphids. Hale *et al.* (2003) tested *Rhopalosiphum padi* (L.) performance on continuously moderately stressed host plants. While the intrinsic rate of increase was reduced on three grass species, it was unaffected on a fourth species of grass, suggesting that, even with closely related host species, effects may not be consistent.

The Pulsed Water Stress Hypothesis suggested by Huberty & Denno (2004) proposed that, though stress-induced increases in nitrogen would be inaccessible to phloem-feeding insects such as aphids during periods of continuous stress, bouts of intermittent stress would allow a recovery of turgor pressure in the plant and allow the insects to take advantage of the improved nutritional quality (Huberty & Denno, 2004). Mody *et al.* (2009) further developed this hypothesis, by showing that the intensity of water stress affected the interaction between aphids and their host plant; the water stress pulse intensity influenced whether aphid performance was enhanced or reduced. These findings explain results observed by Major (1990) on *E. abietinum*, who found that, in intermittent stress treatments, aphid population growth was significantly greater than that on control and continuously stressed treatments. Furthermore, aphids on intermittently stressed plants produced a greater mean number of nymphs per adult per day.

A feature of *E. abietinum* inter-year population dynamics is the suppression of high aphid densities following an outbreak year (Day & Crute, 1990; Day & Kidd, 1998). This results in recovery time which, combined with the fact that new needles are chemically defended from aphid attack for the first three to four months (Jackson & Dixon, 1996), means that even under high aphid populations tree mortality is minimal. This characteristic population dynamic, where densities in years following high populations are reduced, is shared with many other species of arboreal aphid (Dixon, 1973).

A further characteristic of tree-feeding aphids is the ‘see-saw’ effect, whereby high abundance in spring is followed by low abundance in autumn, and *vice-versa* (Dixon, 1985; Dixon & Kindlmann, 1998). This is thought to be driven by aphid effects on host quality and induced defence mechanisms, or through effects on aphid quality passed through generations. In contrast to this, Williams *et al.* (2005) observed that high spring densities of *E. abietinum* did not adversely affect subsequent generations of the aphid. Rather, their results suggested that not only did high spring populations improve host quality of the current year’s needles for the autumn generations, but also failed to induce any chemical defence mechanisms in Sitka spruce.

The study presented here was conducted in order to further investigate and clarify the way in which drought stress affects the fitness and performance of *E. abietinum* on Sitka spruce. Drought is known to improve host quality and it may also decrease plant defences. It might also affect the onset of dormancy in spruce, which would increase the period of susceptibility to *E. abietinum* (Straw, 1995). Relationships between drought stress and individual aphid performance might explain differences between drought treatments in the field.

Aims and Objectives

1. Test whether spring drought stress improves host-plant quality for *E. abietinum*, by assessing the performance of individual *E. abietinum* aphids under controlled conditions.

2. Investigate whether spring drought stress elicits differences in performance through time following bud burst.

3. Establish whether spring-summer drought improves *E. abietinum* performance in the autumn, and determine whether the effects are time-dependent.

Materials and Methods

Drought treatments

Five drought levels were explored in this study:

1. **FC** - Plants maintained at field capacity;
2. **MS** - Plants maintained at 60% of field capacity;
3. **CS** - Plants maintained at 20% of field capacity;
4. **IS1** - Plants subjected to fluctuating stress, whereby pots were allowed to dry out to 20% of field capacity, and were then watered back up to field capacity;
5. **IS2** - Plants subjected to fluctuating stress, whereby pots were allowed to dry out to 30% of field capacity, and were then watered up to 70% of field capacity.

These treatments were maintained using a combination of pot weight and soil moisture content, as established on pots in a greenhouse (see Appendix 2). The soil moisture was measured as percentage volume using an SM200 Soil Moisture Sensor and an HH2 Meter (Delta T Devices, Cambridge). Pots were checked daily to ensure the correct application of the drought treatments.

Experimental design

Sitka spruce saplings (vegetatively propagated, Ident. QSS 04 (0R18TE)) were obtained from the Forestry Commission Delamere Nursery, Cheshire (U.K.) in early 2011 and potted up at Silwood Park, Berkshire (U.K.) in 3L pots. A standard 2:1:1 peat, bark and perlite growing medium, with 20g Osmacote® Plus controlled release granular fertiliser (16%N + 8%P + 11%K + 2%MgO; Scotts Ltd, U.K.), was mixed at Alice Holt Forest Research Station, Surrey (U.K.) and brought to Silwood Park for the potting.

Ten Sitka spruce trees from each of the five drought treatments, totalling 50 plants, were maintained on pallets outdoors without aphids under the appropriate watering regimes. These plants were moved to a CT room at 15 °C, with 70% RH and a 16:8 photoperiod under continued drought treatment one week before the start of the spring suite of experiments, on the 19th April 2011, to acclimatise to the conditions. Pots, and therefore treatments, were intermixed in a fully randomised design. Upon completion of the spring suite of the experiment, all aphids were removed and plants brought back outdoors under continued drought treatment to recover condition.

Plants were moved back into the CT room one week before the start of the autumn suite of experiments, on the 12th September 2011, under continued drought treatment in order to acclimatise.

Three trials were run for each of the suites, starting on the dates as follow:

Experiment 1: Spring suite

1. At bud burst - 27th April 2011 (ST1);
2. Two weeks after bud burst - 10th May 2011 (ST2);
3. Five weeks after bud burst - 07th June 2011 (ST3).

Experiment 2: Autumn suite

1. 19th September 2011 (AT1);
2. 04th October 2011 (AT2);
3. 24th October 2011 (AT3).

At the start of each trial, eight adult *E. abietinum* aphids from the stock culture were caged in gelatin capsules (size 00, Value Healthcare, U.K.; Figure 5.1) on each of the plants. Four were caged on needles on the leader shoot, and four on needles on the side shoot. These were left undisturbed, and after 24 hours each capsule was inspected. If any nymphs had been deposited on the needle within each capsule, one was selected at random and left to establish. An additional nymph was gently moved to and caged on a different needle using a fine paintbrush, two onto the leader shoot and two onto side shoots, and were used to determine the proportion of nymphs surviving seven days after deposition before being removed. All other nymphs and the adult were removed. Where no nymphs had been deposited, the adult was returned and left undisturbed for a further 24 hours, where the process was then repeated until each capsule contained an experimental nymph.

Half of the experimental aphids, two on the leader shoot and two on side shoots, were used to calculate the aphid mean relative growth rate (mRGR), as removing and replacing nymphs has been shown to significantly affect growth rate (Major, 1990). The remaining four were used to establish all other performance indicators.

The remaining four experimental nymphs per plant were left *in situ* to establish and were inspected every 24 hours to monitor development. Once the nymphs reached adulthood, any nymphs deposited (by the adult aphid) were counted, removed from the gelatin capsule, and weighed daily. This permitted several indicators of aphid performance to be explored

All weights were taken on a Sartorius microbalance (Type M3P, last calibrated on the 29th September, 2009).

Aphid performance indicators

The following performance indicators were recorded for each of the three trials in both the experiments.

Aphid mean Relative Growth Rate (mRGR)

Each of the four mRGR nymphs was removed from the plant and weighed, before being returned to the plant. They were then left to feed undisturbed for seven days, before being removed from the plant and re-weighed.

The mRGR for each nymph was calculated using the following formula (Fisher, 1921; Radford, 1967):

$$mRGR\,(\text{mg/mg/day}) = \frac{[\ln(\text{final weight}) - \ln(\text{initial weight})]}{\text{N. of days between weighings}} \tag{I}$$

All were removed after the data was collected. Results are summarised in Table 5.1.

Survival

Each of the four nymphs were re-caged on the day of deposition, and checked again after seven days had passed. Capsules were then checked and survival of the experimental

Figure 5.1: CT room trial capsule set-up. Four adult *E. abietinum* aphids from the stock culture were caged in gelatin capsules on each of the plants. Two were caged on needles on the leader shoot, and two on needles on the side shoot.

nymph recorded. All were removed after the data was collected. Results are summarised in Table 5.2.

Lifespan, generation time and reproductive days

In addition to recording the lifespan of each experimental aphid, the length of time taken for the experimental aphid to start depositing nymphs from its own deposition was recorded, allowing the calculation of the generation time. Furthermore, the number of days between first nymph deposition and the aphid's death, this being the number of reproductive days, was also recorded. Results are summarised in Tables 5.3, 5.4 and 5.5.

Aphid weights

On the first day of nymph deposition by each experimental aphid, the adult was removed from the needle and weighed before being returned to its capsule to obtain the adult weight. Additionally, for each adult aphid, the total nymph weight was divided by the number of nymphs for each daily cohort, giving the mean nymph weight. Results are summarised in Tables 5.6 and 5.7.

Seven-day fecundity

The number of nymphs produced by each experimental aphid from day two to day eight of the aphid's reproductive period were counted. The sum total equates to the seven-day fecundity. Nymphs deposited on the first day of recording were excluded from the calculation, as the length of time the experimental aphid would have been producing nymphs during that first 24 hours was unknown. Results are summarised in Table 5.8.

Intrinsic rate of increase (r_m)

The number of nymphs produced by each experimental aphid was counted for the number of days that it took for the aphid to reach reproductive age. The r_m was calculated using the following formula (Wyatt & White, 1977):

$$r_m = 0.74(\ln F_D/D) \tag{II}$$

where F_D is the number of nymphs produced over a period of time equal to the pre-reproductive period (D). Results are summarised in Table 5.9.

Total reproductive output

The nymphs produced by each experimental aphid were collected and weighed daily. The total weight of all the nymphs equates to the total reproductive output. Results are summarised in Table 5.10.

Statistical analysis

All performance indicators were analysed using linear mixed effects models to take into account pseudoreplication. In cases where an aphid did not meet the requirements for the calculation of a given performance indicator, the aphid was excluded from the analysis of that indicator (e.g. too few reproductive days to calculate seven-day fecundity, or if no nymphs were deposited before death). Survival of the nymphs was analysed with a binomial distribution. Drought was modelled as a fixed effect, while capsule location and tree were modelled as random effects (groups: tree = 50, location = 2; n = 50, estimated d.f. for each parameter = 4).

Comparison of the performance indicators between the three trials in each experiment was conducted in the same manner as per the indicators' analysis within each trial.

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

Experiment 1: Performance in spring

Mean Relative Growth Rate (mRGR)

A significant effect of drought on mRGR was found in all three spring trials (ST1: ${\chi^2}_4 = 48.16$, P< 0.001; ST2: ${\chi^2}_4 = 32.02$, P< 0.001; ST3: ${\chi^2}_4 = 33.34$, P< 0.001; Figure 5.2). There was also a significant difference between trials (t = 4.98, $P < 0.001$), under the IS1 drought treatment (t = 5.24, $P < 0.001$).

At budburst(ST1), nymphs under IS1 drought had a significantly lower mRGR (t = 4.10, $P < 0.001$), whereas those reared under IS2 had a higher mRGR (t = 2.35, $P < 0.05$) when compared with those reared under control FC conditions. In the second trial (ST2), nymphs reared under severe-type drought stress had reduced mRGR (CS: t = 3.01, $P < 0.01$; IS1: t = 2.33, $P < 0.05$). Again, nymphs reared under the IS2 drought level had a significantly higher mRGR (t = 2.06, $P < 0.05$). Intermittent stress had a significant effect on nymph mRGR in ST3. In both treatments, nymphs had a higher mRGR than the FC control (IS1: t = 3.57, $P < 0.001$; IS2: t = 3.96, $P < 0.001$).

Survival

There was no difference in the proportion of surviving nymphs seven days after deposition in ST2 and ST3 (ST2: ${\chi^2}_4 = 1.27$, $P > 0.05$; ${\chi^2}_4 = 5.59$, $P > 0.05$; Figure 5.3). While drought did significantly affect survival in ST1 (${\chi^2}_4 = 12.05$, $P < 0.05$), there was no difference between the treatments and the FC control. Rather, the difference was observed between the CS and IS2 treatments compared with the IS1 treatment (Figure 5.3). This was reflected by differences in the proportion surviving depending on trial (under CS drought, t = 2.02, $P < 0.05$).

Lifespan, generation time and reproductive days

Lifespan was not affected by drought stress (ST1: ${\chi^2}_4 = 5.47$, P> 0.05; ST2: ${\chi^2}_4 = 7.11$, P> 0.05; Figure 5.4(A & B)), except in ST3 (${\chi^2}_4 = 13.17$, P< 0.05; Figure 5.4(C)) where it was reduced under the MS treatment (t = 2.43, $P < 0.05$). Furthermore, lifespan under

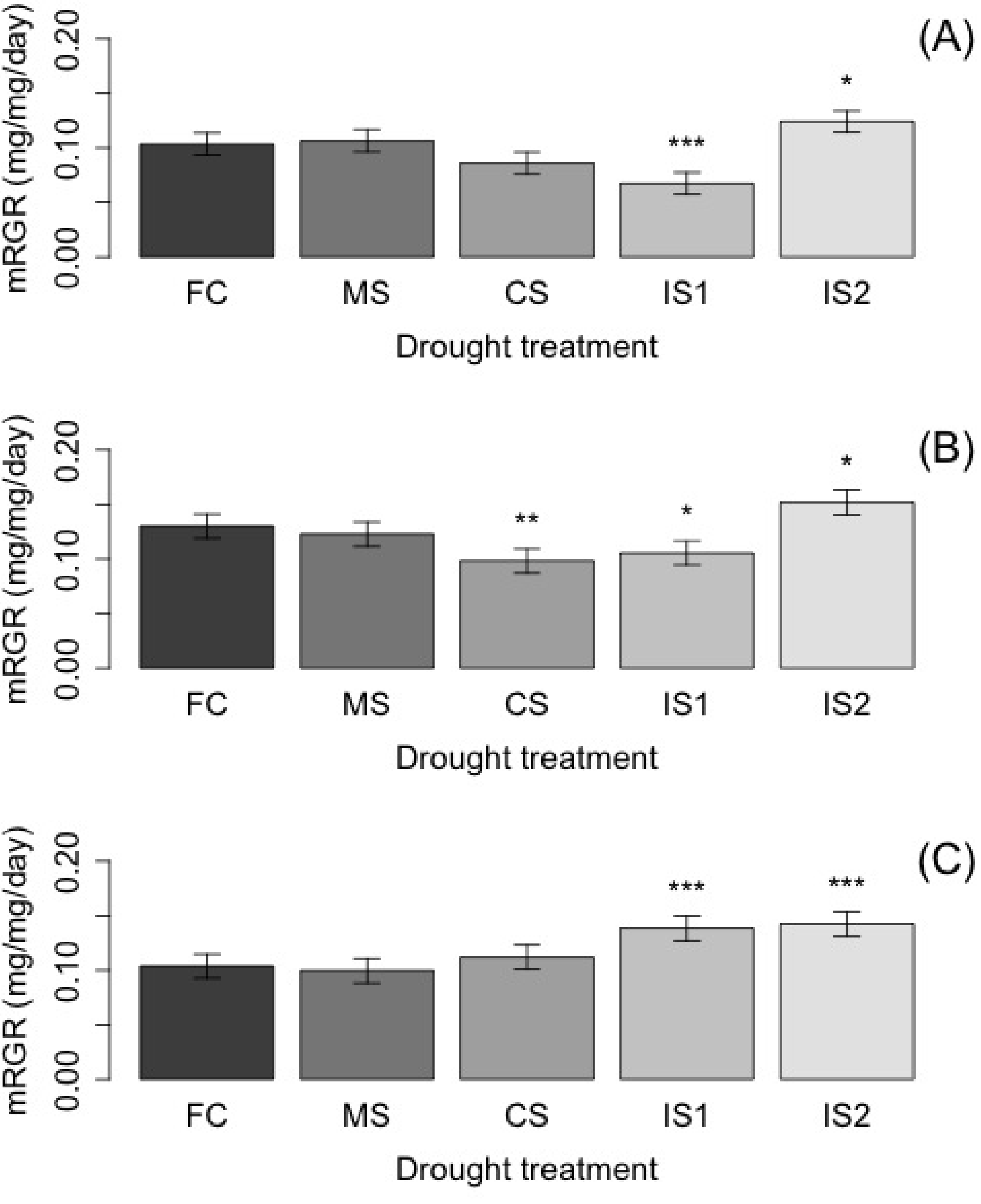

Figure 5.2: The effect of drought stress on mRGR of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

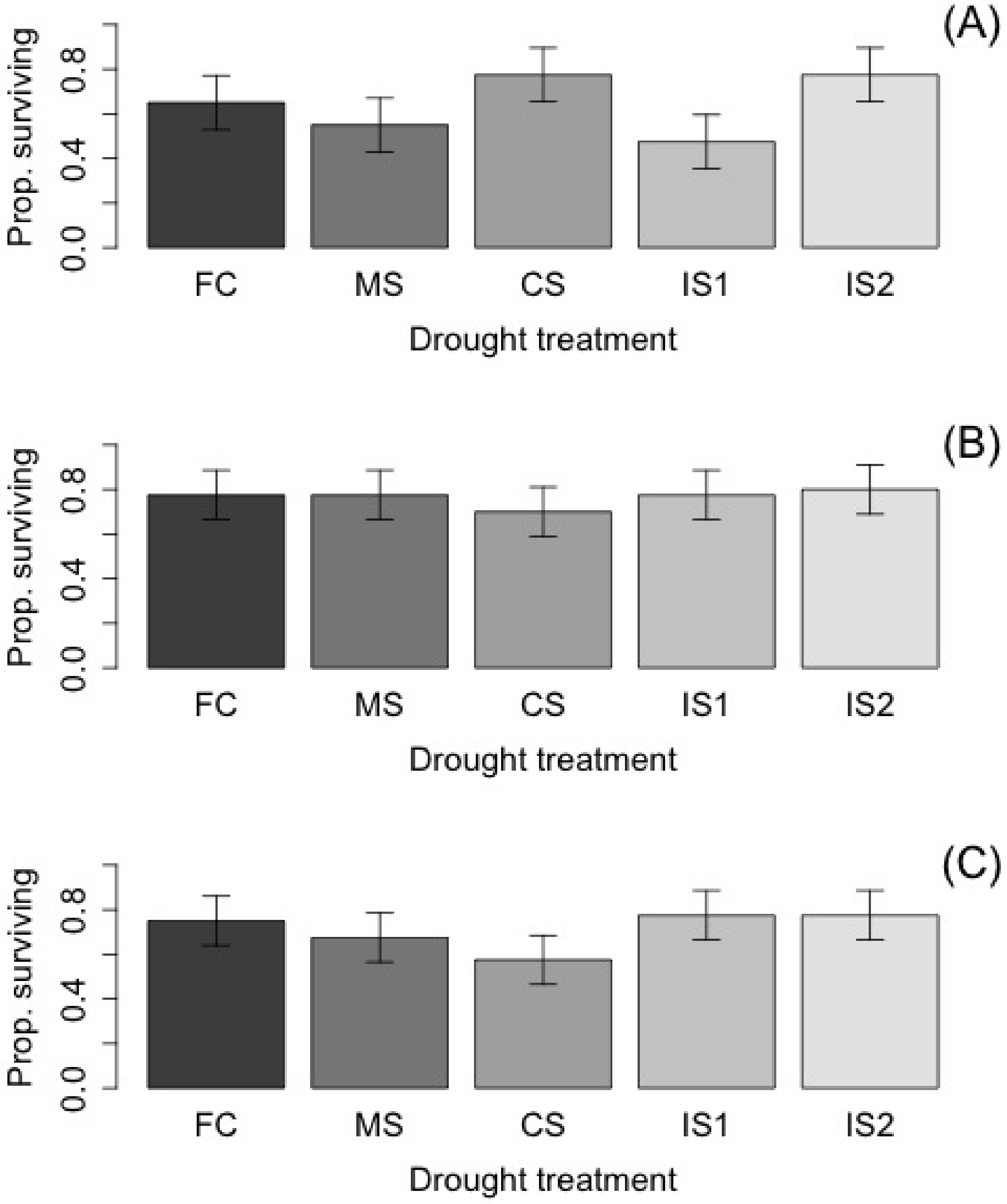

Figure 5.3: The effect of drought stress on survival of *E. abietinum* nymphs on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

MS drought differed between the trials ($t = 2.21$, $P < 0.05$).

Although lifespan may not have been affected, the generation time (ST1: ${\chi^2}_4 = 316.44$, $P < 0.001$; ST2: ${\chi^2}_4 = 763.83$, $P < 0.001$; ST3: ${\chi^2}_4 = 225.71$, $P < 0.001$; Figure 5.5) and number of reproductive days (ST1: ${\chi^2}_4 = 44.82$, $P < 0.001$; ST2: ${\chi^2}_4 = 46.72$, $P < 0.001$; ST3: ${\chi^2}_4 = 34.95$, $P < 0.001$; Figure 5.6) were both significantly affected by drought stress in all three trials.

Under the severe drought treatments, CS and IS1, generation time was increased in all three trials (ST1 - CS: $t = 9.84$, $P < 0.001$; IS1: $t = 9.25$, $P < 0.001$. ST2 - CS: $t = 16.91$, $P < 0.001$; IS1: $t = 19.08$, $P < 0.001$. ST3 - CS: $t = 6.08$, $P < 0.001$; IS1: $t = 6.94$, $P < 0.001$). Conversely, in the ST1 and ST3 trials, generation time of the experimental aphids under IS2 drought treatment was significantly reduced (ST1: $t = 3.96$, $P < 0.001$. ST3: $t = 6.08$, $P < 0.001$). Generation time also differed between the trials ($t = 3.53$, $P < 0.05$) under both the CS ($t = 2.33$, $P < 0.05$) and IS2 ($t = 2.03$, $P < 0.05$) drought treatments.

Although the number of reproductive days was not affected by the trial ($t = 1.01$, $P > 0.05$), it was affected by drought. In both ST1 and ST2, severe stress reduced the number of reproductive days (ST1 - CS: $t = 2.00$, $P < 0.05$; IS1: $t = 3.09$, $P < 0.001$. ST2 - CS: $t = 5.42$, $P < 0.001$; IS1: $t = 4.10$, $P < 0.001$). Furthermore, in ST1, reproductive days were significantly higher for IS2 aphids ($t = 2.91$, $P < 0.01$). In the third spring trial (ST3), it was continuous stress which reduced the number of reproductive days (MS: $t = 2.45$, $P < 0.05$; CS: $t = 3.54$, $P < 0.001$).

Aphid weights

Adult weight

Adult *E. abietinum* weight was increased under drought treatment during all three trials (ST1: ${\chi^2}_4 = 88.26$, $P < 0.001$; ST2: ${\chi^2}_4 = 165.88$, $P < 0.001$; ST3: ${\chi^2}_4 = 70.19$, $P < 0.001$; Figure 5.7). During budburst (ST1), aphids under both CS($t = 3.09$, $P < 0.01$) and IS2 ($t = 7.19$, $P < 0.001$) treatments were affected. During the ST2 trial, CS($t = 3.55$, $P < 0.001$), IS1 ($t = 2.54$, $P < 0.05$), and IS2 ($t = 11.41$, $P < 0.001$) treatments increased adult aphid weight. The same drought treatments also had an effect in ST3 (CS: $t = 2.31$, $P < 0.05$; IS1: $t = 2.54$, $P < 0.05$; IS2: 7.07, $P < 0.001$).

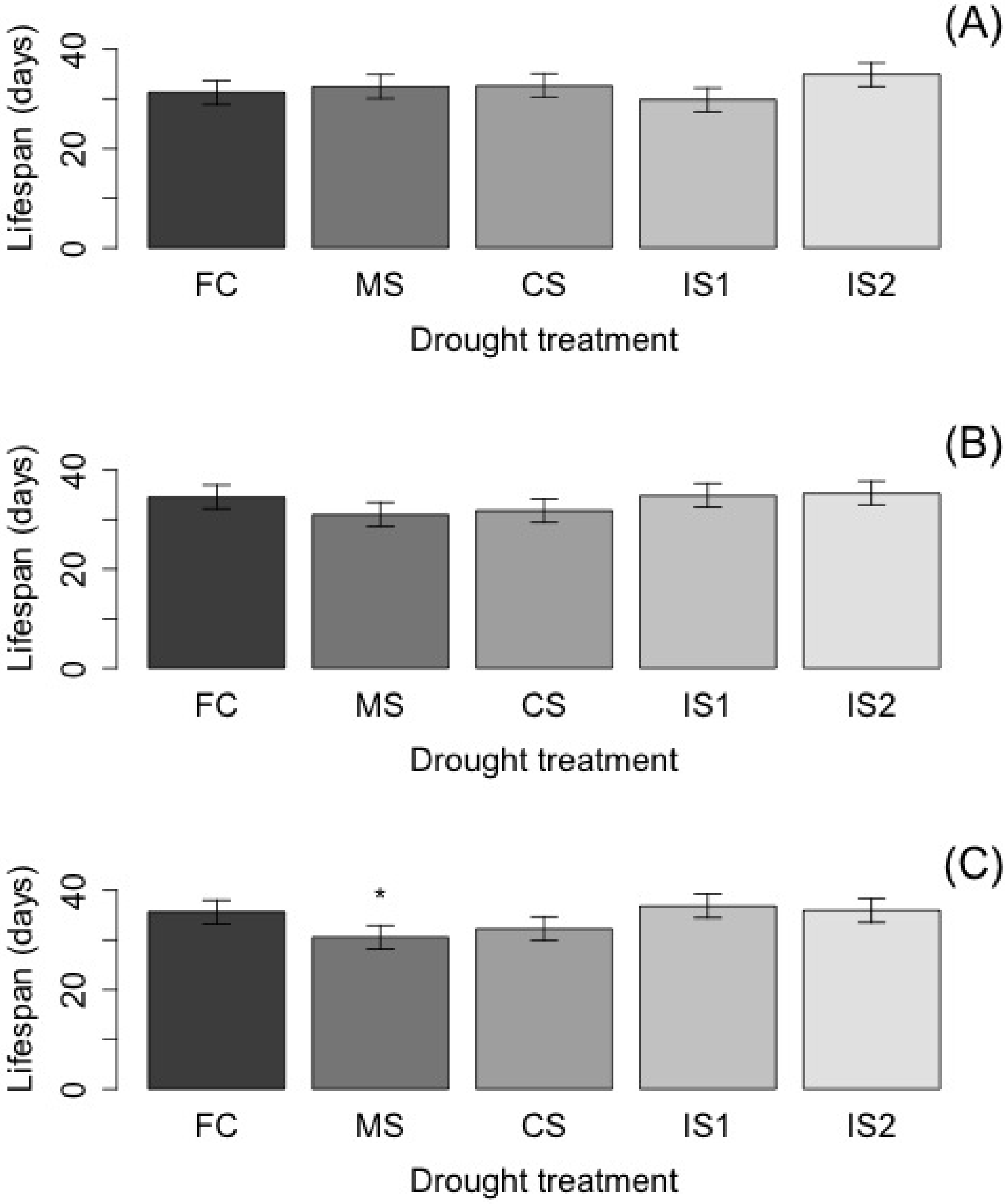

Figure 5.4: The effect of drought stress on lifespan of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

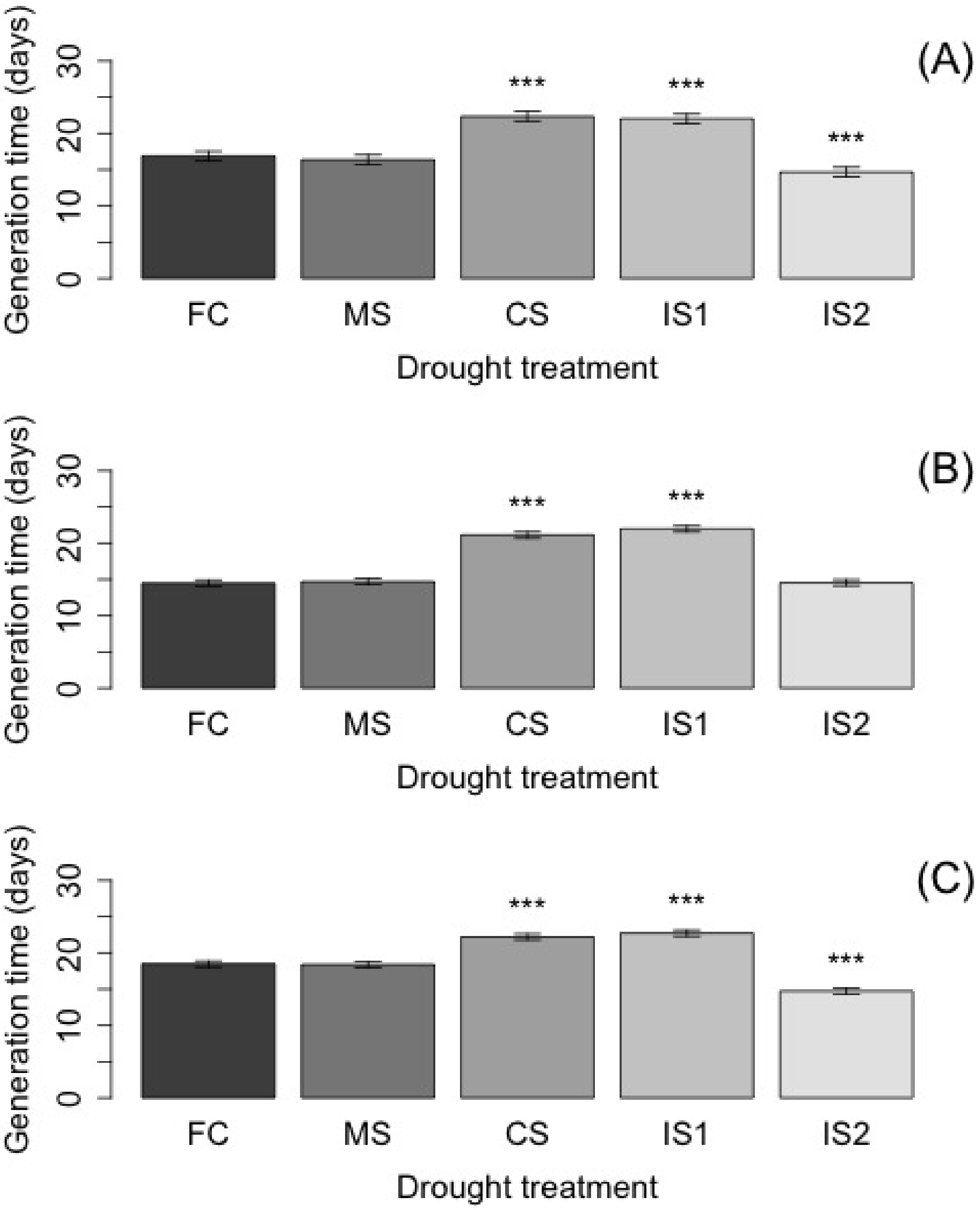

Figure 5.5: The effect of drought stress on generation time of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = P < 0.001; ** = P < 0.01; * = P < 0.05.

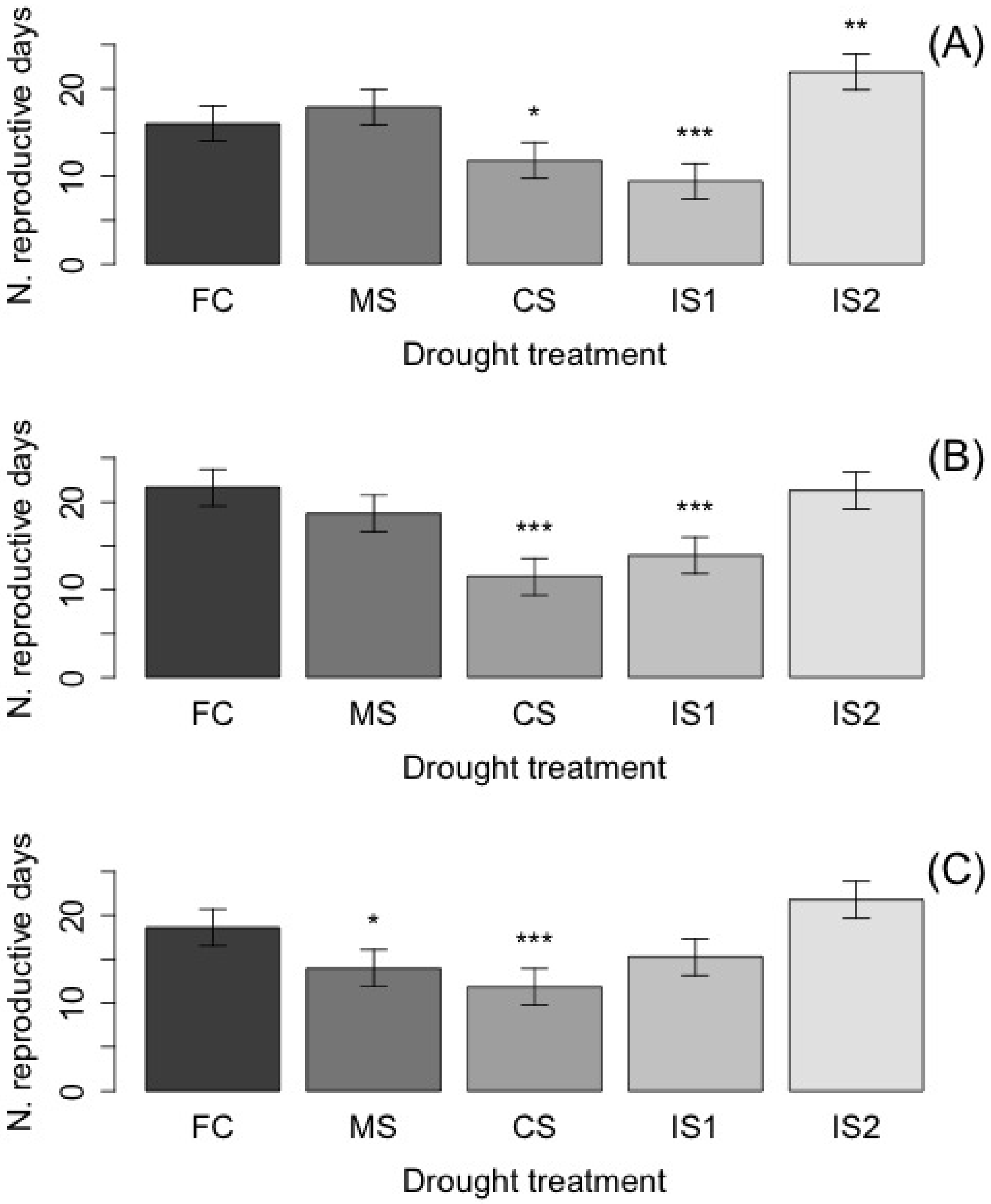

Figure 5.6: The effect of drought stress on number of reproductive days of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

Additionally, there was a difference under both CS ($t = 2.56$, $P < 0.05$) and IS2 ($t = 6.08$, $P< 0.001$) drought treatments between trials ($t = 2.84$, $P < 0.05$).

Mean nymph weight

Although mean nymph weight was affected by drought in all three spring trials (ST1: ${\chi^2}_4 = 1475.20$, $P < 0.001$; ST2: ${\chi^2}_4 = 1233.90$, $P < 0.001$; ST3: ${\chi^2}_4 = 252.40$, $P < 0.001$; Figure 5.8), the nature of the response depended on the drought type.

Mean nymph weight was significantly reduced by severe stress, be it continuous or intermittent (ST1 - CS: $t = 22.89$, $P < 0.001$; IS1: $t = 21.22$, $P < 0.001$. ST2 - CS: $t = 23.14$, $P < 0.001$; IS1: $t = 16.42$, $P < 0.001$. ST3 - CS: $t = 11.23$, $P < 0.001$; IS1: $t = 9.78$, $P < 0.001$). Nymphs weight was also reduced under MS drought during both ST1 ($t = 4.74$, $P < 0.001$) and ST2 ($t = 5.37$, $P < 0.001$). In contrast to this, nymphs deposited under IS2 drought treatment were significantly heavier than those under the FC control (ST1: $t = 8.32$, $P < 0.001$; ST2: $t = 7.47$, $P < 0.001$), and it was under this same drought treatment that there was a difference between trials ($t = 11.84$, $P < 0.001$; IS2: $t = 3.30$, $P < 0.01$).

Seven-day fecundity

Drought stress affected the seven-day fecundity of *E. abietinum* in all three trials (ST1: ${\chi^2}_4 = 31.67$, $P < 0.001$; ST2: ${\chi^2}_4 = 88.87$, $P < 0.001$; ST3: ${\chi^2}_4 = 75.05$, $P < 0.001$; Figure 5.9).

Seven-day fecundity was increased under IS2 drought (ST1: $t = 3.05$, $P < 0.01$; ST3: $t = 4.19$, $P < 0.001$). Furthermore, it was reduced under CS (ST2: $t = 5.42$, $P < 0.01$; ST3: $t = 2.61$, $P < 0.05$) and IS1 (ST1: $t = 5.62$, $P < 0.01$; ST3: $t = 3.40$, $P < 0.001$) severe drought treatments.

Intrinsic rate of increase (r_m)

The r_m was only affected by drought treatment during the ST2 trials (ST1: ${\chi^2}_4 = 4.76$, $P > 0.05$; ST2: ${\chi^2}_4 = 11.20$, $P < 0.05$; ST3: ${\chi^2}_4 = 1.49$, $P > 0.05$). Specifically, r_m was reduced under the CS and IS1 drought treatments ($t = 2.45$, $P < 0.01$) during ST2,

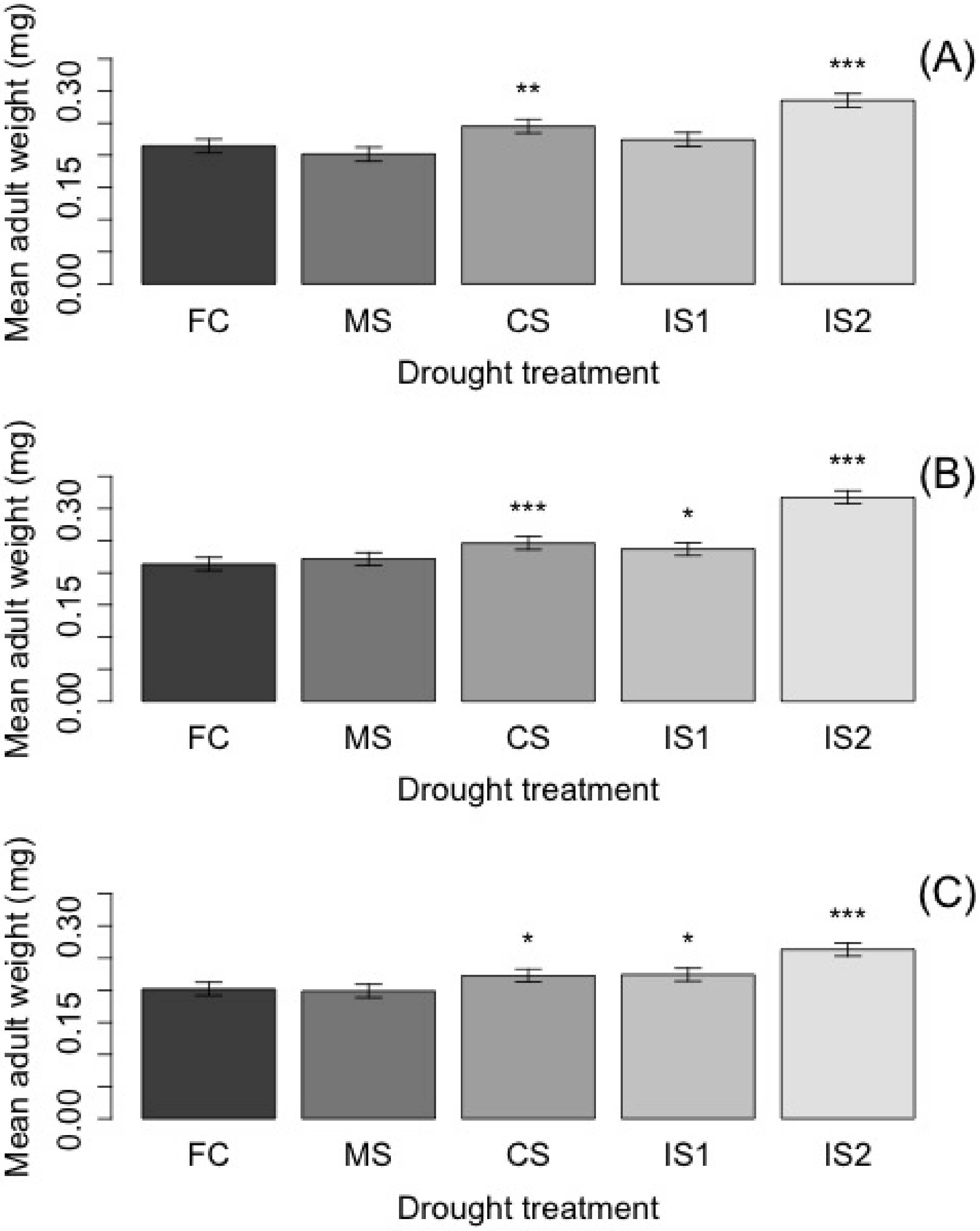

Figure 5.7: The effect of drought stress on adult weight of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

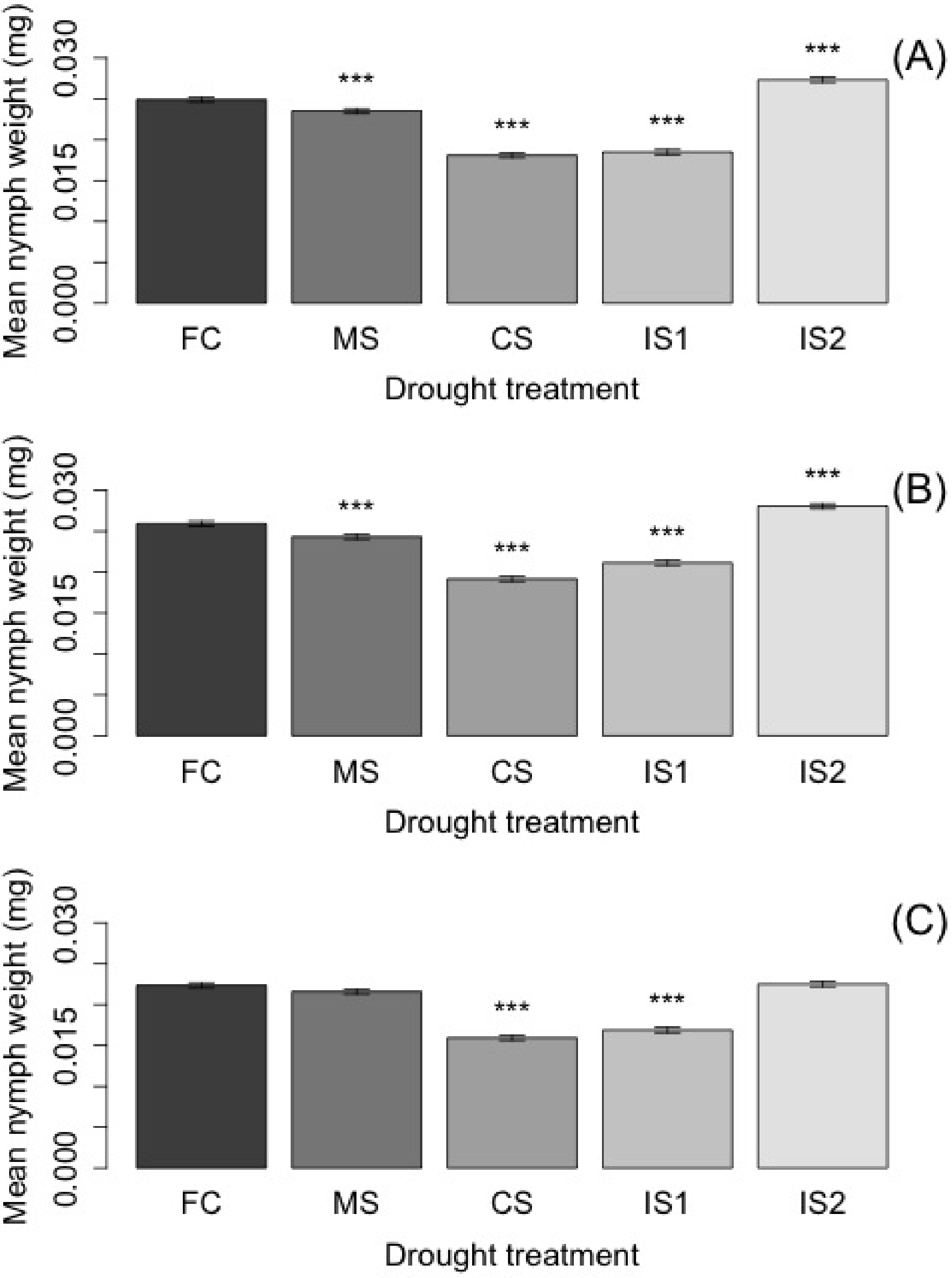

Figure 5.8: The effect of drought stress on mean nymph weight of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

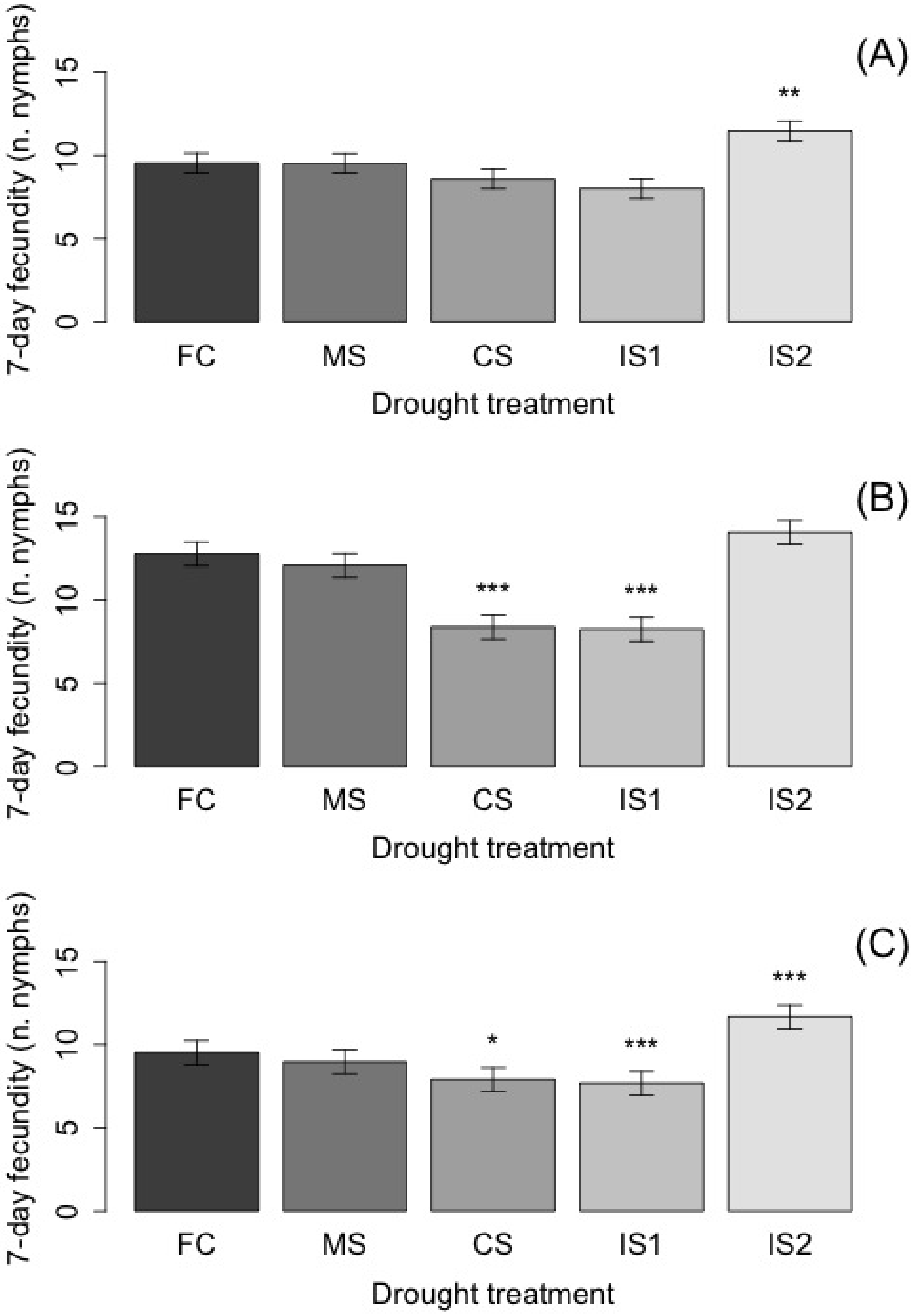

Figure 5.9: The effect of drought stress on seven-day fecundity of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

though there was no significant difference between the two treatments. There was also no difference between the trials ($t = 0.92$, $P > 0.05$).

Total reproductive output

Although total reproductive output did not differ between trials ($t = 0.40$, $P > 0.05$), there was a significant effect of drought treatment in all three trials (ST1: ${\chi^2}_4 = 166.45$, $P < 0.001$; ST2: ${\chi^2}_4 = 197.42$, $P < 0.001$; ST3: ${\chi^2}_4 = 111.54$, $P < 0.001$; Figure 5.11).

Severe drought reduced reproductive output in all three trials (ST1 - CS:4.08, $P < 0.001$; IS1: $t = 4.83$, $P < 0.001$. ST2 - CS:9.32, $P < 0.001$; IS1: $t = 8.29$, $P < 0.001$. ST3 - CS: $t = 4.63$, $P < 0.001$; IS1: $t = 3.41$, $P < 0.001$). Similarly, a reduction was seen under MS drought during both ST2 ($t = 2.61$, $P < 0.01$) and ST3 ($t = 2.23$, $P < 0.01$). In contrast to these, IS2 drought resulted in significantly higher reproductive output during ST1 ($t = 6.62$, $P < 0.001$) and ST3 ($t = 4.81$, $P < 0.001$).

Experiment 2: Performance in autumn

Mean Relative Growth Rate (mRGR)

An effect of drought stress was observed in all three autumn trials (AT1: ${\chi^2}_4 = 16.11$, $P < 0.05$; AT2: ${\chi^2}_4 = 35.39$, $P < 0.001$; AT3: ${\chi^2}_4 = 14.79$, $P < 0.01$; Figure 5.12). Specifically, nymphs on trees subjected to CS-type drought had increased mRGR (AT1: $t = 3.47$, $P < 0.001$; AT2: $t = 5.56$, $P < 0.001$; AT3: $t = 2.73$, $P < 0.01$). Additionally, during AT2, the mRGR of IS2 aphids was also increased ($t = 2.23$, $P < 0.05$). There was, however, no significant difference in the rates between trials ($t = 1.146$, $P > 0.05$).

Survival

The survival rate of *E. abietinum* nymphs was affected by drought during AT1 (${\chi^2}_4 = 12.00$, $P < 0.05$; Figure 5.13(A)). The proportion of surviving nymphs reduced under IS1 drought ($z = 2.03$, $P < 0.05$). No effect on survival was observed in AT2 (${\chi^2}_4 = 1.48$, $P > 0.05$; Figure 5.13(B)) nor in AT3 (${\chi^2}_4 = 0.41$, $P > 0.05$; Figure 5.13(C)). There was also no difference in the proportion across the three treatments ($P > 0.05$).

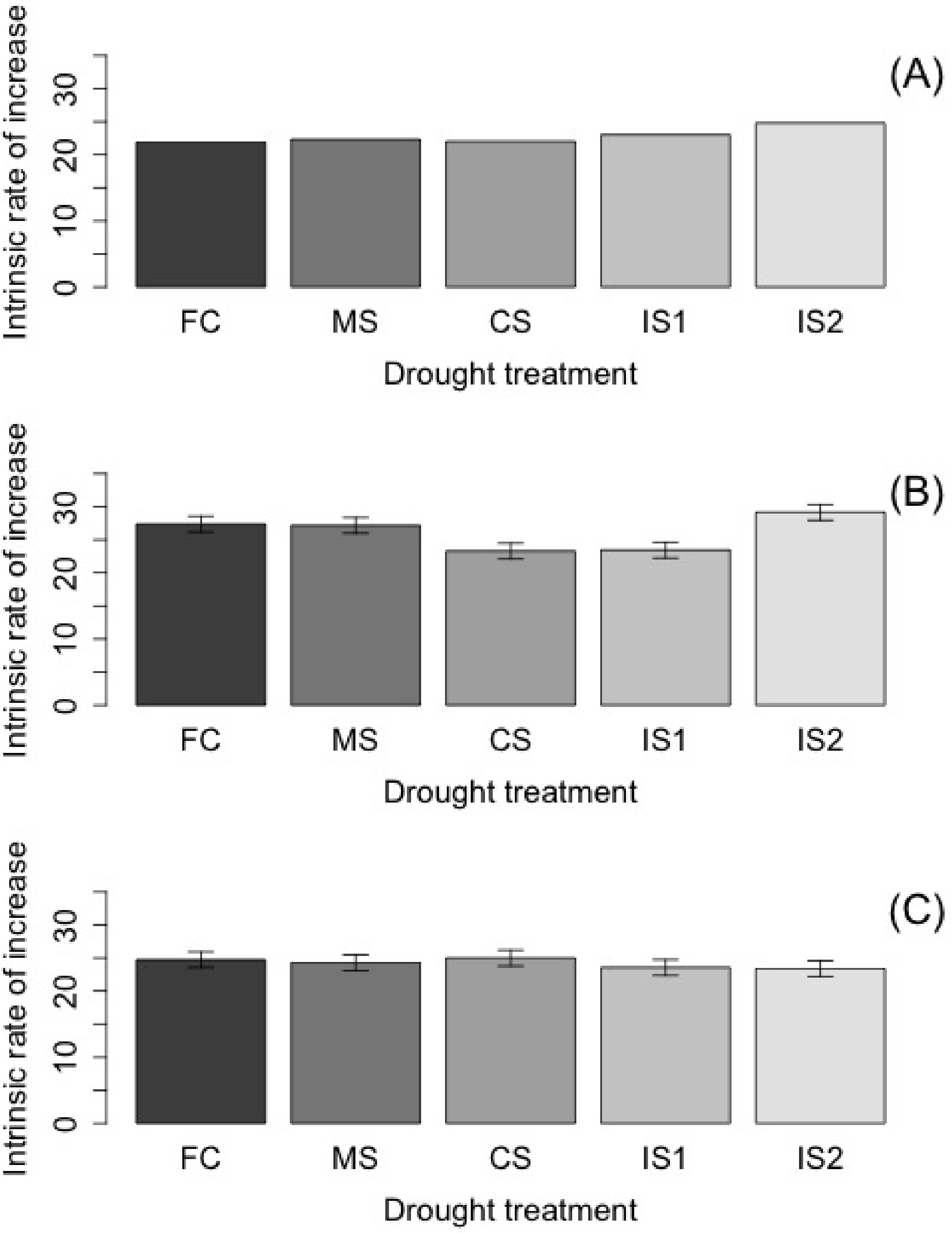

Figure 5.10: The effect of drought stress on the intrinsic rate of increase of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

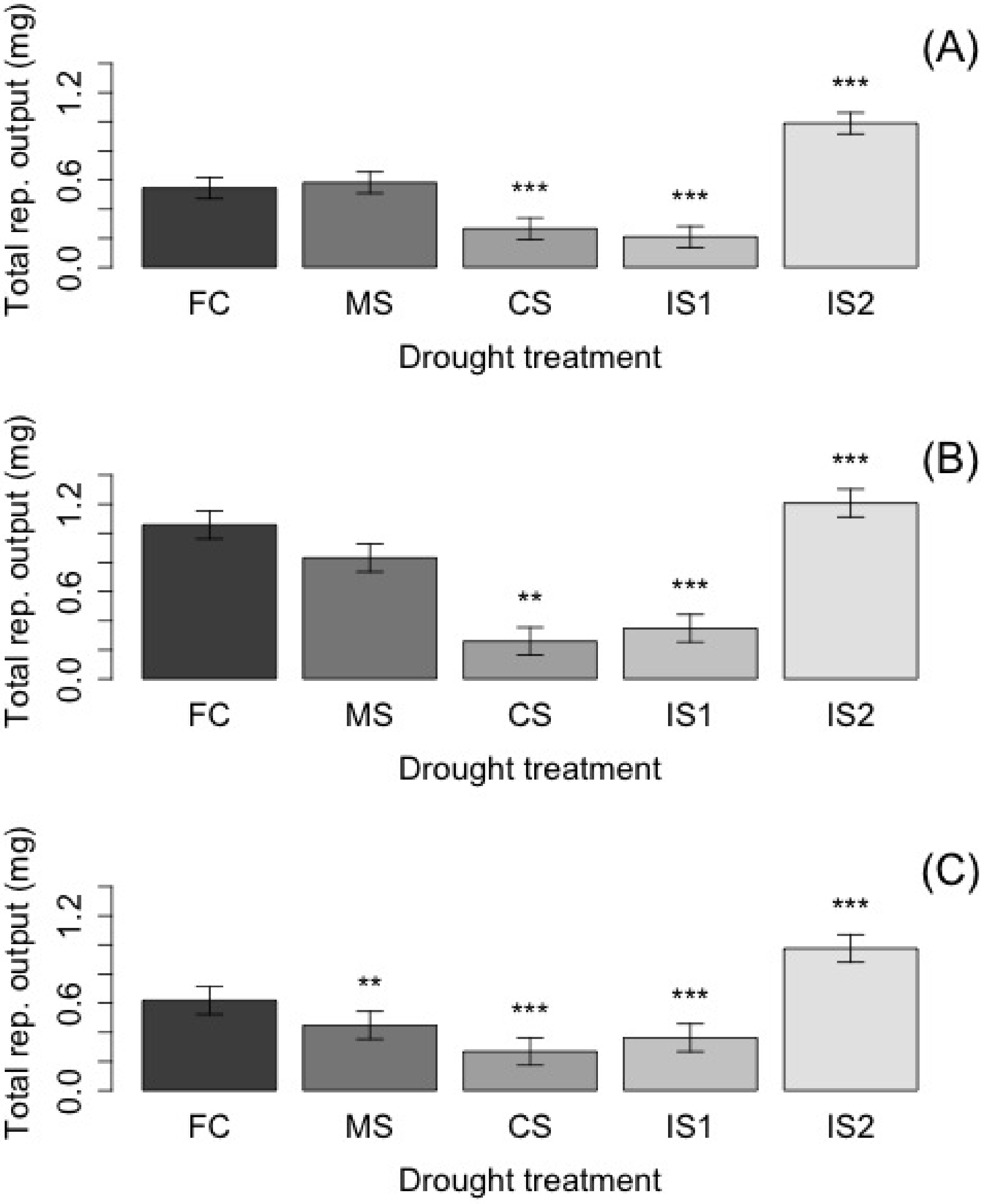

Figure 5.11: The effect of drought stress on total reproductive output of *E. abietinum* on spruce under controlled conditions during spring.

Bars indicate Least Significant Difference. (A) ST1: At bud burst; (B) ST2: Two weeks after bud burst; (C) ST3: Five weeks after bud burst. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = P < 0.001; ** = P < 0.01; * = P < 0.05.

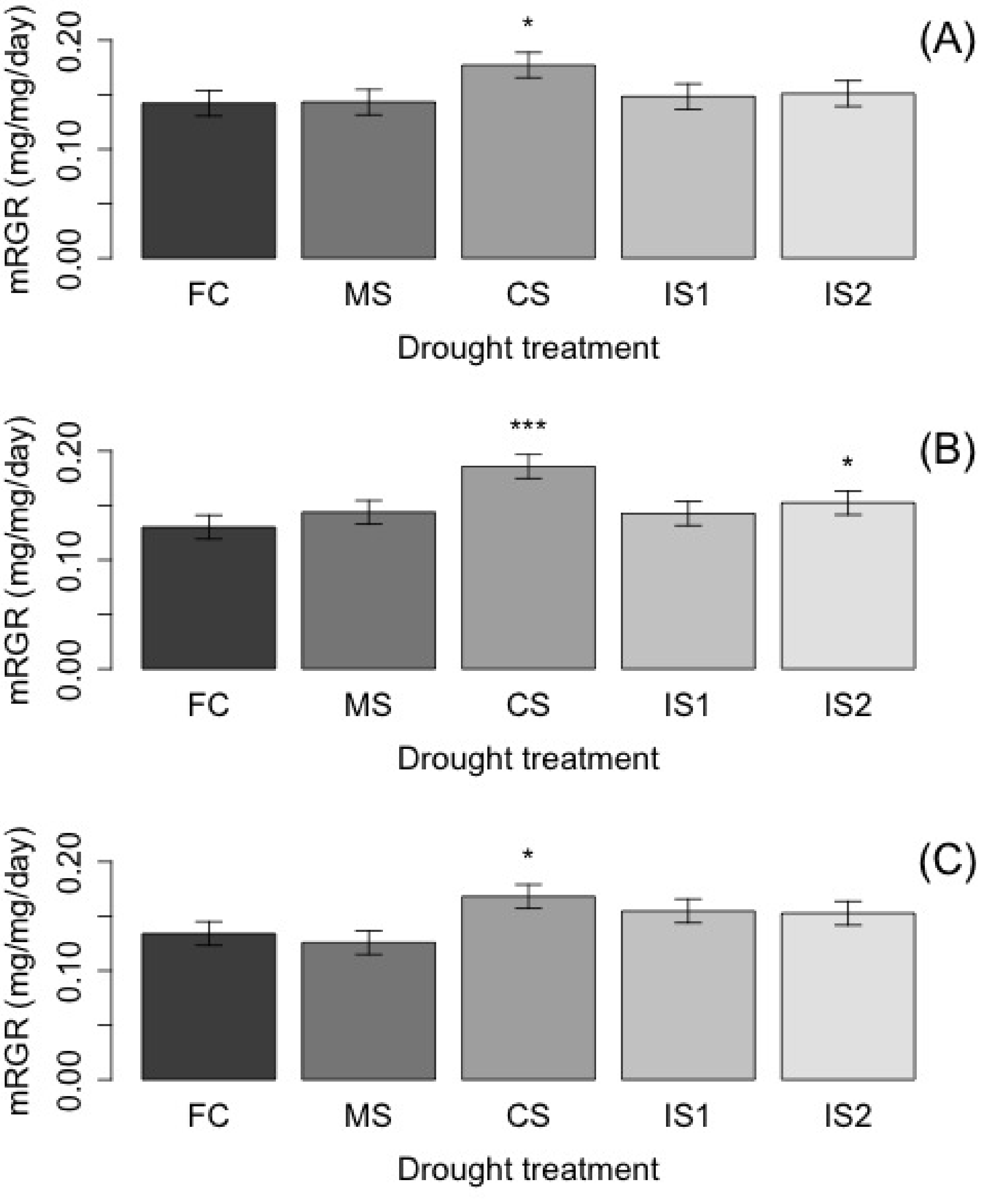

Figure 5.12: The effect of drought stress on mRGR of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

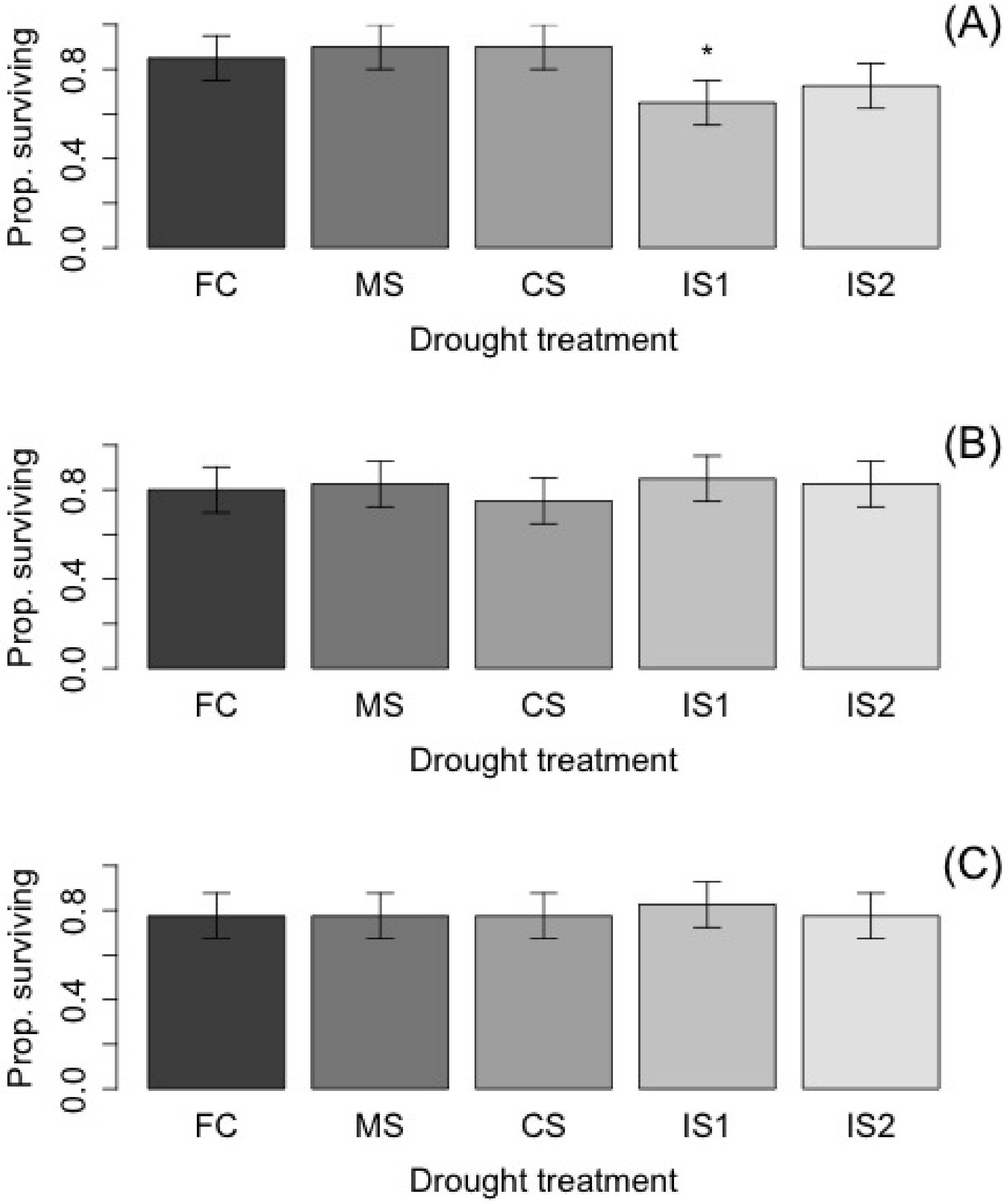

Figure 5.13: The effect of drought stress on survival of *E. abietinum* nymphs on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = P < 0.001; ** = P < 0.01; * = P < 0.05.

Lifespan, generation time and reproductive days

Although lifespan was increased under IS2 drought ($t = 2.64$, $P < 0.01$) during AT2, drought was not found to have a significant effect on the model (${\chi^2}_4 = 5.97$, $P > 0.05$; Figure 5.14(B)). Furthermore, in comparison to the FC control, lifespan was reduced under MS drought ($t = 2.33$, $P < 0.05$) during AT1, though again overall drought was not a significant effect in the model (${\chi^2}_4 = 8.61$, $P > 0.05$; Figure 5.14(A)). There was no effect during AT3 (${\chi^2}_4 = 4.53$, $P > 0.05$; Figure 5.14(C)). Lifespan under MS ($t = 2.60$, $P < 0.01$), CS ($t = 2.29$, $P < 0.05$) and IS1($t = 2.54$, $P < 0.05$) drought treatments were found to differ between trials ($t = 2.06$, $P < 0.05$), even if not within trial.

Generation time showed a less complex response to drought stress, which had an effect in all three trials (AT1: ${\chi^2}_4 = 89.35$, $P < 0.001$; AT2: ${\chi^2}_4 = 25.84$, $P < 0.01$; AT3: ${\chi^2}_4 = 59.94$, $P < 0.001$; Figure 5.15). Severe drought treatments increased the generation time (AT1 - CS: $t = 6.16$, $P < 0.001$; IS1: $t = 3.95$, $P < 0.001$. AT2 - CS: $t = 4.12$, $P < 0.001$; IS1: $t = 2.71$, $P < 0.001$), and this was also true during AT3 under MS drought ($t = 3.22$, $P < 0.01$). In addition to this, a significant difference was found between trials ($t = 6.57$, $P < 0.001$), where both MS ($t = 3.32$, $P < 0.001$) and IS1 ($t = 2.23$, $P < 0.05$) were affected.

Drought also had a significant impact on the number of reproductive days of *E. abietinum* adults in AT1 (${\chi^2}_4 = 21.34$, $P < 0.001$; Figure 5.16(A)) and AT2 (${\chi^2}_4 = 14.63$, $P < 0.01$; Figure 5.16(B)). Despite this, there was no difference between the trials ($t = 0.12$, $P > 0.05$). A reduction in the number of days was observed under severe stress during AT1 (CS: $t = 3.10$, $P < 0.01$; IS1: $t = 2.56$, $P < 0.001$), and though there was no difference between the two treatments in AT2, they again saw a reduction ($t = 2.049$, $P < 0.05$) when compared to the FC control. During AT3, despite an effect of drought (${\chi^2}_3 = 9.33$, $P < 0.05$; Figure 5.16(C)), none of the treatment levels differed from the FC control. However, aphids under MS and IS2 treatments had more reproductive days when they were combined into a single factor level compared to the combined remaining treatments ($t = 2.57$, $P < 0.05$).

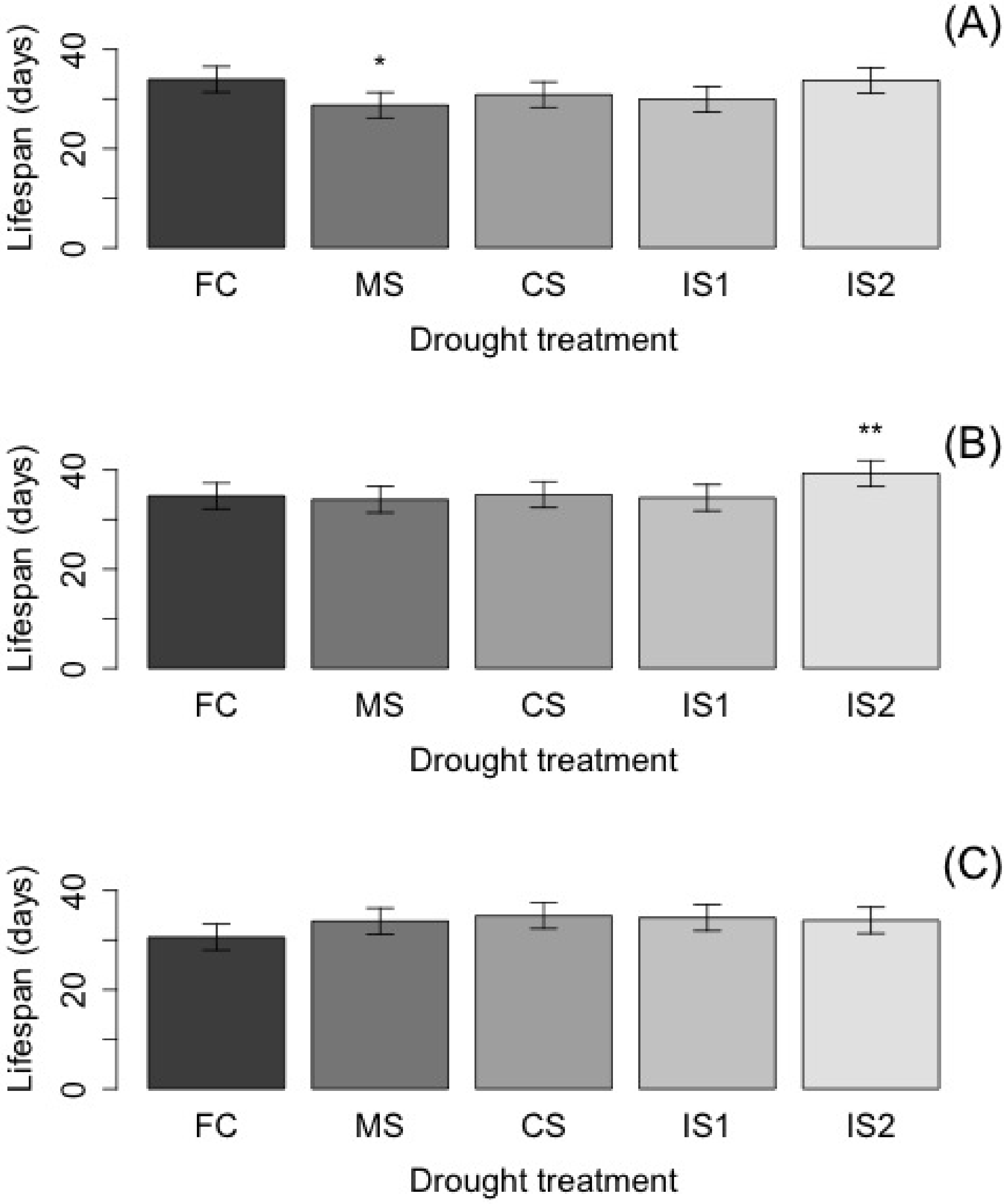

Figure 5.14: The effect of drought stress on lifespan of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

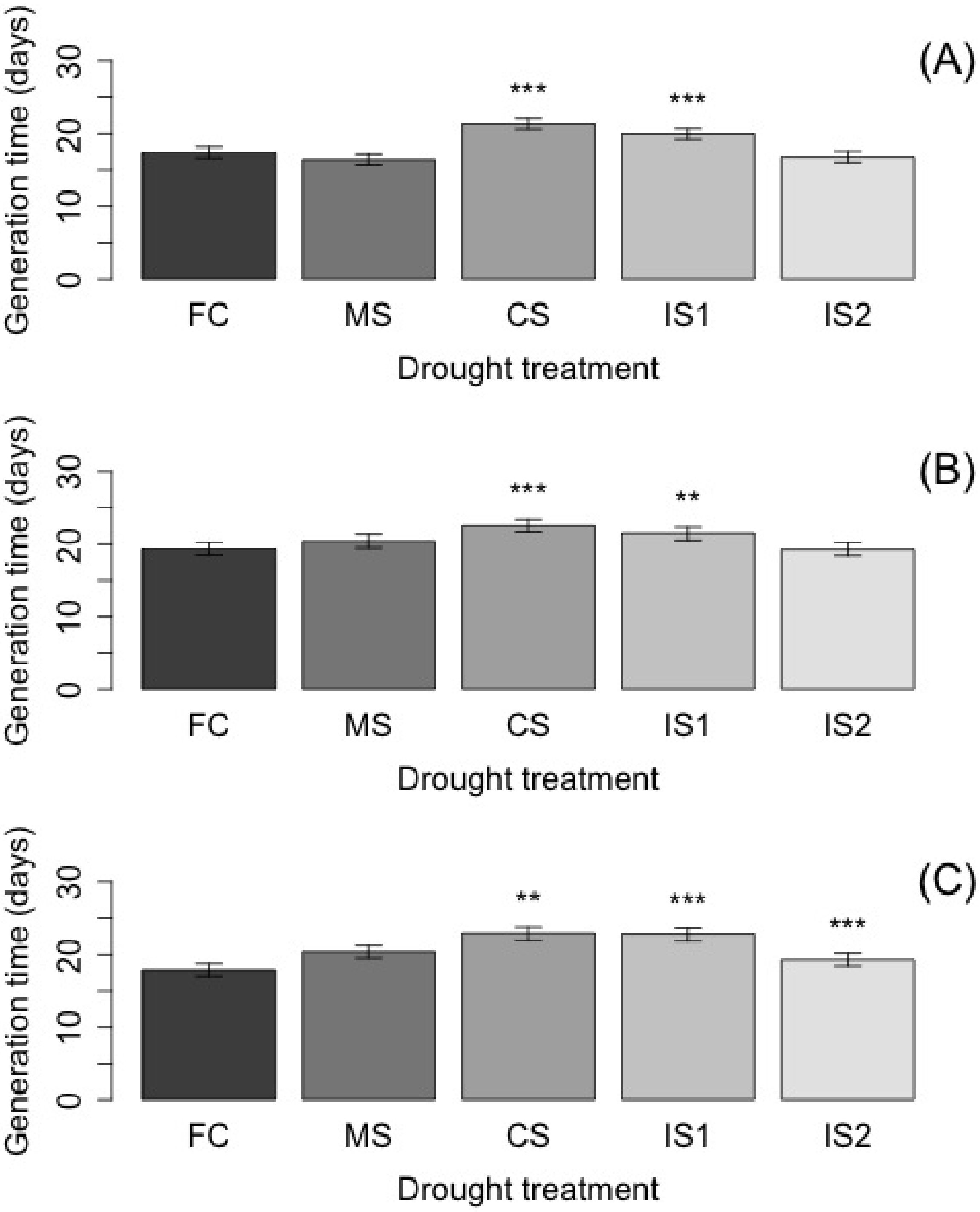

Figure 5.15: The effect of drought stress on generation time of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

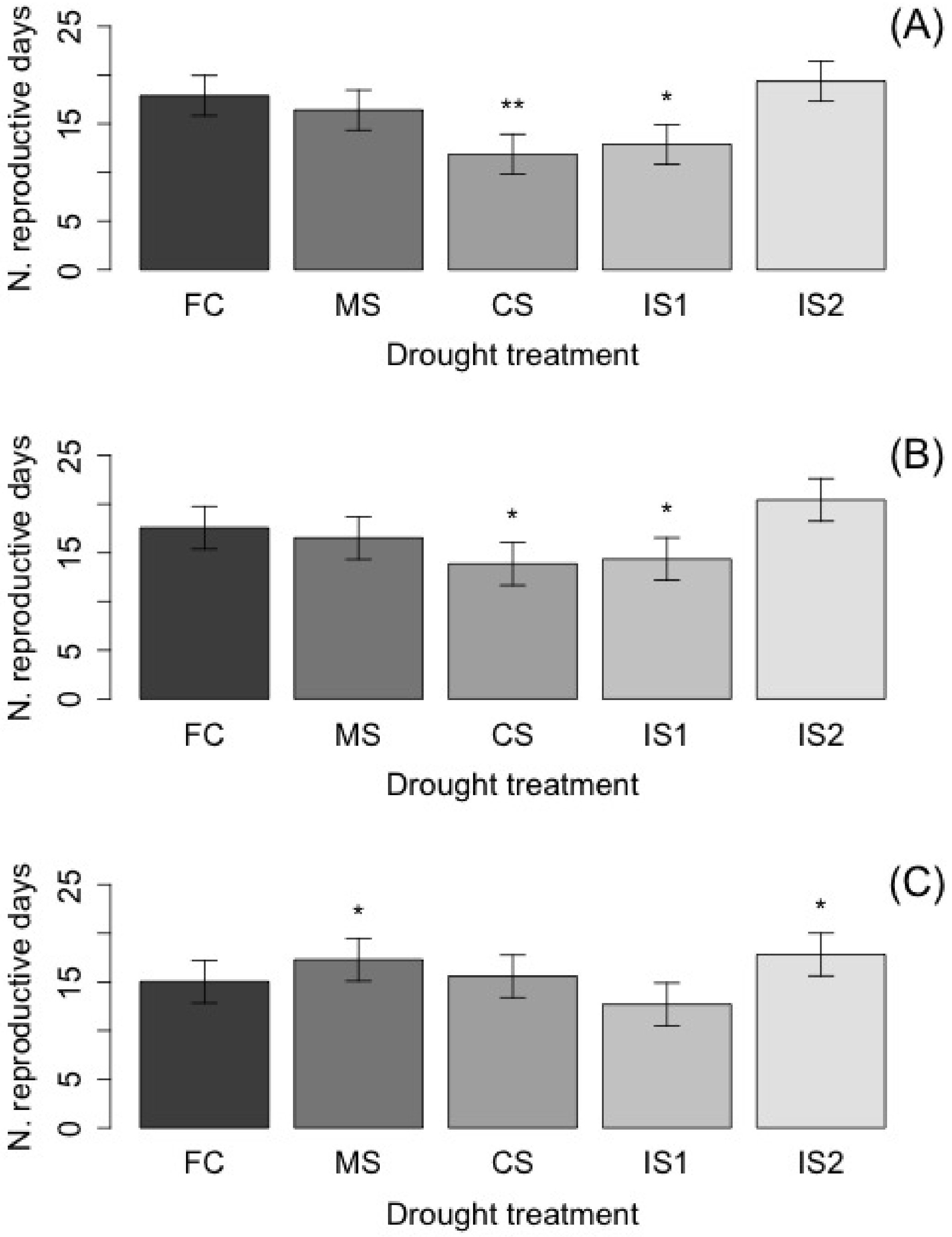

Figure 5.16: The effect of drought stress on number of reproductive days of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

Aphid weights

Adult weight

Although adult weight did not differ between the trials ($t = 0.35$, $P > 0.05$), a within-trial effect of drought was found in all three (AT1: ${\chi^2}_4 = 132.59$, $P < 0.001$; AT2: ${\chi^2}_4 = 91.68$, $P < 0.001$; AT3:${\chi^2}_4 = 85.87$, $P < 0.001$; Figure 5.17).

Although adult weight was consistently increased under IS2 drought (AT1: $t = 5.86$, $P < 0.001$; AT2: $t = 3.27$, $P < 0.01$; AT3: $t = 4.17$, $P < 0.001$), the opposite was true for the remaining drought treatments (AT1 - MS: $t = 2.69$, $P < 0.01$; CS: $t = 3.73$, $P < 0.001$; IS1: $t = 4.23$, $P < 0.001$. AT2 - MS: $t = 2.85$, $P < 0.01$; CS: $t = 4.04$, $P < 0.001$; IS1: $t = 4.79$, $P < 0.001$. AT3 - CS: $t = 4.01$, $P < 0.001$; IS1: $t = 3.58$, $P < 0.001$).

Mean nymph weight

Drought had a significant effect on mean nymph weight in all three autumn trials (AT1: ${\chi^2}_4 = 841.04$, $P < 0.001$; AT2: ${\chi^2}_4 = 2544.70$, $P < 0.001$; AT3:${\chi^2}_4 = 1021.20$, $P < 0.001$; Figure 5.18). The was also a difference between the trials ($t = 9.94$, $P < 0.001$), under CS($t = 2.43$, $P < 0.05$), IS1 ($t = 8.30$, $P < 0.001$) and IS2 ($t = 4.35$, $P < 0.001$) drought treatments.

The same response was shown by mean nymph weight as adult weight. Namely, weight was increased under IS2 drought in all three trials (AT1: $t = 15.05$, $P < 0.001$; AT2: $t = 16.67$, $P < 0.001$; AT3: $t = 9.96$, $P < 0.001$). Furthermore, nymph weight was reduced under the remaining drought treatments (AT1 - MS: $t = 3.58$, $P < 0.001$; CS: $t = 13.86$, $P < 0.001$; IS1: $t = 2.63$, $P < 0.01$. AT2 - MS: $t = 9.65$, $P < 0.01$; CS: $t = 31.05$, $P < 0.001$; IS1: $t = 12.42$, $P < 0.001$. AT3 - MS: $t = 2.46$, $P < 0.01$; CS: $t = 18.07$, $P < 0.001$; IS1: $t = 14.56$, $P < 0.001$).

Seven-day fecundity

Drought stress affected seven-day fecundity in all three trials (AT1: ${\chi^2}_4 = 107.34$, $P < 0.001$; AT2: ${\chi^2}_4 = 26.01$, $P < 0.001$; AT3: ${\chi^2}_4 = 31.84$, $P < 0.001$; Figure 5.19). There was, however, no difference between the trials overall ($t = 2.03$, $P > 0.05$).

Moderate intermittent stress (IS2) increased fecundity in all the trials (AT1: $t = 8.20$,

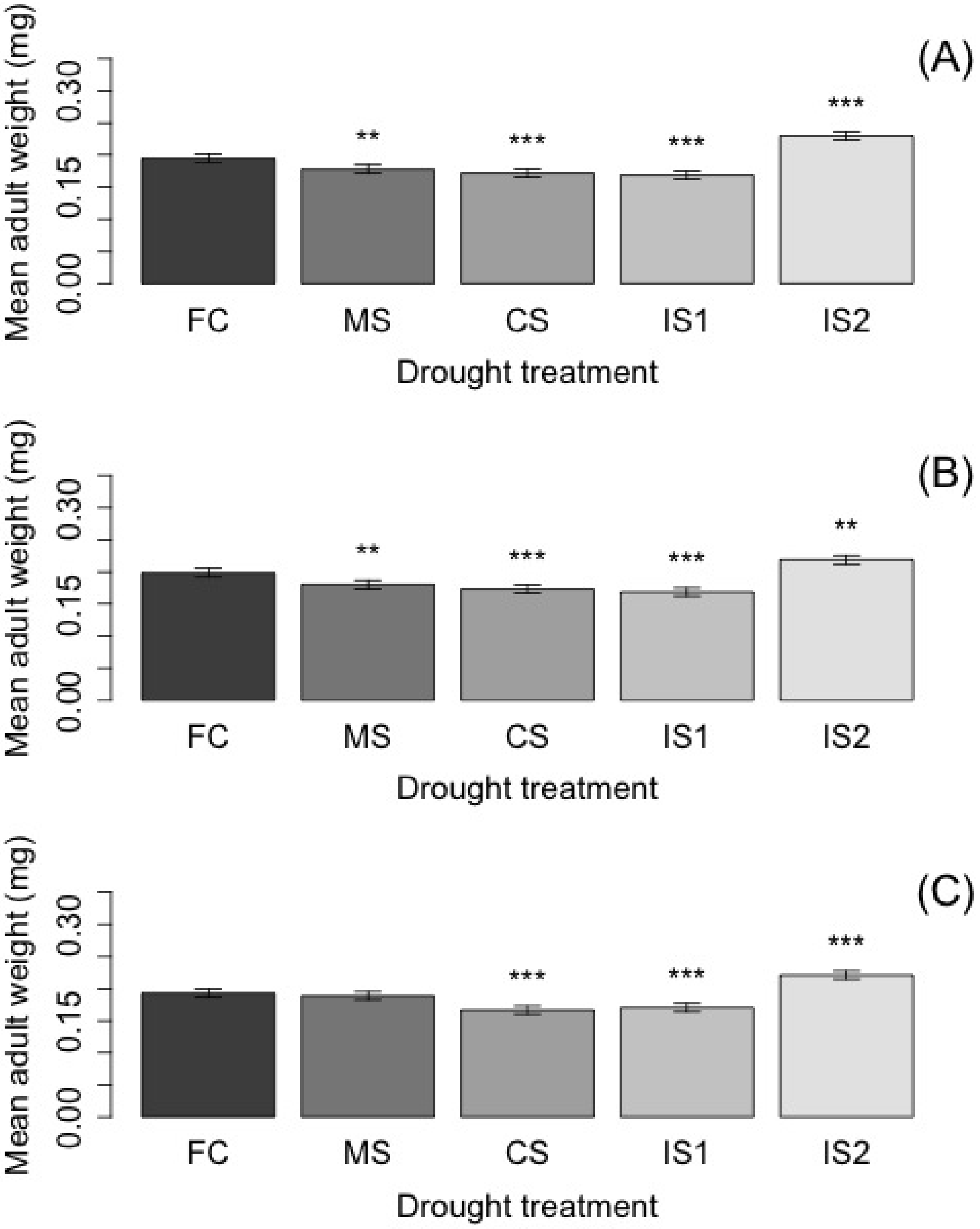

Figure 5.17: The effect of drought stress on adult weight of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

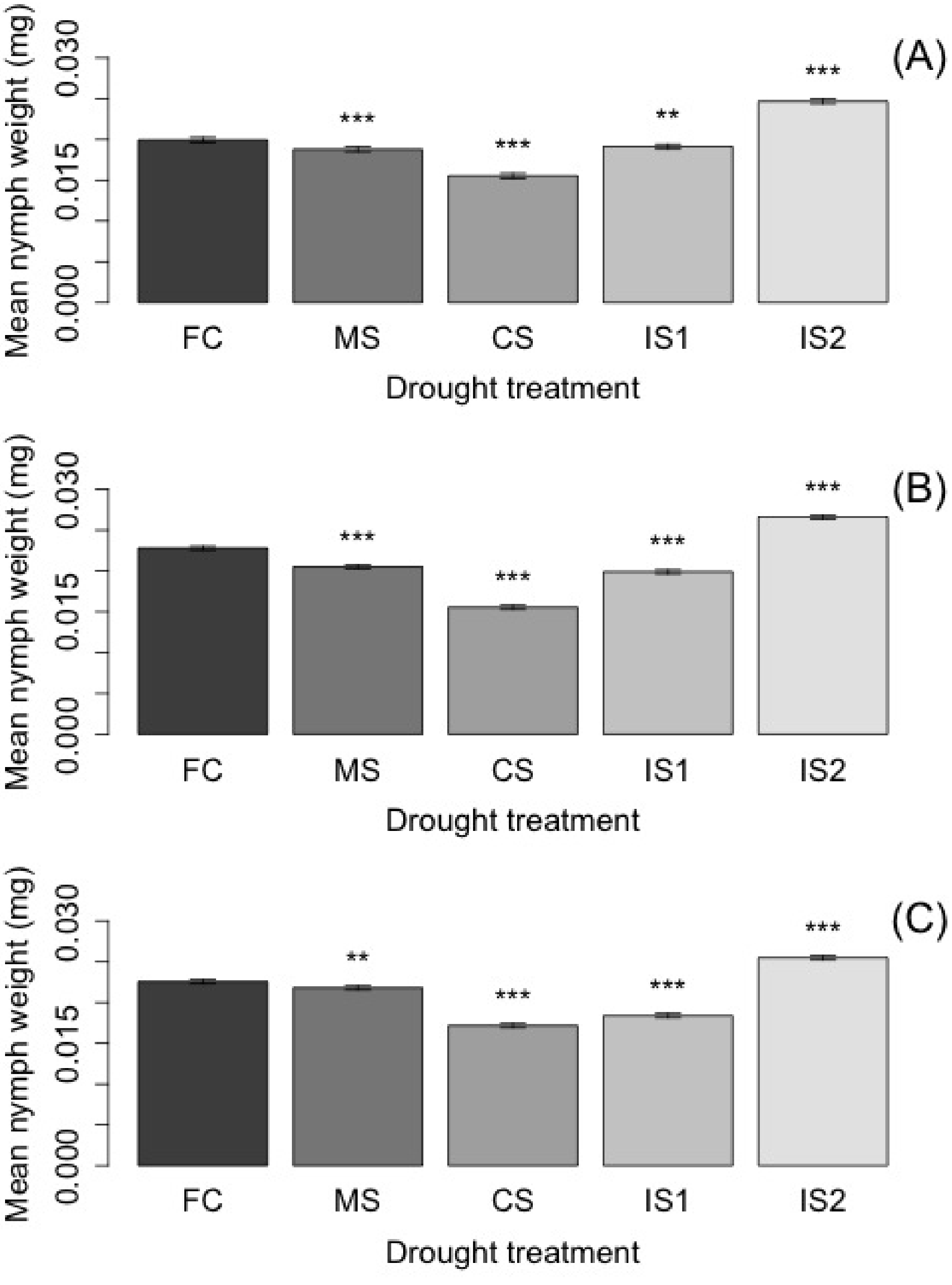

Figure 5.18: The effect of drought stress on mean nymph weight of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

$P < 0.001$; AT2: $t = 3.56$, $P < 0.001$; AT3: $t = 3.22$, $P < 0.01$), and, during AT2, the same was true under the IS1 drought treatment ($t = 3.49$, $P < 0.001$). During AT3, fecundity was reduced under CS drought ($t = 2.13$, $P < 0.05$).

Intrinsic rate of increase (r_m)

The r_m was only significantly affected by drought in the AT1 and AT3 trials (AT1: $\chi^2{}_4 = 42.79$, $P < 0.001$; AT2: $\chi^2{}_4 = 4.14$, $P > 0.05$; AT3:$\chi^2{}_4 = 10.35$, $P < 0.05$; Figure 5.20), though there was no difference in the rate between trials ($t = 1.49$, $P > 0.05$). In both AT1 and AT3, r_m was increased under IS2 drought ($t = 5.51$, $P < 0.001$, and $t = 2.72$, $P < 0.01$, respectively).

Total reproductive output

During the three autumn trials, a significant effect of drought on total reproductive output was observed (AT1: $\chi^2{}_4 = 160.53$, $P < 0.001$; AT2: $\chi^2{}_4 = 75.33$, $P < 0.001$; AT3: $\chi^2{}_4 = 59.76$, $P < 0.001$; Figure 5.21). During AT1, reproductive output was reduced under CS and IS1 drought (CS: $t = 4.21$, $P < 0.001$; IS1: $t = 2.54$, $P < 0.01$), while it was increased under IS2 drought ($t = 7.68$, $P < 0.001$). The same responses were found during AT3 (CS: $t = 2.36$, $P < 0.05$; IS1: $t = 2.19$, $P < 0.05$; IS2: 4.37, $P < 0.001$). During AT2, while the reproductive output of *E. abietinum* was still increased under IS2 ($t = 4.84$, $P < 0.001$), a reduction was only observed under CS drought ($t = 3.43$, $P < 0.001$). There was no difference in the effect of drought between the three trials ($t = 0.72$, $P > 0.05$).

Discussion

Drought typically alters both plants and their environment, and such impacts can be expected to affect the associated phytophagous insects. Despite this, studies reveal a lack of consistency. Sources of variation have been attributed to a range of things, such as differing insect traits (*e.g.* feeding guild (Larsson, 1989; Björkman & Larsson, 1999), or developmental stage (White, 1984)), effects on host plant (*e.g.* type of stress (Huberty & Denno, 2004; Mody *et al.*, 2009), plant growth rates (Jones & Coleman, 1991), or plant taxa (Waring & Cobb, 1992)), as well as in relation to experimental design (ranging from

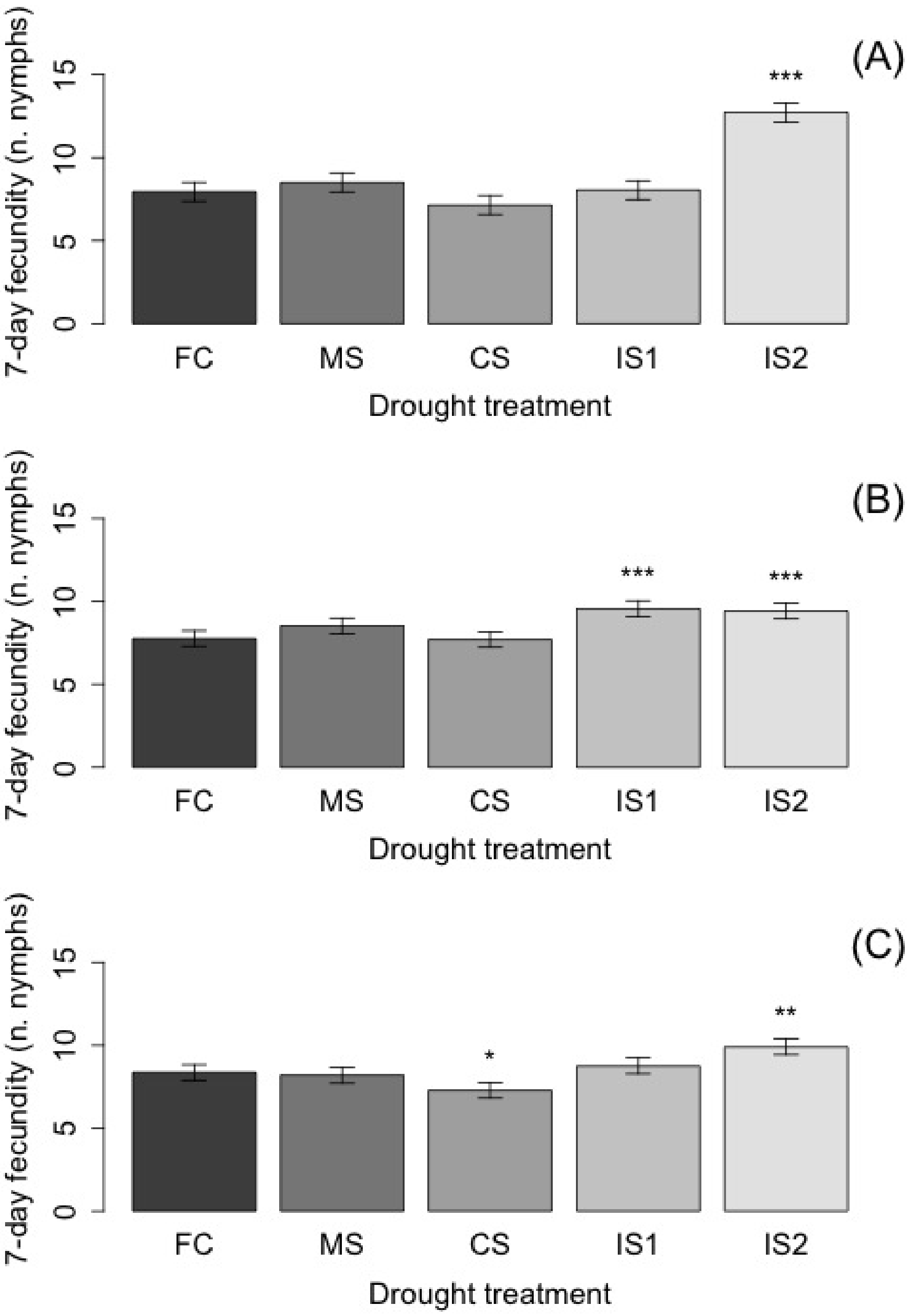

Figure 5.19: The effect of drought stress on seven-day fecundity of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

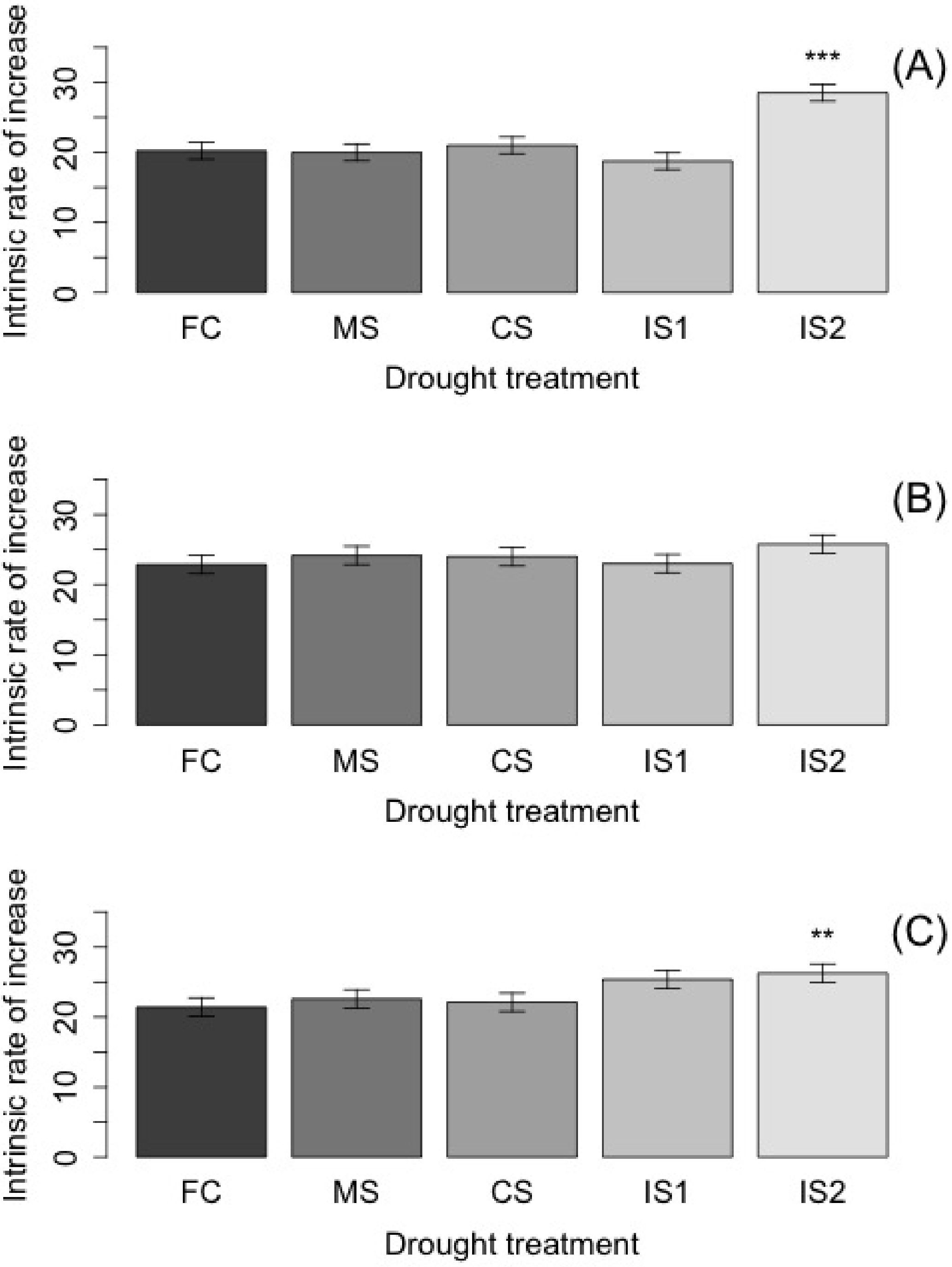

Figure 5.20: The effect of drought stress on the intrinsic rate of increase of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

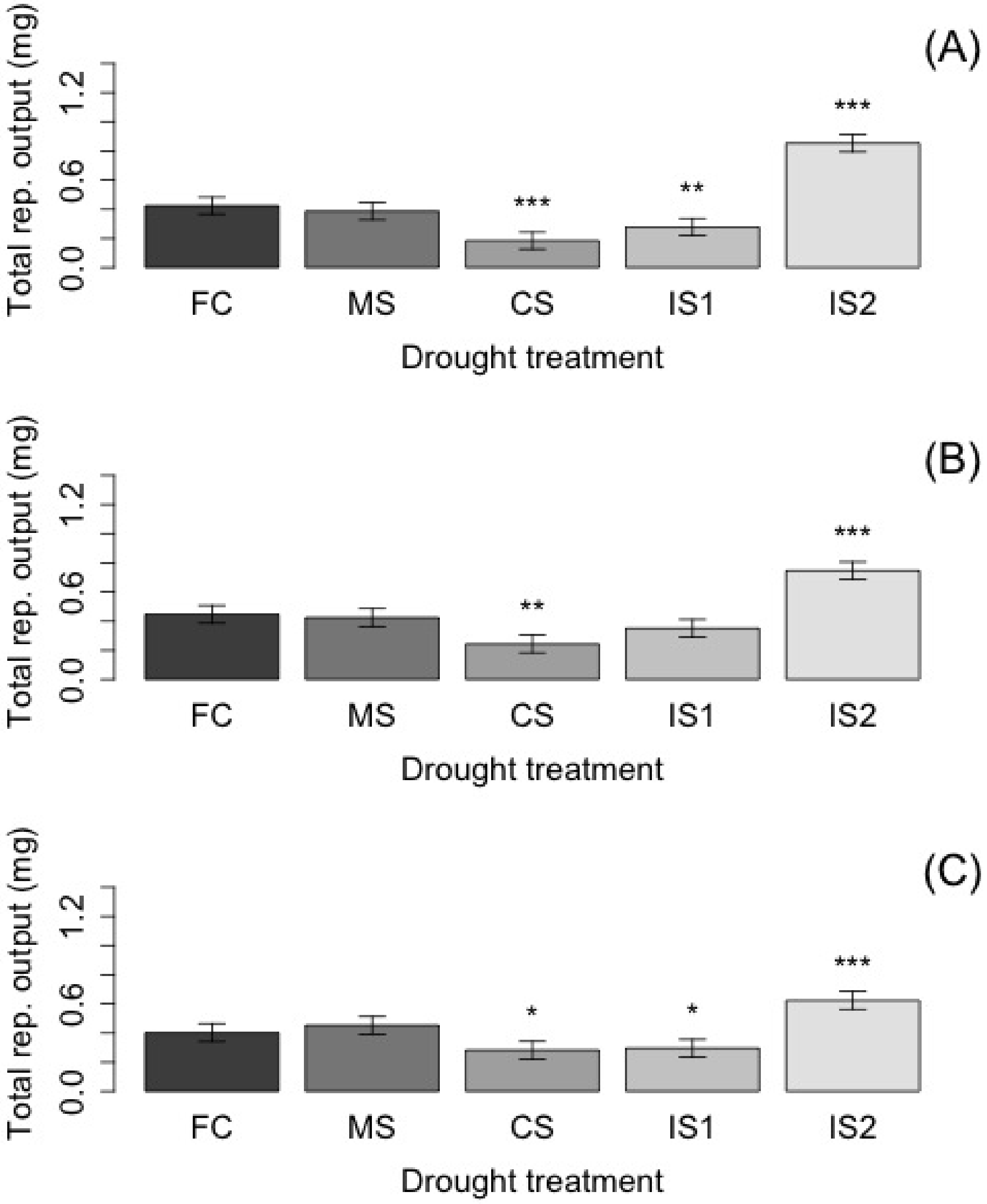

Figure 5.21: The effect of drought stress on total reproductive output of *E. abietinum* on spruce under controlled conditions during autumn.

Bars indicate Least Significant Difference. (A) AT1: 19th September 2011; (B) AT2: 04th October 2011; (C) AT3: 24th October 2011. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

the type of experiment, through replication and duration or timing of stress treatments (Koricheva *et al.*, 1998)). In regard to aphids, two key processes have been used as explanations for observed effects and differences: (1) an increase in plant nutritional quality, with greater nitrogen availability in the phloem sap; and (2) loss of cell turgor or increased sap viscosity, reducing feeding (Wearing, 1972). Huberty & Denno (2004) suggested that continuous stress, despite improving nutritional quality of phloem sap, rendered plant sap less accessible to aphids due to reduced turgor pressure. Mody *et al.* (2009) refined this hypothesis by suggesting that intermittent stress modified the effect on performance, by allowing periods of nutrient availability. The observations in this study are compatible with the modified hypothesis as proposed by Mody *et al.* (2009).

In a meta-analysis, Koricheva *et al.* (1998) found that the relative growth rates of sucking insects was increased on stressed plants. The mRGR is often used as a surrogate measure for host plant quality, which can be difficult to determine directly (Williams *et al.*, 2005). The mRGR response of insect herbivores to drought is by no means uniform. Although sucking insects and aphids may be positively affected, this is not the case for chewing, mining and boring insects (Koricheva *et al.*, 1998; Heijari *et al.*, 2010).

The results obtained in this study show a complex response of this indicator to drought, further compounded by differences between the trials. During spring trials (Experiment 1), moderate intermittent stress was found to positively affect the mRGR of *E. abietinum*, with significant increases in all three trials. The same was also true in mid-autumn (AT2). Responses to severe intermittent drought stress were only found during spring, and interacted with the timing of the studies. Specifically, the mRGR under this drought treatment was found to increase at each of the trials, starting off as significantly lower at bud-burst while being significantly higher five weeks later. Continuous severe stress also had a complex response. Although mRGR under continuous severe drought was decreased two weeks after bud-burst, during the autumn trials (Experiment 2) an increase in mRGR was observed for all trials. The findings of this study support those of Huberty & Denno (2004) and Mody *et al.* (2009), showing the importance of stress intensity and frequency on the performance of insect herbivores.

Williams *et al.* (2005) observed an effect of timing of infestation with *E. abietinum* on mRGR following previous defoliation, highlighting the importance of time of year on aphid performance and attributing it to improved nutritional quality. This may serve to explain why, under continuous severe stress, mRGR was significantly higher during the autumn trials than the spring trials.

Few studies on the effects of drought on aphids have assessed survival rates, though several have been conducted on chewing insects. Kamata & Tanabe (1999), for example, found a reduction in beech caterpillar survivorship on drought stressed beeches, while studies by English-Loeb *et al.* (1997) and Björkman & Larsson (1999) observed no such differences in *Spodoptera exigua* and *Gilpinia hercyniae* larvae respectively.

There was no overall effect of drought on the survival rates of *E. abietinum* nymphs seven days after deposition, with a reduction only observed under severe intermittent stress in early autumn (AT1). Furthermore, during the spring trials, a reduction of the proportion of aphids surviving was observed through time under continuous severe stress even if, within each trial, it did not differ significantly from the FC control.

Simpson *et al.* (2012) had concluded that, at least in the case of *Myzus persicae* Sulzer, drought did not lead to improved aphid survival. This finding matched with Pons & Tatchell (1995), who observed no difference in nymphal mortality in cereal aphids on drought stressed wheat. These contrast with the findings of Björkman (2000), who observed reduced survival in *Adelges abietis* (L.) stem-mothers on Norway spruce (*Picea abies* (L.) Karst.). The findings of the study herein presented suggest that the survival rates are more likely to be affected by plant changes due to season, modulated by drought, and highlights the importance of timing for such studies.

Lifespan, generation time (also known as development of pre-reproductive time) and the number of reproductive days are closely related measures. For example, assuming the same longevity, an increase in generation time will result in a decrease in reproductive days and *vice versa.*

Drought was not found to have an overall effect on lifespan, although moderate stress was found to reduce or increase lifespan depending on the frequency (reductions were made in ST3 and AT1 under continuous stress, and increased in AT2 under intermittent stress). Severe drought stress (be it continuous or intermittent) significantly increased generation time in both spring and autumn, reflected by decreases in the number of reproductive days under these drought treatments. Furthermore, generation time was reduced under moderate intermittent stress, with a consequent increase in the number of reproductive days (true even when not significantly so). This corresponds with the increases in mRGR observed under this drought treatment, and suggests that *E. abietinum* aphids were able to better access any improvements in plant nutritional quality. These findings support the view that a severe enough continuous stress is detrimental to aphid performance (Huberty & Denno, 2004), as well as highlighting the importance of stress magnitude as proposed by Mody *et al.* (2009).

These findings contrast those of Major (1990) and Pons & Tatchell (1995), who observed no difference in generation time of *E. abietinum* and *Rhopalosiphum padi* (L.), respectively, under drought stress. They are, however, supported by Sumner *et al.* (1986), who observed declines in the longevity and reproductive period of *Rhopalosiphum maidis* (Fitch) with increasing levels of drought stress. These inconsistencies may reflect methodological differences. On the other hand, the generation time of the aphid *Aulacorthum solani* (Kalt.) was found to interact with host plant species, suggesting a potentially plant-mediated response.

Aphid size has often been used as an indicator of aphid fitness, and though it is usually a good indicator of fecundity it does not necessarily reflect other performance parameters (Dixon *et al.*, 1982; Leather & Dixon, 1984). The adult weight of *E. abietinum* was consistently found to be higher under moderate intermittent stress. This corresponds with the increased mRGR and decreased generation times observed under this drought treatment, and further supports an improved host quality under moderate intermittent stress. Under severe stress, however, the response was mediated by experiment timing. During the spring, adult weight was found to be greater than the well-watered control, with increases to the generation time. However, the reduced number of reproductive days suggest that despite obtaining a larger size, the aphids did not have increased fitness.

In contrast to this, during the autumn experiments, adult weight was reduced under severe stress despite increases to generation time. The mRGR was increased at this time under continuous severe stress, though not under intermittent severe stress. These findings imply that, during the autumn period, *E. abietinum* reared under severe drought stress may spend a longer period of time between reaching maturity and depositing their first nymph. This is likely due to inaccessibility of nutrition from the host (Dixon, 1985).

Although Major (1990) and Pons & Tatchell (1995) did not observe an effect of drought on adult aphid weight, Aslam *et al.* (2013) found a reduction in the mass of apterous adult *R. padi* on drought-stressed plants. Such differences observed between results are likely to reflect methodological differences. A review by Koricheva *et al.* (1998) highlights the importance of such differences. Furthermore, as reflected by the differences between drought treatments in this study, drought intensity can have a significant impact on the growth (and performance) of the aphids.

Major (1990) observed no effect of drought on nymph weight. In contrast, this study found that nymph weight was consistently reduced under continuous and severe drought treatments, but increased under moderate intermittent stress. Dixon (1985) stated that, generally, larger individuals of many aphid species produced large offspring. While this holds true for the observed results under moderate intermittent stress, and for continuous and severe stress during the autumn, the same cannot be claimed for severe stresses during the spring experiment. In this case, the larger adults produced smaller nymphs. This probably reflects deficiencies in plant nutritional content caused by severe drought.

Aphid size, be it of adults or nymphs, while perhaps not an ideal indicator of aphid performance nonetheless has important implications. Larger individuals are more likely to survive adverse conditions (Dixon, 1985), and are also more likely to be able to avoid or defend against predation and parasitisation attempts (Dixon, 1970). Furthermore, birth weight also affects time to maturity, whereby small nymphs born to small mothers are likely to take longer to develop than their large counterparts (Dixon, 1985).

The total reproductive output (being the total weight of all nymphs produced by the adult aphids) is logically correlated with nymph weight and the number of reproductive days. This holds true for the results observed in this study. Under moderate intermittent stress, heavier nymphs produced over a longer period of time resulted in a higher total reproductive output, whereas the smaller nymphs produced over fewer reproductive days resulted in a reduced total output. This was true for both the spring and autumn trials. Although reproductive output does not provide an indication of potential population size, as it is based on nymph weight rather than number, it may reflect potential population fitness given the implications of aphid size discussed above.

During spring, the same can be said for *E. abietinum* fecundity on severely stressed

Sitka spruce, though severe intermittent stress was not found to have a negative impact during the autumn. Under moderate intermittent stress, however, seven-day fecundity was increased throughout the year. The r_m of *E. abietinum* was also only increased under moderate intermittent stress during the autumn, with no other significant effects of drought observed.

The response of fecundity and r_m to drought stress is not clearly understood, with various studies obtaining contrasting results. Reduced fecundity and r_m on drought stressed plants has frequently been observed for cereal aphids, such as *R. padi* (Sumner *et al.*, 1986; Pons & Tatchell, 1995; Hale *et al.*, 2003). Tariq *et al.* (2012) found that *Brevicoryne brassicae* L. and *M. persicae* had increased fecundity and r_m under continuous moderate stress. In contrast, a study by Simpson *et al.* (2012) found no evidence to support improved *M. persicae* population growth under drought stress, with no differences to fecundity or r_m observed.

Kennedy *et al.* (1958), in experiments with *Aphis fabae*, observed that under severe continuous water stress nymph deposition was reduced despite an improvement in the quality of the phloem sap. They attributed the negative effects to a reduction in the quantity of obtainable phloem sap, due to reduced turgor pressure or increased viscosity of the sap. The study further postulated that previously recorded positive effects may have been associated with either a reduced severity or intermittent water stress in the plant, resulting in a situation where the reduction in obtainable sap was compensated by the improvement in quality (Kennedy *et al.*, 1958). In a later study, McVean & Dixon (2001) observed a similar trend in *Acyrthosiphon pisum* (Harris). On continuously stressed plants, abundance of *A. pisum* was significantly lower after three weeks when compared to watered plants. In the case of *E. abietinum*, Warrington & Whittaker (1990) found a small increase in mean number of aphids on drought stressed trees.

The disparity among the results suggests that the response of fecundity and r_m to drought is affected by drought type and intensity, and by consequence whatever effects that has on host plant quality. Leather & Dixon (1984) found a strong correlation of fecundity with mRGR, and even more so in the case of r_m. This may serve to explain the response observed in the study here presented under moderate intermittent stress, but even so the relationship is not clear. For example, although higher mRGR was observed under this drought treatment during spring, the same was only true for one trial in mid-autumn. Fecundity, however, was increased throughout the year, whereas r_m was only significantly increased in the autumn.

In this study, *E. abietinum* reared under moderate intermittent stress achieved a greater adult weight, and it is generally thought that large aphids are more fecund than their smaller counterparts (Dixon, 1976). This corresponds with the observed increased fecundity under moderate intermittent stress, and to the increases in r_m observed during the autumn. Dixon *et al.* (1982) and Dixon (1985) ascertained that, in aphids, size results as a consequence of the relative effects of temperature, nutrition and birth weight on growth and development rates, and that where conditions are favourable a high increase in numbers can be achieved regardless of weight. The same must be true of the opposite; where conditions are unfavourable, growth and fecundity may be reduced. Hale *et al.* (2003) observed increases in the quality of phloem sap under drought but a reduction in the performance of *R. padi* under the same conditions, concluding that diet quality could not account for the differences. The same may be true in an arboreal setting, though few such studies have been conducted (Koricheva *et al.*, 1998).

Differences in *E. abietinum* performance during spring and autumn were highlighted by Williams *et al.* (2005). In their study, they observed increased mRGR in the autumn following a spring defoliation treatment. Indeed, the mRGR obtained during the study herein presented were higher or comparable (even if not significantly so) for all drought treatments in the autumn when compared to those obtained during the spring. Aphid performance in population development is sensitive to nitrogen concentration in the sap (Dixon, 1985; Douglas, 1993), and it is likely that the presence of *E. abietinum* in spring improves the nutritional quality of Sitka spruce in the autumn (Williams *et al.*, 2005). The amino acid balance of Sitka spruce has been shown to be affected by *E. abietinum* feeding, and aphids reared on chlorotic needles are typically heavier than those reared on green needles (Fisher, 1987). The nutritional quality of phloem sap has also been found to decline following bud-burst and remain low over the summer period (Parry, 1974b; Day & Kidd, 1998). In addition to this, Major (1990) found that drought seemed to promote the rate at which chlorotic bands developed on needles. Furthermore, high *E. abietinum* populations are not thought to induce defensive mechanisms in Sitka spruce (Williams *et al.*, 2005), and similarly drought has not been found to affect induced herbivore resistance in apples attacked by *Spodoptera littoralis*, despite effects on constitutive resistance (Gutbrodt *et al.*, 2012).

Density-dependent processes, nutritional quality, induced plant defences and increased predation have all been used as explanations of poorer performance of broadleaved tree-dwelling aphids on the same host plant, later in the year (Sluss, 1967; Dixon, 1975; Dixon, 1990; Dixon & Barlow, 1979; Liao & Harris, 1985; Wellings *et al.*, 1985; Leather, 1990; Bumroongsook & Harris, 1991). Lewis (1987), as cited by Kidd *et al.* (1990), observed a reduction in Scots pine needle quality following feeding by the aphid *Schizolachnus pineti* leading to reduced aphid performance later in the season. Despite this, *E. abietinum* differs from many other species of arboreal aphids, as its feeding habits do not typically cause mortality despite causing extensive defoliation. Williams *et al.* (2005) suggest that the premature abscission of needles early in the season improve nutritional conditions in the autumn. Similar effects, where such early defoliation leads to increased foliar nutritional quality, has been observed in several studies (Cook *et al.*, 1978; Wagner & Evans, 1985; Leather, 1993; Smits & Larsson, 1999). Such observations are reflected in this study, where performance indicators were typically similar, despite drought treatment, between spring and autumn trials.

Maternal effects could also have influenced the results. These have been well documented in aphids (Dixon *et al.*, 1993; McLean *et al.*, 2009; Tariq *et al.*, 2010), as well as several other insect species (Rossiter, 1991; Rossiter *et al.*, 1993; Wainhouse *et al.*, 2001; Spitzer, 2004). Dixon *et al.* (1993), for example, assessed the influence of the optimal energy allocation model and observed that a mother aphid produced larger offspring towards the end of her life, which achieved greater adult weight, mRGR and potential intrinsic rate of increase than the smaller offspring deposited earlier in the female's life. As such, the size of the offspring varied inversely with the residual reproductive value, whereas the reproductive investment did so positively. It was deduced that excess energy produced by the soma in older mothers was utilised to accelerate offspring growth rate in the remaining undeposited nymphs.

The nutritional environment of the maternal generation has also been shown to affect offspring. For example, the parental host plant of *Lymantria dispar* was shown to influence growth rate, fecundity and dispersal potential in offspring (Rossiter, 1991). In *Hylobius abietis*, significant maternal effects were observed on egg and larval size in relation to both female size and the conifer species on which the weevils were fed (Wainhouse *et al.*, 2001). These studies could suggest that, as the founding mothers used in the CT room experiments of this project were all taken from the stock culture, it could be that the effect of the parental host plant superseded that of the experimental plant for the experimental aphid. Nevertheless, any such effects in this experiment should have been limited as stock aphids were also raised on Sitka spruce, and it is unlikely that differences in Sitka spruce genotype would have a significant impact through maternal effects. Furthermore, these effects should have averaged out because of random selection and allocation of aphids to the treatments.

Current research has shown that aphid interactions with their host plant are complex and often defy attempts at generalisation. In addition to this, there is increasing evidence to suggest that individual performance parameters under controlled conditions may not reflect or predict population densities in the field under altered conditions (Awmack *et al.*, 2004; Pritchard *et al.*, 2007; Mondor *et al.*, 2010). It is probable that detailed understanding of the interactions between individual species and their host plants, rather than any general predictions of response, will arise as the result of such complexities.

Conclusions

1. The response of individual *E. abietinum* to drought stress was complex, and varied both by drought intensity and frequency, as well as through time.

2. Moderate intermittent stress generally improved host plant quality for *E. abietinum* as reflected by individual aphid performance parameters, while severe stress was usually detrimental.

3. Host quality tended to improve through time under moderate intermittent drought stress, but declined under continuous severe stress.

4. Several performance parameters showed differences between trials during spring (six of ten measured parameters). This supports claims that changes to host plant

quality can be observed following bud-burst.

5. There was little evidence to support an effect of drought on bringing forward Sitka spruce dormancy. There were few instances of differences between trial in the autumn (three of ten measured parameters), suggesting that dormancy was not brought forward by the drought treatments.

Summary tables

Table 5.1:

Summary of mRGR (mg/mg/day) of *E. abietinum* nymphs under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 20% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	0.103 ± 0.007	0.106 ± 0.005	0.087 ± 0.006	0.067 ± 0.006	0.124 ± 0.007	48.16	< 0.001
ST2	0.130 ± 0.006	0.123 ± 0.006	0.098 ± 0.008	0.106 ± 0.007	0.152 ± 0.007	32.02	< 0.001
ST3	0.104 ± 0.006	0.100 ± 0.006	0.112 ± 0.007	0.139 ± 0.009	0.142 ± 0.007	33.34	< 0.001
AT1	0.142 ± 0.007	0.143 ± 0.007	0.177 ± 0.008	0.148 ± 0.008	0.151 ± 0.006	16.11	< 0.05
AT2	0.130 ± 0.006	0.144 ± 0.009	0.186 ± 0.007	0.143 ± 0.007	0.152 ± 0.004	35.39	< 0.001
AT3	0.134 ± 0.008	0.126 ± 0.007	0.168 ± 0.008	0.155 ± 0.007	0.153 ± 0.012	14.79	< 0.01

Table 5.2:

Summary of proportion of surviving *E. abietinum* nymphs seven days after deposition under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\hat{p}$ **alive**	$\hat{p}$ **alive**	$\hat{p}$ **alive**	$\hat{p}$ **alive**	$\hat{p}$ **alive**	χ^2	**P**
ST1	0.650	0.550	0.775	0.475	0.775	12.05	< 0.05
ST2	0.775	0.775	0.700	0.775	0.800	1.27	n.s
ST3	0.750	0.675	0.575	0.775	0.775	5.59	n.s.
AT1	0.850	0.900	0.900	0.650	0.725	12.00	< 0.05
AT2	0.800	0.825	0.750	0.850	0.825	1.48	n.s.
AT3	0.775	0.775	0.775	0.825	0.775	0.41	n.s.

Table 5.3:

Summary of lifespan (days) of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	31.30 ± 1.66	32.50 ± 1.66	32.70 ± 1.17	29.83 ± 1.14	34.93 ± 1.60	5.48	n.s.
ST2	34.53 ± 1.52	31.05 ± 1.62	31.78 ± 1.26	34.85 ± 1.45	35.33 ± 1.44	7.11	n.s.
ST3	35.70 ± 1.71	30.60 ± 1.38	32.28 ± 1.34	36.83 ± 1.34	35.98 ± 1.39	13.17	< 0.05
AT1	33.95 ± 1.51	28.75 ± 1.74	30.88 ± 1.55	29.93 ± 1.45	33.75 ± 1.63	8.61	n.s.
AT2	34.75 ± 1.80	34.03 ± 1.76	35.00 ± 1.40	34.38 ± 1.52	39.25 ± 1.55	5.97	n.s.
AT3	30.60 ± 1.67	33.80 ± 1.69	34.93 ± 1.75	34.50 ± 1.23	33.98 ± 1.69	4.53	n.s.

Table 5.4:

Summary of generation time (days) of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	16.88 ± 0.36	16.38 ± 0.40	22.35 ± 0.51	22.03 ± 0.48	14.65 ± 0.17	316.44	< 0.001
ST2	14.50 ± 0.17	14.70 ± 0.17	21.13 ± 0.33	21.98 ± 0.37	14.55 ± 0.19	763.83	< 0.001
ST3	18.48 ± 0.34	18.38 ± 0.38	22.20 ± 0.48	22.73 ± 0.45	14.75 ± 0.18	225.71	< 0.001
AT1	17.40 ± 0.40	16.45 ± 0.35	21.38 ± 0.63	19.95 ± 0.48	16.80 ± 0.36	89.35	< 0.001
AT2	19.38 ± 0.53	20.40 ± 0.64	22.53 ± 0.54	21.45 ± 0.51	19.33 ± 0.51	25.84	< 0.001
AT3	17.83 ± 0.55	20.40 ± 0.57	22.85 ± 0.61	22.75 ± 0.65	19.28 ± 0.43	59.94	< 0.001

Table 5.5:

Summary of number of reproductive days of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	16.03 ± 1.66	17.92 ± 1.45	11.83 ± 0.94	9.46 ± 1.09	21.89 ± 1.31	44.82	< 0.001
ST2	21.65 ± 1.26	18.69 ± 1.44	11.51 ± 1.22	13.92 ± 1.38	21.31 ± 1.37	46.72	< 0.001
ST3	18.62 ± 1.58	13.97 ± 1.34	11.85 ± 1.26	15.24 ± 1.17	21.77 ± 1.27	34.95	< 0.001
AT1	17.89 ± 1.24	16.40 ± 1.59	11.88 ± 1.40	12.87 ± 1.33	19.37 ± 1.39	21.34	< 0.001
AT2	17.57 ± 1.51	16.52 ± 1.47	13.86 ± 1.37	14.36 ± 1.36	20.44 ± 1.40	14.63	< 0.01
AT3	15.03 ± 1.45	17.29 ± 1.32	15.58 ± 1.47	12.70 ± 1.21	17.82 ± 1.35	9.33	< 0.05

Table 5.6:

Summary of adult weights (mg) of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x} \pm$ **SE**	$\bar{x} \pm$ **SE**	$\bar{x} \pm$ **SE**	$\bar{x} \pm$ **SE**	$\bar{x} \pm$ **SE**	χ^2	**P**
ST1	0.214 ± 0.006	0.202 ± 0.006	0.245 ± 0.008	0.224 ± 0.008	0.285 ± 0.008	88.26	< 0.001
ST2	0.214 ±0.007	0.221 ± 0.006	0.2446 ± 0.007	0.237 ± 0.006	0.317 ± 0.005	165.88	< 0.001
ST3	0.202 ± 0.006	0.199 ± 0.005	0.223 ± 0.007	0.224 ± 0.007	0.263 ± 0.006	70.19	< 0.001
AT1	0.195 ± 0.004	0.178 ± 0.003	0.172 ± 0.003	0.169 ± 0.003	0.230 ± 0.006	132.59	< 0.001
AT2	0.199 ± 0.004	0.180 ± 0.003	0.173 ± 0.003	0.169 ± 0.004	0.219 ± 0.006	91.68	< 0.001
AT3	0.193 ± 0.005	0.189 ± 0.005	0.166 ± 0.003	0.170 ± 0.003	0.221 ± 0.007	85.87	< 0.001

Table 5.7:

Summary of mean nymph weight (mg) of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	0.025 ± 0.00019	0.024 ± 0.00019	0.018 ± 0.00018	0.018 ± 0.00028	0.027 ± 0.00014	1475.20	< 0.001
ST2	0.026 ± 0.00015	0.024 ± 0.00011	0.019 ± 0.00027	0.021 ± 0.00023	0.028 ± 0.00020	1233.90	< 0.001
ST3	0.022 ± 0.00031	0.022 ± 0.00040	0.016 ± 0.00047	0.017 ± 0.00043	0.023 ± 0.00038	252.40	< 0.001
AT1	0.020 ± 0.00014	0.019 ± 0.00017	0.016 ± 0.00028	0.019 ± 0.00026	0.025 ± 0.00014	841.04	< 0.001
AT2	0.023 ± 0.00013	0.021 ± 0.00014	0.016 ± 0.00019	0.020 ± 0.00023	0.027 ± 0.00012	2544.70	< 0.001
AT3	0.023 ± 0.00023	0.022 ± 0.00019	0.017 ± 0.00024	0.018 ± 0.00017	0.026 ± 0.00017	1021.20	< 0.001

Table 5.8:

Summary of seven-day fecundity (n. nymphs) of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	9.54 ± 0.34	9.52 ± 0.30	8.58 ± 0.46	8.00 ± 0.44	11.46 ± 0.58	31.67	< 0.001
ST2	12.76 ± 0.48	12.07 ± 0.48	8.36 ± 0.39	8.23 ± 0.34	14.05 ± 0.67	88.87	< 0.001
ST3	9.52 ± 0.31	8.96 ± 0.35	7.89 ± 0.47	7.68 ± 0.34	11.67 ± 0.44	75.05	< 0.001
AT1	7.94 ± 0.31	8.50 ± 0.40	7.14 ± 0.50	8.04 ± 0.53	12.73 ± 0.42	107.34	< 0.001
AT2	7.76 ± 0.32	8.52 ± 0.35	7.70 ± 0.37	9.56 ± 0.38	9.43 ± 0.33	26.01	< 0.001
AT3	8.36 ± 0.39	8.19 ± 0.30	7.28 ± 0.51	8.75 ± 0.33	9.90 ± 0.26	31.84	< 0.001

Table 5.9:

Summary of intrinsic rate of increase of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	21.89 ± 0.95	22.30 ± 1.01	22.00 ± 1.53	23.00 *NA*	24.77 ± 1.02	4.76	n.s.
ST2	27.39 ± 0.88	27.17 ± 0.85	23.29 ± 0.61	23.44 ± 1.28	29.16 ± 1.26	11.20	< 0.05
ST3	24.74 ± 0.65	24.30 ± 1.36	25.00 ± 1.68	26.56 ± 1.19	23.41 ± 0.73	1.49	n.s.
AT1	20.22 ± 1.15	20.00 ± 1.16	21.00 ± 1.67	18.75 ± 1.59	28.53 ± 1.13	42.79	< 0.001
AT2	22.88 ± 1.20	24.17 ± 1.23	24.00 ± 2.43	23.00 ± 1.24	25.78 ± 1.02	4.14	n.s.
AT3	21.43 ± 1.05	22.58 ± 1.77	22.10 ± 0.27	25.38 ± 1.76	26.27 ± 1.19	10.35	< 0.05

Table 5.10:

Summary of total reproductive output (mg) of *E. abietinum* under drought stress, and across trials. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. Spring trials: ST1 = At bud burst (27th April 2011); ST2 = Two weeks after bud burst (10th May 2011); ST3 = Five weeks after bud burst (07th June 2011). Autumn trials: AT1 = 19th September 2011; AT2 = 04th October 2011; AT3 = 24th October 2011.

	FC	MS	CS	IS1	IS2		
Trial	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
ST1	0.54 ± 0.057	0.58 ± 0.048	0.27 ± 0.023	0.21 ± 0.024	0.99 ± 0.066	166.45	< 0.001
ST2	1.06 ± 0.069	0.83 ± 0.069	0.26 ± 0.028	0.35 ± 0.035	1.21 ± 0.081	197.42	< 0.001
ST3	0.62 ± 0.051	0.45 ± 0.046	0.27 ± 0.029	0.36 ± 0.027	0.98 ± 0.061	111.54	< 0.001
AT1	0.43 ± 0.032	0.39 ± 0.039	0.19 ± 0.025	0.28 ± 0.027	0.85 ± 0.061	160.53	< 0.001
AT2	0.45 ± 0.042	0.43 ± 0.037	0.24 ± 0.025	0.35 ± 0.032	0.75 ± 0.054	75.33	< 0.001
AT3	0.40 ± 0.037	0.45 ± 0.038	0.28 ± 0.026	0.30 ± 0.028	0.62 ± 0.048	59.76	< 0.001

Chapter 6

The effects of drought stress on the consumption rates of a conifer specialist and a generalist coccinellid predator of *Elatobium abietinum*

Introduction

Natural enemies are believed to play an important role in driving certain aspects of the population dynamics exhibited by green spruce aphids, *Elatobium abietinum* (Walker) (Homoptera: Aphididae) (Crute & Day, 1990), a major defoliating pest of Sitka spruce (*Picea sitchensis* (Bong.) (Carr.)) in the United Kingdom. The cyclical dynamics result not only from climate and density-dependant processes, which affect both overwinter survival and consequent spring peak size, but also a delayed density-dependent impact of predators and parasitoids (Day & Kidd, 1998). The latter is thought to help maintain low aphid population size in the years following a major outbreak (Day *et al.*, 2010), as well as contributing to driving the population decline following the spring peak (Crute & Day, 1990; Straw, 1995).

As a consequence of climate change, *E. abietinum* is expected to increase in pest status (Straw, 1995). It is therefore important to understand the potential nature of the interactions between this pest species and its predators and parasites. A variety of natural enemy families and species have been found to be associated with *E. abietinum* including, but not limited to, Coccinellidae (and several other Coleopteran families), Hemerobiidae (Neuroptera), Syrphidae (Diptera), and various Hymenopteran parasitoids (including Aphididae and Aphelinidiae) (Austarå *et al.*, 1998; Timms, 2004). Timms (2004) found that, among the various groups of natural enemies, coccinellids were the most abundant, and of the coccinellid species present the larch ladybird, *Aphidecta obliterata* (L.) (Coleoptera: Coccinellidae), was predominant. A similar predominance was also observed by Parry (1992).

Some characteristics exhibited by natural enemies are not fixed and have been shown to be influenced by external factors. These effects can be both direct and indirect, through prey-mediated influences. Furthermore, the responses of different groups of natural enemies can also differ under the same changes, as shown on cereal aphids by Garratt *et al.* (2010).

Host plant quality has the biggest impact on phytophagous insect species, and has been shown to have an effect on tritrophic interactions in agricultural crops (Staley *et al.*, 2011; Banfield-Zanin *et al.*, 2012; Caballero-López *et al.*, 2012). Apart from effects on performance, changes to consumption rates and functional responses have also been observed in response to host plant quality (Aqueel & Leather, 2012).

Prey quality has an important, direct effect on natural enemies. Different aphid species, for example, can have different dietary quality (Blackman, 1967; Parry, 1992). Furthermore, some specialised insect herbivores can sequester secondary defence metabolites from plants, which in turn affect natural enemy performance. Prey quality can also affect predator behaviour. Kalushkov (1999), for example, showed that *Adalia bipunctata* L. (Coleoptera: Coccinellidae) change from extensive to intensive searching behaviour upon finding a suitable species of aphid prey, a change not mirrored following consumption of three species of unsuitable aphid prey.

Environmental cues are also used by natural enemies, and result in increased foraging efficiency. Ladybird larvae have been shown to respond to the presence of aphid honeydew, although the response was not mediated by aphid quality (Purandare & Tenhumberg, 2012). Herbivore-induced plant volatiles are used by parasitoids to help locate hosts for oviposition (Uefune *et al.*, 2012). Evidence for attraction of natural enemies by such semiochemicals is extensive, and there is also evidence to suggest a role for them in the interactions between aphidophagous predators (Tapia *et al.*, 2010).

All the above factors already result in a complex scenario, which can only be further complicated by climate change. Increased atmospheric gases such as CO_2 and sulfur dioxide pollution have been shown to affect aphid performance and vulnerability to predation (Warrington & Whittaker, 1990; Awmack *et al.*, 1997).

Increased temperatures, particularly over the winter, associated with climate change would not only affect pest populations but also natural enemy populations by improving growth rates and performance, as well as increasing overwinter survival rates (Evans *et al.*, 2002; Evans *et al.*, 2013). Additional effects would also be observed in terms of shifting distributions and altered phenology (Jeffs & Lewis, 2013), all of which would affect the interactions between pests and their natural enemies. Both *A. obliterata* and *A. bipunctata* have been shown to respond significantly to temperature, both in terms of consumption rates and development times (Timms & Leather, 2008).

Climate change in the U.K. is predicted to result in an increase in the frequency of summer drought (Murphy *et al.*, 2009). Few studies have been conducted on the effects of drought stress on arboreal aphids (Koricheva *et al.*, 1998), though there have been many on agriculturally important aphid species. In the case of *Rhopalosiphum padi* (L.), aphid numbers were unaffected by drought, though the population demography was changed (Aslam *et al.*, 2013). Parasitism rates were reduced on the droughted plants, suggested to have been associated with the changes in demography as opposed to any physiological changes in the individuals (Pons & Tatchell, 1995; Aslam *et al.*, 2013).

Despite this, drought has repeatedly been shown to have the potential to affect plant chemistry. In brassicaceous plants, the glucosinolate profile was changed thus affecting aphid performance (Khan *et al.*, 2010), and in another study was shown to result in contrasting responses by phytophagous lepidopterans (Gutbrodt *et al.*, 2011). Major (1990) observed a significant difference in monoterpene levels of Sitka spruce under drought, with differences observed between intermittent stress compared to continuous stress and a control. In oak species, drought affected stress-related foliar metabolites in a metabolite- and species-specific manner, in combination with warmer temperatures (Hu *et al.*, 2013).

Drought has already been shown in Chapter 5 to affect various performance indicators of *E. abietinum*. As an example, during spring and early summer, mean nymph weight was reduced under severe drought stress, be it continuous or intermittent. Contrasting this, adult aphid weight under the same treatments was increased. Under moderate intermittent drought stress, the weights of both nymphs and adults were both increased, and this was also true during the autumn following drought treatment.

Coccinellids are important predators of aphids (Hodek, 1973), and are the most abundant predators of *E. abietinum* on spruce (Leather & Owuor, 1996; Timms, 2004). In order to understand their potential in the control of *E. abietinum* under future climate conditions or to make predictions on their interaction, understanding the potential tritrophic effects of drought are essential. *Aphidecta obliterata* (Figure 6.1(B)) is a spruce specialist, and *A. bipunctata* (Figure 6.1(A)), though not always associated with *E. abietinum*, is an arboreal generalist and was found by Leather & Owuor (1996) to be the most abundant predator on Norway spruce, *P. abies*, at Silwood Park (Ascot, U.K.). Furthermore, determining whether any differences may be attributed to changes in host plant or the aphid physiology would also be important, due to the potential influences of host (Timms *et al.*, 2008).

Figure 6.1: (A) *Adalia bipunctata* adult, red morph. (B) *Aphidecta obliterata* adult. Photographs obtained from UK Ladybird Survey).

Aims and Objectives

1. Assess the effect of drought stress on the 24-hour consumption rate of a conifer specialist (*A. obliterata*) and generalist (*A. bipunctata*) coccinellid aphid predator,

as both adults and larvae.

2. Determine whether any effects are attributable to differences between aphid prey, potential changes of host plant material, or an interaction of the two.

Materials and Methods

Plant material

Sitka spruce saplings

Material for arena cuttings

This experiment was conducted in 2012. In 2011, fifty 2-year-old Sitka spruce saplings (vegetatively propagated, Ident. QSS 04 (0R18TE)) were obtained from the Forestry Commission Delamere Nursery, Cheshire (U.K.). These were then potted up at Silwood Park, Berkshire (U.K.) in 3L pots, using a standard 2:1:1 peat, bark and perlite growing medium mixed at Alice Holt Forest Research Station, Surrey (U.K.). The growing medium also had 20g controlled release granular fertiliser mixed in (Osmacote® Plus: 16%N + 8%P + 11%K + 2%MgO; Scotts Ltd, U.K.). They were maintained outdoors on raised pallets under a summer drought regimen, which was assigned randomly:

1. **FC** - Plants maintained at field capacity;
2. **MS** - Plants maintained at 60% of field capacity;
3. **CS** - Plants maintained at 20% of field capacity;
4. **IS1** - Plants subjected to fluctuating stress, whereby pots were allowed to dry out to 20% of field capacity, and were then watered back up to field capacity;
5. **IS2** - Plants subjected to fluctuating stress, whereby pots were allowed to dry out to 30% of field capacity, and were then watered up to 70% of field capacity.

The drought treatments were maintained from the beginning of March 2011 to the end of October 2011. The trees were watered using an automated irrigation system, which was monitored daily to ensure the correct moisture content of the soil. Furthermore, a sealed plastic skirt was applied to each tree, to exclude rainwater. At the end of the drought treatment, the plastic skirts were removed.

In early February 2012, the trees, now 3-year-old saplings, were re-potted into 7L pots using the same growing medium as in the previous year (mixed at Alice Holt Forest Research Station, Surrey, U.K.). They were returned to their pallet, and allowed to re-establish. At the start of March 2012, the plastics skirts were reapplied and the drought treatment started again.

Material for experimental aphid cultures

In mid-January 2012, an additional fifty two-year-old Sitka spruce saplings (vegetatively propagated, Ident. QSS 04 (0R18TE)) were obtained from the Forestry Commission Delamere Nursery and potted up in 3L pots with the same growing medium at Silwood Park. These were kept in a greenhouse under a minimum of 20 °C and a 16:8 photoperiod (with overhead lighting provided by mercury halide and sodium bulbs during the day, to ensure a minimum light intensity of 300 watts/m^2). They were fully watered for two weeks to allow the saplings to establish, before drought treatments were applied, with ten trees per treatment.

They were moved onto raised pallets outdoors at the start of March 2012, under continued drought treatment. They were left outdoors until mid-May 2012, at which they were moved to a CT room at 15 °C, with 70% RH and a 16:8 photoperiod and allowed to acclimatise for two weeks under continued drought treatment.

Barley

Barley (*Hordeum vulgare* (L.)) was grown in large plant pots in John Innes compost in a greenhouse, where the conditions were maintained at a minimum of 20 °C and a 16:8 photoperiod. Overhead lighting was provided by mercury halide and sodium bulbs during the day, to ensure a minimum light intensity of 300 watts/m^2.

Cultures

Aphids

Elatobium abietinum cultures were established on the 2-year-old potted Sitka spruce saplings, ten trees per drought treatment, after they had been moved into the CT room at 15 °C, with 70% RH and a 16:8 photoperiod. The trees were artificially inoculated with cuttings taken from a stock culture raised in buckets of water in a CT room at 15 °C, with 70% RH and a 16:8 photoperiod on cut branches from Alice Holt Forest Research Station, Surrey (U.K.).

Rhopalosiphum padi (L.) cultures were maintained on barley in insect cages in the same CT room as the *E. abietinum* cultures, as a food source for the coccinellid cultures (Timms, 2004).

Coccinellids

Cultures of *A. obliterata* and *A. bipunctata* were maintained in a separate CT room at 15 °C, with 70% RH and a 16:8 photoperiod. They were raised in 14×9.5×26.5cm perspex boxes, each of which had two large muslin-covered holes in the lid. Each box was provided with folded filter paper, to provide an egg-laying surface, and some moistened cotton balls. The boxes were checked daily, with any eggs removed and placed in their own perspex box until hatching. Both adult and larval coccinellids were fed daily *ad libitum* on *R. padi*, which was found to be a suitable substitute for *E. abietinum* (Timms, 2004; Timms & Leather, 2008).

Consumption in a Petri dish

These experiments were all conducted in the coccinellid culture CT room, which was kept at 15 °C, with 70% RH and a 16:8 photoperiod. Aphid numbers offered were selected to represent *ad libitum* availability, as per Timms (2004).

Adult consumption

75 adult *A. obliterata* and *A. bipunctata* were weighed and transferred into individual 9cm Petri dishes and starved for 24 hours. They were then transferred into a new 9cm

Petri dish with Fluon®-coated sides (this was to avoid aphids from walking onto the top of the Petri dish). Each of these new Petri dishes contained 100 approximately 3rd instar *E. abietinum* aphids, sourced from five of the culture trees for each drought treatment. The coccinellids were then left for a further 24 hours to feed before being removed, and the number of aphids consumed in that period of time recorded. Third instar aphids were utilised, as these would not produce additional young during the experiment's duration.

Larval consumption

150 1st instar *A. obliterata* and *A. bipunctata* larvae were weighed and transferred into individual 9cm Petri dishes with Fluon®-coated sides within 12 hours of hatching. Each Petri dish contained 50 approximately 3rd instar *E. abietinum* aphids, sourced from all ten of the culture trees for each drought treatment. The larvae were then left for 24 hours to feed before being removed, and the number of aphids consumed in that period of time recorded. Third instar aphids were utilised, as these would not produce additional young during the experiment's duration.

Consumption on host plant material

The above methodology was repeated again, for both adults and larvae of each coccinellid species, in the presence of Sitka spruce plant material. Again, the experiments were conducted in the coccinellid culture CT room, which was kept at 15 °C, with 70% RH and a 16:8 photoperiod.

A 4cm segment of Sitka spruce side-branch, sourced from the 3-year-old Sitka spruce left outdoors, was placed into each Petri dish and the appropriate number of 3rd instar *E. abietinum* aphids added. Each segment was carefully examined for aphids (which were removed) before being placed in the Petri dishes. Rather than the usual lid, an inverted Petri dish base was attached securely to the top to allow enough space for the spruce needles. The sides of all Petri dish bases were coated with Fluon®. After two hours, any aphids which had not moved onto the spruce needles were moved there using a fine paintbrush and left for a further hour. A coccinellid was then placed onto each spruce segment and left for 24 hours, after which they were removed and the number of aphids consumed recorded. Each needle was carefully removed and examined for aphids and the remaining section of stem carefully examined, to make sure no aphids were missed.

Statistical analysis

The effects of drought stress and host plant material were analysed using linear mixed effects models. Drought was modelled as a fixed effect, while coccinellid weight and the tree from which prey aphids were obtained were modelled as random effects (For each species of adult coccinellid - groups: tree = 5, weight = 75; n = 5, estimated d.f. for each parameter = 4. For each species of coccinellid larvae - groups: tree = 10, weight = 150; n = 10, estimated d.f. for each parameter = 4).

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

Effects on adult coccinellids

Aphidecta obliterata **adults**

While no interactions were observed, a significant effect of both drought (${\chi^2}_4 = 94.11$, $P < 0.001$) and arena 'substrate', where more aphids were consumed in an empty Petri dish (${\chi^2}_1 = 14.64$, $P < 0.001$), were found through model simplification (Table 6.1).

In a Petri dish with no Sitka spruce segment, a greater number of aphids were consumed by *A. obliterata* adults under drought (${\chi^2}_4 = 66.95$, $P < 0.001$). A response was shown under both the severe drought level treatments, CS and IS1 (CS: $t = 4.29$, $P < 0.001$; IS1: $t = 3.75$, $P < 0.001$; Figure 6.2B). The moderate drought level treatments, MS and IS2, however, did not show a significant difference to the FC control (MS: $t = 1.92$, $P > 0.05$; IS2: $t = 0.54$, $P > 0.05$; Figure 6.2B).

While a smaller number of aphids was consumed across the treatments when a segment of Sitka spruce was included in the Petri dish, the responses still showed the same pattern ($\chi^2{}_4 = 66.95$, $P < 0.001$). More aphids were consumed under MS, CS and IS1 drought treatments (MS: $t = 2.12$, $P < 0.05$; CS: $t = 5.19$, $P < 0.001$; IS1: $t = 4.72$, $P < 0.001$; 6.2A), while the IS2 drought treatment showed no difference in consumption ($t = 1.46$, $P > 0.05$; Figure 6.2A).

Adalia bipunctata **adults**

Similarly to *A. obliterata*, a significant effect of both drought ($\chi^2{}_4 = 69.07$, $P < 0.001$) and arena 'substrate', where more aphids were consumed in an empty Petri dish ($\chi^2{}_1 = 163.29$, $P < 0.001$), were found through model simplification for *A. bipunctata* adults (Table 6.1). No interactions between the two were found.

Overall, although *A. bipunctata* adult coccinellids ate a greater number of aphids, the response showed followed the pattern shown by *A. obliterata.* In the absence of a Sitka spruce segment ($\chi^2{}_4 = 58.94$, $P < 0.001$), adult coccinellids presented with aphids reared under the IS2 drought treatments showed no significant difference to the FC drought treatment control ($t = 0.42$, $P > 0.05$; Figure 6.3B), while a significantly greater number of aphids was consumed under the MS, CS and IS1 drought treatments (MS: $t = 2.83$, $P < 0.01$; CS: $t = 6.03$, $P < 0.001$; IS1: $t = 3.95$, $P < 0.05$; Figure 6.3B).

When a host plant segment was included ($\chi^2{}_4 = 32.79$, $P < 0.001$), a greater number of aphids were consumed over 24 hours in both the CS and IS1 drought treatments (CS: $t = 4.70$, $P < 0.001$; IS1: $t = 2.83$, $P < 0.01$; Figure 6.3A). The MS and IS2 drought treatments showed no significant effect on consumption (MS: $t = 1.01$, $P > 0.05$; IS2: $t = 0.04$, $P > 0.05$; Figure 6.3A).

Effects on 1st instar coccinellid larvae

Aphidecta obliterata **larvae**

As was in the case for adult coccinellids, both drought ($\chi^2{}_4 = 146.60$, $P < 0.001$) and the presence of host plant material ($\chi^2{}_1 = 11.76$, $P < 0.001$) were shown to have a significant effect on the number of aphids consumed by 1st instar larvae of *A. obliterata* over 24

Table 6.1:

Summary of the mean consumption rates by adult coccinellids of *E. abietinum* raised under drought stress in 24 hours. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

	A. obliterata		*A. bipunctata*	
Drought treatment	**With host plant**	**Without host plant**	**With host plant**	**Without host plant**
	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**
FC	26.67 ± 1.01	29.75 ± 0.91	45.90 ± 1.23	54.01 ± 0.97
MS	30.21 ± 1.15	32.76 ± 1.08	47.64 ± 1.26	58.58 ± 0.85
CS	35.43 ± 1.20	36.62 ± 0.95	54.02 ± 1.18	63.66 ± 1.14
IS1	34.54 ± 1.35	35.66 ± 1.19	50.77 ± 1.31	60.26 ± 1.36
IS2	24.02 ± 1.26	30.51 ± 1.15	46.02 ± 1.08	53.32 ± 1.04

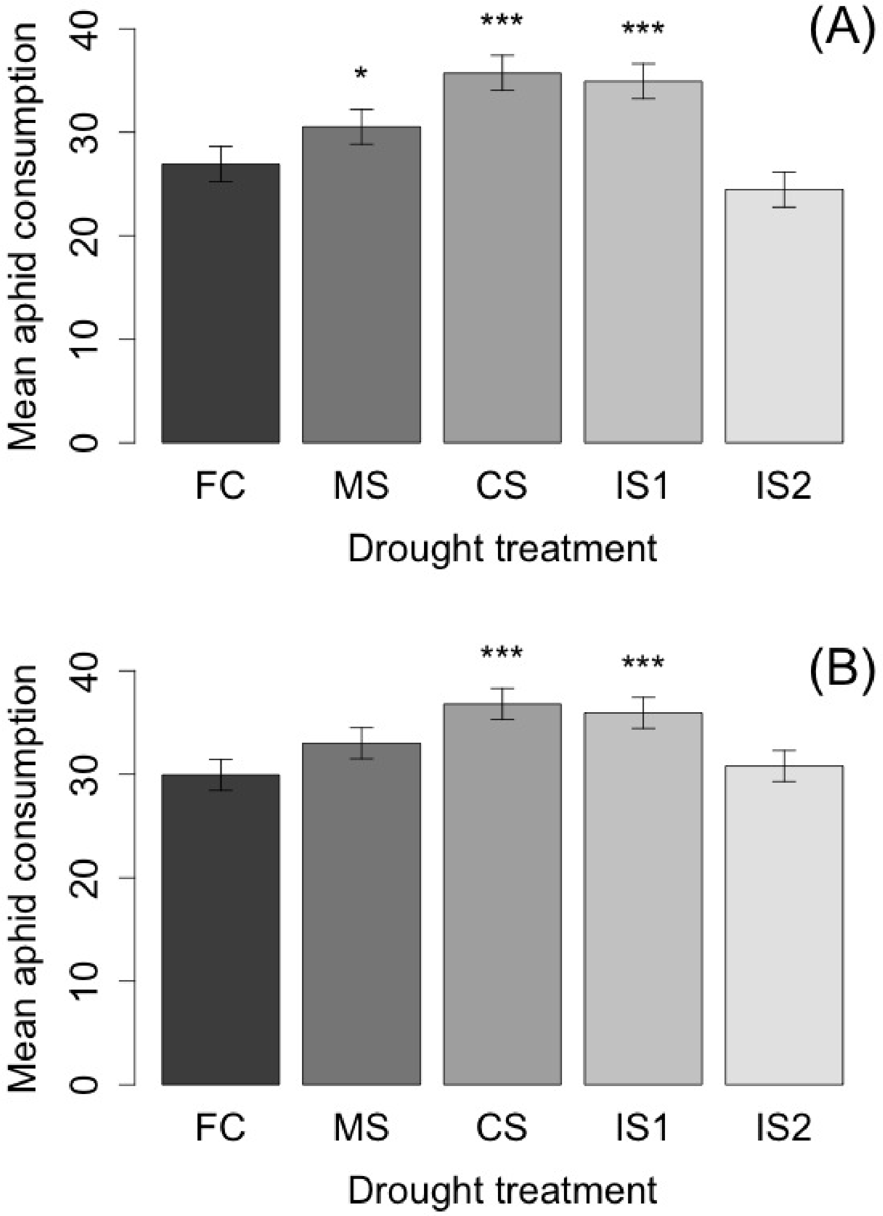

Figure 6.2: The effect of drought stress on 3^{rd} instar *E. abietinum* consumption by *Aphidecta obliterata* adults

(A) Mean number of aphids consumed over 24 hours ± LSD in the presence of a Sitka spruce segment; (B) Mean number of aphids consumed over 24 hours ± LSD with no host plant material. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

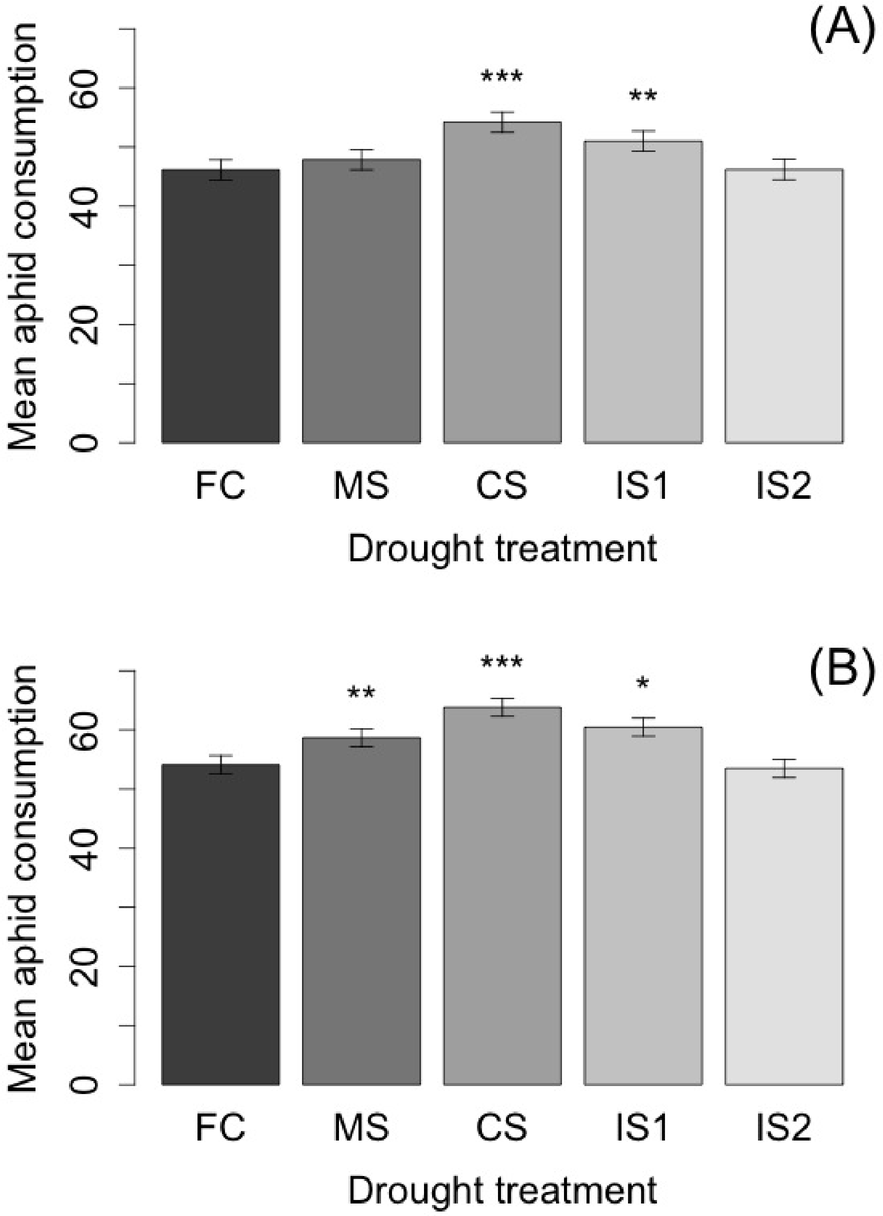

Figure 6.3: The effect of drought stress on 3^{rd} instar *E. abietinum* consumption by *Adalia bipunctata* adults

(A) Mean number of aphids consumed over 24 hours ± LSD in the presence of a Sitka spruce segment; (B) Mean number of aphids consumed over 24 hours ± LSD with no host plant material. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

hours (Table 6.2). Fewer aphids, however, rather than more, were consumed in an empty Petri dish by the larvae.

The consumption rates of the coccinellid larvae did not show as consistent a pattern as the adults of their respective species. In the case of *A. obliterata* in a Petri dish which included a Sitka spruce segment (${\chi^2}_4 = 67.96$, $P < 0.001$), there was no significant difference between the number of aphids eaten by the larvae in both the MS and IS1 drought treatments (MS: $t = 0.91$, $P > 0.05$; IS1: $t = 1.00$, $P > 0.05$; Figure 6.4A) and the larvae in the FC control drought treatment. A greater number of aphids, however, were consumed under the CS drought treatment ($t = 3.73$, $P < 0.001$; Figure 6.4A), while significantly fewer were consumed under the IS2 drought treatment ($t = 4.28$, $P < 0.001$; Figure 6.4A).

In the absence of host plant material, all drought treatments showed a significant difference from the FC drought treatment control (${\chi^2}_4 = 60.66$, $P < 0.001$). More aphids were consumed under the MS, CS and IS1 treatments (MS: $t = 2.74$, $P < 0.01$; CS: $t = 3.27$, $P < 0.001$; IS1: $t = 2.61$, $P < 0.01$; Figure 6.4B), while fewer were eaten under the IS2 drought treatment ($t = 3.33$, $P < 0.001$; Figure 6.4B).

Adalia bipunctata larvae

Similarly to the adults of its species, *A. bipunctata* larvae showed a significant response under both drought (${\chi^2}_4 = 185.34$, $P < 0.001$) and arena 'substrate', where again more aphids were consumed in an empty Petri dish (${\chi^2}_1 = 221.44$, $P < 0.001$), were found through model simplification for *A. bipunctata* adults. No interactions between the two were found.

In the absence of a Sitka spruce segment (${\chi^2}_4 = 100.55$, $P < 0.001$), there was no difference in the number of aphids consumed under the MS treatment ($t = 0.11$, $P > 0.05$; Figure 6.4A) when compared to consumption rates under the FC control. Furthermore, while more aphids were consumed under CS and IS1 drought treatments (CS: $t = 4.86$, $P < 0.001$; IS1: $t = 2.22$, $P < 0.05$; Figure 6.4B), significantly fewer were consumed under the IS2 drought treatment ($t = 4.76$, $P < 0.001$; Figure 6.4B).

When presented with aphids on a Sitka spruce segment (${\chi^2}_4 = 95.63$, $P < 0.001$), *A. bipunctata* larvae showed the same response as that of the adults of their species - while a greater number of aphids were consumed under the CS and IS1 drought treatments (CS: $t = 6.26$, $P < 0.001$; IS1: $t = 4.65$, $P < 0.001$; Figure 6.4A), there was no effect on consumption under the MS and IS2 drought treatments (MS: $t = 0.20$, $P > 0.05$; IS2: $t = 1.72$, $P > 0.05$; Figure 6.4A).

Discussion

The influence of drought stress

Changes to abiotic conditions associated with climate change, such as increased temperature and atmospheric gases, have repeatedly been shown to affect phytophagous insects (Evans *et al.*, 2002). The same is true for drought stress. The Pulsed Water Stress hypothesis (Huberty & Denno, 2004), proposed that intermittent stress would benefit phloem-feeding insects, further refined by Mody *et al.* (2009), who refined this to consider the importance of stress magnitude. A meta-analysis by Koricheva *et al.* (1998), however, indicated that drought reduced the reproductive potential of sucking insects.

The effects of drought on plants themselves range from effects at a cellular level (Heijari *et al.*, 2010), to plant structure (Cregg & Zhang, 2001; Heijari *et al.*, 2010), changes to plant growth (Cregg & Zhang, 2001; Eilmann & Rigling, 2012; Sánchez-Salguero *et al.*, 2012), through to effects on plant chemistry (Major, 1990; Branco *et al.*, 2010; Khan *et al.*, 2011; Hu *et al.*, 2013). Nutrient uptake is also known to be affected by drought (Kreuzwieser & Gessler, 2010) which would affect the quality of phloem sap for phloem-feeding insects such as aphids, much as turgor pressure would affect availability of the sap to the herbivores.

In order to understand the predatory performance of natural enemies under drought stress, it is necessary to first understand the effects on their phytophagous prey. Water stress has been shown to affect the host preference of insects. The wood borer *Tomicus destruens* (Coleoptera: Scolytidae) was found to not only have a preference for well-watered pine, but also to have higher survival rates on those plants when compared to stressed plants (Branco *et al.*, 2010). Another borer, *Hylotropus bajulus* (Coleoptera: Cerambycidae), however, showed no difference in performance between well-watered and

Table 6.2:

Summary of the mean consumption rates by 1st instar coccinellid larvae of *E. abietinum* raised under drought stress in 24 hours. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

	A. obliterata		*A. bipunctata*	
Drought treatment	**With host plant**	**Without host plant**	**With host plant**	**Without host plant**
	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**
FC	10.46 ± 0.22	9.58 ± 0.19	5.34 ± 0.18	7.74 ± 0.21
MS	10.15 ± 0.18	10.57 ± 0.21	5.23 ± 0.20	7.68 ± 0.26
CS	11.83 ± 0.25	10.66 ± 0.24	7.09 ± 0.26	9.31 ± 0.18
IS1	10.80 ± 0.28	10.48 ± 0.20	6.66 ± 0.22	8.46 ± 0.21
IS2	8.94 ± 0.14	8.48 ± 0.23	4.88 ± 0.14	6.19 ± 0.25

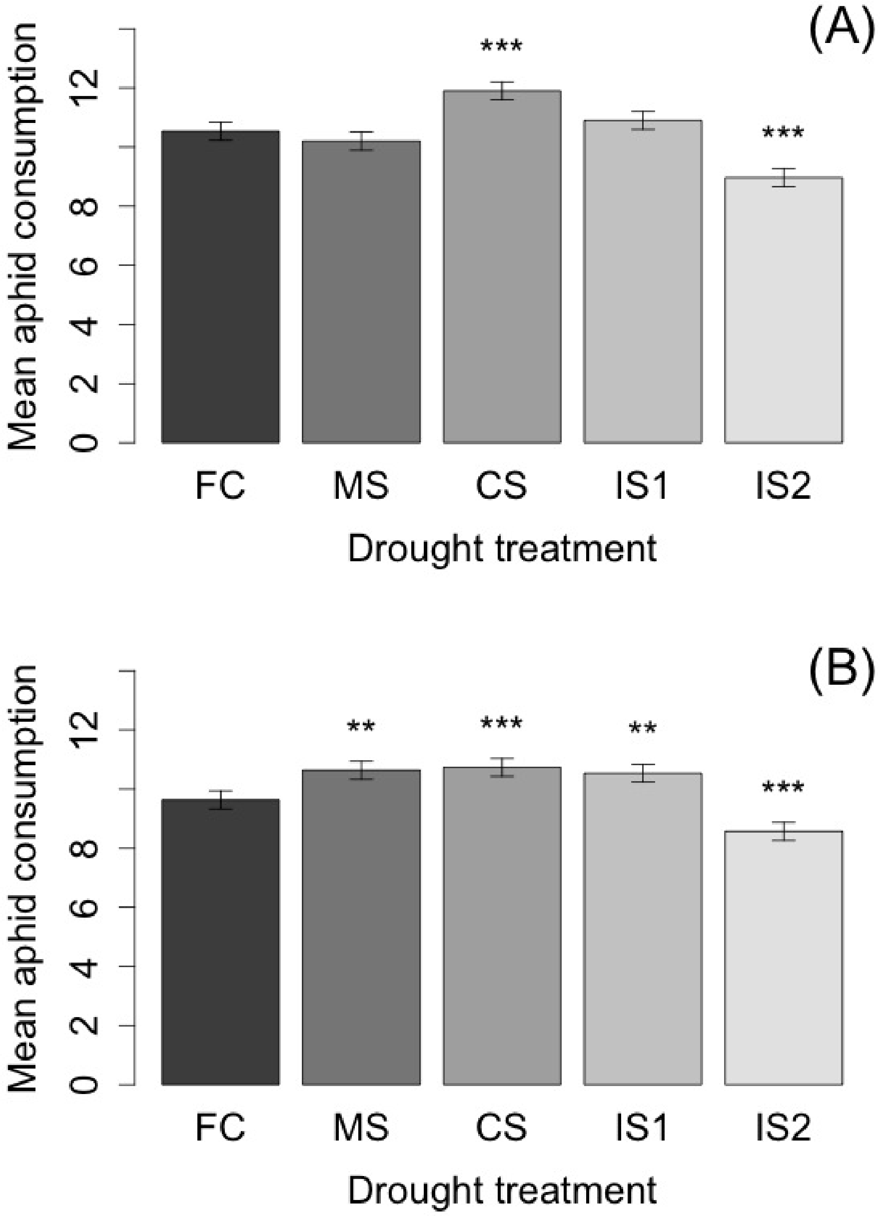

Figure 6.4: The effect of drought stress on on 3rd instar *E. abietinum* consumption by *Aphidecta obliterata* 1st instar larvae

(A) Mean number of aphids consumed over 24 hours ± LSD in the presence of a Sitka spruce segment; (B) Mean number of aphids consumed over 24 hours ± LSD with no host plant material. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

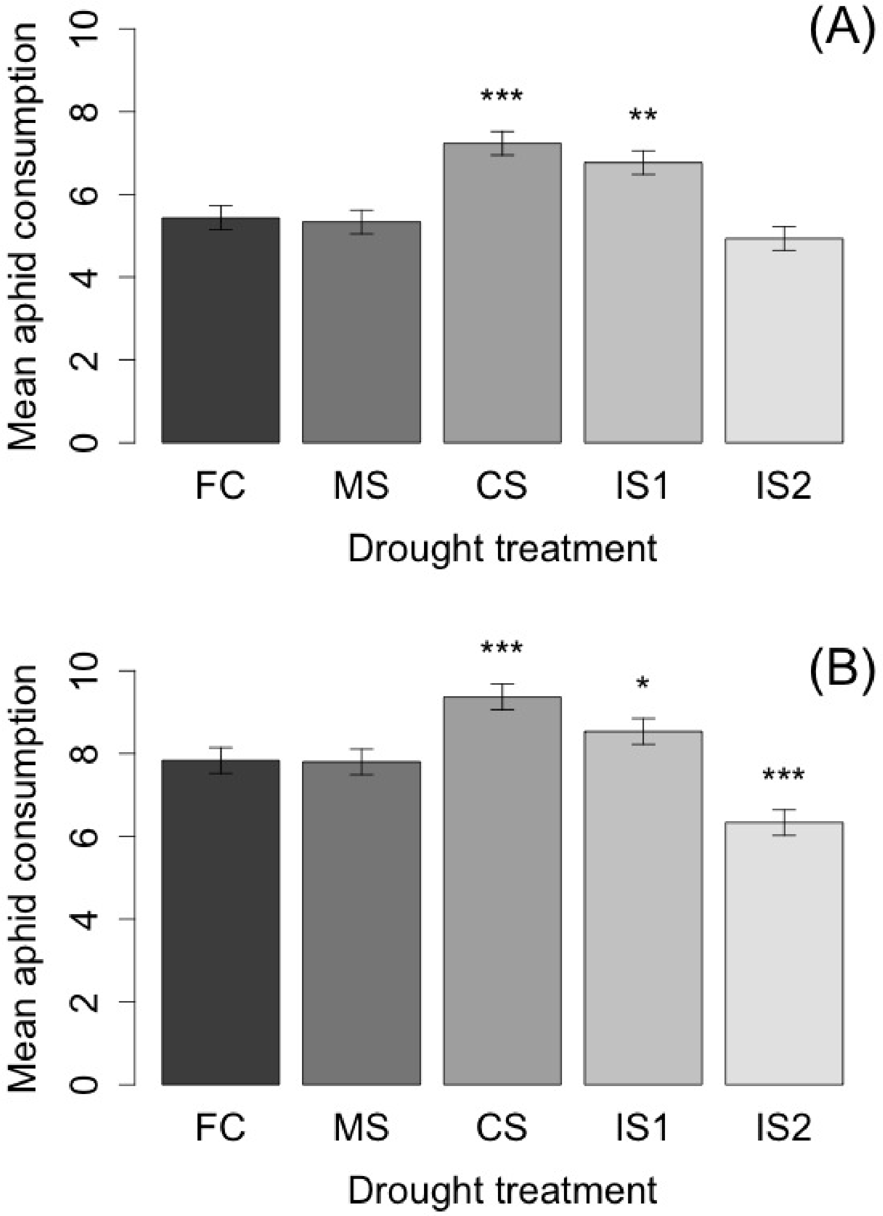

Figure 6.5: The effect of drought stress on on 3rd instar *E. abietinum* consumption by *Adalia bipunctata* 1st instar larvae

(A) Mean number of aphids consumed over 24 hours ± LSD in the presence of a Sitka spruce segment; (B) Mean number of aphids consumed over 24 hours ± LSD with no host plant material. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

stressed pine seedlings (Heijari *et al.*, 2010). On the other hand, leaf-cutting *Atta* ants had a preference for drought stressed plants (Neto *et al.*, 2012). By affecting host preference, and given that drought rarely has an even effect across a tree stand (Mitchell *et al.*, 2013), changes to prey distribution could be observed, which in turn may affect accessibility to natural enemies.

Aphids show inconsistent responses to drought stress. Several studies have observed reductions in growth rate or performance (Kennedy *et al.*, 1958; McVean & Dixon, 2001; Hale *et al.*, 2003), while others have observed improved performance (Simpson *et al.*, 2012). Responses are often species-specific. Khan *et al.* (2010) observed that while the specialist *Brevicoryne brassicae* was less affected by drought stress, the generalist species *Myzus persicae* had larger populations on drought stressed plants. A species-specific response can also be observed in other insect families, such as lepidopterans (Gutbrodt *et al.*, 2011). Interactions between phytophagous insects can also be altered by drought (Staley *et al.*, 2007).

The performance of *E. abietinum* in response to drought stress is discussed in Chapter 5. During a comparable trial in relation to the start of the study presented here (started two weeks after bud burst, on the 10th May 2011), mean nymphal weight was reduced under both continuous and intermittent severe drought stress (CS and IS1), while adult weight was slightly, though significantly, increased under the same treatments. Moderate intermittent stress (IS2) was significantly increased in both cases.

The consumption rates of the coccinellids were significantly higher under the severe stress treatments, for the adults and larvae of both species and regardless of arena substrate. Fewer aphids were consumed under moderate intermittent stress, although the response was only significant for the larvae. These findings, supported by those in Chapter 5, suggest that under severe drought a greater number of aphids must be consumed in order to meet the dietary requirements of natural enemies. It could also be the case, especially for larvae, that the reduction in aphid size may reduce handling time of the prey.

The results observed under moderate intermittent stress are somewhat less clear-cut, although fewer aphids reared under these conditions were consumed by both adults and larvae. Fewer aphids would need to be consumed, as they were larger, however a significant reduction was only observed in the consumption rates of the larvae, suggesting a potential age-dependent effect. It is likely that, due to their larger size, the aphids were more difficult to handle for the small larvae (Dixon, 1970), whereas adults required fewer prey items to become satiated.

The response of plants to drought can be complex. Changes to terpene levels, for example, have been observed in Sitka spruce in response to drought stress. Major (1990) observed higher levels under intermittent stress when compared to continuous and control levels of drought stress. Terpenes function as a defensive secondary metabolite which can also function as volatiles. Herbivore-induced plant volatiles and other semiochemicals are known to affect the behaviour of herbivores and their natural enemies. They can, for example, control host selection for herbivores (Paiva *et al.*, 2011). They can also enhance the ability of natural enemies to locate their prey on a plant (Uefune *et al.*, 2012). Changes to the morphology of conifer seedlings has been recorded in response to drought stress (Cregg & Zhang, 2001), although such changes are not necessarily true in all arboreal settings (Moser *et al.*, 2010). Trees, through their lifetime, are likely to face a diverse range of conditions and stresses, and therefore have means of compensating for these effects.

The consumption rates in empty Petri dishes compared with those with Sitka spruce segments were found to be significantly different. Despite this, the responses followed the same pattern in all cases - more aphids consumed under severe stress, fewer under moderate intermittent stress (even if not significantly so). This would suggest that the differences in consumption rates were driven by differences in the aphids rather than changes to the host plant structure. It is unclear, however, whether drought-induced changes to the chemistry of the Sitka spruce host plants played a role through the aphids.

The plant segments provided in the Petri dish arenas during the experiments were taken from plants which had undergone a previous year's worth of drought treatment, in order to reflect any changes to needle morphology. Given that the consumption rates followed the same patterns in both arenas, the implication is that there were no significant differences in the morphology of the segments. At the very least, any changes would not have affected the searching behaviour of the coccinellids.

The influence of 'substrate' - Sitka spruce segment vs. empty Petri dish

Functional response studies are often carried out in empty Petri dishes (on the dish surface, e.g. Hassell *et al.* (1977); Leather & Owuor (1996)). While this does reduce the number of uncontrolled variables and allows for comparison between previous standardised Petri dish studies, the realised functional response exhibited by the predator in a natural environment may not conform to the observed results. The same holds true in the case of consumption rates. Given that it is the realised response and consumption rate which, in terms of potential biological control, are the most pertinent, comparing the two scenarios is important as several factors are altered by the presence of host material. Two such factors are effects of herbivore behaviours and search time.

Phytophagous insect activity differs when in the presence of host plant material in comparison to an empty substrate surface. As an example, *Tetranychus urticae* mites were found by Everson (1980) to be inactive on bean leaves, but active in empty Petri dishes. The same applies in the case of aphids, which are comparatively immobilised during feeding due to the insertion of their stylets into the plant tissue in order to access the phloem (Dixon, 1973). Feeding cannot take place, however, if there is no plant material and as such they may be able to respond to predator disturbance more promptly - in order to escape from attack, a feeding aphid must first remove its stylets from the plant before reacting. While many species walk away, *E. abietinum* exhibits a dropping response to disturbance (Day *et al.*, 2006). Though this may be of benefit in the presence of a Sitka spruce segment the same does not hold true in an empty Petri dish, where the behaviour would not remove the aphid from the immediate vicinity of the predator.

Beyond the effects on herbivore behaviour, search time for the predator on host plant material must inevitably be increased in comparison with an empty Petri dish. This results from an increased search area and, in the cases where the host plant is a coniferous species, each needle must be searched individually.

In this experiment, significant differences were observed in aphid consumption rates in all cases dependent on the arena substrate conditions. In the case of adult coccinellids of both species, a greater number of aphids were consumed in the empty Petri dish arenas.

The difference was less pronounced for *A. obliterata* adults, with only a difference of 8.3% in the mean consumption rates of the two substrate types. *Adalia bipunctata*, on the other hand, nearly doubled the difference, consuming 18.5% more aphids in an empty Petri dish. The responses of the 1st instar larvae, on the other hand, were species-dependent. Considerably more aphids, 25.5% more, were eaten by *A. bipunctata* larvae. In contrast to this, and to what was observed with the adults, *A obliterata* larvae consumed 4.6% fewer aphids in an empty Petri dish. Not only that, but *A. obliterata* larvae consumed a greater number of aphids in both cases than did *A. bipunctata*.

The observed results of this study suggest that *E. abietinum* were able to take advantage of the presence of host plant material in all cases except when predated upon by 1st instar *A. obliterata* larvae. Furthermore, *A. bipunctata* were less able to compensate for the presence of plant material, and although adults of this species consumed a greater number of aphids than the spruce specialist, their larval counterparts performed worst than the *A. obliterata* larvae. Although the results deal with consumption rates over 24 hours rather than functional responses, the findings presented here contrast with those of Timms *et al.* (2008), who observed no difference between the specialist and generalist coccinellids.

Limitations of the experiment, and potential for future research

The potential differences in consumption rates between male and female coccinellids were not considered, as the sex of the individuals was not determined and used as a variable. In general, male coccinellids are typically smaller than females of their species (Hodek, 1973), and this is true for both *A. obliterata* and *A. bipunctata* (Timms *et al.*, 2008). Despite the differences in weight, which would lead one to expect a reduced consumption rate by males, Timms *et al.* (2008) did not find any significant differences in average consumption of *E. abietinum* by *A. obliterata*. That said, Hemptinne *et al.* (1996), did observe a lower intake by males of *A. bipunctata* in comparison to females of the same species. The weight of both larval and adult coccinellids used in the experiment were assessed and no significant differences were found, suggesting that such effects should have balanced out.

The functional response, or changes to it, of *A. obliterata* and *A. bipunctata* feeding on *E. abietinum* under drought stress were also not explored. A Holling Type II response was observed by Timms (2004), the typical response to increasing prey density exhibited by predators (Hassell *et al.*, 1977). Awmack *et al.* (1997) observed a decreased response to aphid alarm pheromones by *Aulacorthum solani* (Kalt.) under elevated CO_2 levels comparable with those predicted for the end of century. Such changes in climate and conditions could not only affect the behaviour of phytophagous insect, but may also do so for their natural enemies, thus the potential for a change in functional response in response to drought may exist and would be of interest to explore.

Major (1990) observed changes to the levels of monoterpenes in Sitka spruce needles under different levels of drought stress. Such data was not collected as part of this experiment, and given the ability of secondary plant metabolites to affect not only the phytophagous insects (Kidd *et al.*, 1990) but also tritrophic interactions (Aqueel & Leather, 2012; Banfield-Zanin *et al.*, 2012), it would be inappropriate to conclude whether foliar terpenoid compounds in the host plant affected the consumption rate response without such data.

Conclusions

1. The consumption rate of *E. abietinum* by *A. obliterata* and *A. bipunctata* coccinellids is increased under severe drought stress, for both adults and 1st instar larvae.
2. Under moderate intermittent stress, the consumption rate is reduced.
3. The response patterns were similar in both empty Petri dishes and with Sitka spruce material provided. As such, differences in the consumption rates are likely to result from differences in the aphids, rather than any changes to host plant material.

Chapter 7

General Discussion

Forests in the U.K. provide a valuable commodity, not just for recreational purposes or as habitats for associated wildlife, but also as a timber resource and associated employment. In terms of climate change mitigation, forest cover provides a carbon sink that contributes to helping the U.K. meet international targets. The financial return in the forest industry, however, is not high, and as such any losses caused by environmental and pest problems risk reducing economic viability. Softwood timber is a low-value product, such that any input costs (both fertilisers and pest management means) must be minimised to ensure a return. One way to mitigate the effects of pests is to understand their ecology and relationship with their host plants.

Sitka spruce, *Picea sitchensis* (Bong.) Carr., is the most important forestry conifer species in the U.K., and is highly productive in the current maritime climate (Halsall *et al.*, 2006). It is currently attacked by several insect pests. The large pine weevil, *Hylobius abietis* (L.), is one of the most important and is a pest of restocking sites, sometimes causing high levels of sapling mortality (Heritage, 1996). It can, however, be protected against, as the susceptible stage can be chemically treated before planting out (Heritage, 1997; Rose, 2002), while higher levels of sap production in older trees produce a protective effect (Tomlin & Borden, 1997).

The green spruce aphid (*Elatobium abietinum* Walker), is another major pest, and provides a more pressing and complex problem for forest managers, however. This aphid, due to its feeding behaviour, causes significant damage to Sitka spruce of all ages. By feeding on one-year-old and older needles, even older trees can sustain damage, and this can be particularly problematic as Randle & Ludlow (1998) indicated that a tree's ability to recover decreases with age. With increasing stand age, chemical control of the pest becomes a less viable solution in reducing pest levels. Even though mortality caused by the aphid is rare, growth can be greatly reduced under *E. abietinum* infestation. Given that forestry is a long-term investment, with Sitka spruce stands requiring roughly forty years to mature (Moore, 2011), the potential financial cost of *E. abietinum* as a pest may exceed economically viable limits.

Climate change predictions stipulate an increasing frequency and intensity of extreme events, likely to be matched by an increased frequency and severity of pest attack. It is important to understand how predicted climate change may alter the current situation, in order to establish appropriate management plans or mitigation techniques. This is necessary in order to meet timber requirements while maximising financial profit.

Sitka spruce is a major component species in many drought-prone areas, particularly in Scotland (Green & Ray, 2009). Several arboreal pathogenic diseases have already been identified as likely to increase in severity and frequency in drought-prone forests as a result of climate change (Green & Ray, 2009), and *E. abietinum* has similarly been identified as a potential problem under the same conditions (Straw, 1995). It is therefore important to factor the effects and damage from such organisms into future climate change adaptation strategies in order to sustainably and economically manage British forestry. However, plant response varies both spatially and temporally and the mechanisms which allow this are poorly understood (Pritchard *et al.*, 2007).

Relatively few studies have been published which address the impact of water limitation on arboreal aphids. Knowledge on the influence of drought stress on both *E. abietinum* population dynamics and the interaction with spruce is currently limited, and results vary. Warrington & Whittaker (1990) found that while drought had a significant direct effect on Sitka spruce, it had no interaction with *E. abietinum.* Major (1990), on the other hand, found increased population densities under intermittent drought stress. The work for this thesis was carried out in order to investigate the effect of drought stress on *E. abietinum* in a systematic manner. The effects on host tolerance were also assessed, while the effects of differing drought frequency and intensity were also considered as part of the study. In order to better elucidate the response, the effects were assessed at two levels: (1) aphid population densities on young, potted Sitka spruce; and (2) individual aphid performance under controlled laboratory conditions.

The effects of drought on spring population development and aphid performance

In the UK, spring population density tends to be responsible for the majority of the damage caused by *E. abietinum* to Sitka spruce. Several factors are known to affect the population dynamics of this aphid species. Climate, in particular, is an important overriding factor. For example, winter temperatures affect overwinter survival, which in turn governs spring population peak size (Straw, 1995). The predicted increase in winter temperatures is already expected to cause an increase in overwinter survival, leading to more frequent and severe aphid outbreaks. In addition to this, host plant quality affects the performance of aphids on their host and therefore mediates population potential. Drought is one factor that could affect host plant quality and therefore affect the population dynamics and performance of spruce aphids.

One potential impact of spring-summer drought could be alterations to the timing and size of the spring population peak. Such changes would arise from changes to host plant quality leading to improved performance and more rapid aphid population development. In order to elucidate such a response, aphid densities were monitored non-intrusively in a nursery environment while individual performance was investigated under controlled laboratory conditions (Chapters 3 and 5).

It was established in the field work conducted for Chapter 3 that, while different drought frequencies and intensities did not alter population development rates, there were changes to the nature of the aphid peak behaviour. Trees reared under moderate intermittent stress consistently maintained higher aphid population densities for longer following the spring peak, suggesting an improved host quality under this type and level of drought. Furthermore, although the size of the peak was not affected following a first instance of spring drought, the peak on trees under moderate intermittent stress was achieved later and was greater in a second year of drought; the rate of population development was nonetheless not affected when compared with other drought levels.

Measures such as mean relative growth rate (mRGR), fecundity and intrinsic rate of increase have frequently been used as proxies for aphid population potential and as indicators for host plant quality (Leather & Dixon, 1984). Aphid performance in the spring, as explored in Chapter 5, showed that the response of *E. abietinum* on an individual level to drought stress was not only complex, but affected by drought intensity and frequency, as well as timing. Moderate intermittent stress generally improved host quality for the aphids, while severe stress (both continuous and intermittent) was typically detrimental. Furthermore, host quality tended to improve through time under moderate intermittent stress, but declined under continuous severe stress.

A significant finding, therefore, of this study, is that *E. abietinum* spring performance and population density is affected not only by drought stress, but by the frequency and intensity of that stress. These results are in accordance with the findings of Price (1991)'s Plant Vigor Hypothesis (PVH), as well as Huberty & Denno (2004) and Mody *et al.* (2009)'s Pulsed Water Stress Hypothesis (PWSH). In terms of the PVH, severe stress was typically detrimental. A positive effect was only generally observed under an intermittent, albeit moderate, drought treatment level, in accordance with the PWSH. Specifically, it is probable that improved nutritional quality of phloem sap was not accessible to the aphid when drought was severe, even if it was intermittent, whereas a moderate intermittent stress achieved a balance between improved quality and availability that suited *E. abietinum.*

The improved individual performance shown under moderate intermittent drought stress by *E. abietinum* suggest that a difference in population development rate should have been observed, especially when considered in combination with the detrimental effects of severe drought stress on performance. This was not observed, however, suggesting that individual aphid performance measures may not reliably reflect the observed dynamics in the field, in accordance with the observations raised by Awmack *et al.* (2004) and Pritchard *et al.* (2007), and that some other mechanism may be at work. On the other hand, they do lend support to the observation that moderate intermittent drought stress may prolong the period of high aphid density.

There are two implications of these findings. The first of these is that under moderate intermittent drought, as may occur under reduced precipitation but not necessarily a full drought event, damage by *E. abietinum* to Sitka spruce may be increased. During extreme drought events, however, damage levels caused by *E. abietinum* are unlikely to change. Despite this, it must be remembered that Sitka spruce prefers moist conditions for optimal growth and therefore such an event could cause direct damage to the tree, adding to effects of aphid damage. Such potential effects were assessed in terms of needle retention (Chapter 3) and plant growth (Chapter 4), and are discussed further in a later section (see 'Host tolerance under drought and aphid infestation').

The second implication is a potential influence of drought stress on aphid migration. This in itself is twofold. First, migration may be delayed under moderate intermittent drought. This is because alate production, necessary for migration, has been found to be affected by host nutritional quality *inter alia* (Dixon, 1985), and thus if conditions were favourable on the original host plant alate production may be delayed. This would result in a potentially shorter period of time in which aphid populations could develop and cause damage to newly-infested trees following aphid migration. Second, if plant conditions are favourable during the period of aphid migration, larger infestations on previously uninfested plants may observed, leading to greater damage. Such changes to migration dynamics are important to forest management on a national scale. Alate aphids are known to be carried over great distances during the migration period (Parry, 1973; Straw, 1995), and the morphs are typically deposited after the spring peak (Dixon, 1973; Carter & Halldórsson, 1998). Although the timing of the peak may not be altered under limited water availability, aphids migrating into an area where trees are being submitted to a moderate intermittent stress will find favourable conditions for population development (given that this level of drought has been shown to prolong the period of suitable host quality), allowing for an increased population density to be developed and the potential for greater damage.

Autumn host quality under limited water availability

The spring population peak of *E. abietinum* follows an improvement of host plant quality during budburst. A similar improvement in host plant quality can be observed in the autumn, when the Sitka spruce enters dormancy and again becomes favourable for aphid population development (Carter & Nichols, 1988). In the UK, autumn peaks are typically smaller than those observed in the spring and are comparatively uncommon, but can still cause severe defoliation (Carter, 1989). It is speculated that drought may advance dormancy and improve host plant quality for *E. abietinum* in the autumn. Work conducted as part of Chapters 3 and 5 sought to investigate whether this may indeed be the case.

Elatobium abietinum in the field consistently exhibited a small, albeit short-lived, autumn peak under severe continuous drought stress (Chapter 3). Such a peak was not shown under the remaining drought levels, although at the end of the first season of drought application population densities in the remaining drought treatments had started to show some indications of recovery. These findings may initially appear to suggest that dormancy is indeed advanced under continuous severe drought stress. The performance measures obtained in Chapter 5, however, do not support this. Although mRGR was improved under continuous severe stress in the autumn, no other measures showed significant improvements in this treatment when compared to a well-watered control. While this finding does suggest an improved host plant quality, few performance parameters showed differences between the time-staggered trials providing no conclusive evidence for an advancement in Sitka spruce dormancy.

In combination, the findings of this work suggest an improved autumn host plant quality under continuous severe stress though no advancement in autumn dormancy as a result of drought. Given that an increase in the frequency of severe summer drought has been predicted for the UK by Murphy *et al.* (2009), it is probable that such peaks of *E. abietinum* density in the autumn are likely to become more common. An increase in the incidence of such peaks may have severe repercussions for Sitka spruce growth. Straw *et al.* (1998a), for example, noted that autumn defoliation appeared to have a greater affect on tree growth based on studies conducted in Iceland, where peak population levels are observed in the autumn rather than spring. Although defoliation rates and growth increment reductions may be similar following both UK spring and Icelandic autumn peaks, autumn defoliation may result in the exhaustion of plant reserves and a decrease in winter photosynthetic potential (Straw *et al.*, 1998a).

The results of the work presented for this thesis suggest that under severe continuous stress both a spring and autumn peak will be exhibited by *E. abietinum*. This will mean that, in years of drought, Sitka spruce will be subjected to two population peaks rather than one, with an increase in the potential for defoliation, damage and therefore growth reductions. This has serious implications for forest management. As the forestry industry already works on marginal profits, such aphid-related damage may render a Sitka spruce stand economically non-viable, especially if direct damage is caused on the trees by drought. Furthermore, although mortality is rare under *E. abietinum* infestation, it is more likely to occur following severe or complete autumn defoliations rather than spring defoliations (Straw *et al.*, 1998a).

Host tolerance under drought and aphid infestation

The ability of *E. abietinum* to cause extensive defoliation is what renders this particular aphid such an important forest pest species, given the potential for said defoliation to impact on Sitka spruce growth. As such, a great deal of interest and study has recently been dedicated to this in order to improve forest management. Given that it is predicted that *E. abietinum* attack frequency and intensity are expected to increase under climate change (Straw, 1995), it is also important to understand how related abiotic factors, such as drought, are likely to affect the interaction between the pest and its Sitka spruce host, given the importance of the tree species to forest industries not just in the UK but also throughout Europe.

Reductions in growth increments and increased needle loss as a result of aphid attack have repeatedly been demonstrated (Straw *et al.*, 1998a; Straw *et al.*, 1998b; Straw *et al.*, 2000; Straw & Green, 2001; Straw *et al.*, 2002; Straw *et al.*, 2005). Warrington & Whittaker (1990) found that drought caused similar reductions to Sitka spruce growth as aphid infestation, though the effects were not additive. The investigation into needle retention in Chapter 3 appear to support such findings. Percentage needle loss did not differ, overall, between the five drought treatments, but was significantly increased on those trees which had been inoculated with aphids. This would suggest that Sitka spruce has insufficient plasticity to show additional needle loss effects to drought stress after aphid attack, similarly to the findings of Warrington & Whittaker (1990).

The growth increment analyses in Chapter 4, however, did not replicate what would have been expected from the extensive literature. Neither height nor collar diameter increments or totals were found to be affected by drought and aphid attack. Total mid-point diameter, on the other hand, was reduced, though increment growth was not. These results appear counter-intuitive and are not corroborated by existing literature.

Several factors could be at play, which may go some way to explain the aberrant findings of Chapter 4. Similarly to the findings of Warrington & Whittaker (1990), it may be that there were no additive effects between drought and aphid infestation. The effects of each may have been severe enough that insufficient capacity remained to show further growth loss. This does not seem likely, as some difference would at least be expected in those treatments were water was withheld or aphids applied and the well-watered aphid-less control. Another potential reason may be that the lack of differences in growth reflect some methodological problem. Given that differences in aphid population density and performance were established in response to drought stress in Chapters 3 and 5, it is unlikely that this may have arisen due to issues with the drought treatments, as the findings suggested a difference in host quality at points in a season. There may be some other mechanism which regulated host plant quality in comparison to plant growth, though further investigation would be required to determine what such a mechanism could be.

Ramírez & Verdugo (2009) suggest that tolerance and resistance to aphids is affected by drought stress, though the trade-off between the two remains unaffected; tolerance showed no cost under limited water availability, while resistance elicited a cost in growth. The overall observation was that herbivore damage under drought caused compensatory growth, while trees with resistance to aphid pests grew less when aphids were not present. Tolerance in branch length for trees under drought stress was approximately twice that of well-watered control trees. Their study was conducted on deciduous poplar (*Populus* spp.) hybrids, which are fundamentally different from conifers. Despite this, simultaneous *E. abietinum* attack and drought stress may cause a general plant response in Sitka spruce, with compensatory growth under drought and aphid attack and resistance to aphids reducing growth in their absence. Tolerance and resistance expression may be resource dependent (Katjiua & Ward, 2006), and as such drought stress could mediate such a response. Chapter 3's findings that overall needle loss was unaffected by drought treatment, with reductions associated instead primarily with aphid presence, lends some support to such a mechanism being in effect. Further investigation into such a relationship and underlying mechanisms would be necessary in order to determine whether this is indeed the explanation for the aberrancy of the findings of the thesis, at least in relation to Sitka spruce growth.

Potential implications for natural enemies

As previously discussed, the forestry industry functions around marginal profits, and represents an increased financial investment with increasing stand age. Control by chemical means is often not economically viable, and pest damage can result in a reduction of already low financial returns. As such, control provided by natural enemies of insect pests is an important factor to be considered as part of forest management (Timms, 2004).

Natural enemies are as likely as their prey items to be affected by climate change, thus altering the nature of their interactions. In the case of *E. abietinum*, however, it is speculated that the influence of natural enemies on the population dynamics of the aphid will not increase under altered climactic conditions (Straw, 1995). It is nonetheless important to understand how changed abiotic conditions will affect the relationship, to inform management practices. The experiments conducted in Chapter 6 sought to provide an initial exploration of how the effects of drought on Sitka spruce and *E. abietinum* might affect natural enemies, by investigating the impact on the consumption rates of a coccinellid predator.

The first finding of the study is that any differences in the consumption rates of the aphid are in all likelihood a consequence of differences between the aphids, rather than changes to host plant material. This can be surmised as the response patterns were similar in both empty Petri dishes and in those where a Sitka spruce segment was provided. The overall lack of difference in Sitka spruce growth as a result of drought observed in Chapter 4 may support this, however there may have been differences in needle structure or density which were not assessed.

The second important finding of Chapter 6 was a change in consumption rates as a result of drought. Consumption rates of coccinellid predators feeding on 3rd instar *E. abietinum* were increased under severe stress, but decreased under moderate intermittent stress. These findings correspond and are supported by those of Chapter 5, where aphid nymphs were found to be smaller under severe stress and larger under moderate intermittent stress. Similarly, adult aphids were found to be larger under moderate intermittent stress, with those reared under severe stress larger in spring but smaller during the autumn. These findings have implications for potential control, by affecting the ability of natural enemies to function as control agents.

Prey size is known to have an important influence on predation and parasitisation rates (Sih, 1987; Sabelis, 1992). In the case of predators, a larger number of smaller prey items are required to satiate a predator of a given size than would be required with larger prey items (Roger *et al.*, 2000). However, larger prey items may also be more efficient at fending off attacks by natural enemies, both predators and parasitoids (Rotheray, 1989). To summarise, prey size affects the handling time and capture efficiency of natural enemies. This is important, as it is these two factors that primarily influence a species' performance as a control agent. Where *E. abietinum* individuals are larger, fewer may be attacked by natural enemies; should they also occur in greater numbers, then the impact on and pressure applied to the pest populations will be reduced overall and damage caused by the pest will increase. Conversely, smaller aphid individuals may result in increased pressure and impact, with the potential for reduced damage.

The observations made in Chapters 6, in combination with those of Chapter 5, would suggest that not only can drought stress be expected to cause changes to the dynamics between *E. abietinum* and its natural enemies, but that the nature of the changes will depend on the frequency and intensity of the drought. Under reduced precipitation, as reflected by the moderate intermittent drought treatment, it is likely that control by natural enemies may be rendered comparatively more inefficient or ineffective. Larger aphids as would be expected under these conditions can be expected to not only satiate predators more rapidly, but also defend themselves from attack more readily. As such, it is likely that consumption rates and predation pressure will decrease, allowing larger populations to be maintained and greater damage to be incurred by the host Sitka spruce.

In the case of severe stress, as would be experienced in the predicted drought events, predation pressure is likely to increase. This may serve to relieve potential damage to Sitka spruce hosts caused by *E. abietinum*, though increased direct damage to the trees as a result of severe drought could also be expected. Such direct impacts have already been observed in Sitka spruce (Green & Ray, 2009). Natural enemies of *E. abietinum* do not reduce the spring peak, but instead play a role in the summer decline and suppression of population densities after the peak in combination with changes to host quality (Hussey, 1952; Leather & Kidd, 1998). More importantly, natural enemies have been shown to suppress or at least reduce the size of the autumn peak (Austarå *et al.*, 1998). Given that *E. abietinum* can be expected to be smaller in size during the autumn following severe drought stress (Chapter 5), and that an increased consumption rate by coccinellid predators has been shown in Chapter 6 under such conditions, an additional effect could be a suppression of the autumn peak observed in Chapter 3 under continuous severe drought conditions.

Future research

Although the research conducted for this thesis suggests a positive effect of moderate intermittent drought stress on *E. abietinum* populations and a detrimental effect of severe drought stress, several aspects require further investigation. This will be necessary in order to elucidate the mechanisms behind the response, and to establish more clearly how the pest aphid populations will respond to the multiple stressors associated with climate change.

Individual aphid performance will contribute to the population dynamics of the aphid species. Although such measures were investigated under controlled laboratory conditions, such values were not established for populations in the field. An attempt was made (refer to Appendix B), but high mortality due to methodological limitations resulted in low replication. Valuable information would be provided by a more in-depth study once a suitable caging method is found; the caged aphids would be subjected to more similar conditions as their non-caged counterparts, including fluctuating temperatures and exposure to the elements, and therefore their performance measures may tie more closely in with the observations of field populations.

A major limitation of this thesis is that analyses were not conducted on plant chemistry or physiology. As phloem-feeders, aphid performance and population development is tied closely with host plant quality, and therefore such investigations may aid a more clear explanation of *E. abietinum*'s response to drought stress. Nitrogen-Phosphorus-Potassium (NPK) analyses of needles have previously been used to provide indications of sap nutritional quality (Straw & Green, 2001; Williams *et al.*, 2005). Advances in technology and techniques mean that increasingly refined measures can be taken. For example, amino acid composition within sieve elements of the phloem can now be determined through the combination of laser-induced florescence and high-sensitivity capillary electrophoresis (Gattolin *et al.*, 2007; Gattolin *et al.*, 2008). Temporal changes to amino acid composition could be followed using such a technique, which could then be linked to aphid performance.

Terpenes are secondary defensive compounds produced by conifers, and changes in their concentration have been linked with the both success of insect pests (Cates *et al.*, 1982), and in host-location behaviour by both pests and their natural enemies. Terpene concentrations have previously been assessed in Sitka spruce (Major, 1990; Williams *et al.*, 2005), sometimes in relation to drought stress. Little information regarding aphid responses to terpenoid compounds in droughted arboreal settings is currently available, and a study in this regard would add to the limited literature available. Furthermore, an understanding of the effects of drought on such secondary compounds may also help explain changes in the dynamics between insect pests and their natural enemies.

Physiological measures, such as leaf water potential, would also provide additional information that may explain aphid responses to drought. It has been shown repeatedly that improvements to host quality will not necessarily correspond with improvements to aphid performance (Hale *et al.*, 2003). This is attributed to problems in phloem accessibility when turgor pressure is too low (Huberty & Denno, 2004).

Information on the chemical and physiological responses of host Sitka spruce plants and more detailed information on aphid performance in the field, when combined with the population observations (Chapter 3) and performance indicators (Chapter 5) obtained in this study, would prove useful in formulating a better understanding of *E. abietinum* response to drought. If additional information relating to the effects of drought and *E.*

abietinum infestation on Sitka spruce growth was also then incorporated, more informed forest management decisions could be made. Although no growth effects were observed in this thesis (Chapter 4), changes to needle structure or below-ground (*i.e.* root) growth and biomass may result as a consequence of drought stress, and such changes may then filter through as effects on plant growth and aphid behaviour.

Population dynamics of *E. abietinum*, and effects on Sitka spruce, are likely to be different in mature forests when compared to the responses of the potted saplings used for this thesis. Although such research would be valuable in terms of management strategies, it would be logistically complex to accomplish. First and foremost, manipulations of water availability would be practically impossible. Rather, sites under existing drought would have to be located, ideally along a stress gradient, and these would need to be similar enough in other abiotic factors for comparisons to be drawn. If this were not the case, interpreting any obtained results would be complex due to the presence of confounding variables. Non-direct means of aphid monitoring may also be required, as direct means would require access to the forest canopy, which requires scaffolds and other such specialised equipment. This would still be secondary, however, to locating sites under suitable drought stress. Despite such complexities observations made in mature forests would prove useful, as the time spent as small saplings is limited in comparison to the that spent as larger, mature trees.

Some insight is provided by the work in Chapter 6 on potential effects of drought on the interaction between *E. abietinum* and its natural enemies. It is, however, clear that further work will be required before an in-depth understanding can be reached on the effects of drought in this regard. The work in this thesis should be extended to assess the consumption rates (or parasitisation rates, in the case of parasitoids), functional responses, and development rates of natural enemies, including not only coccinellids but also parasitoid wasps, as well as other predatory insects such as hoverflies *inter alia.* Many different families attack *E. abietinum*, and Timms (2004) showed the potential of natural enemies as control agents can be affected by abiotic changes, such as temperature. Development rates and behaviour of the natural enemies may be altered under drought stress, and in order to assess their potential role in limiting populations it is necessary to know how drought will affect them both directly and through effects on their prey items.

The frequency of drought events is expected to increase as a result of climate change (Murphy *et al.*, 2009), and this implies other simultaneous changes to abiotic factors. As such, in order to obtain a clearer understanding of the effect of climate change on *E. abietinum* it will be essential to conduct research on the effects of drought stress in combination with other stressors, particularly increases in temperature and changes to atmospheric gas levels. Although each stressor has been shown to affect aphid performance or populations, few studies have addressed the effects of multiple stressors occurring simultaneously, though more have been conducted on plants. The research herein presented was conducted solely on the effects of limited water availability; aphid performance was observed under controlled laboratory conditions (Chapter 5), while in the field experiment all trees were subjected to the same abiotic conditions (refer to Appendix A). As such, the response of *E. abietinum*, or its interactions with the Sitka spruce host and natural enemies, under altered overall conditions can not be reliably deduced. A broader understanding of the effects of multiple stressors will be essential for future forest management strategies, and for appropriate mitigation of the pest in order to maximise profits within the forest industry.

Overall conclusions

In conclusion, this thesis has demonstrated that *E. abietinum* on Sitka spruce is likely to show a response to drought stress, the nature of which will be mediated by the frequency and intensity of the drought events. The effects have been shown at multiple scales. The response of aphid populations over two drought-affected seasons were assessed, and individual aphid performance was monitored across a single season. Overall, *E. abietinum* were shown to respond positively to moderate intermittent stress, with a detrimental effect of severe stress. The effect of drought stress on coccinellid predator consumption rates reflect these findings, especially when considered in combination with aphid size under drought. Damage is therefore likely to increase under moderate intermittent stress, though potential changes to damage levels under severe stress remain unclear. This thesis has also highlighted the need for further investigation into the effects of drought on arboreal aphids, the effects of multiple abiotic stressors occurring simultaneously, and the consequences of these on natural enemies of the aphid pests. The forestry industry runs on marginal profits and long time-scales. Detailed knowledge and understanding of the response of insect pests will be essential in formulating economically viable management strategies, and in mitigating the potential effects of *E. abietinum* as a pest species on Sitka spruce under changing climate.

Bibliography

Abrams, M. D. (2011) Adaptations of forest ecosystems to air pollution and climate change. *Tree Physiology*, **31**: 258–261.

Adler, L. S., DeValpine, P., Harte, J., & Call, J. (2007) Effects of long-term experimental warming on aphid density in the field. *Journal of the Kansas Entomological Society*, **80**: 156–168.

Albert, K. R., Mikkelsen, T. N., Michelsen, A., & Ro-Poulsen, H. (2011) Interactive effects of drought, elevated CO_2 and warming on photosynthetic capacity and photosystem performance in temperate heath plants. *Journal of Plant Physiology*, **168**: 1550–1561.

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, K., Rigling, A., Breshears, D. D., Hogg, E. H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S. W., Semerci, A., & Cobb, N. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, **259**: 660–684.

Amano, T., Smithers, R. J., Sparks, T. H., & Sutherland, W. J. (2010) A 250-year index of first flowering dates and its response to temperature changes. *Proceedings of the Royal Society B*, **277**: 2451–2457.

Anderegg, W. R. L., Plavcová, L., Anderegg, L. D. L., Hacke, U. G., Berry, J. A., & Field, C. B. (2013) Drought's legacy: multiyear hydraulic deterioration underlies widespread aspen forest die-off and portends increased future risk. *Global Change Biology*, **19**: 1188–1196.

Aqueel, M. A. & Leather, S. R. (2012) Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids. *Annals of Applied Biology*, **160**: 6–15.

Arend, M., Kuster, T., Günthardt-Goerg, M. S., & Dobbertin, M. (2011) Provenance-specific growth responses to drought and air warming in three European oak species (*Quercus robur*, *Q. petraea* and *Q. pubescens*). *Tree Physiology*, **31**: 287–297.

Aslam, T. J., Johnson, S. N., & Karley, A. J. (2013) Plant-mediated effects of drought on aphid population structure and parasitoid attack. *Journal of Applied Entomology*, **137**: 136–145.

Austarå, O., Carter, C., Eilenburg, J., Halldórsson, G., & Harding, S. (1998) A conspectus of potential natural enemies found in association with the green spruce aphid in north-west European spruce plantations. In: Day, K. R., Halldórsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 53–60.

Awmack, C. S. & Leather, S. R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**: 817–844.

Awmack, C. S. & Leather, S. R. (2007) Growth and Development. In: van Emden, H. F. & Harrington, R. (eds.). *Aphids as Crop Pests.* CAB International, Wallingford, UK. pp. 135–152.

Awmack, C. S., Woodcock, C. M., & Harrington, R. (1997) Climate change may increase vulnerability of aphids to natural enemies. *Ecological Entomology*, **22**: 366–368.

Awmack, C. S., Harrington, R., & Lindroth, R. L. (2004) Aphid individual performance may not predict population responses to elevated CO_2 or O_3. *Global Change Biology*, **10**: 1414–1423.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D.,

& Whittaker, J. B. (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8**: 1–16.

Banfield-Zanin, J. A., Rossiter, J. T., Wright, D. J., Leather, S. R., & Staley, J. T. (2012) Predator mortality depends on whether its prey feeds on organic or conventionally fertilised plants. *Biological Control*, **63**: 56–61.

Bansal, S., Hallsby, G., Löfvenius, M. O., & Nilsson, M.-C. (2013) Synergistic, additive and antagonistic impacts of drought on herbivory on *Pinus sylvestris*: leaf, tissue and whole-plant responses and recovery. *Tree Physiology*, **33**: 451–463.

Barigah, T. S., Bonhomme, M., Lopez, D., Traore, A., Douris, M., Venisse, J.-S., Chochard, H., & Badel, E. (2013) Modulation of bud survival in *Populus nigra* sprouts in response to water stress-induced embolism. *Tree Physiology*, **33**: 261–274.

Barnes, J. D., Eamus, D., Davison, A. W., Ro-Poulsen, H., & Mortensen, L. (1990) Persistent effects of ozone on needle water loss and wettability in Norway spruce. *Environmental Pollution*, **63**: 345–363.

Bates, D., Maechler, M., & Bolker, B. (2012) *lme4: Linear mixed-effects models using S4 classes*. R package version 0.999999-0.

Bejer-Peterson, B. (1962) Peak years and regulation of numbers in the aphid *Neomyzaphis abietina* Walker. *Oikos*, **13**: 155–168.

Bertin, S., Perks, M. P., Straw, N., Bertin, J.-M., & Mencuccini, M. (2010) Green spruce aphid infestations cause larger growth reductions to Sitka spruce under shade. *Tree Physiology*, **30**: 1403–1414.

Björkman, C. (1998) Opposite, linear and non-linear effects of plant stress on a galling aphid. *Scandinavian Journal of Forest Research*, **13**: 177–183.

Björkman, C. (2000) Interactive effects of host resistance and drought stress on the performance of a gall-making aphid living on Norway spruce. *Oecologia*, **123**: 223–231.

Björkman, C. & Larsson, S. (1999) Insects on drought-stressed trees: four feeding guilds in one experiment. In: INRA (ed.). *Physiology and Genetics of Tree-Phytophage Interactions*. Les Colloques, n.90. INRA, Paris, France. pp. 323–335.

Blackman, R. L. (1967) The effects of different aphid foods on *Adalia bipunctata* L. and *Coccinella 7-punctata* L. *Annals of Applied Biology*, **59**: 207–219.

Blackman, R. L. & Eastop, V. F. (1994) *Aphids on the world's trees: An identification and information guide.* CAB International, Wallingford, UK.

Branco, M., Pereira, J. S., Mateus, E., Tavares, C., & Paiva, M. R. (2010) Water stress affects *Tomicus destruens* host pine preference and performance during the shoot feeding phase. *Annals of Forest Science*, **67**: 608.

Broadmeadow, M. S. J., Webber, J. F., Ray, D., & Berry, P. M. (2009a) An assessment of likely future impacts of climate change on UK forests. In: Read, D. J., Freer-Smith, P. H., Morison, J. I. L., Hanley, N., West, C. C., & Snowdon, P. (eds.). *Combating climate change - A role for UK forests. An assessment of the potential of the UK's trees and woodlands to mitigate and adapt to climate change.* The Stationery Office, Edinburgh, UK. pp. 67–98.

Broadmeadow, M. S. J., Morecroft, M. D., & Morison, J. I. L. (2009b) Observed impacts of climate change on UK forests to date. In: Read, D. J., Freer-Smith, P. H., Morison, J. I. L., Hanley, N., West, C. C., & Snowdon, P. (eds.). *Combating climate change - A role for UK forests. An assessment of the potential of the UK's trees and woodlands to mitigate and adapt to climate change.* The Stationery Office, Edinburgh, UK. pp. 50–66.

Bumroongsook, S. & Harris, M. K. (1991) Nature of the conditioning effect on pecan by blackmargined aphid. *Southwestern Entomologist*, **16**: 267–275.

Caballero-López, B., Blanco-Moreno, J. M., Pérez-Hidalgo, N., Michelena-Saval, J. M., Pujade-Villar, J., Guerrieri, E., Sánchez-Espigares, J. A., & Sans, F. X. (2012) Weeds, aphids, and specialist parasitoids and predators benefit differently from organic and conventional cropping of winter cereals. *Journal of Pest Science*, **85**: 81–88.

Carter, C. I. (1977) Impact of the green spruce aphid on growth: can a tree forget its past? *Forestry Commission Research and Development Paper 116*, **Forestry Commission**: Edinburgh.

Carter, C. I. (1989) The 1989 outbreak of the green spruce aphid, *Elatobium abietinum. Forestry Commission Research Information Note 161*, **Forestry Commission**: Edinburgh.

Carter, C. I. & Halldórsson, G. (1998) Origins and background to the green spruce aphid in Europe. In: Day, K. R., Halldórsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 1–10.

Carter, C. I. & Nichols, J. F. A. (1988) The green spruce aphid and Sitka spruce provenances in Britain. *Forestry Commission Occasional Paper 19*, **Forestry Commission**: Edinburgh.

Cates, R. G., Redak, R. A., & Henderson, C. B. (1982) Patterns in defensive natural product chemistry. Douglas Fir and Western Spruce Budworm interactions. In: Hedin, P. A. (ed.). *Plant Resistance To Insects.* ACS Symposium Series 208. American Chemical Society, Washington DC, USA. pp. 1–19.

Centritto, M., Brilli, F., Fodale, R., & Loreto, F. (2011) Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (*Populus nigra*) saplings. *Tree Physiology*, **31**: 275–286.

Chandler, J. W. & Dale, J. E. (1990) Needle growth in Sitka spruce (*Picea sitchensis*): effects of nutrient deficiency and needle position within shoots. *Tree Physiology*, **6**: 41–56.

Chiu, M.-C., Chen, Y.-H., & Kuo, M.-H. (2012) The effect of experimental warming on a low-latitude aphid, *Myzus varians. Entomologia Experimentalis et Applicata*, **142**: 216–222.

Cockfield, S. D. & Potter, D. A. (1986) Interaction of Euonymus scale (Homoptera: Diaspididae) feeding damage and severe water stress on leaf abscission and growth of *Euonymus fortunei. Oecologia*, **71**: 41–46.

Cook, J. L., Houseweart, M. W., Kulman, H. M., & Thompson, L. C. (1978) Foliar mineral differences related to sawfly defoliation of white spruce. *Environmental Entomology*, **7**: 780–781.

Crawley, M. J. (2007) *The R Book*. 1st edition. John Wiley & Sons Ltd., Chichester, UK.

Cregg, B. M. & Zhang, J. W. (2001) Physiology and morphology of *Pinus sylvestris* seedlings from diverse sources under cyclic drought stress. *Forest Ecology and Management*, **154**: 131–139.

Crous, K. Y., Zaragoza-Castells, J., Löw, M., Ellsworth, D. S., Tissue, D. T., Tjoelker, M. G., Barton, C. V. M., Gimeno, T. E., & Atkin, O. K. (2011) Seasonal acclimation of leaf respiration in *Eucalyptus saligna* trees: impacts of elevated atmospheric CO_2 and summer drought. *Global Change Biology*, **17**: 1560–1576.

Crous, K. Y., Zaragoza-Castells, J., Ellsworth, D. S., Duursma, R. A., Lãw, M., Tissue, D. T., & Atkin, O. K. (2012) Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric CO2 and summer drought. *Plant, Cell & Environment*, **35**: 966–981.

Crute, S. & Day, K. R. (1990) Understanding the impact of natural enemies on spruce aphid populations through simulation modelling. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 329–337.

Davis, J. A., Radcliffe, E. B., & Ragsdale, D. W. (2006) Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environmental Entomology*, **35**: 1461–1468.

Day, K. R. & Crute, S. (1990) The abundance of spruce aphid under the influence of an oceanic climate. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 25–33.

Day, K. R. & Kidd, N. A. C. (1998) Green spruce aphid population dynamics: Effects of climate, weather and regulation. In: Day, K. R., Halldorsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 41–52.

Day, K. R. & McClean, S. I. (1991) Influence of the green spruce aphid on defoliation and radial stem growth of Sitka spruce. *Annals of Applied Biology*, **119**: 415–423.

Day, K. R., Halldorsson, G., Harding, S., & Straw, N. A., eds. (1998) *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK.

Day, K. R., Docherty, M., Leather, S. R., & Kidd, N. A. C. (2006) The role of generalist insect predators and pathogens in suppressing green spruce aphid populations through direct mortality and mediation of aphid dropping behaviour. *Biological Control*, **38**: 233–246.

Day, K. R., Ayres, M. P., Harrington, R., & Kidd, N. A. C. (2010) Interannual dynamics of aerial and arboreal green spruce aphid populations. *Population Ecology*, **52**: 317–327.

Dent, D. (1991) *Insect Pest Management.* CAB International, Wallingford, UK.

Ditmarová, L., Kurjak, D., Palmroth, S., Kmeť, J., & Střelcová, K. (2009) Physiological responses of Norway spruce (*Picea abies*) seedlings to drought stress. *Tree Physiology*, **30**: 205–213.

Dixon, A. F. G. (1970) Factors limiting the effectiveness of the coccinellid beetle, *Adalia bipunctata* (L.), as a predator of the sycamore aphid, *Drepanosiphum platanoides* (Schr.) *Journal of Animal Ecology*, **39**: 739–751.

Dixon, A. F. G. (1973) *Biology of Aphids.* Studies in Biology 44. Edward Arnold Ltd., London, UK.

Dixon, A. F. G. (1975) Effect of population density and food quality on autumnal reproductive activity in the sycamore aphid, *Drepanosiphum platanoides* (Schr.) *Journal of Animal Ecology*, **44**: 297–304.

Dixon, A. F. G. (1976) Reproductive strategies of the alate morphs of the bird-cherry aphid, *Rhopalosiphum padi* (L.) *Journal of Animal Ecology*, **45**: 817–830.

Dixon, A. F. G. (1985) *Aphid Ecology.* Blackie & Son Ltd., Glasgow, UK.

Dixon, A. F. G. (1987) Parthenogenetic reproduction and the rate of increase in aphids. In: Minks, A. K. & Harrewijn, P. (eds.). Vol. 2A. *Aphids: Their Biology, Natural Enemies, and Control.* Elsevier, Amsterdam, Netherlands. pp. 269–287.

Dixon, A. F. G. (1990) Population dynamics and abundance of deciduous tree-dwelling aphids. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 11–23.

Dixon, A. F. G. & Barlow, N. D. (1979) Population regulation in the lime aphid. *Zoological Journal of the Linnaen Society*, **67**: 225–237.

Dixon, A. F. G. & Kindlmann, P. (1998) Population dynamics of aphids. In: Dempster, J. P. & McLean, I. F. G. (eds.). *Insect Populations in Theory and Practice.* Kluwer Academic Publishers, Dordrecht, Netherlands. pp. 207–230.

Dixon, A. F. G., Chambers, R. J., & Dharma, T. R. (1982) Factors affecting size in aphids with particular reference to the black bean aphid, *Aphis fabae. Entomologia Experimentalis et Applicata*, **32**: 123–128.

Dixon, A. F. G., Kundu, R., & Kindlmann, P. (1993) Reproductive effort and maternal age in iteroparous insects using aphids as a model group. *Functional Ecology*, **7**: 267–272.

Docherty, M., Salt, D. T., & Holopainen, J. K. (1997) The impacts of climate change and pollution on forest pests. In: Watt, A. D., Stork, N., & Hunter, M. D. (eds.). *Forests and Insects.* Chapman & Hall, London, UK. pp. 229–247.

Douglas, A. E. (1993) The nutritional quality of phloem sap utilized by natural aphid populations. *Ecological Entomology*, **18**: 31–38.

Dumbleton, L. G. (1932) Report on spruce-aphid investigation for the year ending December 1930. *New Zealand Journal of Science and Technology*, **13**: 207–220.

Eamus, D., Barnes, J. D., Mortensen, L., Ro-Poulsen, H., & Davison, A. W. (1990) Persistent stimulation of CO_2 assimilation and stomatal conductance by summer fumigation in Norway spruce. *Environmental Pollution*, **63**: 365–379.

Eilmann, B. & Rigling, A. (2012) Tree-growth analyses to estimate tree species' drought tolerance. *Tree Physiology*, **32**: 178–187.

English-Loeb, G., Stout, M. J., & Duffrey, S. S. (1997) Drought stress in tomatoes: changes in plant chemistry and potential non-linear consequences for insect herbivores. *Oikos*, **79**: 456–468.

Evans, E. W., Carlile, N. R., Innes, M. B., & Pitigala, N. (2013) Warm springs reduce parasitism of the cereal leaf beetle through phenological mismatch. *Journal of Applied Entomology*, **137**: 383–391.

Evans, H., Straw, N., & Watt, A. (2002) Climate Change: Implications for Insect Pests. In: Broadmeadow, M. (ed.). *Climate Change: Impacts on UK Forests* Bulletin 125. Forestry Commission, Edinburgh, UK. pp. 99–118.

Everson, P. (1980) The relative activity and functional response of *Phytoseiulus persimilis* (Acarina: Phytoseiidae) and *Tetranychus urticae* (Acarina: Tetranychidae): the effect of temperature. *Canadian Entomologist*, **112**: 17–24.

Eyles, A., Robinson, A. P., Smith, D., Carnegie, A., Smith, I., Stone, C., & Mohammed, C. (2011) Quantifying stem growth loss at the tree-level in a *Pinus radiata* plantation to repeated attack by the aphid, *Essigella californica. Forest Ecology and Management*, **261**: 120–127.

Fawcett, R. G. & Collis-George, N. (1967) A filter-paper method for determining the moisture characteristics of soil. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **7**: 162–167.

Fisher, M. (1987) The effect of previously infested spruce needles on the growth of the green spruce aphid, *Elatobium abietinum*, and the effect of the aphid on the amino acid balance of the host plant. *Annals of Applied Biology*, **111**: 33–41.

Fisher, M. & Dixon, A. F. G. (1986) Role of photoperiod in the timing of dispersal in the green spruce aphid *Elatobium abietinum. Journal of Animal Ecology*, **55**: 657–667.

Fisher, R. A. (1921) Some remarks on the methods formulated in a recent article on "The quantitative analysis of plant growth." *Annals of Applied Biology*, **7**: 367–372.

Fox, J. & Weisberg, S. (2011) *An R Companion to Applied Regression.* 2nd edition. Sage. Thousand Oaks, CA.

Galiano, L., Martínez-Vilalta, J., Sabaté, S., & Lloret, F. (2012) Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. *Tree Physiology*, **32**: 478–489.

Galvez, D. A., Landhäusser, S. M., & Tyree, M. T. (2011) Root carbon reserve dynamics in aspen seedlings: does simulated drought induce reserve limitation? *Tree Physiology*, **31**: 250–257.

Gardiner, B., Leban, J.-M., Auty, D., & Simpson, H. (2011) Models for predicting wood density of British-grown Sitka spruce. *Forestry*, **84**: 119–132.

Garratt, M. P. D., Wright, D. J., & Leather, S. R. (2010) The effects of organic and conventional fertilizers on cereal aphids and their natural enemies. *Agricultural and Forest Entomology*, **12**: 307–318.

Gate, I. M., McNeill, S., & Ashmore, M. R. (1995) Effects of air pollution on the searching behaviour of an insect parasitoid. *Water, Air and Soil Pollution*, **85**: 1425–1430.

Gattolin, S., Newbury, J., Bale, J., Barrett, D., & Pritchard, J. (2007) Quantitative analysis of amino acids composition in the sap of individual sieve elements of wheat (*Triticum aestivum*). *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology*, **146**: 277–278.

Gattolin, S., Newbury, H. J., Bale, J. S., Tseng, H., & Barrett, D. A. (2008) A diurnal component to the variation in sieve tube amino acid content in wheat. *Plant Physiology*, **147**: 912–921.

Ge, Z. M., Kellomäki, S., Peltola, H., Zhou, X., Wang, K. Y., & Väisänen (2011) Impacts of changing climate on the productivity of Norway spruce dominant stands with a mixture of Scots pine and birch in relation to water availability in southern and northern Finland. *Tree Physiology*, **31**: 323–338.

Godfray, H. C. J., Hassell, M. P., & Holt, R. D. (1994) The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. *Journal of Animal Ecology*, **63**: 1–10.

Gordh, G. & Headrick, D. H. (2001) *A Dictionary of Entomology.* CAB International, Wallingford, UK.

Green, S. & Ray, D. (2009) Potential impacts of drought and disease on forestry in Scotland. In: *Research Note FCRN004: Forest Research (Forestry Commission).* HMSO.

Green, S., Hendry, S. J., & Redfern, D. B. (2008) Drought damage to pole-stage Sitka spruce and other conifers in north-east Scotland. *Scottish Forestry*, **62**: 10–18.

Grinnan, R., Carter Jr, T. E., & Johnson, M. T. J. (2013) The effects of drought and herbivory on plant-herbivore interactions across 16 soybean genotypes in a field experiment. *Ecological Entomology*, **38**: 290–302.

Gruber, A., Strobl, S., Veit, B., & Oberhuber, W. (2010) Impact of drought on the temporal dynamics of wood formation in *Pinus sylvestris*. *Tree Physiology*, **30**: 490–501.

Gunderson, C. A., Edwards, N. T., Walker, A. V., O'Hara, K. H., Campion, C. M., & Hanson, P. J. (2012) Forest phenology and a warmer climate - growing season extension in relation to climatic provenance. *Global Change Biology*, **18**: 2008–2025.

Guo, X.-Y., Zhang, X.-S., & Huang, Z.-Y. (2010) Drought tolerance in three hybrid poplar clones submitted to different watering regimes. *Journal of Plant Ecology*, **3**: 79–87.

Gutbrodt, B., Mody, K., & Dorn, S. (2011) Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos*, **120**: 1732–1740.

Gutbrodt, B., Dorn, S., & Mody, K. (2012) Drought stress affects constitutive but not induced herbivore resistance in apple plants. *Arthropod-Plant Interactions*, **6**: 171–179.

Hale, B. K., Bale, J. S., Pritchard, J., & Masters, G. J. (2003) Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): A mechanistic analysis. *Ecological Entomology*, **28**: 666–677.

Halldórsson, G., Docherty, M., Oddsdóttir, E. S., & Day, K. R. (2001) The performance of different populations of the green spruce aphid (*Elatobium abietinum* Walker) at different temperatures. *Icelandic Agricultural Sciences*, **14**: 75–84.

Halsall, L., Gilbert, J., Matthews, R., & Fairgrieve, M. (2006) United Kingdom: New forecast of softwood availability. In: *Forestry and British Timber: PF2005*. Forestry Commission.

Harrington, R., Bale, J. S., & Tatchell, G. M. (1995) Aphids in a changing climate. In: Harrington, R. & Stork, N. E. (eds.). *Insects in a changing environment.* Academic Press, London, UK. pp. 125–155.

Hartley, S. E. & Lawton, J. H. (1990) Damage-induced Changes in Birch Foliage: Mechanisms and Effects on Insect Herbivores. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 147–155.

Hassell, M. P., Lawton, J. H., & Beddington, J. R. (1977) Sigmoid functional responses by invertebrate predators and parasitoids. *Journal of Animal Ecology*, **46**: 249–262.

Hazell, S. P., Neve, B. P., Groutides, C., Douglas, A. E., Blackburn, T. M., & Bale, J. S. (2010) Hyperthermic aphids: insights into behaviour and mortality. *Journal of Insect Physiology*, **56**: 123–131.

Heijari, J., Nerg, A. M., Holopainen, J. K., & Kainulainen, P. (2010) Wood borer performance and wood characteristics of drought-stressed Scots pine seedlings. *Entomologia Experimentalis et Applicata*, **137**: 105–110.

Heliövaara, K. & Väisänen, R. (1990) Changes in Population Dynamics of Pine Insects Induced by Air Pollution. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 209–218.

Hemptinne, J.-L., Dixon, A. F. G., & Lognay, G. (1996) Searching behaviour and mate recognition by males of the two-spot ladybird beetle, *Adalia bipunctata. Ecological Entomology*, **21**: 165–170.

Heritage, S. (1996) Protecting plants from damage by the large pine weevil, and black pine beetles. *Forestry Commission Research Information Note 268*, **Forestry Commission**: Edinburgh.

Heritage, S. (1997) Protecting plants from weevil damage by dipping or spraying before planting using aqueous insecticides. *Forestry Commission Research Information Note 270*, **Forestry Commission**: Edinburgh.

Herms, D. A. & Mattson, W. J. (1992) The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*, **67**: 283–335.

Hodek, I. (1973) *Biology of Coccinellidae.* Academia, Prague, Czech Republic.

Hopmans, P., Collett, N. C., Smith, I. W., & Elms, S. R. (2008) Growth and nutrition of *Pinus radiata* in response to fertilizer applied after thinning and interaction with defoliation associated with *Essigella californica. Forest Ecology and Management*, **255**: 2118–2128.

Hsiao, T. C. (1973) Plant responses to water stress. *Annual Review of Plant Physiology*, **24**: 519–570.

Hsiao, T. C., Acevedo, E., Fereres, E., & Henderson, D. W. (1976) Stress metabolism - water stress, growth, and osmotic adjustment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **273**: 479–500.

Hu, B., Simon, J., & Rennenberg, H. (2013) Drought and air warming affect the species-specific levels of stress-related foliar metabolites of three oak species on acidic and calcareous soil. *Tree Physiology*, **33**: 489–504.

Huberty, A. F. & Denno, R. F. (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, **85**: 1383–1398.

Hunt, E. D., Hubbard, K. G., Wilhite, D. A., Arkebauer, T. J., & Dutcher, A. L. (2009) The development and evaluation of a soil moisture index. *International Journal of Climatology*, **29**: 747–759.

Hussey, N. W. (1952) A contribution to the bionomics of the green spruce aphid (*Neomyzaphis abietina* Walker). *Scottish Forestry*, **6**: 121–130.

Ibáñez, H., Ballester, A., Muñoz, R., & Quiles, M. J. (2010) Chlororespiration and tolerance to drought, heat and high illumination. *Journal of Plant Physiology*, **167**: 732–738.

Inouye, M. & Yamaguchi, H. (1955) Aphid problems of the Todo-fir plantations in Hokkaido. 1. The relationship of ants and other organisms to certain aphid pests on Todo-fir plantations at Hononai Iburi Province in Hokkaido. *Government Forest Experiment Station, Hokkaido Branch Special Report*, **3**: 28–41.

Isebrands, J. G., McDonald, E. P., Kruger, E., Hendrey, G., Percy, K., Pregitzer, K., Sober, J., & Karnosky, D. F. (2001) Growth responses of *Populus tremuloides* clones to interacting elevated carbon dioxide and tropospheric ozone. *Environmental Pollution*, **115**: 359–371.

Jackson, D. & Dixon, A. F. G. (1996) Factors determining the distribution of the green spruce aphid, *Elatobium abietinum*, on young and mature needles of spruce. *Ecological Entomology*, **21**: 358–364.

Jackson, G. E., Irvine, J., & Grace, J. (1995) Xylem cavitation in Scots pine and Sitka spruce saplings during water stress. *Tree Physiology*, **15**: 783–790.

Jactel, H., Petit, J., Desprez-Loustau, M.-L., Delzon, S., Piou, D., Battisti, A., & Koricheva, J. (2012) Drought effects on damage by forest insects and pathogens: a meta-analysis. *Global Change Biology*, **18**: 267–276.

Jarvis, N. J. & Mullins, C. E. (1987) Modelling the effects of drought on the growth of Sitka spruce in Scotland. *Forestry*, **60**: 13–30.

Jarvis, P. G. (1994) Capture of carbon dioxide by coniferous forests. In: Monteith, J. L., Scott, R. K., & Unsworth, M. H. (eds.). *Resource capture by crops.* Nottingham University Press, Loughborough, UK. pp. 351–374.

Jarvis, P. G., Clement, R. J., Grace, J., & Smith, K. A. (2009) The role of forests in the capture and exchange of energy and greenhouse gases. In: Read, D. J., Freer-Smith, P. H., Morison, J. I. L., Hanley, N., West, C. C., & Snowdon, P. (eds.). *Combating climate change - A role for UK forests. An assessment of the potential of the UK's trees and woodlands to mitigate and adapt to climate change.* The Stationery Office, Edinburgh, UK. pp. 21–47.

Jeffs, C. T. & Lewis, O. T. (2013) Effects of climate-warming on host-parasitoid interactions. *Ecological Entomology*, **38**: 209–218.

Johnson, N. E. (1965) Reduced growth associated with infestations of Douglas fir seedlings by *Cinara* species (Homoptera: Aphidae). *Canadian Entomologist*, **97**: 113–119.

Jones, C. G. & Coleman, J. S. (1991) Plant stress and insect herbivory: toward an integrated perspective. In: Mooney, H. A., Winner, W. E., & Pell, E. J. (eds.). *Repsonse of Plants to Multiple Stresses*. Academic Press, San Diego, USA. pp. 249–280.

Kalushkov, P. (1999) The effect of prey quality on searching behaviour of *Adalia bipunctata* and its susceptibility to insecticides. *Entomologia Experimentalis et Applicata*, **92**: 277–282.

Kamata, N. & Tanabe, H. (1999) Performance of beech caterpillar (*Syntypistis punctatella* (Motschulsky)) on water-stressed beech. In: INRA (ed.). *Physiology and Genetics of Tree-Phytophage Interactions*. Les Colloques, n.90. INRA, Paris, France. pp. 313–322.

Karnosky, D. F. (2003) Impacts of elevated CO_2 on forest trees and forest ecosystems: knowledge gaps. *Environment International*, **29**: 161–169.

Karnosky, D. F., Skelly, J. M., Percy, K. E., & Chappelka, A. H. (2007) Perspectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests. *Environmental Pollution*, **147**: 489–506.

Katjiua, M. L. J. & Ward, D. (2006) Resistance and tolerance of *Terminalia sericea* trees to simulated herbivore damage under different soil nutrient and moisture conditions. *Journal of Chemical Ecology*, **32**: 1431–1443.

Kennedy, J. S., Lamb, K. P., & Booth, C. O. (1958) Responses of *Aphis fabae* Scop. to water shortage in host plants in pots. *Entomologia Experimentalis et Applicata*, **1**: 274–291.

Khan, M. A. M., Ulrichs, C., & Mewis, I. (2010) Influence of water stress on the glucosinolate profile of *Brassica oleracea* var. *italica* and the performance of *Brevicoryne brassicae* and *Myzus persicae*. *Entomologia Experimentalis et Applicata*, **137**: 229–236.

Khan, M. A. M., Ulrichs, C., & Mewis, I. (2011) Water stress alters aphid-induced glucosinolate response in *Brassica oleracea* var. *italica* differently. *Chemoecology*, **21**: 235–242.

Kidd, N. A. C. (1991) The implications of air pollution for conifer aphid population dynamics: a simulation analysis. *Journal of Applied Entomology*, **111**: 166–171.

Kidd, N. A. C., Smith, S. D. J., & Lewis, G. B. (1990) Interactions between Host-plant Chemistry and the Population Dynamics of Conifer Aphids. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 183–193.

Kimmins, J. P. (1996) *Forest Ecology: A foundation for sustainable management.* Prentice Hall Inc., New Jersey, USA.

Kirschbaum, M. U. F., Fischlin, A., Cannel, M. G. R., Galinski, W., & Cramer, W. P. (1996) Climate change and impacts on forests. In: Watson, R. T., Zinyowera, M. C., Moss, R. H., & Dokken, D. J. (eds.). *Climate change 1995. Impacts, adaptations and mitigation of climate change: Scientific technical analyses. Contribution of working group II to the second assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge, UK.

Koricheva, J. (2002) Meta-analysis of sources of variation in fitness costs of plant anti-herbivore defences. *Ecology*, **83**: 176–190.

Koricheva, J., Larsson, S., & Haukioja, E. (1998) Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual Review of Entomology*, **43**: 195–216.

Korner, C. (2003) Carbon limitation in trees. *Journal of Ecology*, **91**: 4–17.

Kreuzwieser, J. & Gessler, A. (2010) Global climate change and tree nutrition: influence of water availability. *Tree Physiology*, **30**: 1221–1234.

Kuo, M.-H., Chiu, M.-C., & Perng, J.-J. (2006b) Temperature effects on life history traits of the corn leaf aphid, *Rhopalosiphum maidis* (Homoptera: Aphididae) on corn in Taiwan. *Applied Entomology and Zoology*, **41**: 171–177.

Kuo, M.-H., Lu, W.-N., Chiu, M.-C., Kuo, Y.-H., & Hwang, S.-H. (2006a) Temperature-dependent development and population growth of *Tetraneura nigriabdominalis* (Homoptera: Pemphigidae) on three host plants. *Journal of Economic Entomology*, **99**: 1209–1213.

Larsson, S. (1989) Stressful times for the plant stress-insect performance hypothesis. *Oikos*, **56**: 277–283.

Larsson, S. & Björkman, C. (1993) Performance of chewing and phloem-feeding insects on stressed trees. *Scandinavian Journal of Forest Research*, **8**: 550–559.

Leather, S. R. (1990) The Role of Host Quality, Natural Enemies, Competition and Weather in the Regulation of Autumn and Winter Populations of the Bird Cherry Aphid. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 35–44.

Leather, S. R. (1993) Early season defoliation of bird cherry influences autumn colonisation by the bird cherry aphid, *Rhopalosiphum padi*. *Oikos*, **66**: 43–47.

Leather, S. R. & Dixon, A. F. G. (1984) Aphid growth and reproductive rates. *Entomologia Experimentalis et Applicata*, **35**: 137–140.

Leather, S. R. & Kidd, N. A. C. (1998) The quantitative impact of natural enemies and the prospect for biological control. In: Day, K. R., Halldorsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 61–70.

Leather, S. R. & Owuor, A. (1996) The influence of natural enemies and migration on spring populations of the green spruce aphid, *Elatobium abietinum* Walker (Hom., Aphididae). *Journal of Applied Entomology*, **120**: 529–536.

Liao, H.-T. & Harris, M. K. (1985) Population growth of the blackmargined aphid on pecan in the field. *Agriculture, Ecosystems and Environment*, **12**: 253–261.

Liu, C.-C., Liu, Y.-G., Guo, K., Zheng, Y.-R., Li, G.-Q., Yu, L.-F., & Yang, R. (2010) Influence of drought intensity on the response of six woody karst species subjected to successive cycles of drought and rewatering. *Physiologia Plantarum*, **139**: 39–54.

Lukac, M., Calfapietra, C., Lagomarsino, A., & Loreto, F. (2010) Global climate change and tree nutrition: effects of elevated CO_2 and temperature. *Tree Physiology*, **30**: 1209–1220.

Major, E. J. (1990) Water Stress in Sitka Spruce and its Effect on the Green Spruce Aphid *Elatobium abietinum.* In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects.* Intercept Ltd., Andover, UK. pp. 85–93.

Marchin, R., Zeng, H., & Hoffmann, W. (2010) Drought-deciduous behavior reduces nutrient losses from temperate deciduous trees under severe drought. *Oecologia*, **163**: 845–854.

Maschinski, J. & Whitham, T. G. (1989) The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *American Naturalist*, **134**: 1–19.

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., & Yepez, E. A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist*, **178**: 719–739.

McKay, H. M. & White, I. M. S. (1996) Fine root electrolyte leakage and moisture content: indices of Sitka spruce and Douglas-fir seedling performance after desiccation. *New Forests*, **13**: 139–162.

McLane, S. C., Daniels, L. D., & Aitken, S. N. (2011) Climate impacts on lodgepole pine (*Pinus contorta*) radial growth in a provenance experiment. *Forest Ecology and Management*, **262**: 115–123.

McLean, A. H., Ferrari, J., & Godfray, H. C. J. (2009) Effects of the maternal and pre-adult host plant on adult performance in the pea aphid, *Acyrthosiphon pisum. Ecological Entomology*, **34**: 330–338.

McNeill, S. & Whittaker, J. B. (1990) Air Pollution and Tree-Dwelling Aphids. In: Watt, A. D., Leather, S. R., Hunter, M. D., & Kidd, N. A. C. (eds.). *Population Dynamics of Forest Insects*. Intercept Ltd., Andover, UK. pp. 195–208.

McVean, R. I. K. & Dixon, A. F. G. (2001) The effect of plant drought-stress on populations of the pea aphid *Acyrthosiphon pisum. Ecological Entomology*, **26**: 440–443.

Meehl, G. & Tebaldi, C. (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, **305**: 994–997.

Mishra, A. K. & Singh, V. P. (2010) A review of drought concepts. *Journal of Hydrology*, **391**: 202–216.

Misson, L., Degueldre, D., Collin, C., Rodriguez, R., Rocheteau, A., Ourcival, J.-M., & Rambal, S. (2011) Phenological responses to extreme droughts in a Mediterranean forest. *Global Change Biology*, **17**: 1036–1048.

Mitchell, P. J., Battaglia, M., & Pinkard, E. A. (2013) Counting the costs of multiple stressors: is the whole greater than the sum of the parts? *Tree Physiology*, **33**: 447–450.

Mody, K., Eichenberger, D., & Dorn, S. (2009) Stress magnitude matters: different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology*, **34**: 133–143.

Mondor, E. B., Awmack, C. S., & Lindroth, R. L. (2010) Individual growth rates do not predict aphid population densities under altered atmospheric conditions. *Agricultural and Forest Entomology*, **12**: 293–299.

Moore, J. (2011) Wood properties and uses of Sitka spruce in Britain. In: *Forestry Commission Research Report FCRP015*. Forestry Commission, Edinburgh, UK.

Moser, G., Leuschner, C., Hertal, D., Hölscher, D., Köhler, M., Leitner, D., Michalzik, B., Prihastanti, E., Tjitrosemito, S., & Schwendenmann, L. (2010) Response of cocoa trees (*Theobroma cacao*) to a 13-month desiccation period in Sulawesi, Indonesia. *Agroforestry Systems*, **79**: 171–187.

Murphy, J. M., Sexton, D. M. H., Jenkins, G. J., Boorman, P. M., Booth, B. B. B., Brown, C. C., Clark, R. T., Collins, M., Harris, G. R., Kendon, E. J., Betts, R. A., Brown, S. J., Howard, T. P., Humphrey, K. A., McCarthy, M. P., McDonald, R. E., Stephens, A., Wallace, C., Warren, R., Wilby, R., & Wood, R. A. (2009) *UK Climate Projections Science Report: UKCP09*. Met Office, Hadley Centre, Exeter, UK.

Musolin, D. L., Tougou, D., & Fujisaki, K. (2010) Too hot to handle? Phenological and life-history responses to simulated climate change of the southern green stink bug *Nezara viridula* (Heteroptera: Pentatomidae). *Global Change Biology*, **16**: 73–87.

Nakai, A. & Kisanuki, H. (2011) Stress responses of *Salix gracilistyla* and *Salix subfragilis* cuttings to repeated flooding and drought. *Journal of Forest Research*, **16**: 465–472.

Narasimhan, B. & Srinivasan, R. (2005) Development and evaluation of Soil Moisture Deficit Index (SMDI) and Evapotranspiration Deficit Index (ETDI) for agricultural drought monitoring. *Agricultural and Forest Meteorology*, **133**: 69–88.

Neto, J. D. R., Pinho, B. X., Meyer, S. T., Wirth, R., & Leal, I. R. (2012) Drought stress drives intraspecific choice of food plants by *Atta* leaf-cutting ants. *Entomologia Experimentalis et Applicata*, **144**: 209–215.

Nichols, J. F. A. (1987) Damage and performance of the green spruce aphid, *Elatobium abietinum* on twenty spruce species. *Entomologia Experimentalis et Applicata*, **45**: 211–217.

Nielsen, C., Eilenberg, J., Harding, S., Oddsdottir, E., & Halldórsson, G. (2001) Geographical distribution and host range of entomophthorales infecting the green spruce aphid *Elatobium abietinum* Walker in Iceland. *Journal of Invertebrate Pathology*, **78**: 72–80.

Norby, R. J., Warren, J. M., Iversen, C. M., Medlyn, B. E., & McMurtrie, R. E. (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Science USA*, **107**: 19368–19373.

Nzokou, P. & Cregg, B. M. (2010) Morphology and foliar chemistry of containerized *Abies fraseri* (Pursh) Poir. seedlings as affected by water availability and nutrition. *Annals of Forest Science*, **67**: 602.

Ohtaka, C. & Ishikawa, H. (1991) Effects of heat treatment on the symbiotic system of an aphid mycetocyte. *Symbiosis*, **11**: 19–30.

Olesinski, J., Lavigne, M. B., & Krasowski, M. J. (2011) Effects of soil moisture manipulations on fine root dynamics in a mature balsam fir (*Abies balsamea* L. Mill.) forest. *Tree Physiology*, **31**: 339–348.

Osier, T. L. & Lindroth, R. L. (2006) Genotype and environment determine allocation to and costs of resistance in quaking aspen. *Oecologia*, **148**: 293–303.

Paiva, M. R., Mateus, E., Santos, M. H., & Branco, M. R. (2011) Pine volatiles mediate host selection for oviposition by *Thaumetopoea pityocampa* (Lep., Notodontidae). *Journal of Applied Entomology*, **135**: 195–203.

Parry, W. H. (1971) Differences in the probing behaviour of *Elatobium abietinum* feeding on Sitka and Norway spruce. *Annals of Applied Biology*, **69**: 177–185.

Parry, W. H. (1973) Observations on the flight periods of aphids in a Sitka spruce plantation in north eastern Scotland. *Bulletin of Entomological Research*, **62**: 391–399.

Parry, W. H. (1974b) The effects of nitrogen levels in Sitka spruce needles on *Elatobium abietinum* populations in northeastern Scotland. *Oecologia*, **15**: 305–320.

Parry, W. H. (1974a) Damage caused by the green spruce aphid to Norway and Sitka spruce needles. *Annals of Applied Biology*, **77**: 113–120.

Parry, W. H. (1977) The effects of nutrition and density on the production of alate *Elatobium abietinum* on Sitka spruce. *Oecologia*, **30**: 367–375.

Parry, W. H. (1992) A comparison of *Aphidecta obliteratae* (L.) (Col., Coccinellidae) populations feeding on *Elatobium abietinum* (Walker) and on *Adelges cooleyi* (Gillette). *Journal of Applied Entomology*, **114**: 280–288.

Pons, X. & Tatchell, G. M. (1995) Drought stress and cereal aphid performance. *Annals of Applied Biology*, **126**: 19–31.

Port, G. R. & Thompson, J. R. (1980) Outbreaks of insect herbivores along motorways in the United Kingdom. *Journal of Applied Ecology*, **17**: 649–656.

Powell, W. & Parry, W. H. (1976) Effects of temperature on overwintering populations of the green spruce aphid *Elatobium abietinum. Annals of Applied Biology*, **82**: 209–219.

Price, P. W. (1991) Plant vigor hypothesis and herbivore attack. *Oikos*, **62**: 244–251.

Pritchard, J., Griffiths, B., & Hunt, E. J. (2007) Can the plant-mediated impacts on aphids of elevated CO_2 and drought be predicted? *Global Change Biology*, **13**: 1616–1629.

Prittinen, K., Pusenius, J., Koivunoro, K., & Roininen, H. (2003) Genotypic variation in growth and resistance to insect herbivory in silver birch (*Betula pendula*) seedlings. *Oecologia*, **137**: 572–577.

Purandare, S. R. & Tenhumberg, B. (2012) Influence of aphid honeydew on the foraging behaviour of *Hippodamia convergens* larvae. *Ecological Entomology*, **37**: 184–192.

R Development Core Team (2012) *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing. Vienna, Austria.

Radford, P. J. (1967) Growth analysis formulae - their use and abuse. *Crop Science*, **7**: 171–175.

Ramírez, C. C. & Verdugo, J. A. (2009) Water availability affects tolerance and resistance to aphids but not the trade-off between the two. *Ecological Research*, **24**: 881–888.

Randle, T. J. & Ludlow, A. R. (1998) Growth costs to plantations: modelling the cumulative effect of defoliation by *Elatobium abietinum.* In: Day, K. R., Halldorsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 32–40.

Roger, C., Coderre, D., & Boivin, G. (2000) Differential prey utilization by the generalist predator *Coleomegilla maculata lengi* according to prey size and species. *Entomologia Experimentalis et Applicata*, **94**: 3–13.

Rose, D. (2002) *Control of the large pine weevil, Hylobius abietus, L.* PhD thesis. Imperial College London.

Rossiter, M. C. (1991) Environmentally-based maternal effects: a hidden force in insect population dynamics? *Oecologia*, **87**: 288–294.

Rossiter, M. C., Cox-Foster, D. L., & Briggs, M. A. (1993) Initiation of maternal effects in *Lymantria dispar*: Genetic and ecological components of egg provision. *Journal of Evolutionary Biology*, **6**: 577–589.

Rotheray, G. E. (1989) *Aphid Predators.* Naturalists' Handbooks 11. The Richmond Publishing Co. Ltd, Slough, UK.

Ryan, M. G. (2011) Tree responses to drought. *Tree Physiology*, **31**: 237–239.

Sabelis, M. W. (1992) Predatory arthropods. In: Crawley, M. J. (ed.). *Natural Enemies.* Blackwell Publishing Ltd, Oxford, UK. pp. 225–264.

Samuel, C. J. A., Fletcher, A. M., & Lines, R. (2007) *Choice of Sitka Spruce Seed Origins for Use in British Forests.* Forestry Commission, Edinburgh, UK.

Sánchez-Salguero, R., Navaro-Cerrillo, R. M., Camarero, J. J., & Fernández-Cancio, A. (2012) Selective drought-induced decline of pine species in southeastern Spain. *Climatic Change*, **113**: 767–785.

Seaby, D. A. & Mowat, D. J. (1993) Growth changes in 20-year-old Sitka spruce *Picea sitchensis* after attack by the green spruce aphid *Elatobium abietinum. Forestry*, **66**: 371–379.

Siemens, D. H., Lischke, H., Maggiulli, N., Schurch, S., & Roy, B. A. (2003) Cost of resistance and tolerance under competition: the defence-stress benefit hypothesis. *Evolutionary Ecology*, **17**: 247–263.

Sih, A. (1987) Predators and prey lifestyles: An evolutionary and ecological overview. In: Kerfoot, W. C. & Sih, A. (eds.). *Predation: Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, Hanover, USA. pp. 203–224.

Simpson, K. L. S., Jackson, G. E., & Grace, J. (2012) The response of aphids to plant water stress - the case of *Myzus persicae* and *Brassica oleracea* var *capitata. Entomologia Experimentalis et Applicata*, **142**: 191–202.

Skirvin, D. J., Perry, J. N., & Harrington, R. (1997) The effect of climate change on aphid-coccinellid interaction. *Global Change Biology*, **3**: 1–11.

Sluss, R. R. (1967) Population dynamics of the walnut aphid, *Chromaphis juglandicola* (Kalt.) in northern California. *Ecology*, **48**: 41–58.

Smits, A. & Larsson, S. (1999) Effects of previous defoliation on pine looper larval performance. *Agricultural and Forest Entomology*, **1**: 19–26.

Spitzer, B. W. (2004) Maternal effects in the soft scale insect *Saissetia coffeae* (Hemiptera: Coccidae). *Evolution*, **58**: 2452–2461.

Staley, J. T. & Hartley, S. E. (2002) Host-mediated effects of feeding by winter moth on the survival of *Euceraphis betulae. Ecological Entomology*, **27**: 626–630.

Staley, J. T., Mortimer, S. R., Masters, G. J., Morecroft, M. D., Brown, V. K., & Taylor, M. E. (2006) Drought stress differentially affects leaf-mining species. *Ecological Entomology*, **31**: 460–469.

Staley, J. T., Mortimer, S. R., Morecroft, M. D., Brown, V. K., & Masters, G. J. (2007) Summer drought alters plant-mediated competition between foliar- and root-feeding insects. *Global Change Biology*, **13**: 866–877.

Staley, J. T., Girling, R. D., Stewart-Jones A. Poppy, G. M., Leather, S. R., & Wright, D. J. (2011) Organic and conventional fertilizer effects on a tritrophic interaction: parasitism, performance and preference of *Cotesia vestalis*. *Journal of Applied Entomology*, **135**: 658–665.

Stevens, M. T., Waller, D. M., & Lindroth, R. L. (2007) Resistance and tolerance in *Populus tremuloides*: genetic variation, costs and environmental dependency. *Evolutionary Ecology*, **21**: 829–847.

Straw, N., Fielding, N., Green, G., & Price, J. (2006) Seasonal changes in the distribution of green spruce aphid *Elatobium abietinum* (Walker) (Homoptera: Aphididae) in the canopy of Sitka spruce. *Agricultural and Forest Entomology*, **8**: 139–154.

Straw, N. A. (1995) Climate change and the impact of green spruce aphid, *Elatobium abietinum* (Walker), in the UK. *Scottish Forestry*, **49**: 134–145.

Straw, N. A. & Green, G. (2001) Interactions between green spruce aphid (*Elatobium abietinum* (Walker)) and Norway and Sitka spruce under high and low nutrient conditions. *Agricultural and Forest Entomology*, **3**: 263–274.

Straw, N. A., Halldórsson, G., & Benedikz, T. (1998a) Damage sustained by individual trees: Empirical studies on the impact of the green spruce aphid. In: Day, K. R., Halldórsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 15–31.

Straw, N. A., Fielding, N. J., Green, G., & Coggan, A. (1998b) The impact of green spruce aphid, *Elatobium abietinum* (Walker), on the growth of young Sitka spruce in Hafren Forest, Wales: pattern of defoliation and effect on shoot growth. *Forest Ecology and Management*, **104**: 209–225.

Straw, N. A., Fielding, N. J., Green, G., & Price, J. (2000) The impact of green spruce aphid, *Elatobium abietinum* (Walker), on the growth of young Sitka spruce in Hafren Forest, Wales: effects on height, diameter and volume. *Forest Ecology and Management*, **134**: 97–109.

Straw, N. A., Fielding, N. J., Green, G., & Price, J. (2002) The impact of green spruce aphid, *Elatobium abietinum* (Walker), on the growth of young Sitka spruce in Hafren Forest, Wales: delayed effects on needle size limit wood production. *Forest Ecology and Management*, **157**: 267–283.

Straw, N. A., Fielding, N. J., Green, G., & Price, J. (2005) Defoliation and growth loss in young Sitka spruce following repeated attack by the green spruce aphid, *Elatobium abietinum* (Walker). *Forest Ecology and Management*, **213**: 349–368.

Straw, N. A., Timms, J. E. L., & Leather, S. R. (2009) Variation in abundance of invertebrate predators of the green spruce aphid *Elatobium abietinum* (Walker) (Homoptera: Aphididae) along an altitudinal transect. *Forest Ecology and Management*, **258**: 1–10.

Straw, N. A., Williams, D. T., & Green, G. (2011) Influence of sticky trap color and height above ground on capture of alate *Elatobium abietinum* (Hemiptera: Aphididae) in Sitka spruce plantations. *Environmental Entomology*, **40**: 120–125.

Sumner, L. C., Eikenbary, R. D., & Johnson, R. C. (1986) Survival and reproduction of *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae) on winter wheat during simulated drought stress. *Journal of the Kansas Entomological Society*, **59**: 561–563.

Tanaka, S., Denlinger, D. L., & Wolda, H. (1987) Daylength and humidity as environmental cues for diapause termination in a tropical beetle. *Phsyiological Entomology*, **12**: 213–224.

Tapia, D. H., Morales, F., & Grez, A. A. (2010) Olfactory cues mediating pre-searching behaviour in interacting aphidophagous predators: are semiochemicals key factors in predator-facilitation? *Entomologia Experimentalis et Applicata*, **137**: 28–35.

Tariq, M., Wright, D. J., & Staley, J. T. (2010) Maternal host plant effects on aphid performance: contrasts between a generalist and specialist species on Brussels sprout cultivars. *Agricultural and Forest Entomology*, **12**: 107–112.

Tariq, M., Wright, D. J., Rossiter, J. T., & Staley, J. T. (2012) Aphids in a changing world: testing the plant stress, plant vigour and pulsed stress hypotheses. *Agricultural and Forest Entomology*, **14**: 177–185.

Theobald, F. V. (1914) Notes on the green spruce aphid (*Aphis abietina* Walker). *Annals of Applied Biology*, **1**: 22–36.

Thomas, R. C. & Miller, H. G. (1994) The interaction of green spruce aphid and fertilizer application on the growth of Sitka spruce. *Forestry*, **67**: 329–341.

Timms, J. E., Oliver, T. H., Straw, N. A., & Leather, S. R. (2008) The effects of host plant on the coccinellid functional response: is the conifer specialist *Aphidecta obliterata* (L.) (Coleoptera: Coccinellidae) better adapted to spruce than the generalist *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae)? *Biological Control*, **47**: 273–281.

Timms, J. E. L. (2004) *Factors affecting natural control of the green spruce aphid, Elatobium abietinum (Walker).* PhD thesis. Imperial College London.

Timms, J. E. L. & Leather, S. R. (2008) How do the consumption and development rates of the conifer specialist *Aphidecta obliterata* respond to temperature, and is it better adapted to limited prey than a generalist? *Annals of Applied Biology*, **153**: 63–71.

Tomlin, E. S. & Borden, J. H. (1997) Thin bark and high density of outer resin ducts: interrelated resistance traits in Sitka spruce against the white pine weevil (Coleoptera: Curculionidae). *Journal of Economic Entomology*, **90**: 235–239.

Townend, J. (1993) Effects of elevated carbon dioxide and drought on the growth and physiology of clonal Sitka spruce plants (*Picea sitchensis* (Bong.) Carr.) *Tree Physiology*, **13**: 389–399.

Uefune, M., Kugimiya, S., Sano, K., & Takabayashi, J. (2012) Herbivore-induced plant volatiles enhance the ability of parasitic wasps to find hosts on a plant. *Journal of Applied Entomology*, **136**: 133–138.

Valladares, G. R. & Hartley, S. E. (1994) Effects of scale on detecting interactions between *Coleophora* and *Eriocrania* leaf miners. *Ecological Entomology*, **19**: 257–262.

Van Nouhuys, S. & Lei, G. C. (2004) Parasitoid-host metapopulation dynamics: the causes and consequences of phenological asynchrony. *Journal of Animal Ecology*, **73**: 526–535.

Vaz, M., Pereira, J. S., Gazarini, L. C., David, T. S., David, J. S., Rodrigues, A., Maroco, J., & Chaves, M. M. (2010) Drought-induced photosynthetic inhibition and autumn recovery in two Mediterranean oak species(*Quercus ilex* and *Quercus suber*). *Tree Physiology*, **30**: 946–956.

Visser, M. E. & Holleman, L. J. M. (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society B*, **268**: 289–294.

Vucic-Pestic, O., Ehnes, R. B., Rall, B. C., & Brose, U. (2011) Warming up the system: higher predator feeding rates but lower energetic efficiencies. *Global Change Biology*, **17**: 1301–1310.

Wagner, M. R. & Evans, P. D. (1985) Defoliation increases nutritional quality and allelochemicals of pine seedlings. *Oecologia*, **67**: 235–237.

Wainhouse, D., Ashburner, R., & Boswell, R. (2001) Reproductive development and maternal effects in the pine weevil *Hylobius abietis*. *Ecological Entomology*, **26**: 655–661.

Walther, G.-R. (2010) Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**: 2019–2024.

Waring, G. L. & Cobb, N. S. (1992) The impact of plant stress on herbivore population dynamics. In: Bernays, E. A. (ed.). Vol. 4. *Insect-Plant Interactions*. CRC Press, Boca Raton, USA. pp. 167–226.

Warren, J. M., Norby, R. J., & Wullschleger, S. D. (2011) Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology*, **31**: 117–130.

Warrington, S. & Whittaker, J. B. (1990) Interactions between Sitka spruce, the green spruce aphid, sulphur dioxide pollution and drought. *Environmental Pollution*, **65**: 363–370.

Watt, A. D., Lindsay, E., Leith, I. D., Fraser, S. M., Docherty, M., Hurst, D. K., Hartley, S. E., & Kerslake, J. (1996) The effects of climate change on the winter moth, *Operophtera brumata*, and its status as a pest on broadleaved trees, Sitka spruce and heather. *Aspects of Applied Biology*, **45**: 307–316.

Watt, A. D., Flückiger, W., Leith, I. D., & Lindsay, E. (1998) Atmospheric pollution, elevated CO_2 and spruce aphids. In: Day, K. R., Halldorsson, G., Harding, S., & Straw, N. A. (eds.). *The Green Spruce Aphid in Western Europe: Ecology, Status, Impacts and Prospects for Management.* Forestry Commission, Edinburgh, UK. pp. 86–96.

Way, D. A. (2011) The bigger they are, the harder they fall: CO_2 concentration and tree size affect drought tolerance. *Tree Physiology*, **31**: 115–116.

Wearing, C. H. (1972) Responses of *Myzus persicae* and *Brevicoryne brassicae* to leaf age and water stress in Brussels sprouts grown in pots. *Entomologia Experimentalis et Applicata*, **15**: 61–80.

Wellings, P. W., Chambers, R. J., Dixon, A. F. G., & Aikman, D. P. (1985) Sycamore aphid numbers and population density. I. Some patterns. *Journal of Animal Ecology*, **54**: 411–424.

West, C. C. & Morison, J. I. L. (2009) Climate Trends and Projections. In: Read, D. J., Freer-Smith, P. H., Morison, J. I. L., Hanley, N., West, C. C., & Snowdon, P. (eds.). *Combating climate change - A role for UK forests. An assessment of the potential of the UK's trees and woodlands to mitigate and adapt to climate change.* The Stationery Office, Edinburgh, UK. pp. 16–20.

White, T. C. R. (1969) An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology*, **50**: 905–909.

White, T. C. R. (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, **63**: 90–105.

Whittaker, J. B. (2001) Insects and plants in a changing atmosphere. *Journal of Ecology*, **89**: 507–518.

Williams, D. T., Straw, N. A., & Day, K. R. (2005) Performance of the green spruce aphid, *Elatobium abietinum* (Walker) on previously defoliated Sitka spruce. *Agricultural and Forest Entomology*, **7**: 95–105.

Wratten, S. D., Edwards, P. J., & Dunn, I. (1984) Wound-induced changes in the palatability of *Betula pubescens* and *B. pendula*. *Oecologia*, **61**: 372–375.

Wyatt, I. J. & White, P. F. (1977) Simple estimation of intrinsic increase rates for aphids and Tentranychid mites. *Journal of Applied Ecology*, **14**: 757–766.

Yoder, J. A., Christensen, B. S., & Keeney, G. D. (2010) Enhanced tolerance to water stress in adults of the South India strain of the seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae), as a product of large body size. *European Journal of Entomology*, **107**: 271–275.

Zhou, X. L., Harrington, R., Woiwood, I. P., Perry, J. N., Bale, J. S., & Clark, S. J. (1995) Effects of temperature on aphid phenology. *Global Change Biology*, **1**: 303–313.

Appendix A

Differences in microclimate and atmospheric conditions at the field trial site

Background

Aphids have repeatedly been shown to be affected by a wide range of abiotic factors, and these, in part, help drive the population dynamics of a species. Temperature is arguably the most important abiotic factor affecting *Elatobium abietinum* at both the individual and population level (Harrington *et al.*, 1995), and aphids and herbivorous insects in general (Bale *et al.*, 2002).

At the population level, the size of the population peak, and therefore the damage caused, depends on overwinter survival of the aphids, which in turn is governed by winter temperatures (Powell & Parry, 1976; Leather, 1990; Evans *et al.*, 2002). The timing of the population peak is also affected by thermal input (Day & Kidd, 1998). On the individual scale, temperature controls *E. abietinum* development and performance, affecting fecundity, growth rates and mortality (Harrington *et al.*, 1995).

Due to the importance of temperature, it was necessary to determine whether there were any differences in the climactic conditions experienced by the experimental trees on each pallet. Furthermore, due to the failure of several sensors in the first year, and all in the second, it was important to determine whether any differences existed between the microclimactic and atmospheric conditions at the field site. This was necessary to ensure that temperature was not a confounding variable in the field trial.

Aims and Objectives

1. Establish whether a difference in temperature and conditions was experienced by the trees on the different pallets in the field trial of the study.
2. Determine whether a significant difference in the microclimate of the trees and ambient conditions existed during the study.

Materials and Methods

Microclimate

A DS1921 Thermochron iButton temperature sensor (HomeChip, U.K.) was attached to one randomly selected plant per pallet (Figure A.1). These were programmed to take a temperature reading once an hour between the 25th April 2010 and the 3rd October 2010. The readings were then averaged out to obtain a daily mean temperature. Any iButtons which failed to record throughout this period were removed from the analyses.

Ambient conditions

Weather data were obtained from the Silwood Weather Station (Silwood Park, Berkshire, U.K.). Temperature readings were then averaged out to obtain a daily mean temperature for comparison with the iButton 'microclimate' data. All iButton data was also averaged out to obtain a daily mean for comparison.

Statistical analysis

The iButton temperature data for the pallets were analysed using ANOVA. A generalised linear model was used to analyse and compare between the iButton and Silwood Weather Station data.

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Figure A.1: A DS1921 Thermochron iButton temperature sensor used to record temperature on the trees in the field experiment. These were attached to one randomly selected plant per pallet, and were set to take a temperature reading once per hour.

Results

Microclimate

There were no significant differences in the readings between the pallets ($F_{21,3520} = 1.184$, $P > 0.05$), nor was there interaction between pallet and date ($F_{1,3520} = 0.808$, $P > 0.05$).

Ambient conditions

There was no significant difference between the mean daily temperatures recorded by the iButtons and the Silwood Weather Station ($t_{322} = 0.664$, $P > 0.05$).

Discussion and Comments

The results herein presented indicate that there was no difference in microclimate for each pallet, and that there was also no difference between the microclimactic and atmospheric conditions. This reflects the selection of the site's location, which was done in order to avoid any differences in temperature and shading, which has also been shown to affect aphid populations (Bertin *et al.*, 2010). Furthermore, due to the experimental trees' comparatively small size (2 and 3-year-old saplings), it is unlikely that any effects of drought on microclimate could be established.

These findings imply that the conditions experienced by the aphids on the trees were not significantly different from each other across the field site, such that the climactic conditions, and particularly temperature, should not have had a confounding effect on population development.

It is entirely plausible, however, that drought stress may affect climactic conditions in a mature forest. *Elatobium abietinum* has been repeatedly shown to affect Sitka spruce growth and development (Straw *et al.*, 1998a; Straw *et al.*, 1998b; Straw *et al.*, 2000; Straw *et al.*, 2005; Bertin *et al.*, 2010). Changes to height, shoot length and extension, and needle retention under *E. abietinum* infestation would probably affect conditions in the canopy, and, if this was further compounded by drought, differences between drought levels might also have been seen.

Conclusions

1. All pallets in the field trial experienced the same climatic conditions.
2. It was therefore justified, where necessary, to make use of the Silwood Weather Station data in any analyses, as this presented a more complete data set.

Appendix B

Performance of *Elatobium abietinum* in a nursery setting

Background

Limitations to the effectiveness of individual performance parameters in predicting or mirroring what is observed in the field have been discussed in previous studies. Major (1990) noted that, under drought stress, a lack of differences between individual performance did not match final *E. abietinum* populations in the field. Similarly, Mondor *et al.* (2010) observed that changes to CO_2 or O_3 levels, associated with climate change, did not affect the growth and development of individual *Acyrthosiphon pisum* aphids on *Trifolium pratense*, despite observed reductions of population size under enhanced CO_2.

An attempt was made to assess the performance of individual *Elatobium abietinum* aphids on the Sitka spruce during the field trial in 2010. It was hoped that, by doing this, a comparison could be drawn between the observed performances of the aphids in the CT room trials and what was effectively occurring in the field.

Aims and Objectives

1. Assess differences in individual aphid mean relative growth rate (mRGR) in the field trial and CT room trials of the study.

Materials and Methods

Adult aphids obtained from a stock culture (established on cut Sitka spruce branches from Hafren Forest, Wales, and maintained in a CT room at 15 °C, with 70% RH and a 16:8 photoperiod) were caged in gelatin capsules (size 00, Value Healthcare, U.K.) on needles of one aphid-inoculated tree on each pallet in the nursery experiment (see Chapter 3). Two aphids were caged on needles on the leader shoot and two on needles on the side shoot, before being left undisturbed for 24 hours. The capsules were then inspected, and where nymphs had been deposited, all but one were removed along with the adult. If a nymph had not been deposited the adult was returned and left for a further 24 hours. This step was repeated until each capsule contained an experimental nymph.

Each of the experimental nymphs was removed from the plant and weighed on a Sartorius microbalance (Type M3P, last calibrated on the 29th September, 2009), before being carefully returned to the plant of origin to feed undisturbed for seven days. After this time, each capsule was inspected and the survival of the nymph recorded. The mean relative growth rate (mRGR) was calculated for each nymph which had survived after they were removed from the plant and re-weighed, using the formula (Fisher, 1921; Radford, 1967):

$$mRGR\,(\text{mg/mg/day}) = \frac{[\ln(\text{final weight}) - \ln(\text{initial weight})]}{\text{N. of days between weighings}} \tag{I}$$

Unfortunately, the use of capsules outdoors was not successful, as any form of moisture (including morning dew) caused damage and resulted in no viable data points being recorded. Furthermore, condensation inside the capsule was found to be a problem, as this not only caused moisture build-up but also affected the aphid and distorted results.

The above methodology was repeated using 200 μl Eppendorf tubes instead of gelatin capsules. The lids were detached, and a hole punched into them to fit around the needle. While the cages were now no longer damaged by water, condensation was still found to be a problem (albeit less so).

An attempt was made at removing the bottom section of the Eppendorf tubes and covering them in gauze to allow for passage of air, but this rendered the tubes too short for the needles. Further attempts using larger Eppendorf tubes was abandoned, as they were too heavy to be supported by the spruce needles.

Statistical analysis

Survival of the aphid nymphs was analysed using a linear mixed effect model with binomial error, to account for pseudoreplication. Drought was modelled as a fixed effect, while capsule location and tree location (*i.e.* pallet) were modelled as random effects (number of observations = 100, groups: pallet = 25, location = 2; n = 25, estimated d.f. for each parameter = 4). Mortality was extremely high (Table B.1), but some minimal mRGR data were collected and these were analysed using Student's T-tests. Both the mRGR and survival results should, however, be interpreted with extreme caution and are presented here merely as indications.

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

There was no significant effect of drought treatment on the survival rates of the *E. abietinum* nymphs over seven days ($\chi^2{}_4 = 1.340$, $P > 0.05$). Furthermore, there was no effect of either MS, CS or IS1 drought treatment on the mRGR of the nymphs when compared to the FC control (MS: $t_{3.76} = 1.814$, $P > 0.05$; CS: $t_{2.92} = 0.047$, $P > 0.05$; IS1: $t_{1.81} = 0.355$, $P > 0.05$). A T-test could not be conducted with the IS2 data, because only a single data point was obtained for that drought treatment.

Discussion and Comments

The results observed in this experiment appear to match those presented in Appendix C - namely, there was no effect of drought stress on the survival or mean relative growth

Table B.1:

Mean RGR of *Elatobium abietinum* nymphs in a nursery trial and summary of T-tests under five different drought treatments.

Drought level	**N**	**mRGR** $\bar{x} \pm$ **SE**
FC	3	0.123 ± 0.036
MS	3	0.207 ± 0.028
CS	2	0.125 ± 0.021
IS1	2	0.178 ± 0.058
IS2	1	0.195 ± NA

rate (mRGR) of *E. abietinum* nymphs on Sitka spruce. They do not, however, match observed performance measures shown in Chapter 5, where a more complex relationship between aphid performance and drought stress was recorded. This reflects the findings of Major (1990) and Mondor *et al.* (2010), who both suggested that individual aphid performance may not necessarily reflect what happens in the field.

Incongruence between observations under controlled laboratory conditions and those in the field have been found in several studies. Staley & Hartley (2002) found that, though survival rates of *Euceraphis betulae* aphids feeding on damaged or undamaged *Betula pendula* showed similar responses in a field and greenhouse trial, the feeding preference of the same aphids was significantly different between field and laboratory conditions. Similarly, in feeding preference comparisons conducted on birch-feeding caterpillars in laboratory and field conditions, Hartley & Lawton (1990) suggested a general trend, but also observed that results varied depending both on the way the experiment was conducted as well as species used.

It has also been proposed that interactions, palatability and feeding preference may be more easily demonstrated in the smaller scales typical of controlled laboratory conditions, as opposed to those at the larger field scale (Wratten *et al.*, 1984; Valladares & Hartley, 1994).

While feeding preference may not be immediately relevant to the study herein presented, a similar effect may be applicable to individual performance measures under different experimental conditions, including drought stress. Furthermore, additional factors often apply under field conditions that are not applicable in a laboratory setting, such as differences in microclimate (though these were not apparent in the field trial, see Appendix A), presence of natural enemies, and variations in host plant quality induced by previous herbivory. Any of a variety of biotic and abiotic factors could cause disturbances to experimental aphids in the field, which are reduced under the controlled environment of a CT room. All these factors combined could explain why the population size of a herbivore in the field may not reflect growth rates of individuals under laboratory conditions.

Appendix C

Performance on *E. abietinum* under laboratory conditions in a 'pilot' study

Background

Assessing the performance of individual aphids under controlled conditions can provide valuable indications of population development potential, often on a finer scale than might be achievable under field conditions. An extensive array of measures of performance, some more accurate as predictors than others, have been widely used in studies on both agricultural and arboreal aphids species (e.g. Kennedy *et al.* (1958); Fisher (1987); Warrington & Whittaker (1990); McVean & Dixon (2001); Williams *et al.* (2005); Mody *et al.* (2009)).

The following study was conducted in 2010, and was a precursor to the more detailed series of trials run in 2011. It could not be included in the analysis of that data, as it was incomparable.

Aims and Objectives

1. Investigate the effect of different levels of drought stress on the fitness and performance of *Elatobium abietinum* on Sitka spruce.

Materials and Methods

This experiment was run in 2010, and the same methodology described in Chapter 5 was used. Only one trial was run.

Ten Sitka spruce plants from each of the five drought treatments were maintained outdoors without aphids. These plants were moved to a CT room at 15 °C, with 70% RH and a 16:8 photoperiod on May 26th 2010, under continued drought treatment, to acclimatise to the conditions, and were intermixed in a fully randomised design. On June 3rd 2010 eight adult *E. abietinum* aphids from the stock culture were caged in gelatin capsules (size 00, Value Healthcare, U.K.) on each of the plants, and the performance recorded in the same way as described in Chapter 5.

The performance indicators assessed were:

- Mean Relative Growth Rate (mRGR);
- Survival seven days after nymph deposition;
- Lifespan;
- Generation time;
- Reproductive days;
- Adult weight;
- Mean nymph weight;
- Seven-day fecundity;
- Intrinsic rate of increase (r_m); and
- Total reproductive output.

Statistical analysis

Aphid mRGR, generation time, lifespan, adult weight, mean nymph weight, seven-day fecundity, total reproductive output and reproductive days were analysed using linear mixed effect models. In cases where an aphid did not meet the data requirements for the calculation of a given performance indicator, the aphid was excluded from the analysis of that indicator (e.g. too few days for seven-day fecundity, or did not produce nymphs before dying). Survival of the nymphs from the mRGR experiments was also analysed using a linear mixed effect model, with a binomial distribution. Drought was modelled as a fixed effect, while capsule location and tree were modelled as random effects (groups: tree = 50, location = 2; n = 50, estimated d.f. for each parameter = 4).

All statistical analyses were carried out using the statistical program, R (version 2.11.0, R Development Core Team (2012)). Linear mixed effect models were plotted using the '`lme4`' package (Bates *et al.*, 2012), and were checked for significance using the '`car`' package (Fox & Weisberg, 2011). Model simplification was carried out and tested with anova where appropriate, as per Crawley (2007).

Results

The mean values and results for the analyses of the performance indicators are summarised in Table C.1.

While drought stress did not have an effect on either lifespan ($\chi^2{}_4 = 5.977$, $P > 0.05$, Figure C.1(B)), the number of reproductive days($\chi^2{}_4 = 5.229$, $P > 0.05$, Figure C.2(B)) or survival of the nymphs seven days after deposition ($\chi^2{}_4 = 3.633$, $P > 0.05$, Figure C.1(A)), there was a significant effect on the generation time ($\chi^2{}_3 = 8.624$, $P < 0.05$, Figure C.2(A)). Although MS and IS2 drought treatments had no effect (MS: $t = 0.21$, $P > 0.05$; IS2: $t = 0.15$, $P > 0.05$), generation time was significantly longer under the CS and IS1 treatments ($t = 2.16$, $P < 0.05$), though there was no significant difference between them.

The mRGR of nymphs showed no response to drought stress ($\chi^2{}_4 = 5.479$, $P > 0.05$, Figure C.3(A)). There was also no effect on both seven-day fecundity ($\chi^2{}_4 = 2.175$, $P > 0.05$, Figure C.4(A)) and the intrinsic rate of increase ($\chi^2{}_4 = 4.441$, $P > 0.05$, Figure C.4(B)). The total reproductive output, however, was significantly affected ($\chi^2{}_2 = 6.801$, $P < 0.05$, Figure C.4(C)). The MS, IS1 and IS2 drought treatments had no effect ($t = 1.37$, $P > 0.05$), with no difference between them. However, a significantly lower total reproductive output was achieved under CS drought treatment ($t = 2.60$, $P < 0.01$).

Adult aphid weight was generally significantly affected by drought stress ($\chi^2{}_4 = 19.842$, $P < 0.001$, Figure C.3(B)). While aphids reared under the MS drought treatment were no heavier than those raised under the FC drought treatment ($t = 1.12$, $P > 0.05$), those raised under CS and IS1 drought treatments were significantly heavier ($t = 3.05$, $P < 0.001$), though there was no difference in their response. Adult aphids reared under IS2 drought were also significantly heavier ($t = 4.09$, $P < 0.001$).

Conversely, mean nymph weight was typically reduced under drought stress (${\chi^2}_3 = 9.049$, $P < 0.05$, Figure C.3(C)). Nymphs deposited under both MS and IS2 drought treatments were no lighter than those under the FC drought ($t = 1.40$, $P > 0.05$), but those deposited under CS and IS1 drought treatments were significantly so (CS: $t = 2.27$, $P < 0.05$; IS1: $t = 2.73$, $P < 0.01$).

Discussion and Comments

The observations in this study are compatible with the modified plant water stress hypothesis as proposed by Mody *et al.* (2009): under severe stress, regardless of whether it be continuous or intermittent, aphids took a longer period of time to start producing nymphs and produced lighter nymphs than aphids in other treatments, despite gaining a greater adult weight. Total reproductive output was also reduced under continuous severe drought stress, attributable to the reductions in nymph weight. On the more moderately intermittently stressed plants, which had a smaller stress amplitude, aphids attained a greater adult weight, and otherwise did not perform significantly worse than the well-watered control in any of the performance parameters.

There are, however, limitations to be considered when interpreting the results presented here. The experiments were carried out late in the season, when host plant quality was declining, and this led to the staggered trials run in 2011.

Major (1990) found that, despite significant differences in final *E. abietinum* population size on well-watered, continuously stressed and intermittently stressed trees, there was no significant difference between individual aphid performance in the three treatments. The author suggested that these findings might have reflected a methodological problem, and the same may be applicable to this current study. Furthermore, Mondor *et al.* (2010) concluded in their study that under altered atmospheric conditions associated with climate change the population size of a herbivore may not be readily predicted

Table C.1:

Summary of individual *E. abietinum* aphid performance indicators on Sitka spruce under five different drought levels. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity.

	FC	MS	CS	IS1	IS2		
Indicator	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	$\bar{x}$ ± **SE**	χ^2	**P**
mRGR (mg/mg/day)	0.118 ± 0.015	0.097 ± 0.016	0.136 ± 0.014	0.107 ± 0.016	0.138 ± 0.016	5.479	n.s.
Lifespan (days)	30.158 ± 1.478	27.486 ± 1.423	27.919 ± 1.478	32.000 ± 1.618	29.081 ± 1.230	5.977	n.s.
Generation time (days)	15.394 ± 0.612	15.190 ± 0.557	17.189 ± 0.471	17.256 ± 0.620	15.621 ± 0.634	8.624	< 0.05
Reproductive days	14.763 ± 1.429	12.297 ± 1.645	10.730 ± 1.421	14.744 ± 1.580	13.459 ± 1.335	5.229	n.s.
Adult weight (mg)	0.170 ± 0.009	0.191 ± 0.011	0.218 ± 0.012	0.218 ± 0.007	0.244 ± 0.006	19.842	< 0.001
Mean nymph weight (mg)	0.0286 ± 0.0007	0.0272 ± 0.0007	0.0259 ± 0.0010	0.0254 ± 0.0008	0.0272 ± 0.0007	9.049	< 0.05
7-day fecundity (n. nymphs)	5.357 ± 0.322	4.833 ± 0.328	4.789 ± 0.302	5.067 ± 0.299	5.385 ± 0.412	2.175	n.s.
r_m	10.412 ± 0.875	9.714 ± 0.675	12.500 ± 1.615	11.231 ± 1.161	10.353 ± 0.707	4.441	n.s.
Total reproductive output (mg)	0.313 ± 0.030	0.241 ± 0.029	0.196 ± 0.023	0.270 ± 0.028	0.270 ± 0.025	6.801	< 0.05
	$\hat{p}$ **alive**	$\hat{p}$ **alive**	$\hat{p}$ **alive**	$\hat{p}$ **alive**	$\hat{p}$ **alive**	χ^2	**P**
Survival	0.600	0.650	0.525	0.725	0.600	3.633	n.s.

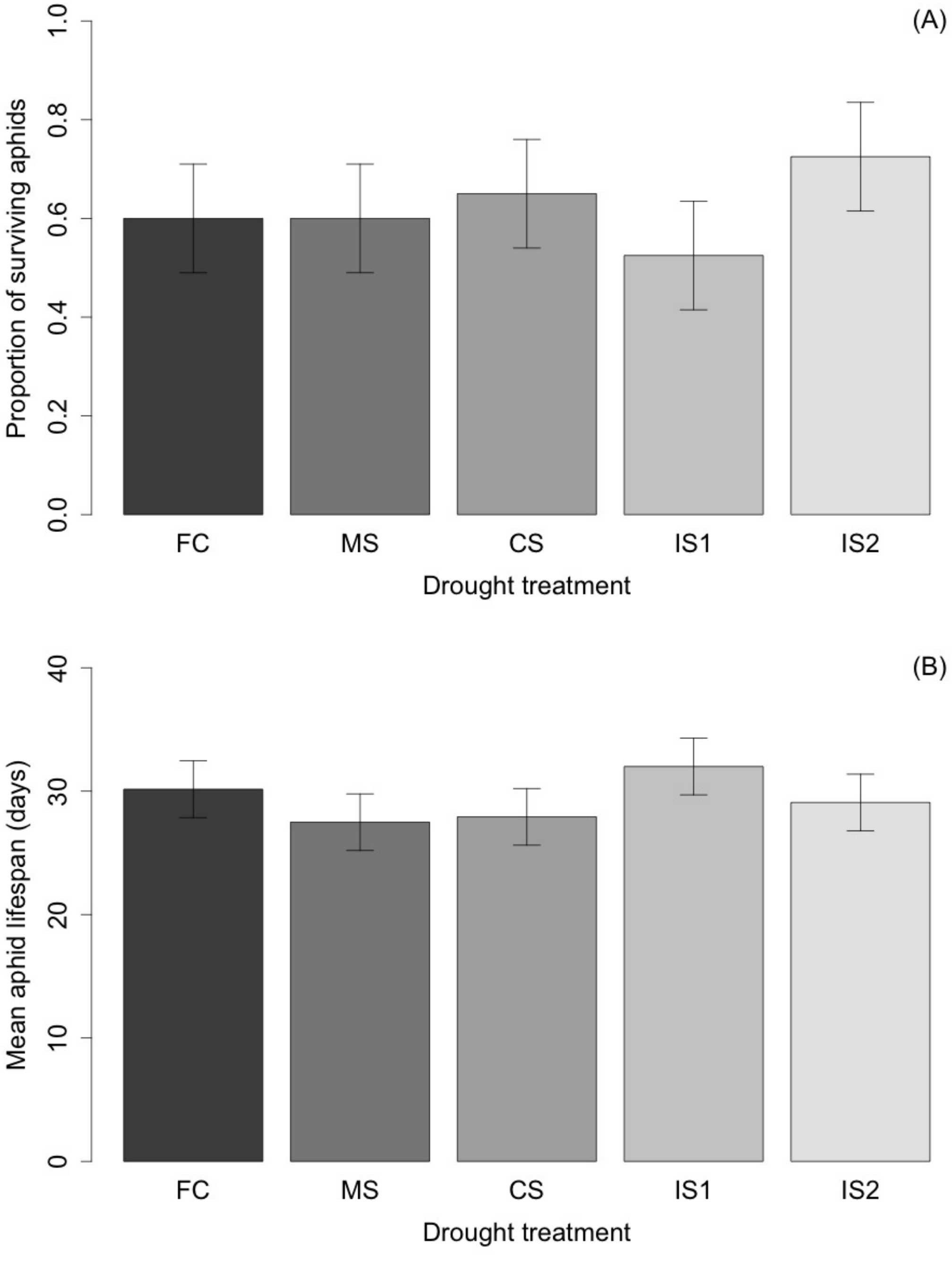

Figure C.1: The effect of drought stress on the survival (A) and lifespan (B) of *E. abietinum* on spruce under controlled conditions (15 °C, 70% RH, 16:8 LD). Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

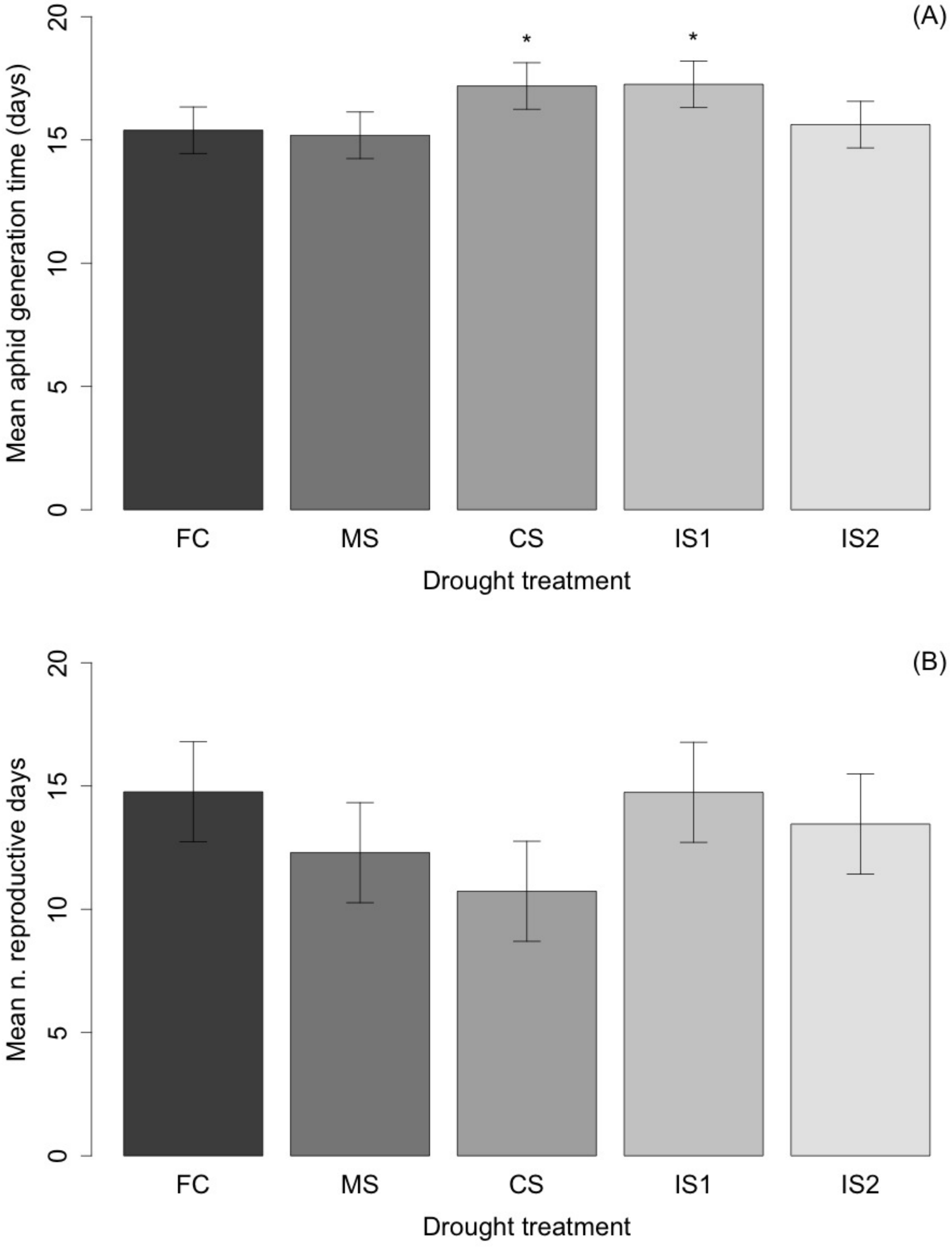

Figure C.2: The effect of drought stress on the generation time (A) and number of reproductive days (B) of *E. abietinum* on spruce under controlled conditions (15 °C, 70% RH, 16:8 LD).

Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

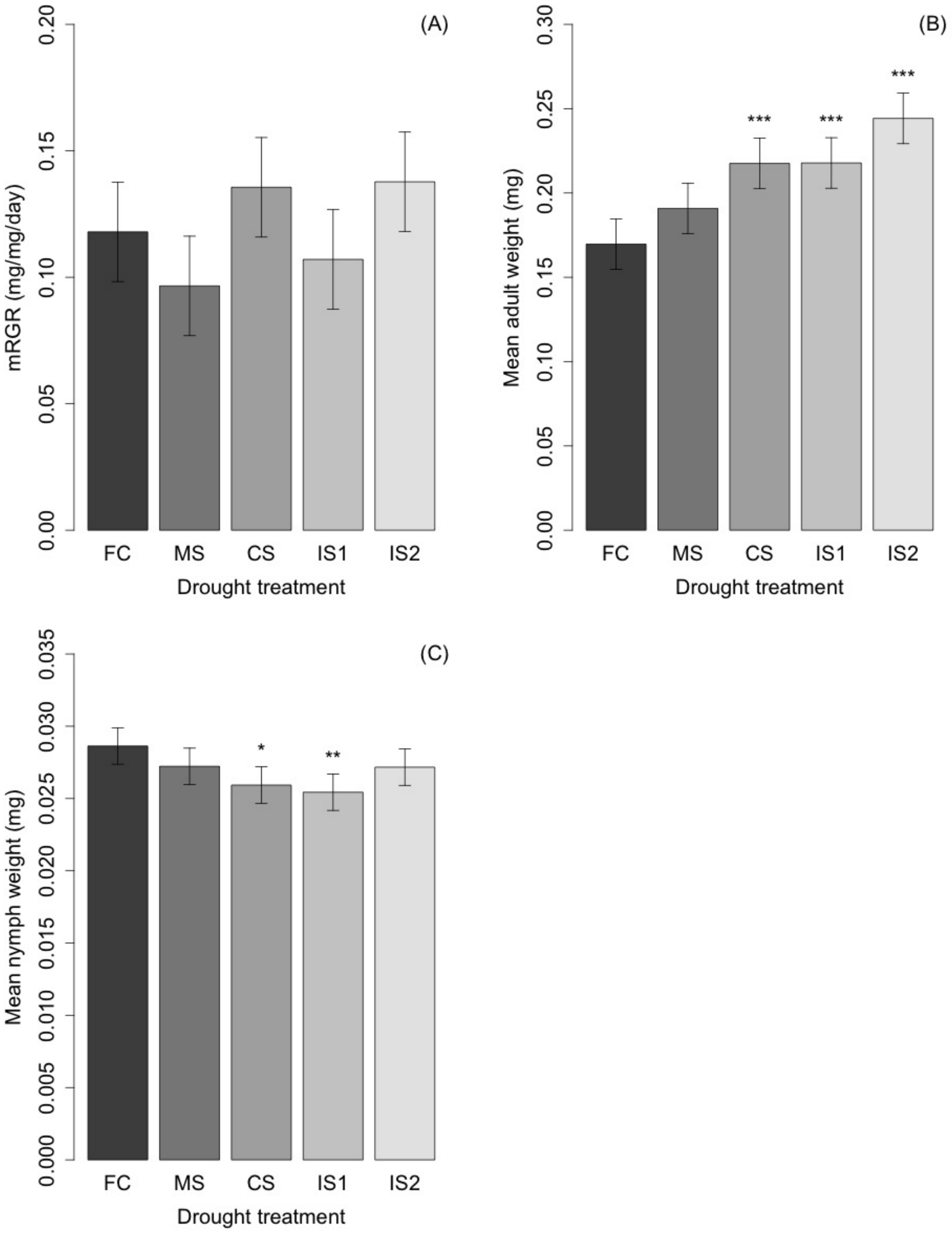

Figure C.3: The effect of drought stress on the mRGR (A), and adult (B) and mean nymph (C) weights of *E. abietinum* on spruce under controlled conditions (15 °C, 70% RH, 16:8 LD).

Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

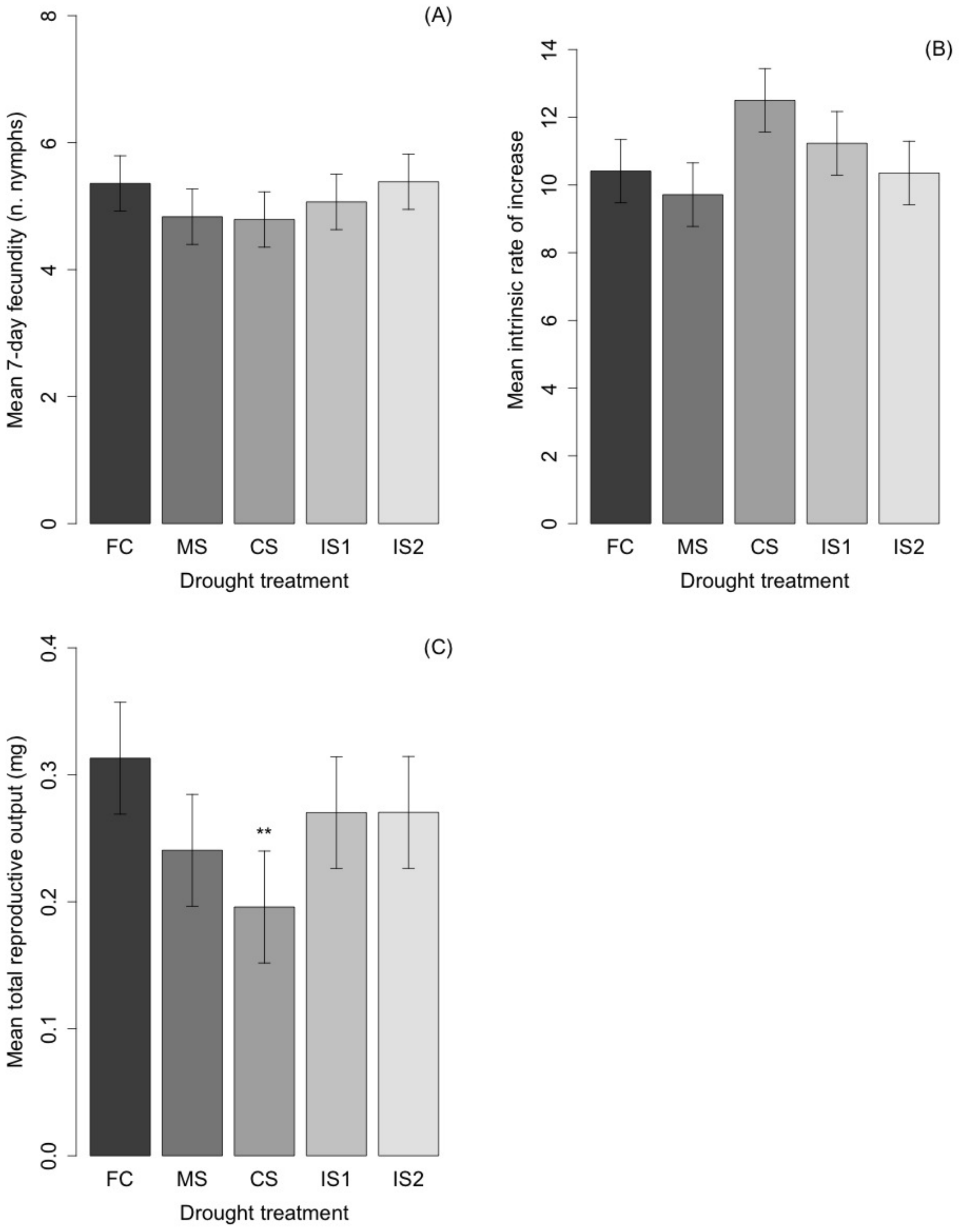

Figure C.4: The effect of drought stress on seven-day fecundity (A), intrinsic rate of increase (B) and total reproductive output (C) of *E. abietinum* on spruce under controlled conditions (15 °C, 70% RH, 16:8 LD).

Bars indicate Least Significant Difference. FC = field capacity; MS = 60% field capacity; CS = 20% field capacity; IS1 = allowed to fluctuate from field capacity to 20% field capacity; IS2 = allowed to fluctuate from 70% to 30% field capacity. *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

from growth rates of individual organisms. The same may also be true for the effects of drought. The potential influence of maternal effects, well documented in aphids (Dixon *et al.*, 1993; McLean *et al.*, 2009; Tariq *et al.*, 2010), should also be kept in mind despite efforts to reduce or control for this in the protocol of this experiment.

Host plant quality has been shown to affect herbivorous insects at both the individual and population scale (Awmack & Leather, 2002), and though it is predicted that drought stress will affect the host quality and suitability of Sitka spruce for *E. abietinum* it is possible that such changes would have a stronger impact on performance in the autumn or year following the initial drought stressed season. Furthermore, as conditions in a CT room are comparatively favourable to the aphids and provide shelter from climatic fluctuations as well as predators, the test aphids may have been able to overcome some host effects.

Finally, *E. abietinum* feed on one year old and older needles only, and would thus not have been subjected to any physical changes in needles structure that drought stress may cause to current needle growth.

Conclusions

1. In general, drought stress did not appear to affect overall aphid performance.
2. Severe drought stress, be it continuous or intermittent, seemed to negatively affect aphid performance, whereas there was some indication of a positive effect of a moderate intermittent stress.