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SUSTAINABLE FOREST MANAGEMENT

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Fertilisation and forest health: preventing or offsetting biotic leaf loss in eucalypt plantations

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Fertilisation and forest health: preventing or offsetting biotic leaf loss in eucalypt plantations

Prepared for the

Forest and Wood Products Research and Development Corporation

by

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EXECUTIVE SUMMARY

The objective of the project was to develop tools that can be used by managers of *Eucalyptus globulus* plantations to prevent or offset the effects of defoliation events on plantation productivity. The role of fertilising was specifically investigated. In order to achieve the objective, a number of steps were necessary. These are summarised below, with key outcomes.

1. Identify the effects of fertilising on growth responses to defoliation by insects and pathogens

Defoliation by pests (insects, mammals, pathogens) is a common occurrence in eucalypt plantations in Australia. We undertook two experiments (one in an *E. globulus* plantation experiencing insect defoliation, and one subjected to artificial defoliation) that demonstrated that fertilising, particularly with nitrogen, reduced the effect of defoliation on stem growth. The diameter and height increment of trees where the upper crowns were defoliated by insects (*Gonipterus scuttelatus*) was reduced when defoliation removed more than 10% of leaf area. Fertilising defoliated trees with 300 kg ha^{-1} N maintained stem growth at the level of undefoliated, unfertilised trees, and improved crown condition.

The artificial defoliation study allowed us to examine the role of pattern, frequency and severity of defoliation, as well as rate and timing of fertiliser application. It was concluded that:

- Upper crown defoliation (including removal of aplical foliage from throughout the crown) should be of more concern to managers than lower-crown defoliation
- More frequent defoliation events had a greater effect on stem growth, particularly if the defoliation was from the upper crown
- Stem growth is likely to be reduced more as defoliation severity increases
- Higher rates of N application (300 vs 100 kg ha^{-1}) improved stem growth more following defoliation
- While pre-defoliation fertiliser application gave best results, there is the potential to use post-defoliation fertilising to offset growth losses associated with defoliation.

In both experiments, application of N fertiliser promoted phase change from juvenile to adult foliage. Foliar N concentrations and total phenolics following artificial defoliation suggested that trees that had been fertilised with N may be more attractive to some insect species. However, given that the objective of growing eucalypt plantations is generally to maximise growth and hence vigour, then an important question is whether crowns can be restored following a defoliation event so that vigour can be maintained, irrespective of whether there is an increased risk of future defoliation events. Our results indicate that this is the case.

The potential for nitrogen and phosphorus fertilisation to prevent chronic injury to *E. globulus* with Mycosphaerella leaf disease (MLD) was tested in a third experiment. Trees fertilised before an MLD epidemic suffered a small, but significant increase in the amount of defoliation due to MLD in the upper crown. However, those trees refoliated more rapidly after the MLD epidemic had passed. Fertilisation before the

MLD epidemic had no effect on the progression of phase change from the susceptible juvenile foliage to the resistant adult foliage.

2. Examine the physiological processes that govern growth responses to defoliation or infection

Understanding the physiological processes underlying growth responses to pest attack is critical for the modelling of growth responses to defoliation. We undertook three experiments to examine physiological responses to defoliation.

It was determined that photosynthesis of trees with MLD declines linearly with increasing disease severity, whereas photosynthetic rates increase following artificial defoliation. In trees with MLD, photosynthesis was also reduced in asymptomatic leaf tissue. Changes in photosynthetic rates were attributed to changes in the rates of biochemical reactions in the leaves, and changes in CO_2 transfer resistances. There was some suggestion that stomatal control was less effective in trees more affected by MLD, which has implications for the impact of the combination of MLD and water stress.

Artificial defoliation (in autumn or spring) resulted in increases in photosynthetic rate. Where defoliation was in autumn, this increase was delayed until the following spring, suggesting that autumn defoliation events may have a greater impact on tree productivity than spring defoliation events. The increases in photosynthetic rate were linked to increases in chlorophyll concentration and changes in light utilisation efficiency. A general increase in leaf thickness/density was observed in fertilised trees in this experiment which may reduce the attractiveness of foliage to defoliating insects, effectively counteracting the effect of the increases in foliar N and total phenolic concentrations that were observed.

A pot experiment was undertaken to compare the physiological responses of two ecualypt species to artificial defoliation or infection by two foliar pathogens (*Mycospharella* spp. or *Phaeophleospora eucalypti*). *E. globulus* and *E. grandis* responded differently to pest attack/defoliation. The changes in patterns of biomass production associated with artificial defoliation and the two diseases varied between eucalypts, although reductions in photosynthetic rates in both eucalypt species following defoliation or infection were associated with similar changes in the biochemical reactions of photosynthesis. The study provided information for parameterising the growth model. It also allowed us to conclude categorically that, although *P. eucalypti* is much easier to use in glasshouse inoculation studies than is *Mycosphaerella*, it should not be substituted because of the different physiological responses associated with the two pathogens.

At a canopy level, pest attack can affect both light interception and canopy light utilisation efficiency (CLUE). Light interception is affected directly by changes in leaf area. Changes in CLUE are determined by photosynthetic responses to pest attack, and by resource availability/patterns of resource allocation. We undertook a glasshouse study with *E. globulus* defoliated to remove 60% of leaf area, or infected with the foliar pathogen *Phaeophleospora eucalypti,* to examine the effects of pest attack on photosynthetic processes and resource allocation, and ultimately to improve our capacity to model responses to pest attack. Both infected and defoliated *E. globulus* seedlings maintained or increased leaf dry mass and leaf area compared

with the control plants, suggesting that maintaining levels of light interception in response to pest attack is a priority in this species. This was achieved at the expense of total dry mass per plant, and specifically root, stem and branch dry mass. Hence pest attack has implications for resource storage in the roots, which could affect the plant's capacity to compensate for future stresses such as drought or further pest attack.

Different modes of attack, which may have similar effects on total dry mass, may affect patterns of allocation within the crowns in different ways than have implications for light interception. The potential reduction in CLUE of infected plants, associated with the downregulation of photosynthetic processes that we observed, may have been offset by increases in leaf dry mass and the upregulation of photosynthesis in asymptomatic leaves. There was remobilisation of N from the roots to the leaves of infected and defoliated plants, and new N entering the plant also was preferentially partitioned to leaves, with less in root and stem material of treated than control plants. This strategy, while it may optimise CLUE, leaves plants vulnerable to N depletion in the event of further pest attack.

Tree vigour can be improved by reducing resource limitation, and there is evidence that pest attack has less effect on growth and biomass production when plants are growing vigorously. Consistent with this, our results demonstrated that loading plants with N prior to pest attack allowed plants to maintain whole plant dry mass at a level similar to that of control plants. An important aspect of this may be that root dry mass, and the root:shoot ratio, was not affected by infection or defoliation in plants where N loading occurred prior to treatment.

3. Develop tools for forest managers and researchers

The major tool developed in this project was a decision support system that could predict the effects of pest attack on productivity. Little work has been done on modeling the impacts of leaf damage or defoliation on tree growth. At the leaf scale the process of infection and plant response is well known, but how this scales to whole canopy production and on to tree performance in subsequent years is more problematic and less studied. The process-based model CABALA has many of the components at suitable scale and degree of generality necessary to model the impacts of loss of effective leaf area on forest production. Carbon-water- nitrogen cycles are linked so that simultaneous impacts of leaf area loss on biomass allocation, transpiration and water use and nitrogen reallocation can be assessed. While the model allows for different spacing arrangements, in its published form it makes assumptions of uniform leaf area density and a fixed attenuation pattern of photosynthetic capacity throughout the crown. CABALA was developed further to extend the light interception and photosynthetic sub-modules of CABALA to better suit them to assessing the impacts of leaf area damage and loss. This forest health module will be available as part of the next release of CABALA.

The potential usefulness of a hand-held chlorophyll meter (Minolta 502 SPAD) was examined for *E. globulus* and *E. nitens*. Good relationships existed between chlorophyll content and a SPAD-derived chlorophyll index (CI). The relationship between foliar N content and CI was weak. It was concluded that the SPAD is suitable for determining foliar chlorophyll content of *E. globulus* and *E. nitens* as a 'generic' indicator of stress, but caution is required for it to be used directly to detect

N deficiency in young crowns. It has potential as a management tool for optimising fertiliser regimes in plantations of these species, but in instances where differences in chlorophyll content are likely to be small, extraction methods are preferable to the use of meters such as the SPAD.

Methods of inoculating plants with *Mycosphaerella* for glasshouse studies were developed. The *Mycosphaerella* species that infect southern Australian eucalypt plantation species are very difficult to inoculate with, because they generally do not produce spores in culture. A method was developed that resulted in spore production from *Mycosphaerella cryptica* in culture, which provides the option for future glasshouse studies where genetic variation in the pathogen can be controlled. Methods that resulted in successful infection of *E. globulus* using infected material collected from the field were also developed. These methods will improve the success of inoculations in future glasshouse studies involving *Mycosphaerella* species.

Foliar oedema, or non-pathogenic blister-like protruberances on leaves, can develop on eucalypt foliage when plants are grown under high relative humidity conditions such as are often experienced in glasshouses. The cellular structure resembles lenticels which are often associated with waterlogged tissue. The presence of lenticel-like structures on *E. globulus* foliage reduced rates of photosynthesis, primarily through reductions in the rate of electron transport, indicating that plant material with foliar oedema should not be used for glasshouse experiments. Photosynthetic rate of afflicted *E. nitens* foliage was not affected, probably because *E. nitens,* because of its cellular structure, is less susceptible to development of oedema.

Future research directions

At the end of this project there are a number of questions remaining, related primarily to the physiological responses of the host to pest attack. In order to refine the forest health module in Cabala and better improve predictions of growth responses to pest attack, the following questions require addressing:

- What are the effects of pest attack on resource partitioning (nutrients, carbohydrates) within the tree, as related to time of year, severity and frequency of attack, and tree age?
- What effect does the interaction of stresses (eg. insect defoliation, nutrient and water stress) have on responses to pest attack?
- What factors govern photosynthetic responses to pest attack? For example, why is photosynthetic upregulation delayed in response to autumn byt not spring defoliation?

Experimental work in the current project was based in Tasmania, and while we endeavoured to conduct experiments in such a way that results would be generally applicable, validation of results under different environmental conditions is important.

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1. Introduction

The eucalypt plantation estate in Australia comprises close to 720 000 ha, of which around 65% is *Eucalyptus globulus* Labill. (Parsons and Gavran 2005). Insect defoliation, infection by foliar pathogens, and/or mammal browsing occurs to some degree in most plantations. The effects of infection or defoliation on growth, and ultimately economic returns, are poorly understood. Most studies have been short-term in nature and restricted to a small number of sites, with a limited number of treatments in terms of severity, pattern and frequency of attack.

While many foliar pathogens are found on eucalypt leaves, the most serious problems in Australian plantations are caused by *Mycosphaerella* species, and in particular two species, *M. nubilosa* and *M. cryptica* (Carnegie et al. 1997). *Mycosphaerella* leaf disease (MLD) is characterised by necrotic lesions, foliar discoloration and, in severe cases, tissue blighting (Park 1988a) followed by premature leaf senescence. Epidemics have been observed in north west Tasmania, Victoria and south west Western Australia. Browsing in eucalypt plantations is caused by browsing mammals such as brushtail possum (*Trichosurus vulpeca*) and red-bellied pademelon (*Thylogale billardierii*) (O'Reilly-Wapstra et al. 2002), and by insects such as eucalypt leaf beetles (*Chrysoptharta* spp. and *Paropsis* spp.), scarab beetles (*Heteronyx* spp.), eucalypt snout weevels (*Gonipterus scutellatus*), gumleaf skeletoniser (*Urabe largens*) and autumn gum moth (*Mnesampela privata*) (Elliott and de Little 1984; Loch and Floyd 2001).

Managing leaf diseases and browsing in plantations is very difficult. Chemical control is usually cost-prohibitive and may be associated with environmental problems. Selection for genetic resistance is possible, as resistance to some foliar pathogens and to browsing is under strong genetic control (Milgate et al. 2005; O'Reilly-Wapstra et al 2002; Rapley 2005). However it is known that that genetic resistance may break down under certain growth conditions (eg. high soil fertility) (O'Reilly-Wapstra et al. 2005). There is some evidence that better nutrition may help prevent or offset the effects of infection or defoliation (Carnegie and Ades 2001; Stone 2001), although silvicultural treatments intended to improve tree vigour may also directly influence herbivore populations and hence future browsing (Bruyn et al. 2001; Lou and Baldwin 2004; Prudic et al. 2005). However maintaining an optimal nutrient status is perhaps the silvicultural option with the most promise for preventing or offsetting stem growth losses following foliar attack. It is well known that fertilising, in the absence of other site limitations, promotes crown development (Smethurst et al. 2003).

It is well-established that defoliation can reduce plant growth (Candy et al. 1992; Carnegie et al. 1997; Cerasoli et al. 2004b; Elek 1997; Kulman 1971; Wills et al. 2004). The impact on growth is related to severity (Pinkard 2003), pattern (Collett and Neumann 2002) and frequency (Wills et al. 2004) of attack, and may also be a function of the causal agent (pathogen or herbivore) (Ayres 1992). Overlying this is the role of site factors in determining growth responses. The complexity of the system makes it difficult to predict the longer-term outcomes of foliar attack. 'Best guess' modelling in north western Tasmania for high productivity sites grown for solid wood estimated that 50% infection with Mycosphaerella spp. over two years (age 2 and 3) would reduce net present value from \$1069 ha^{-1} to \$60 ha^{-1} (T. Wardlaw, Forestry Tasmania, pers. comm.), and for a high productivity site in Western Australia grown for pulp it was estimated that a single 50% defoliation event would reduce returns at harvest by \$424 ha^{-1} (M. Battaglia, Ensis,

pers. comm.). There is also the potential for wood quality as well as volume to be affected by defoliation events, which is particularly important when stands are grown for clearwood. Premature branch death associated with severe defoliation can lead to kino traces in wood following pruning, and the tip dieback that is often observed with upper crown defoliation can result in forked stems that make stem selection for pruning difficult (Beadle et al. 1994; Wardlaw 2001).

In the absence of long term growth data, dynamic models with a physiological basis have been used successfully to predict the long-term effects of silvicultural treatments and changing environmental conditions (Battaglia and Sands 1998), and have the potential to be used to predict the effects of defoliation on growth.

Objectives and report structure

The key objective of this project was to develop management tools for forest growers to prevent or offset the effects of infection and/or defoliation on plantation productivity. There were eleven components to the research, divided into three sections: (1) effects of fertilising on growth responses to defoliation and infection; (2) physiological processes governing growth responses to defoliation or infection; and (3) tools for forest managers and researchers. The components within each section are summarised below.

The report has been prepared as a series of stand-alone reports and papers, which have been summarised into a management context in the Executive Summary. The reports and papers have been edited to remove repetition.

Effects of fertilising on growth responses to defoliation and foliar pathogens

- *Effects of fertilising with nitrogen and phosphorus on growth and crown condition of* Eucalyptus globulus *Labill. experiencing insect defoliation*
  Libby Pinkard, Craig Baillie, Vin Patel and Caroline Mohammed Forest Ecology and Management (in press) **(Chapter 2)**

- *Effects of N nutrition on short-term growth of young* Eucalyptus globulus *Labill. subjected to artificial defoliation.*
  Libby Pinkard, Craig Baillie, Vin Patel, Michael Battaglia, Phillip Smethurst, Caroline Mohammed, Tim Wardlaw and Christine Stone. Forest Ecology and Management (2006) (in press) **(Chapter 3)**

- *Can fertilisation with nitrogen and phosphorus limit growth impacts from Mycosphaerella leaf blight in a young Eucalyptus globulus plantation?*
  Tim Wardlaw, Forestry Tasmania Technical Report 6/2005, Division of Forest Research and Development **(Chapter 4)**

Physiological processes that govern growth responses to defoliation or infection

- *Photosynthesis of* eucalyptus globulus *labill. With mycosphaerella leaf disease*
  Libby Pinkard and Caroline Mohammed (2006), New Phytologist, 170: 119–127 **(Chapter 5)**

- *Influence of defoliation and nitrogen application on photosynthetic processes of young* Eucalyptus globulus *Labill.*
  Libby Pinkard, Michael Battaglia and Caroline Mohammed **(Chapter 6)**

- *Comparison of physiological responses of two plantation eucalypt species to artificial defoliation and foliar infection caused by species of* Mycosphaerella *and* Phaeophleospora eucalypti
  Audrey Quentin, final project report for the Agriculture degree of ESTIPA Rouen (France) (separate report)

- *Photosynthesis and nitrogen allocation in* Eucalyptus globulus *Labill. seedlings subjected to artificial defoliation or infection with* Phaeophleosopora eucalypti.
  Libby Pinkard, ZiHong Xu, Miriam Paul and Caroline Mohammed **(Chapter 7)**

Tools for forest managers and researchers

- *Modelling light interception, photosynthetic and growth impacts of defoliation*
  Michael Battaglia and Peter Sands **(Chapter 8)**

- *Chlorophyll and nitrogen determination for plantation-grown Eucalyptus nitens and* E. globulus *using a non-destructive meter*
  Libby Pinkard, Vin Patel and Caroline Mohammed (2006) Forest Ecology and Management 223:211 – 217 **(Chapter 9)**

- *Working with* Mycosphaerella *in inoculation studies*
  Alieta Eyles, Malcolm Hall, Libby Pinkard and Anna Smith **(Chapter 10)**

- *Formation of environmental response lenticel-like structures (ERLS) on leaves of* Eucalyptus nitens *and* Eucalyptus globulus *seedlings grown under glasshouse conditions*
  Libby Pinkard, Warwick Gill and Caroline Mohammed (2006) Tree Physiology (26: 989 - 999) **(Chaper 11)**

2. Effects of fertilising with nitrogen and phosphorus on growth and crown condition of *Eucalyptus globulus* Labill. experiencing insect defoliation

Libby Pinkard, Craig Baillie, Vin Patel and Caroline Mohammed

Introduction

Herbivore damage in eucalypt plantations can result in loss of stem growth, premature senescence of branches, stem and tip dieback and development of multiple leaders. While reductions in stem growth resulting from defoliation may directly affect harvestable volume at the end of a rotation (Candy et al. 1992), the stem and tip dieback and development of multiple leaders may be important in determining the suitability of stems for clearwood production (Beadle et al. 1994; Neilsen and Pinkard 1999), a rapidly-expanding use of eucalypt plantations. Even within pulpwood regimes, the presence of factors such as multiple leaders may increase harvesting costs. Despite their potential importance, the effects of defoliation on factors other than stem growth have received little attention.

A number of studies have investigated the effects of insect browsing on eucalypt stem growth. For example, mild or severe sawfly (*Perga affinis*) damage on *Eucalyptus globulus* Labill. was found to reduce basal area by 16 – 31% (Jordon et al. 2002). In native *Eucalyptus regnans* F. Muell forest, insect defoliation reduced wood volume by 30% after 8 years, and in three-year-old *Eucalyptus nitens* (Deane and Maiden) Maiden plantations removal of more than 50% of new-season's growth reduced stem growth for two years (Elek 1997). Defoliation that involves disbudding has a greater effect on stem growth (Candy et al. 1992), as does chronic defoliation (Wills et al. 2004).

There is some evidence to suggest that eucalypts growing on more productive sites are better able to withstand foliar attack by insects or pathogens than those experiencing some level of stress (Stone 2001). The site factor that can be most easily manipulated silviculturally is nutrient availability. There is ample evidence that eucalypts can respond to fertiliser applications, particularly of nitrogen (N), by increasing leaf area and hence stem growth (Smethurst et al. 2003). It has been observed that, in an area experiencing Mycosphaerella leaf disease, *E. globulus* growing on soils with a higher nutritional status had healthier crowns than neighbouring trees growing under less fertile conditions (T. Wardlaw, Forestry Tasmania, pers. comm.). In addition, crown recovery following pruning to remove live branches is more rapid on more fertile sites (Pinkard 2003). While there is considerable evidence that increasing foliar N concentrations can make foliage more susceptible to herbivory from some insect pests (Bruyn et al. 2001; Cipollini et al. 2002; Prudic et al. 2005), any treatment that can improve a host's capacity to compensate for leaf loss associated with pest damage offers a potential tool to managers to reduce the effects of herbivory on stem growth.

We undertook an experiment to test the hypothesis that fertiliser application would moderate the effects of insect herbivory on stem growth of plantation-grown *E. globulus*. Specific objectives were to (a) identify a threshold level of damage above which growth was affected; (b) determine the interaction of fertiliser and defoliation; and (c) determine whether defoliation or fertiliser application affected stem properties that might be important for clearwood production.

Methods

Site

The experiment was established in south-eastern Tasmania at Barnback (43°02' 48" S, 146° 48' 26'' E). Mean annual maximum and minimum temperatures and rainfall, measured at the closest Australian Bureau of Meteorology station (~20 km away), are 17.0°, 5.8° and 755 mm respectively. The site is ex-wet sclerophyll eucalypt forest that was cleared, windrowed and burnt before being ripped and mounded for planting. Soils at the site are gradational over Triassic mudstone, with predominantly clay loam topsoil overlying light or light medium clays.

E. globulus seedlings were planted in winter 2001 at 1100 stems ha^{-1}, and were fertilised with 24.7 g nitrogen (N) and 11.3 g phosphorus (P) per seedling approximately six weeks after planting. At the start of the experiment, in May 2004, seedlings had a mean height and diameter at breast height (DBH, 1.3 m) of 4.99 m and 4.92 cm, respectively. The trees had been subjected to low to severe defoliation by *Gonipterus scutellatus* in the summer and autumn prior to the establishment of the experiment. This insect targets juvenile foliage of *E. globulus*, leaving adult foliage largely intact.

Treatments and experimental design

The experiment was established with a 2 x 2 N x P factorial design. The rate of N application was 300 kg ha^{-1} broadcast as urea, and P was applied as triple superphosphate at 100 kg ha^{-1} P in a twin spot application (either side of the tree). Three blocks of four plots were selected at the site, based on slope and aspect. The plots were 20 x 30 m, with an internal measurement plot of 12 x 22 m (approximately 27 trees). Treatments were allocated randomly to plots within blocks. Fertiliser was applied in May 2004.

Measurements

Tree height, diameter at 1.3 m (DBH), height to the green crown base and height to the junction of juvenile and adult foliage were measured immediately before fertiliser application, and five, nine and 12 months after fertilising. Crown condition was assessed using the Crown Damage Index (Stone et al. 2003):

$$CDI = \frac{D_s x D_i}{100} + \frac{N_s x N_i}{100} + \frac{C_s x C_i}{100}$$

where *D* denotes defoliation, *N* denotes necrosis and *C* denotes foliage discoloration, *i* is the estimated percentage of leaves in the crown affected by each type of damage, and *s* is the average percentage of damage to the affected leaves. The crowns were divided vertically into thirds, and a separate assessment was done for each third. Visual standards assisted in uniform assessment (Stone et al. 2003). Assessments were undertaken by the same two people each time, and the assessors were calibrated against each other before the start of each assessment.

A soil description and chemical characterisation was undertaken prior to treatment application. Soil profile descriptions were done in each block, and soil was sampled by horizon. At three-monthly intervals following the application of fertiliser, 20 0 – 10 cm soil samples were taken from the inter-row of each plot and bulked. These were processed to determine soil water content, total carbon (total C) and potassium chloride (KCl)-extractable ammonium (NH_4) and nitrate (NO_3)-N. Once per year, P

concentration was determined using the calcium chloride method. At the end of 12 months, soil from 10 – 30 cm and 30 – 60 cm depths in selected treatments were sampled and water content, total C, NH_4, NO_3, EC, pH and P were determined.

Data analysis

Height and DBH increment, and stem volume, were calculated for each measurement time. Stem volume was calculated using the following equation (Opie 1976):

$$V = \frac{DBH^2 H}{10^{\left(4.762 - \frac{5613}{(DBH+127)^2}\right)}}$$

where *DBH* is in cm, *H* denotes the height in m, and *V* is the volume in m^3. The defoliation component of the CDI was used to class trees into four defoliation classes (0-10, 10.1-20, 20.1-30, >30%) for each measurement time. The percentage of juvenile foliage per tree was calculated for each measurement time as:

$$\%Juvenile = \frac{H_j}{H}$$

where H_j is the height to the juvenile/adult foliage boundary, and *H* is total tree height.

Analysis of variance, with time as a factor and initial tree height, DBH or stem volume as a covariate, was used to explore differences between treatments in height increment, diameter increment, stem volume of H_j. Group regression analysis was used to explore the relationship between height or diameter increment and time, with either treatment or defoliation class as the group (McPherson 1990b). Analysis of variance was used to determine differences in height or diameter increment between defoliation classes and fertiliser treatments, 12 months after fertiliser application. The live crown ratio of trees 12 months after fertilising was calculated as:

$$LCR = \frac{H - H_g}{H}$$

where H_g is height to the first green branch. Differences between treatments were determined using analysis of variance.

The change in crown condition after 12 months was calculated as:

$$D_c = D_{c12} = D_{c0}$$

where D_c is change in defoliation class, D_{c0} is the defoliation class at the start of the experiment, and D_{c12} is the defoliation class 12 months after fertilising. A negative value indicated improved crown condition, and a positive value indicated poorer crown condition compared to the start of the experiment. The number of trees per plot with improved or worse condition greater than one defoliation class was then determined, and analysis of variance was used to examine differences between treatments.

Of those trees with changed crown condition, the change for better or worse was calculated as:

$$C_c = D_{12} - D_0$$

where C_c is the change in defoliation score, D_0 is the defoliation score at the start of the experiment and D_{12} is the defoliation score 12 months after fertiliser application.

Analysis of variance was used to determine differences between treatments in defoliation score. Genstat was used for all analyses.

Results

The number of trees per plot in each defoliation class was similar between fertiliser treatments at the start of the experiment (Figure 2.1A). Approximately 40% of trees per plot had defoliation scores less than 10%, and there were approximately 10% of trees per plot with a defoliation score greater than 30%.

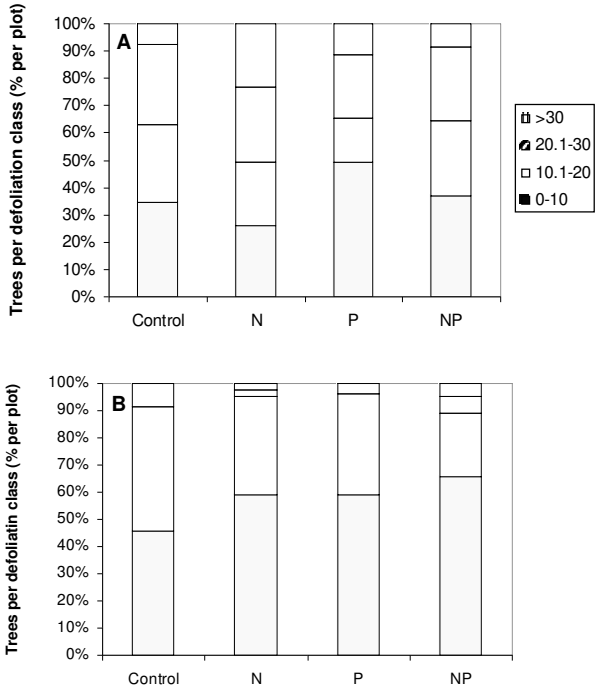

Figure 2.1. Mean percentage of trees per defoliation class (A) prior to the application of either N (300 kg ha^{-1}), P (100 kg ha^{-1}) or N and P, and (B) 12 months after fertiliser application.

Table 2.1. The slopes and intercepts of the relationship between height or diameter increment and time for three-year-old *E. globulus* trees fertilised with either N (300 kg ha^{-1}), P (100 kg ha^{-1}) or the combination of N and P. The relationship is of the form: increment= a + b*time. * denotes significant differences between treatment (P < 0.001).

Treatment	Intercept (a)	Slope (b)	R^2	P
Height increment				
Control	-0.15	0.109	0.63	<0.001
N	-0.18	0.126*		
P	-0.16	0.123		
N+P	-0.18	0.131*		
Diameter increment				
Control	-0.02	0.118	0.73	<0.001
N	-0.07	0.164*		
P	-0.07	0.135*		
N+P	-0.13	0.208*		

Effects of fertiliser on stem growth

Fertiliser application affected both height and diameter increment of *E. globulus* (Figure 2.2). Diameter increment was more affected than height increment. Applications of N

or the combination of N and P resulted in the largest increases in diameter and height increment, and stem volume.

Linear regression provided the best fits to the relationship between diameter or height and time. The slope of the relationship between height increment and time was significantly greater (Table 2.1, $P < 0.001$) than the control in treatments with N, and the slope of the relationship between diameter increment and time was significantly greater with any fertiliser treatment, suggesting longer-term changes in the growth trajectory following fertiliser application, such that stem growth would be increased by fertiliser, and particularly N, application.

Effects of defoliation on stem growth

Mean diameter increment decreased with increasing severity of defoliation (Figure 2.3A). The greatest reduction in diameter increment was observed between 9 and 12 months after the start of the experiment, which was the main period of growth. Over this period, there was very little diameter growth of trees in the >30% defoliation class, and significantly reduced diameter growth in the 20.1-30% defoliation class ($P < 0.001$). The effect of defoliation class on height increment was less obvious (Figure 2.3B).

Table 2.2. The slopes and intercepts of the relationship between height or diameter increment and time for *E. globulus* with four classes of defoliation. The relationship is of the form: increment = a + b*defoliation class. * denotes significant differences between defoliation classes ($P < 0.001$).

Defoliation class	**Intercept (a)**	**Slope (b)**	**R^2**	**P**
Height increment				
0-10	-0.061	0.166	0.67	<0.001
10.1-20	-0.079	0.153		
20.1-30	-0.065	0.125*		
>30	-0.006	0.091*		
Diameter increment				
0-10	-0.196	0.130	0.64	<0.001
10.1-20	-0.215	0.126		
20.1-30	-0.275	0.097*		
>30	-0.019*	0.048*		

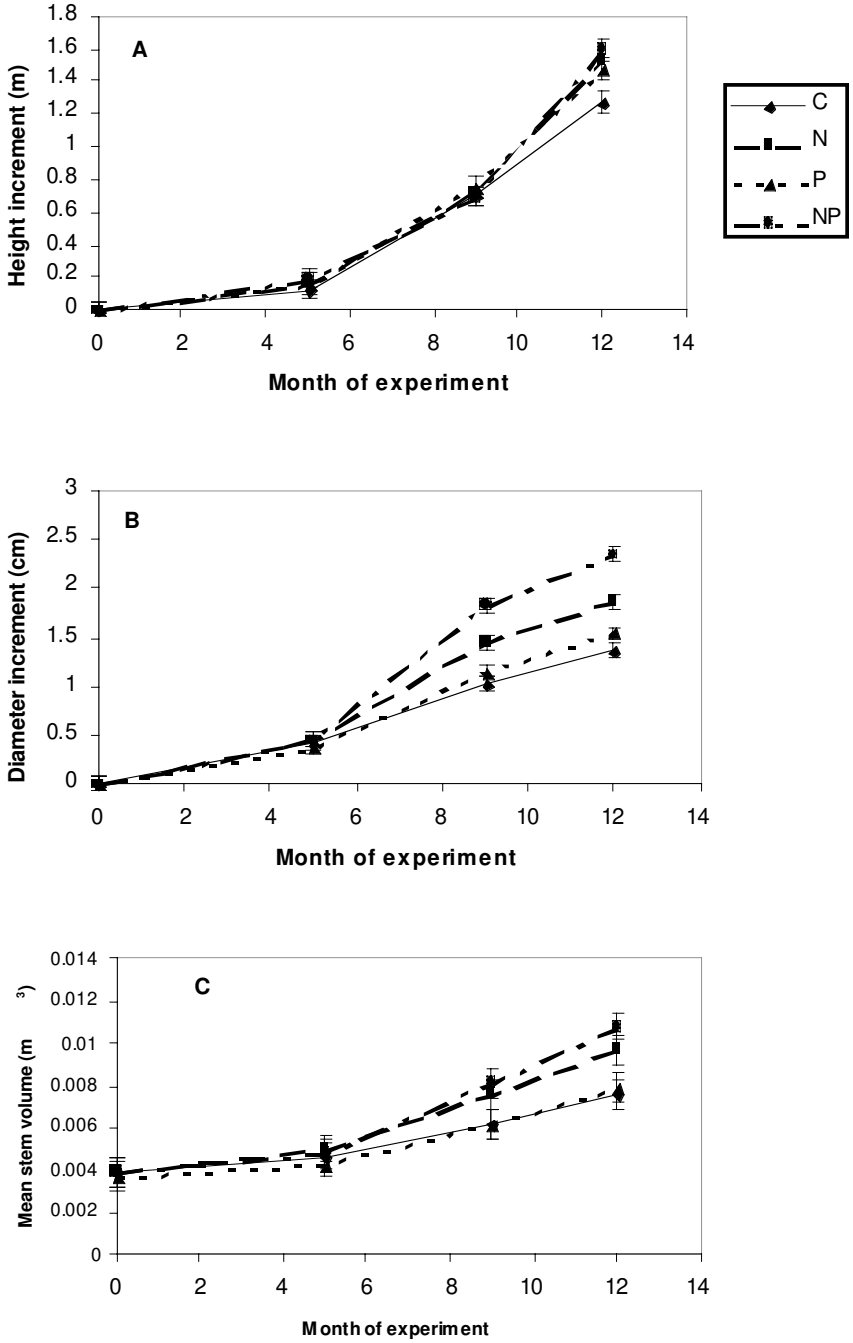

Figure 2.2. (A) mean height increment (m), (B) mean diameter increment (cm), and (C) mean stem volume of *E. globulus* in the 12 months following applications of N (300 kg ha^{-1}), P (100 kg ha^{-1}) or the combination of N and P. Error bars indicate standard errors.

The slope of the linear relationship between height or diameter increment and time were significantly reduced by defoliation scores >20% (Table 2.2), compared to the 0-10% defoliation class ($P < 0.001$). This suggests significant reductions in stem growth and a change in the growth trajectory when defoliation levels were greater than 20%.

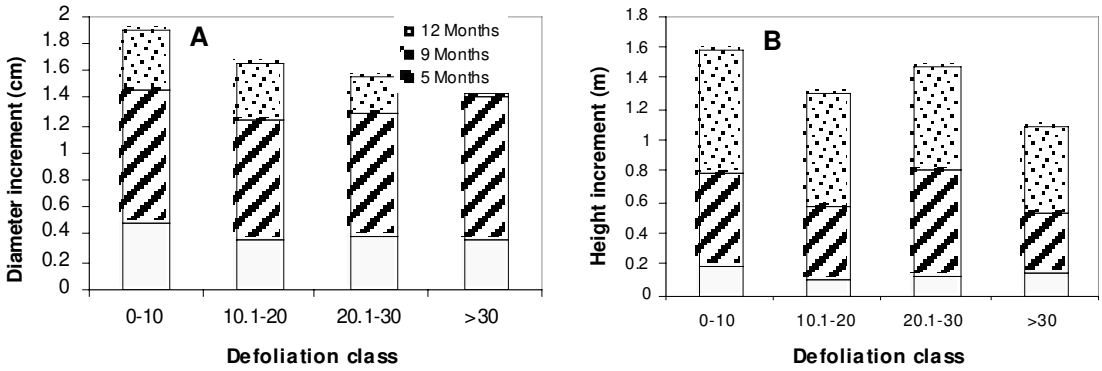

Figure 2.3. (A) mean diameter increment (cm) and (B) mean height increment (m) of *E. globulus* experiencing four classes of insect defoliation, five, nine and 12 months into the experiment.

Interaction between fertiliser and defoliation

Both diameter and height increment in unfertilised plots were reduced when defoliation was greater than 10% (Figure 2.4, $P < 0.001$), 12 months into the experiment. The combination of N and P increased both diameter and height increment of trees with a defoliation score of <10%. The application of N+P increased diameter increment in all defoliation classes to at least the level of that observed for unfertilised trees with a defoliation class of 0-10%. Application of N alone also maintained diameter increment at levels similar to those of unfertilised trees with low levels of defoliation. Application of P alone did not.

In general, height increment of unfertilised trees was reduced by defoliation scores >10% ($P < 0.001$), and fertilising with N, P or N+P increased height increment in any defoliation class to at least the level of unfertilised trees with a defoliation score <10% (Figure 2.4B).

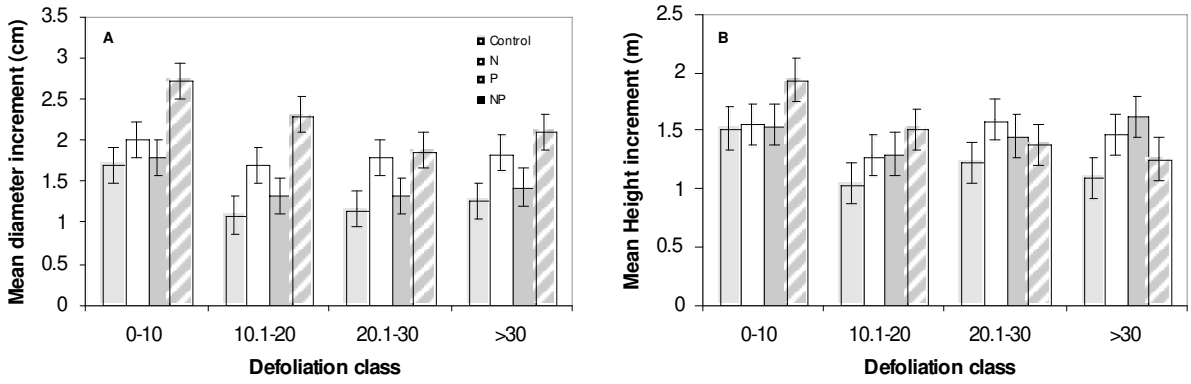

Figure 2.4. (A) mean diameter increment (cm) and (B) mean height increment (m) of *E. globulus* with four classes of insect defoliation, 12 months after the application of N (300 kg ha^{-1}), P (100 kg ha^{-1}) or the combination of N and P.

Effects of fertilising on crown condition

There was an overall improvement in crown condition over the period of the experiment, with an increase in the number of trees per plot with defoliation scores <20% (Figure 2.1B). Twelve months into the experiment, there was a trend towards an increasing number of trees per plot with improved crown condition where N alone had been applied (Figure 2.5A).

In those trees showing improvement, there was a trend towards a greater improvement in crown condition where N alone was applied (Figure 2.5C).

There was significantly less worsening in crown condition where N alone was applied ($P < 0.05$, Figure 2.5D). This was associated with the reduction in the percentage of juvenile foliage per crown, observed in the N-only treatment (Figure 2.6).

Application of N alone had no effect on the live crown ratio of *E. globulus* 12 months after fertilising. However, treatments that included P resulted in a small but significantly greater live crown ratio (Figure 2.7, $P < 0.05$).

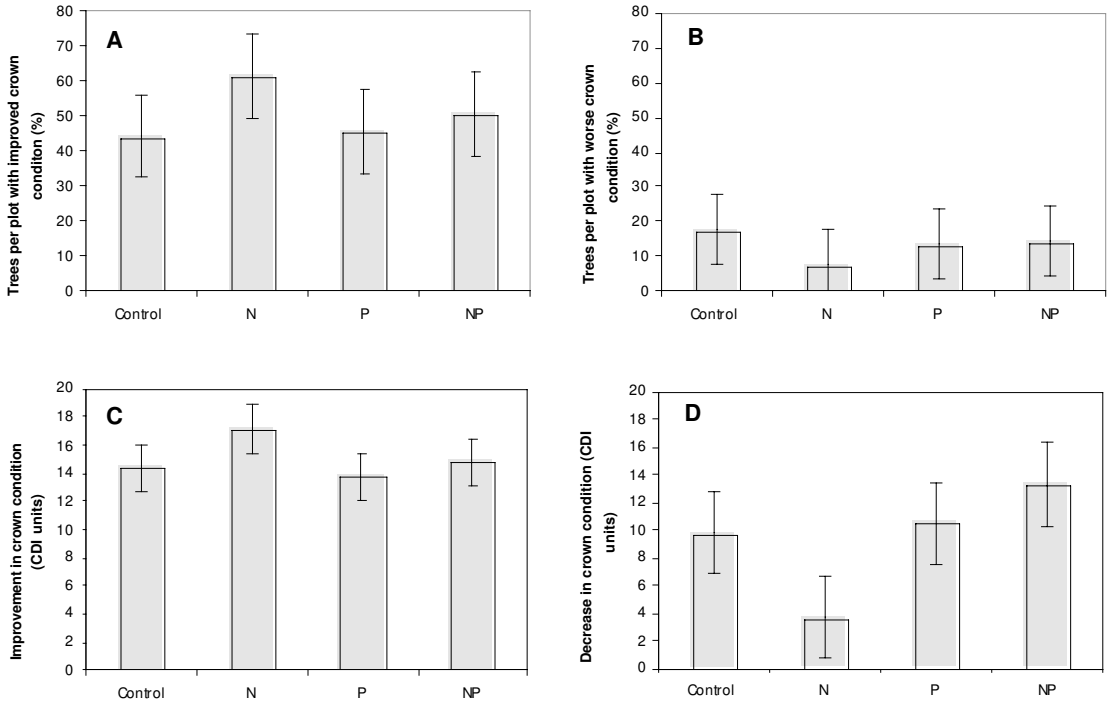

Figure 2.5. (A) the percentage of *E. globulus* trees per plot with improved crown condition, and (B) the percentage of trees per plot with worse condition 12 months after fertilising with N (300 kg ha^{-1}), P (100 kg ha^{-1}) or the combination of N and P; and (C) of those trees with improved condition, the average crown improvement, and (D) of those trees with worse condition, the average worsening of crown condition.

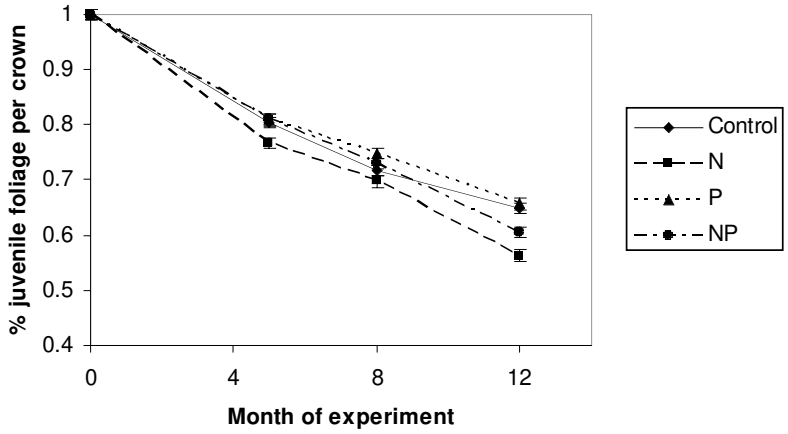

Figure 2.6. Mean percentage of juvenile foliage per crown of *E. globulus* fertilised with N (300 kg ha^{-1}), P (100 kg ha^{-1}) or the combination of N and P, over the 12 months following fertiliser application.

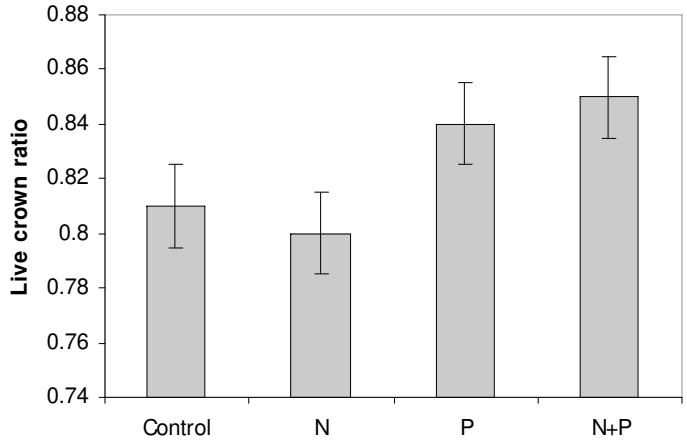

Figure 2.7. Live crown ratio of *E. globulus* trees 12 months after fertilising with N (300 kg ha^{-1}), P (100 kg ha^{-1}) or the combination of N and P.

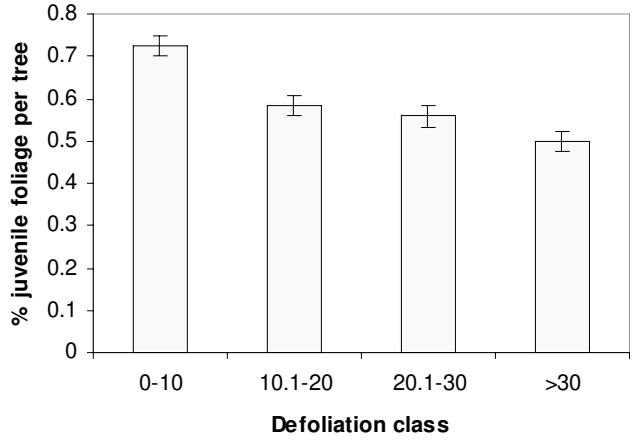

Figure 2.8. Mean percentage of juvenile foliage per crown of *E. globulus* with four classes of insect defoliation, 12 months into the experiment.

Effects of defoliation on crown condition

The percentage of juvenile foliage per tree decreased significantly ($P < 0.001$) as level of defoliation increased, 12 months into the experiment (Figure 2.8). Defoliation class had no effect on the live crown ratio.

Discussion

Defoliation from insects and browsing mammals is a common occurrence in eucalypt plantations in Australia. We have demonstrated that the effects of even severe (incidence x severity of up to 50%) insect defoliation on growth can be moderated or negated through fertiliser application. This offers a potential operationally-feasible management strategy for forest growers.

While greater than 10% defoliation reduced stem growth after 12 months, only defoliation levels of 20% or more affected the shape of the growth curve over time. The 20% defoliation threshold observed in this study is similar to defoliation thresholds

observed in other studies of insect and artificial defoliation of eucalypts (Pinkard 2003; Rapley 2005; Smith 2005). In studies where foliage was removed from the lower crown, particularly following canopy closure, greater levels of defoliation (eg. 50%) were tolerated before stem growth was affected (Pinkard and L. 2000). The change in the slope of the growth curve when defoliation was greater than 20% suggests that defoliation above that level will change the growth trajectory over time. However longer-term monitoring is required.

Growth responses to defoliation are likely to be affected by site and environmental conditions. This has been demonstrated in pruning studies with *E. globulus* (Pinkard 2003), where growth reductions following pruning were more severe on poorer quality sites. While there were no obvious signs of nutrient stress at the site, the strong growth response to fertiliser, and particularly the combination of N and P, suggests that nutrient supply was limiting growth. Our results indicate that fertilising can moderate the effects of even severe defoliation by allowing defoliated trees to increase stem growth above the level that would be possible without fertiliser application. Application of N (with or without P) was particularly important in improving stem growth, although P application seemed to play a role in maintaining height growth. Nitrogen supply is an important determinant of leaf development in eucalypts (Smethurst et al. 2003). The increase in diameter increment of defoliated and fertilised trees relative to control trees suggests that trees in this treatment increased leaf area. There is a strong relationship between stem diameter or sapwood cross-sectional area and leaf area in many species (Shinozaki et al. 1964a; Shinozaki et al. 1964b) that has also been demonstrated for *E. globulus* (Pinkard 2003). The trend towards an increased number of trees per plot with improved crown condition, and a greater improvement in that crown condition, that was observed following N application, supports this conclusion. Responses to N may not be as dramatic as those we observed if other site conditions, such as water or temperature stress, are influencing growth. The role of fertilising under conditions of multiple stress requires further investigation.

The insect responsible for the initial defoliation at the site was *G. scutellatus*. There was little subsequent defoliation throughout the experiment. *G. scutellatus* preferentially feeds on juvenile foliage. The reduction in the proportion of juvenile foliage per crown with N application suggests that trees fertilised with N would be less likely to sustain severe levels of damage from *G. scutellatus* in the future. However they may be more at risk from insects that target adult foliage. Jordan et al. (1999) determined that phase change in *E. globulus* is under strong genetic control. Our results suggest that there may be an environmental component to the initiation of phase change. Williams et al. (2004) also suggested an environmental component in control of transition to adult foliage in *E. nitens*. The reduction in the proportion of juvenile foliage per crown was not related to increased height growth associated with the treatment, because similar height growth also was recorded for the N+P treatment while the reduction in the proportion of juvenile foliage was less in response to the N+P than the N treatment. If fertilising with N promotes phase change to adult foliage, then this may be a useful tool in the management of the impacts on growth of insects and foliar pathogens that target juvenile foliage.

Phosphorus application had no effect on the proportion of juvenile foliage. In contrast, (Williams et al. 2004) found that P application promoted phase change in *E. nitens* at two sites. It is unclear whether the different results from the two experiments is a

function of species differences or growing conditions experienced by the two species. Further experimentation is required to better understand the role of fertilising in promoting phase change under a range of growing conditions.

The reduction in the percentage of juvenile foliage per tree with increasing defoliation severity may reflect the fact that trees were close to the transition to adult foliage at the time of defoliation, such that new growth consisted primarily of adult foliage. Hence more severely-defoliated trees would have a greater proportion of their crown in adult foliage once regrowth had occurred. It is unclear whether this response would be observed in younger plantations following severe defoliation.

The live crown ratio gives an indication of crown lift and hence the development of dead branches in the lower crown. Approximately 20% of the crown length of unfertilised trees comprised dead branches 12 months after defoliation. This was not influenced by defoliation, suggesting that defoliation was not contributing to premature branch senescence. A different pattern of defoliation (eg. of the lower rather than the upper crown) may have provided a different result. There was no indication from our study that defoliation affected stem properties that may have a bearing on clearwood production, but factors such as tip die-back that were observed may have longer-term consequences, such as development of forks, that were not easily observable over the timeframe of our experiment.

In conclusion, fertilising with N may offer a management tool to eucalypt growers concerned with loss of productivity following defoliation. Because N application appears to improve crown condition and increase leaf production following defoliation, it also has potential as a management tool for reducing the effects of foliar diseases on stem growth. While no adverse effects in terms of increased herbivore browsing were observed from fertilising in this experiment, other studies have noted that applications of N can increase the attractiveness of foliage to some species of herbivore (eg. (Bruyn et al. 2001; Forkner and Hunter 2000; Prudic et al. 2005), through an increase in protein levels and reductions in concentrations of secondary metabolites thought to play a role in plant defence against foliar attack (O'Reilly-Wapstra et al. 2005). Further research is required to examine this for *E. globulus*.

3. Effects of nitrogen nutrition on growth of young *Eucalyptus globulus* Labill. subjected to artificial defoliation

Libby Pinkard, Craig Baillie, Vin Patel, Michael Battaglia, Phillip Smethurst, Caroline Mohammed, Tim Wardlaw and Christine Stone

Introduction

Several studies with *E. globulus* have suggested that removal of more than 20% of leaf area can result in long-term changes in stem growth (Rapley 2005; Smith 2005), although this is likely to be influenced by pattern and frequency of defoliation (Pinkard 2003; Wills et al. 2004). Both browsing mammals (O-Reilly-Wapstra et al. 2002) and a number of insects (Elliott and de Little 1984; Loch and Floyd 2001) defoliate *E. globulus* plantations in Australia and are potential threats to productivity. Operationally and economically feasible methods of minimising growth losses from defoliation in *E. globulus* plantations are required.

Silvicultural options for minimising the effects of herbivore damage in eucalypt plantations are limited. Chemical control is often not economically, environmentally or operationally feasible. It has been established that resistance to browsing is under genetic control (O'Reilly-Wapstra et al. 2005). However that genetic control can break down under different nutrient regimes, suggesting that it will be variable depending on site factors. Deployment of resistant genotypes does not currently offer management solutions for existing plantations.

It has been suggested that alleviation of site stress may indirectly affect responses to herbivory by allowing more rapid crown recovery following a defoliation event (Stone 2001). There is anecdotal evidence (T. Wardlaw, Forestry Tasmania, pers. comm.) that *E. globulus* growing in an ashbed following a hot fire shows less crown damage from foliar pathogens and faster rates of crown recovery than trees growing in adjacent, unburnt soil, suggesting that soil nutrition may play a role in crown recovery. Nutrition is one site factor that is relatively easy to manipulate silviculturally, and it may offer a management option to deal with herbivore damage. There is a strong relationship between nitrogen (N) availability and leaf area development in *E. globulus* (Smethurst et al. 2003) that makes N an obvious nutrient to investigate with respect to crown recovery after browsing, although other nutrients are thought to play a role in plant defence against foliar attack (Lambert and Turner 1977; Marschner 1986).

We undertook a study to test the hypothesis that applications of N would reduce the effect of defoliation on *E. globulus* growth. The specific questions asked were:

- Does defoliation reduce stem growth?
- If so, what level of defoliation reduces growth?
- Does pattern or frequency of defoliation influence responses?
- Does fertiliser application influence responses to defoliation?
- If so, is rate or timing of fertiliser application important?
- Does defoliation or N application influence leaf chemistry?

Because it is difficult to regulate levels of natural defoliation in the field, artificial defoliation was used. The results were used to explore management options.

Methods

Site details

The experiment was established in south-eastern Tasmania at Barnback (43°02' 80''S 146° 46' 40''E). The site is ex-wet sclerophyll eucalypt forest, that was cleared, windrowed and burnt before being ripped and mounded. Mean annual maximum and minimum temperatures and rainfall recorded at the closest Australian Bureau of Meteorology site (~ 20 km away) are 17.0°, 5.8° C and 755 mm, respectively. It has gradational soils over Triassic mudstone with predominantly clay loam topsoils overlying light or light medium clays. *E. globulus* seedlings were planted in September 2003 at 1100 stems ha^{-1}, and were fertilised with 24.7 g nitrogen (N) and 11.3 g phosphorus (P) (as diammonium phosphate) per seedling approximately six weeks after planting. At the start of the experiment, in March 2004, seedlings had a mean height and diameter (15 cm) of 0.92 m and 1.66 cm, respectively.

Treatments and experimental design

Three blocks were selected at the site. Two of these sloped gently to the south east with soil approximately 90 cm deep, and the third sloped gently to the south west with approximately 55 cm soil depth. Within each block, six plots of 30 x 20 m were marked. Each had an internal measurement plot of 25 x 12 m (approximately 30 trees). The N fertiliser treatments outlined in Table 3.1 were allocated randomly to the plots, with one replicate of each fertiliser treatment per block. N was applied as urea. P (as triple superphosphate) was applied across the site, at a rate of 100 kg P ha^{-1}. It was applied into a spade slit either side of the stem and approximately 15 cm from the stem, to about 5 cm depth.

Table 3.1. Pre- and post-defoliation N fertiliser treatments applied in the experiment. Treatments were applied at the plot level.

Treatment No.	**Fertiliser regime (kg N ha^{-1})**		
	Pre-defoliation 1	**Pre-defoliation 2**	**Post-defoliation**
1	0	0	0
2	0	0	300
3	100	0	0
4	100	0	300
5	100	200	0
6	100	200	300

Because the trees were small at the start of the experiment, only 100 kg N ha^{-1} was applied in March prior to the first defoliation event. In N treatments 5 and 6 (table 1), a further 200 kg N ha^{-1} was applied in August prior to the second defoliation event.

The post-defoliation application of N was done in January 2005. The Pre-defoliation 1 application involved applying urea as a surface application in a 15 – 80 cm circle around the tree. The Pre-defoliation 2 and Post-defoliation applications were hand broadcast across plots.

The defoliation treatments applied in the experiment are outlined in Table 3.2. The treatments were randomly applied to trees within plots, and there were three replicates of each defoliation treatment per plot. The autumn 2004 defoliation involved removing either 50% of the tissue of every second leaf (25% total leaf area removed), or 50% of the tissue of three out of four leaves (38% of total leaf area removed). The spring 2004

defoliation involved removing leaf area from 50% of the crown length, by either removing all leaf area from the upper 50% of the crown length plus apical growth from the remaining crown (out-in defoliation), or removing all leaf area from the lower 50% of the crown length, excluding apical growth (bottom-up defoliation). The autumn 2005 defoliation involved again removing all leaf area from the lower 50% of crown length, excluding apical growth. Defoliation did not involve disbudding. It was done using hand snippers.

Table 3.2. Autumn and spring defoliation treatments applied in the experiment.

Treatment No.	Defoliation (% total leaf area)		
	Autumn 2004	**Spring 2004**	**Autumn 2005**
1	0	0	0
2	0	50, out-in (O-I)	0
3	0	50, bottom-up (B-U)	0
4	25	0	0
5	38	0	0
6	25	50, out-in	0
7	25	50, bottom-up	50, bottom-up

Measurements

Stem growth

Tree height was measured prior to application of treatments, and then after 3, 6, 9, 12, 16 and 20 months. Diameter at 15 cm height was measured 3, 6 and 9 months after the start of the experiment. Diameter at 1.3 m height (diameter at breast height, DBH) was measured 9, 12, 16 and 20 months after the start of the experiment. The height to the junction of juvenile foliage with adult foliage was recorded for each measurement time.

Foliar and soils analysis

Leaf samples were collected from plots with nitrogen treatments 1, 3 and 6 (Table 3.1), for trees with defoliation treatments 1 and 7 (Table 3.2), 16 months after the start of the experiment. Approximately 10 leaves were collected per tree, from the upper third of the crown and 3 – 5 leaf pairs from branch tips. The samples were placed onto ice immediately, and frozen at -80° C within two hours of collection. Each leaf was divided into half down the midrib. One half was used for analysis of foliar N and P concentrations. Leaf area was determined using a planimeter (Delta-T Devices, Hoddeston, Herts, UK). Leaves were dried at 65° C for 48 hours, weighed, and ground in a hammer mill. The samples were prepared for analysis using the single acid-hydrogen peroxide technique (Lowther 1980), and total N and P were measured using a continuous flow colorimetric autoanalyser (McLeod 1992). The specific leaf area (SLA) of the leaves (single-sided area:dry mass ratio) was used to convert from weight-based to area-based values.

The second half of each leaf was used for analysis of total phenolics. Samples were prepared and extracted using the method detailed in Hagerman (1995). The modified Prussian blue assay was used (Graham 1992) to determine total phenolics.

A soils description and chemical characterisation was undertaken prior to treatment application. Soil profile descriptions were done in each block by auger boring, mid-block, and soil was sampled by horizon. At three-monthly intervals following the

application of N treatments, 20 0 – 10 cm soil samples were taken from the inter-row of each plot and bulked. For all soil samples, total N and P concentrations were determined by horizon by wet digestion (soil TKN, (Rayment and Higginson 1992). Levels of ammonium (NH_4) and nitrate (NO_3) in solution were determined using KCl and $CaCl_2$ extractions (Lowther 1980). Total soil carbon contents were determined on a plot-wise basis by loss on ignition (LOI).

Data analysis

The relationship between diameter at 15 cm height and DBH was determined for the nine month measurement using linear regression. The equation describing the relationship was used to estimate diameter at 15 cm height for the remaining measurement times. Cumulative diameter (15 cm) and height increment were calculated for each measurement time. Analysis of variance with time as a factor was used to determine the effects of N treatment and defoliation treatment on diameter and height increment. Group linear regression analysis (McPherson 1990) was used to determine differences in slope and intercept of the relationship between diameter or height increment and time, with either N treatment or defoliation treatment as the group. This method provides estimated regression equations under different models where groups are present in the data. The procedure tests the hypotheses that

1. the regression lines have common slopes allowing for the possibility that they have different intercepts; and
2. the same line applies to all groups.

The effect of N treatment or defoliation treatment on the proportion of juvenile and adult foliage, on foliar N, P and total phenolic concentrations, and on soil N and P concentrations and total C, after 16 months was determined using analysis of variance.

Results

Effect of fertilising on soil N and P

(Smethurst et al. 2004) proposed, for *E. nitens*, 6 mg g^{-1} and 3 mg g^{-1} as critical concentrations of total N and P, respectively, and 0.05 mM and 0.1 mM as critical concentrations of NH_4 and NO_3, respectively, in soil solution. Based on these critical values, the surface soil total N and P concentrations observed prior to the start of the experiment suggested that this site would be highly responsive to N application, and that the onset of N deficiency would occur between age 1 and 2 years (Figure 3.1 A-E). LOI values (8.9% +/- 0.9%; $p < 0.05$) indicated that total carbon levels were adequate (Figure 3.1F).

Soil samples taken five weeks post-fertiliser application did not reflect changes in concentrations due to fertilisation. This was a function of sampling strategy. The first fertiliser application was to the base of trees, whilst soil samples were taken within the unfertilised inter-rows. Soil solution levels of both NO_3 and NH_4 at this time were above the critical concentrations suggested by Smethurst et al. (2004), however these concentrations fell to below critical levels at the following sampling 21 weeks after application (3 weeks prior to the second pre-defoliation N application) (Figure 3.1A and C). Samples were again from the unfertilised inter-rows, and although measured soil concentrations were below the critical threshold, all trees had received an application of 125 g $tree^{-1}$ of 20:10:0 at planting, plus 100 kg N ha^{-1} in treatments 3 to 6 as a spot

application. Thus the trees had access to higher soil N and P concentrations at the tree base where the fertiliser had been applied.

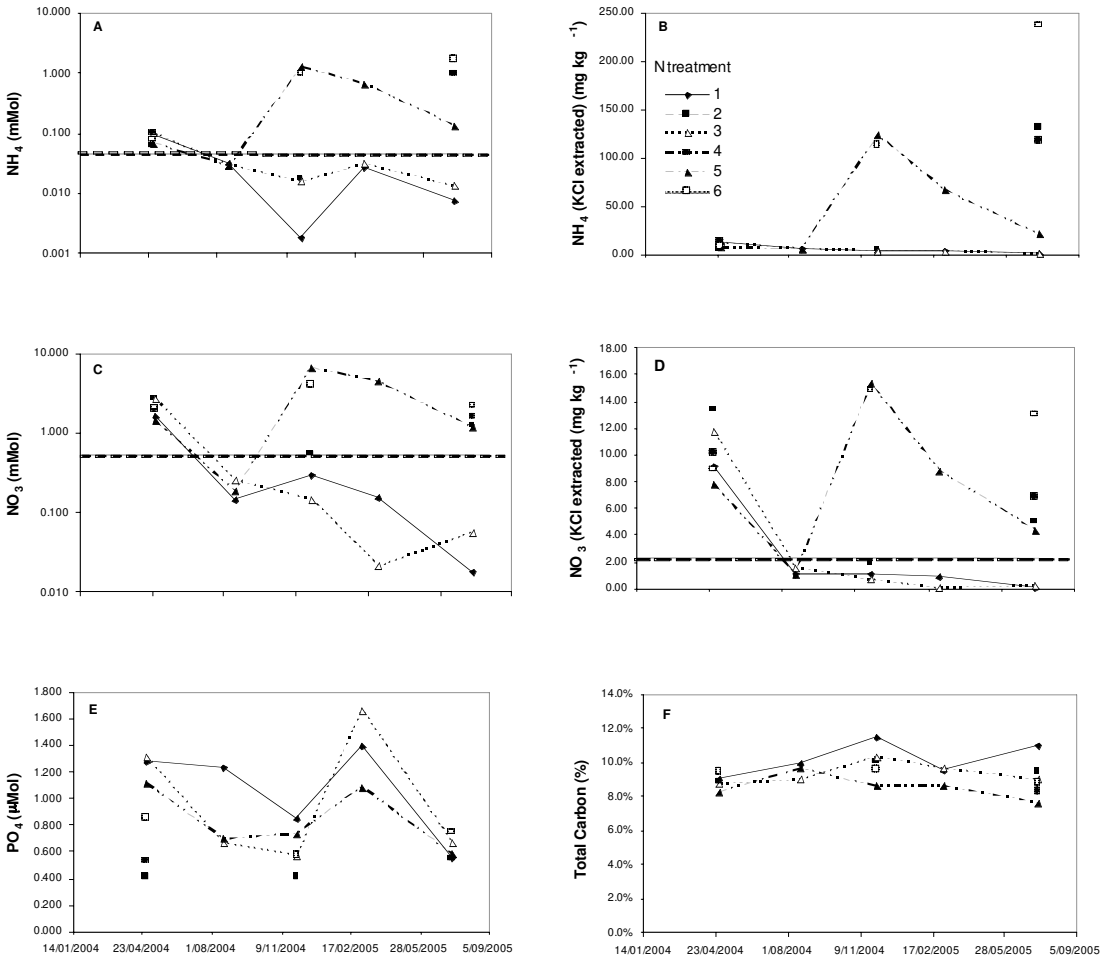

Figure 3.1. Concentrations of NH_4 in soil solution (A) and total (B); NO_3 (C) in soil solution and (D) total; PO_4 in soil solution (E) and total C (F) at Barnback over the first 16 months of the experiment, for the six N treatments (see Table 3.1). Dotted lines indicate critical levels as defined by Smethurst et al. (2004).

As fertiliser in the second pre-defoliation N application in treatments 5 and 6 was broadcast rather than spot-applied, increases in soil N concentrations were observed in these treatments. Increases were also observed in soil samples taken 19 weeks after the post-defoliation application of 300 kg N ha^{-1}.

KCl-extractable NO_3 at the beginning of the experiment was well above the critical concentration defined by Smethurst et al. (2004) of 1.0 mg kg^{-1} (Figure 3.1D). However, at the sampling 3 weeks prior to the second pre-defoliation N application, the concentrations in the unfertilised interrows had fallen to close to the critical concentration. Significant increases in NO_3 concentrations were observed at the next sampling 10 weeks after the second pre-defoliation fertiliser application. Increases also were observed in treatments 15 weeks after receiving the post-defoliation N application. These patterns of change also were observed in KCl-extractable NH_4 (Figure 3.1B).

$CaCl_2$-extracted P was analysed at the time of initial fertilisation. Concentrations across all treatments were 0.725 uM +/- 0.26 uM ($P < 0.05$), close to the critical concentration

proposed by (Mendham et al. 2002) of 0.5 uM (Figure 3.1E). The application of 125 g tree^{-1} of 20:10:0 at planting, plus 50 kg ha^{-1} at trial establishment to all trees should have overcome any potential P-deficiency issues.

Does defoliation reduce stem growth?

In the absence of N application, defoliation reduced both diameter and height increment in the experiment. The slope of the relationship between diameter increment and time was significantly reduced by defoliation treatments 2 (autumn 25%), 4 (spring O-I), 6 (autumn 25%+spring O-I) and 7 (autumn 25%+spring B-U +autumn B-U) (Table 3.3) ($R^2 = 0.91$, $P < 0.001$). The slope of the relationship between height increment and time was significantly reduced by defoliation treatments 3 (autumn 38%), 4 (spring O-I), 6 (autumn 25% + spring O-I) and 7 (autumn 25%+spring B-U+autumn B-U)($R^2 = 0.89$, $P < 0.001$), suggesting that the growth trajectories over time may be changed.

Table 3.3. Slope and intercept of the relationship between diameter or height increment and time for *E. globulus*, for the seven defoliation treatments in unfertilised plots. The relationship is of the form: increment = a + b ^{x} time. * indicates significantly different from the control (* $P < 0.05$, ** $P < 0.01$).

Treatment	Intercept (a)	Slope (b)	R^2	P
Diameter increment				
Control	1.58	4.18	0.91	< 0.001
Autumn 25%	2.91	3.64*		
Autumn 38%	2.15	3.71		
Spring out-in	3.43	3.48*		
Spring bottom-up	2.63	3.96		
Autumn 25%, spring out-in	3.15	3.41**		
Autumn 25%, spring bottom-up, autumn bottom-up	2.77	3.47**		
Height increment				
Control	-22.2	27.0	0.89	< 0.001
Autumn 25%	-21.9	24.5		
Autumn 38%	-17.6	22.6*		
Spring out-in	-13.4	23.4*		
Spring bottom-up	-20.0	25.6		
Autumn 25%, spring out-in	-14.3	21.4**		
Autumn 25%, spring bottom-up, autumn bottom-up	-18.3	22.9*		

What level of defoliation reduces stem growth?

A comparison between two levels of defoliation was undertaken with the autumn 2004 defoliation (treatments 2 and 3). In the absence of fertilising, removal of 25% or 38% of leaf area from throughout the crown in the first autumn of growth reduced both diameter and height increment to a similar level over the course of the experiment (Figure 3.2). Height increment was more affected than diameter increment, with defoliation reducing mean height increment by 17% 20 months after treatment.

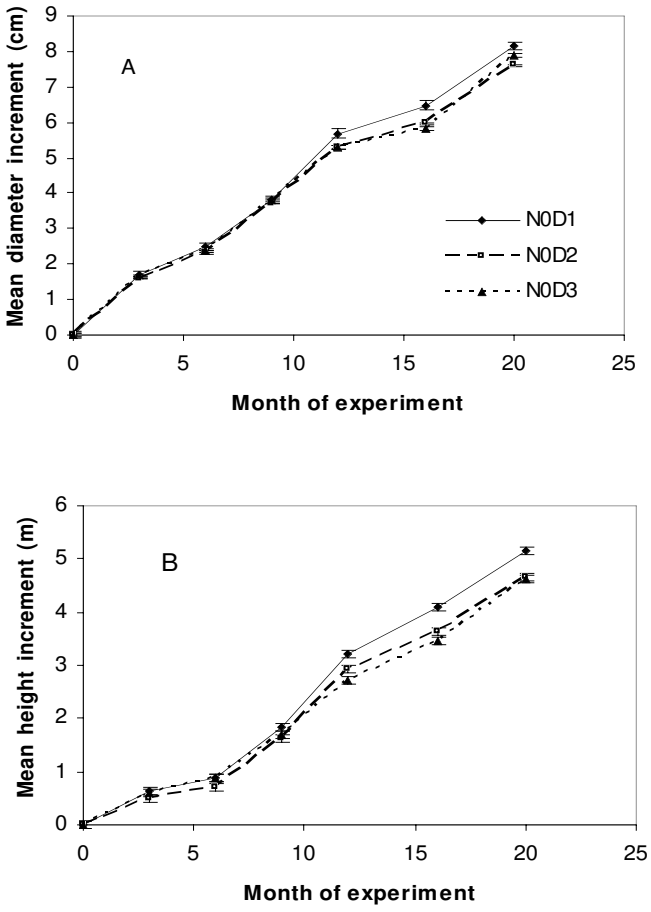

Figure 3.2. (A) mean diameter increment and (B) mean height increment of unfertilised *E. globulus* defoliated in autumn 04 to remove 0% (D1), 25% (D2) or 38% (D3) of leaf area from throughout the crown. Error bars indicate standard errors ($P < 0.001$).

Does pattern of defoliation influence growth responses?

Three patterns of defoliation were examined in the experiment: leaf removal throughout the crown in autumn (treatment 2), and either spring out-in (treatment 4) or spring bottom-up (treatment 5) defoliation. The analysis of pattern of defoliation was only done on the spring out-in and bottom-up data to remove timing of defoliation as a potential confounding factor.

In the absence of fertilising, a single bottom-up defoliation event had no effect on diameter or height increment (Figure 3.3). In contrast, spring out-in defoliation had reduced diameter increment by 12% and height increment by 17% compared to the control at the end of the experiment.

Does frequency of defoliation influence growth responses?

This question was examined by comparing growth responses of unfertilised trees that had been defoliated (a) once or twice (defoliation treatments 4 and 6) or (b) once or three times (defoliation treatments 4 and 7) with those of undefoliated trees. The first

comparison had out-in defoliation as the second defoliation event, and frequency of defoliation affected both diameter and height increment in this comparison (Figure 3.4 A, B). In the first comparison, mean diameter increment was reduced by 14% by both defoliation treatments compared to the control (Figure 3.4A). Mean height increment was reduced over the 20 months of the experiment by 15% and 28% for the 1 and 2-defoliation treatments, respectively (Figure 3.4B). The second comparison had bottom-up defoliation as the second and third defoliation events (Figure 3.4C, D). Single defoliation had no significant effect on diameter or height increment, but triple defoliation reduced diameter increment by 15% and height increment by 26% at the end of the experiment. Despite the greater frequency of defoliation in the bottom-up than the out-in defoliation treatments, multiple defoliation involving out-in defoliation had a greater effect on stem growth than did multiple defoliations involving bottom-up defoliation.

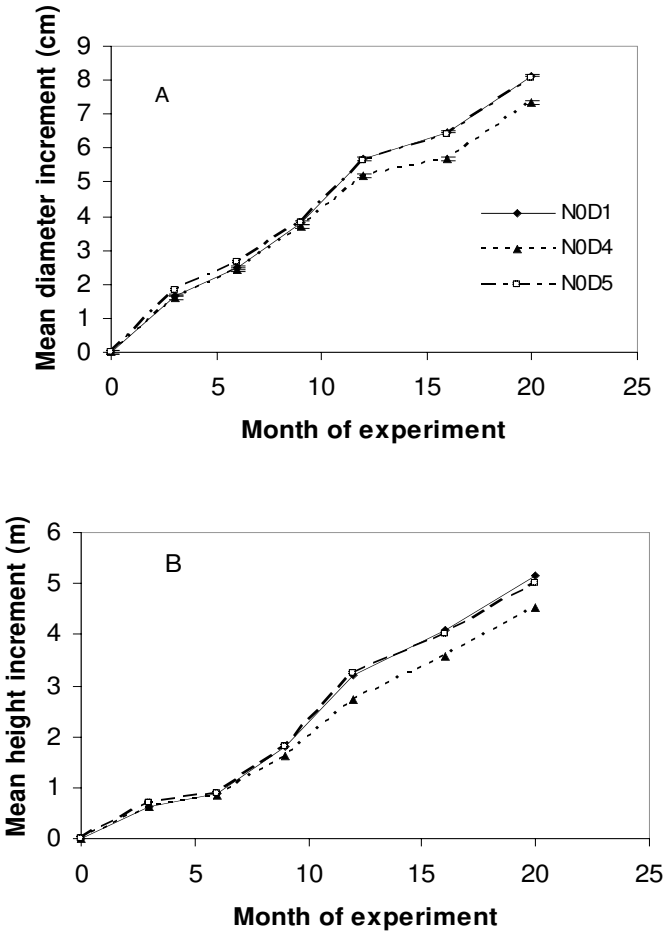

Figure 3.3. (A) mean diameter increment and (B) mean height increment of unfertilised *E. globulus* defoliated either in an out-in pattern (D4) or a bottom-up pattern (D5) in spring 05. D1 is the undefoliated control. Error bars indicate standard errors ($P < 0.001$).

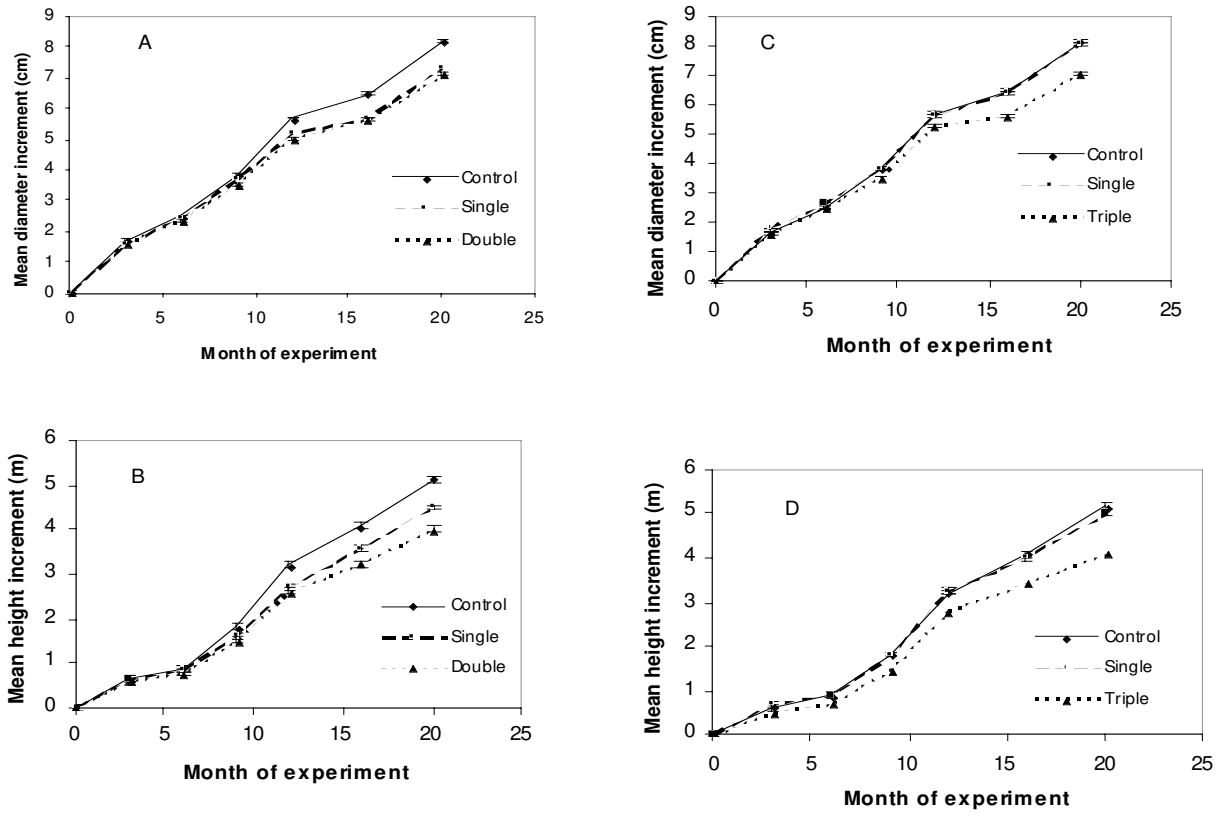

Figure 3.4. The effect of frequency of defoliation on mean diameter (A, C) and height (B, D) increment of *E. globulus*. Results are presented for control trees (D1); trees experiencing either a single (D4) or double (D6) defoliation including spring out-in defoliation (A, B) ; and for trees experiencing either a single (D4) or triple (D7) defoliation including spring bottom-up defoliation (C, D). Error bars indicate standard errors ($P < 0.001$).

Did fertilising affect growth at this site?

Mean diameter increment of undefoliated trees was 7% greater, and mean height increment was 7% less after 20 months, in plots where 300 kg ha^{-1} N had been applied at the start of the experiment than in unfertilised plots ($P < 0.05$) (data not presented). The slopes and intercepts of the relationship between diameter or height increment and time did not change as a result of N treatment (Table 3.4).

Table 3.4. Slope and intercept of the relationship between diameter or height increment and time for *E. globulus*, for undefoliated trees in the six N treatments. N treatment had no effect on the slope or intercept of the relationship. The relationship is of the form: increment = $a + b^{x}$ time

Variable	Intercept (a)	Slope (b)	R^2	P
Diameter increment	1.89	4.20	0.95	<0.001
Height increment	-20.82	25.33	0.91	<0.001

Does rate of fertiliser application influence responses to defoliation?

In order to answer this question, the most severe single defoliation treatment (treatment 4, spring O-I) was compared with the undefoliated treatment. Pre-defoliation fertiliser application (100 or 300 kg ha^{-1}) significantly increased diameter increment of defoliated trees compared to unfertilised, defoliated trees (Figure 3.5A). Trees fertilised with 300 kg ha^{-1} N had a mean diameter increment 6% greater than unfertilised, undefoliated trees at the end of the experiment ($P < 0.05$). Fertilising improved height increment of defoliated trees compared to that of undefoliated trees, but height increment was still 15% lower than that of unfertilised, undefoliated trees at the end of the experiment (Figure 3.5B).

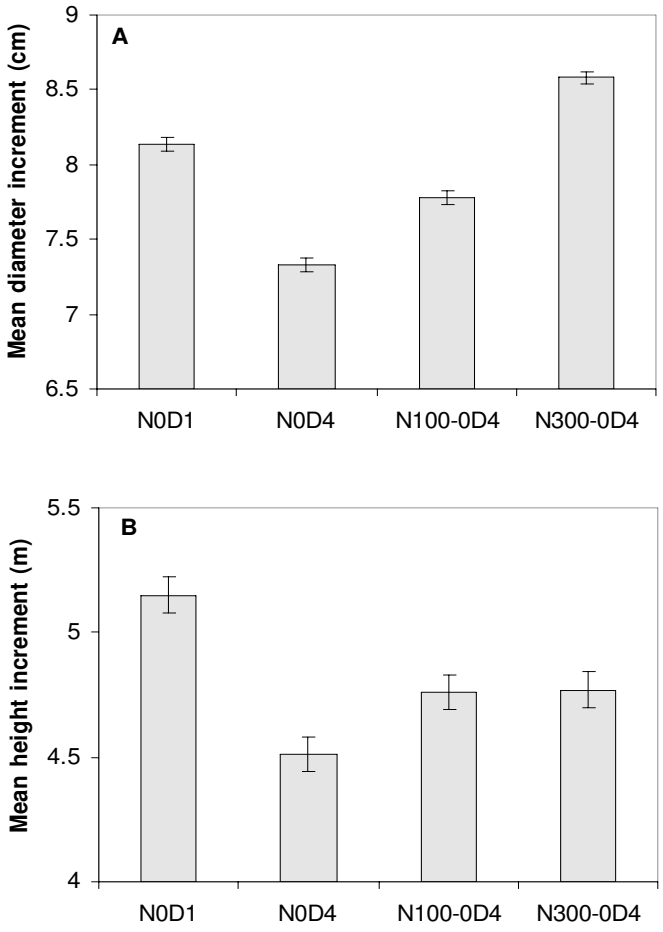

Figure 3.5. Effect of applications of 100 or 300 kg ha^{-1} N to undefoliated *E. globulus* and trees experiencing spring out-in (D4) defoliation, on (A) mean diameter increment and (B) mean height increment. D1 is the control. Error bars indicate standard errors ($P < 0.001$).

Is pre- or post-defoliation fertiliser application preferable?

Timing of fertiliser application with respect to timing of defoliation had an effect on diameter increment (Figure 3.6A). Pre-defoliation applications of N to trees in the spring out-in defoliation treatment increased diameter increment and height increment compared to that of post-defoliation applications of N (Figure 3.6B).

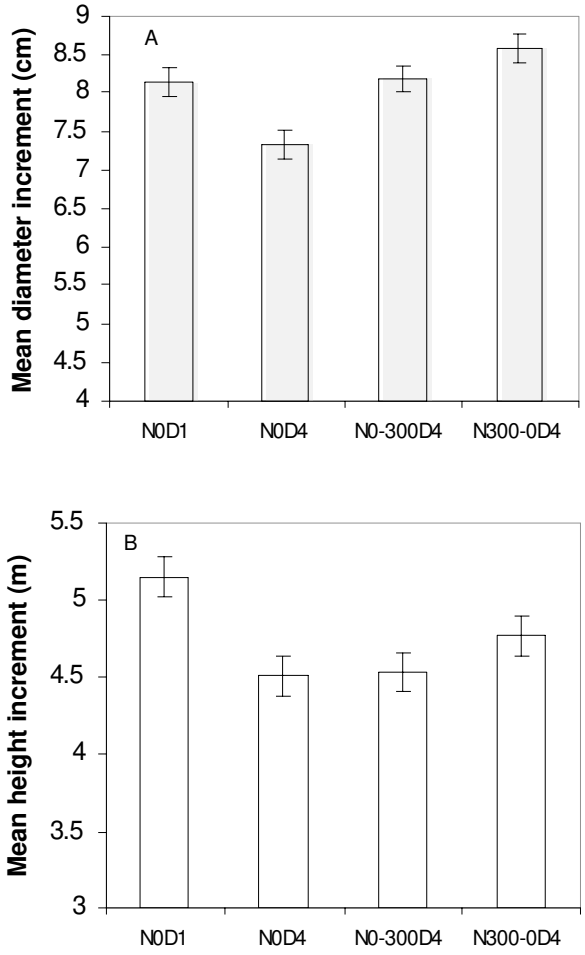

Figure 3.6. Effect of pre- or post-defoliation applications of N on *E. globulus* diameter increment (A) or height increment (B). D1 is the control, and D4 is spring out-in defoliation. Error bars indicate standard errors ($P < 0.001$).

Influence of fertiliser and defoliation on foliar chemistry

Foliar N concentrations 16 months into the experiment increased with increasing rate of N application (Table 3.5), and were 20% greater in N treatment 6 than in the control ($P < 0.05$). This pattern was not observed in response to increasing N where trees had been defoliated. In N treatment 6, defoliated trees had 25% less foliar N than undefoliated trees ($P < 0.05$). Foliar P concentrations were unaffected by N treatment or defoliation treatment.

Total phenolic concentrations were significantly influenced by N treatment ($P < 0.05$). Where N had been applied, total phenolics were between 17 and 48% lower than in unfertilised plots (Table 3.5).

Influence of fertiliser and defoliation on crown characteristics

No adult foliage was present until approximately 12 months into the experiment (tree age 19 months). The proportion of crown length in adult foliage was significantly increased by early applications of N ($P < 0.05$) (Figure 3.7). Later applications of N had no significant effect on the proportion of adult foliage. Both 100 and 300 kg N ha^{-1} resulted in a similar increase in the proportion of the crown length in adult foliage. Defoliation treatment did not influence the ratio of adult to juvenile foliage.

Table 3.5. Foliar nitrogen (N) and phosphorus (P) concentrations measured at Barnback in response to applications of N and artificial defoliation. The N and defoliation treatments are detailed in Tables 3.1 and 3.2. Different letters denote significant differences from the control ($P < 0.05$).

N Treatment	Defoliation treatment	N (g m^{-2})	P (g m^{-2})	Total Phenolics (mg g^{-1})
1 (control)	1 (control)	4.17[a]	0.21[a]	14.40[a]
	7 (bottom-up x 3)	3.90[a]	0.22[a]	13.04[a]
3 (100/0/0)	1 (control)	4.20[a]	0.24[a]	6.53[b]
	7 (bottom-up x 3)	4.57[a]	0.25[a]	7.54[b]
6 (100/200/300)	1 (control)	4.99[b]	0.25[a]	12.64[a]
	7 (bottom-up x 3)	3.98[a]	0.20[a]	10.01[b]

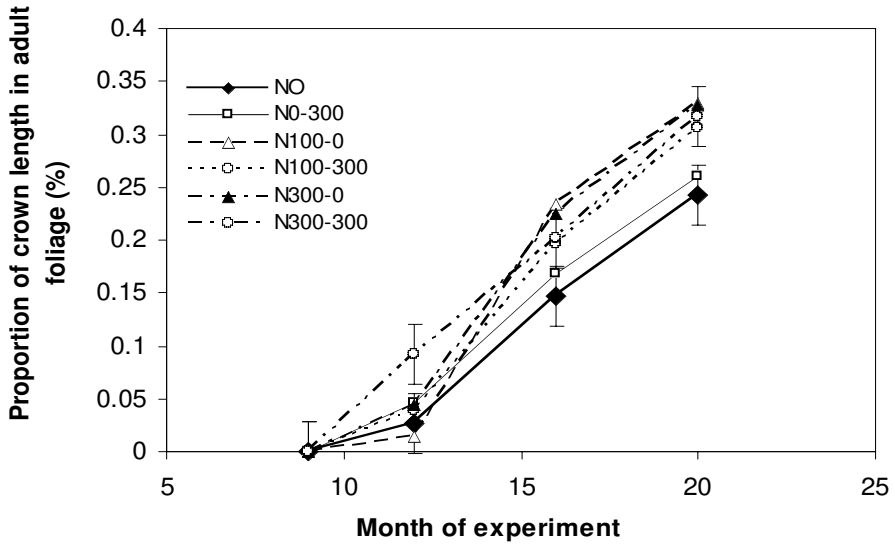

Figure 3.7. Proportion of the crown length in adult foliage (%) in the six N treatments at Barnback. Error bars indicate least squares standard errors ($P < 0.05$).

Discussion

This experiment has demonstrated that fertilising with nitrogen can help *E. globulus* maintain stem growth following defoliation, even on a high productivity site. Given that there are often financial and environmental constraints on controlling or preventing attacks by pests and pathogens in eucalypt plantations, this finding offers an operationally-feasible management option to forest growers.

The first defoliation treatment in the experiment was applied at a very young age (7 months). As has been found with other studies of pre-canopy closure defoliation of plantation eucalypts, removal of even 25% of leaf area reduced stem growth (Pinkard 2003; Rapley 2005; Smith 2005). Post-canopy closure defoliation has been found in other studies to have less effect on stem growth than pre-canopy closure defoliation (Pinkard and Beadle 2000), but our results suggest that this is likely to be influenced by the pattern of defoliation. Other studies also have concluded that pattern of defoliation

is important in determining growth responses to defoliation (Collett and Neumann 2002), and bottom-up defoliation has generally been found to have less effect than removal of leaves from the upper crown. The lack of response to bottom-up defoliation in this study reflects the very rapid growth rates and concomitant crown development that occurred over the period of the experiment. On a slower-growing site bottom-up defoliation may have a greater effect on stem growth.

It is not surprising that more frequent defoliation events increased the effect of defoliation on stem growth, because crown recovery time is more restricted as the frequency of defoliation increases. Continuous defoliations over a period of months, or more than one defoliation event in a year, are realistic scenarios that it is difficult to mimic with artificial defoliation studies. However the result suggests that more frequent defoliation events should be of more concern to forest managers than less frequent or one-off defoliation events. It also appears that *E. globulus* can withstand more frequent defoliation if the pattern is bottom-up rather than out-in.

While fertiliser application did not affect the growth of undefoliated trees in this experiment, the fertiliser response following defoliation suggests that there was an N limitation developing at the site. This was supported by the soil N concentrations measured during the experiment. The extra N applied allowed trees that had been defoliated to rapidly rebuild their crowns, as illustrated by the increase in diameter increment compared to the diameter increment of defoliated but unfertilised trees. There is a strong relationship in many species between stem diameter or sapwood cross-sectional area and leaf area (Shinozaki et al. 1964a; Shinozaki et al. 1964b) that has also been demonstrated for *E. globulus* (Pinkard 2003). Hence greater diameter increment suggests greater leaf area development.

The higher foliar N concentrations that we observed may have resulted in increased photosynthetic rates that would have influenced growth rates. It has been observed in many studies that there is a strong relationship between photosynthetic rate and foliar N concentration (Evans 1989) although this may not be as strong in *E. globulus* (Close et al. 2004). The lower foliar N concentrations in defoliated compared to undefoliated trees suggest that defoliation affected canopy N reserves, and may have affected the capacity for nutrient uptake in defoliated trees.

There was an indication that a higher rate of N application had a greater effect on stem growth following defoliation. This difference may become more obvious as the trees age and site resources become more limited. Similarly, it would be expected that, on sites with an N limitation, applying a higher rate of N would result in greater stem growth following defoliation than would lower levels of N application.

There was a clear indication from our study that pre-defoliation applications of N resulted in greater stem growth following defoliation than did post-defoliation applications. This suggests that sites with an inherently greater N availability will be better able to withstand defoliation events. Sites at high risk of defoliation could be managed to maintain high available N. However if this is not feasible, our results indicate that there is a response to post-defoliation N application, particularly in diameter increment.

There is evidence from other studies that phase change from juvenile to adult foliage in *E. globulus* is under strong genetic control (Jordan et al. 1999). Our results suggest that phase change may also be influenced by environmental factors. Williams et al. (2004) also concluded that environment plays a role in governing phase change in *Eucalyptus nitens*. While further research is required to clarify this in *E. globulus*, fertilising with N offers a possible management tool when dealing with defoliators that target juvenile foliage.

There have been many studies of the relationship between fertiliser application and subsequent attractiveness to pests and pathogens (eg.Bruyn et al. 2001; Cipollini et al. 2002; Prudic et al. 2005). Two main hypotheses have been formulated to explain this relationship: the plant stress hypothesis that proposes that physiologically stressed plants become more susceptible to defoliation, and the plant vigour hypothesis that proposes that plants that grow vigorously are more susceptible to defoliation (Bruyn et al. 2001). Many studies have found in favour of the second hypothesis, concluding that plants that have been fertilised are more susceptible to attack by herbivores or foliar pathogens (Forkner and Hunter 2000; Lower and Orians 2003; O'Reilly-Wapstra et al. 2005; Prudic et al. 2005; Rekhi et al. 2004), but some have also found in favour of the first hypothesis (Fox and Morrow 1992; Stone 2001). The decrease in total phenolics and increase in foliar N concentration that we observed may indicate that fertilising will increase risk of attack from some foliar pests, but this requires further research. However, given that the objective of growing eucalypt plantations is generally to maximise growth, and hence vigour, then an important question is whether crowns can be restored following a defoliation event so that vigour can be maintained, irrespective of whether there is an increased risk of future defoliation events. Our experiment has determined that this is the case. Research by O'Reilley-Wapstra et al. (2005) found that fertilising with N overcame genetic resistance to browsing by the mammal *Trichosurus vulpeculaf* which targets very young *E. globulus* plantations. This highlights the complex nature of herbivore/nutrient interactions, and suggests that care must be taken when identifying the appropriate timing of fertiliser application with respect to moderating the effects of browsing, so that browsing problems are not compounded.

In conclusion, artificial defoliation studies do not necessarily mimic what occurs in the real world, but they can provide valuable insights into processes that can then be used to develop management strategies. Because it is very difficult to control levels of defoliation or infection from foliar pathogens in the field, artificial defoliation studies are useful in the development and validation of forest growth models.

4. Can fertilisation with nitrogen and phosphorus assist in the recovery of *Eucalyptus globulus* after a *Mycosphaerella* leaf disease epidemic?

Timothy J. Wardlaw

Introduction

Infection by *Mycosphaerella nubilosa* resulting in necrotic leaf lesions and defoliation of juvenile foliage on *E. globulus* is called Mycosphaerella leaf disease (MLD). Young *E. globulus* plantations in the Circular Head area of north-western Tasmania are at high risk of severe MLD. An MLD epidemic in 2001 resulted in severe defoliation (>50% leaf area loss) of nearly half of the 1-year-old plantations (Forestry Tasmania, unpublished health surveillance records). Such epidemics are likely to occur at regular intervals in that area.

Measurements made in one plantation that had suffered severe defoliation following the 2001 MLD epidemic indicated that the trees planted in windrows of heaped and burnt logging debris were much less affected by MLD than trees growing in the intervening bays (Wardlaw 2002). Trees growing in the bays between windrows had progressed to chronic injury in response to MLD. This was typified by the premature loss of leaves in the lower crown and greatly reduced apical growth resulting in trees developing rounded crowns. Very few trees in the bays had by gone through the phase change from MLD-susceptible juvenile to MLD-resistant adult foliage by age 2. By contrast trees growing windrows had maintained vigour despite defoliation by MLD. These windrow trees retained leaves in the lower crown, had strong apical growth and a high proportion had progressed through the phase change from juvenile to adult foliage.

The progression from acute to chronic injury in response to MLD is likely to result in severe adverse growth impacts in terms of both the extent and duration growth retardation. The delayed progression from juvenile to adult foliage is likely to further compound the adverse impact of MLD by exposing the trees to an extended susceptible period.

Nutrient supply is likely to be a major difference between trees growing in windrows and those growing in the bays. The greater nutrient reserves in the windrows may allow trees to maintain vigour during MLD epidemics and escape chronic injury. If supplemental fertiliser application in the bays can duplicate this windrow-effect it may be a more viable alternative for minimising adverse impacts of MLD than fungicide protection.

Routinely, secondary fertiliser application, when required, is applied during autumn when trees are about 2½ years-old. However, secondary fertiliser application at this age may be too late for trees growing in nutrient-limited soils to avoid chronic injury after MLD epidemics. Observations in made in Christmas Hills compartment 32E (planted spring 2000) could detect little visual difference between windrow and bay trees during autumn of the first growing season (mid-March 2001). However, noticeable visual differences between windrow and bay trees were observed by spring at the beginning of the second growing season (after an MLD epidemic in winter 2001). These field observations suggest that fertiliser applications may need to commence in spring at the

beginning of the second growing season if chronic injury after MLD epidemics is to be avoided.

This study investigates whether spring or autumn application of nitrogen and phosphorus, either just before or just after an MLD epidemic can maintain growth rates and prevent affected trees from progressing to chronic injury.

Materials and methods

Site

The study was done in Christmas Hills compartment 33B (40°54′60″ S 144°59′50″ E) situated about 20 km west of Smithton, in northwestern Tasmania (Figure 1). The soil at the site is a brown dermosol (type 6.2 in Grant *et al.* 1995) and the site slopes gently from east to west. Site preparation prior to planting involved stump removal, pushing logging debris into windrows, burning the windrows and finally deep ripping and mound ploughing the planting rows. Pre-plant herbicide application was done on 5th October 2001 using Round-up Bio-active® (3 litres ha^{-1}), Brushoff® (20 g ha^{-1}) and the penetrant, Pulse® (200ml 100 $litres^{-1}$ water) applied using a tractor-mounted sprayer. The compartment was planted in November 2001 with *E. globulus* containerised seedlings originating from "Red" and "4.3(1)" seedlots of Bawden's seedorchard. The seedling were planted at a density of 1086 stems / ha^{-1} with an espacement of 2.3 metres (along rows) x 4.0 metres (between rows). A post-planting herbicide application was done between December 2002 – January 2003 using a tractor-mounted shrouded sprayer. Round-up® (3.5 litres ha^{-1}), Brush-off® (50 g ha^{-1}) and the surfactant Freeway® (200ml 100 $litres^{-1}$ water) were sprayed between the planting mounds, and Lontrel (5 litres ha^{-1}) with the surfactant Freeway® (100ml 100 $litres^{-1}$ water) was sprayed on the planting mounds. An aerial spraying operation was done on 17th December 2002 to control an above-threshold population of the leaf beetle *Chrysophtharta agricola* (Coleoptera: Chrysomelidae) using the insecticide Fastac-duo® (250 ml product ha^{-1}).

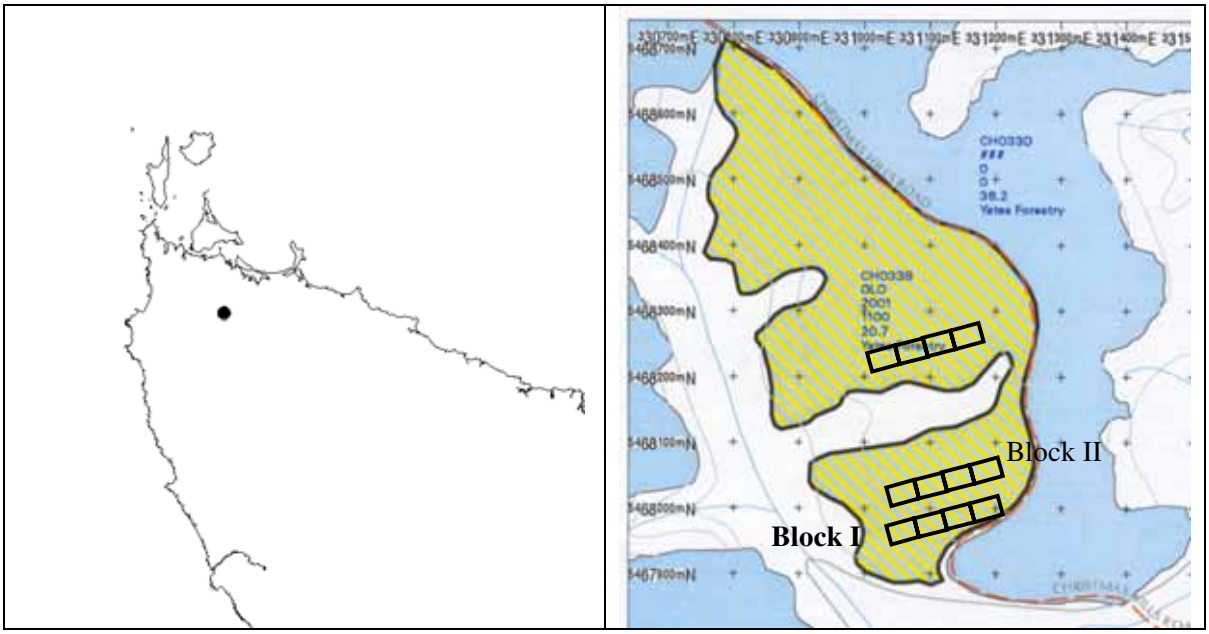

Figure 4.1. Location of the study site (Christmas Hills compartment 033b) showing the layout of the 12 plots in three blocks each of four plots.

There was a severe epidemic of MLD in the general area in the 2001-2 growing season. The study site escaped this epidemic because it had not been planted until after the peak period of the epidemic, which occurred during winter-spring 2001. The study site was, however, exposed to a smaller MLD epidemic in winter – early spring at the beginning of the 2002-3 growing season.

Design of trial

The trial was set out in three replicates (Blocks I-III in Figure 4.1) each containing four 88-tree plots comprising 8 rows of 11 trees. Each plot within a replicate was randomly allocated one of four treatments:

(i) Spring fertilising at age 1 year (November 2002).
(ii) Autumn fertilising at age 1.5 years (April 2003).
(iii) Spring fertilising at age 2 years (November 2003)
(iv) Autumn fertilising at age 2.5 years (April 2004)

The fertiliser treatments involved applying 270 g triple superphosphate (= 70 kg $P.ha^{-1}$) and 180 g. urea (= 100 kg $N.ha^{-1}$) by hand around the base of each tree in the treatment plot. One year after the initial fertiliser application supplementary fertilising of $100kg.ha^{-1}$ of urea was done for each treatment for the duration of the trial.

In treatments (i) and (ii) the fertiliser was applied before the small MLD epidemic that occurred in winter-spring 2003. In these two treatments the trial was testing the effectiveness of the fertiliser treatments as a preventative / protective measure to help the trees escape the growth impacts resulting from the MLD epidemic. In treatments (iii) and (iv) the fertiliser was applied after the winter-spring MLD epidemic. In these two treatments the trail was testing whether spring or autumn fertiliser treatments were effective as remedial treatments to help the trees recover after the epidemic.

Measurement of growth and crown condition

Each 88-tree plot contained an internal measurement sub-plot of 24 trees comprising four rows each of six trees. This provided a treated buffer of two rows either side of the measurement sub-plot and three trees at either end of the measurement sub-plots.

Measurements of total tree height and stem diameter were done. Stem diameter measurements were done initially at 10 cm above ground level and then at breast height (DBH) once trees were large enough. Height was measured using a height pole and diameter using calipers (10 cm above ground level) or diameter tape (DBH). Measurements were done twice yearly commencing November 2002 and concluding January 2005. A further measurement of height and diameter was done in May 2005 to detect whether or not there was a growth response to fertilisation. An additional plot, configured in the same way at the treatment plots, was appended to the western end of each of the three blocks. These three plots were all in unfertilised sections of the compartment and thus represented the base condition from which a fertiliser response could be detected.

The measurement of defoliation assessment used four methods applied at different times throughout the trial. This was because certain crown assessment methods can only be practically applied at particular stages of crown development. For example the crown damage index (*sensu* Stone *et al.* 2003) is only designed for 1-2 year-old trees, which still have conical crowns. The defoliation measurements used are summarised in Table 4.1.

Foliar and soil nutrient levels

In June 2004 a pole pruner was used to remove a 30-cm long shoot from the upper 1/3 of the crown of five randomly selected trees in each plot. Fully expanded leaves from the current growing season were stripped from each shoot and bulked to give a single sample for each of the 12 plots. Leaves were oven-dried at 65°C, ground to pass through a 2mm sieve, digested in sulphuric acid and hydrogen peroxide and then analysed for total nitrogen and phosphorus by flow injection analysis using a Lachat Instruments QuickChem 8000.

In May 2005 a soil auger was used to obtain a soil sample of the top 15 cm of the soil profile in the centre of each of the 15 trial plots (12 fertilised plots plus three unfertilised plots). Soil samples were oven dried and 65°C, ground and sieved through a 2 mm sieve.

Data analysis

Analysis of variance of plot-mean data for each independent variable was used to test the significance of differences among the design (block) and treatment (fertiliser timing) effects. The independent variables chosen for analysis were: height; diameter (10cm and breast height), defoliation and proportion of trees that had started producing adult phase foliage. ANOVA was also used to test the significance of differences among blocks and fertiliser treatments in foliar nitrogen and phosphorus concentrations. All ANOVAs were done using plot mean data. The significance of differences among treatment comparisons was tested using a least significant difference range test. Separate analyses were done for each remeasurement and the resultant treatment means were plotted as time-series to visually inspect the whether any of the treatments resulted in differing growth trajectories.

Table 4.1. Summary of the defoliation measurements done at each of the five remesurements of the fertiliser timing trial.

Assessment date	Defoliation measure	Details of measure
Nov 2002	Crown damage index (CDI)	An ocular assessment of: (i) volume of defoliation (complete or partial removal of leaf tissue); (ii) volume and severity of necrotic leaf lesions; (iii) volume and severity of leaf discolouration. Each of these assessments were aided by photographic visual standards. The crown damage index was calculated from these assessments using the formula given in Stone *et al.* (2003).
Jun 2003	Crown damage index (CDI)	
Nov 2003	Volume of top-down and bottom - up defoliation	Total tree height (h); height to bottom of refoliation after top-down defoliation (h_{br}); height to bottom of top-down defoliation (h_{bd}); height to top of bottom-up defoliation (h_{td});Crown width across rows (d_1); crown width along rows (d_2). These measurements were used to calculate four conic volumes (Figure 2): total crown volume; volume of top-down defoliation; volume of refoliation; volume of bottom-up defoliation. Total defoliation was calculated as the sum of top-down and bottom-up defoliation as a percentage of total crown volume.
Jun 2004	Volume of top-down and bottom-up defoliation	Separate assessments of top-down and bottom-up defoliation. Assessments used an ocular estimate of defoliation made with the assistance of diagrammatic visual standards. Total defoliation was calculated as the sum of top-down and bottom-up defoliation.
Jan 2005	Crown lifting	Height to base of live crown as a percentage of total tree height

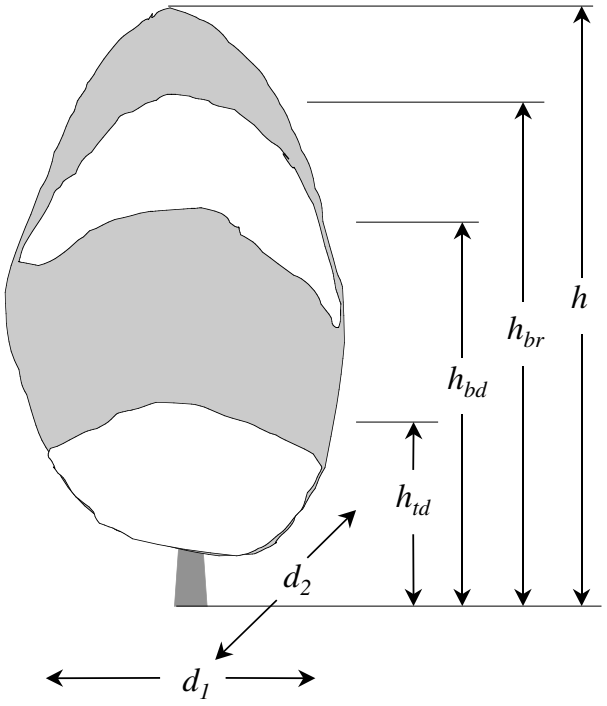

Figure 4.2. Diagram showing the measurements made in the November 2003 remeasurement to determine the volume of top-down and bottom-up defoliation (see Table 4.1 for explanation of the codes).

Results

Defoliation

There were no differences in the severity of defoliation among the three blocks until the November 2003 remeasurement when trees in block II suffered significantly ($F_{2,9}$ = 5.51, MSE = 7.92, Pr = 0.027) more defoliation than the other two blocks. In June 2004 the defoliation was the reverse of the situation at November 2003 remeasurement with trees in block II having significantly less defoliation than trees in block III. By the final defoliation assessment in January 2005 trees in block III had significantly ($F_{2,9}$ = 4.55, MSE = 10.90, Pr = 0.043) more bottom-up defoliation than the other two blocks.

The time series of average defoliation among each of the four treatments all followed a parallel trajectory and clearly show the peak period of MLD activity between the May 2003 and November 2003 remeasurements (Figure 4.3). There were no significant differences in the average overall defoliation (top-down and bottom-up) among the four treatments at any time during the trial. However, there was a significant difference ($F_{3,11}$ = 4.2, MSE = 2.63, P = 0.046) in the amount of top-down defoliation after the winter-spring 2003 MLD epidemic: Trees receiving the spring 2002 fertiliser treatment suffered 17-37% more top-down defoliation than the later fertiliser treatments. Despite this, the amount of top-down defoliation as a result of the winter-spring 2003 MLD epidemic was <20% across all of the treatments. Trees in plots that were fertilised with N and P prior to the winter-spring 2003 MLD epidemic did show greater crown

recovery immediately after the epidemic than trees in plots that were not fertilised until after the epidemic (Figure 4.3). However, these differences in crown recovery were not statistically significant.

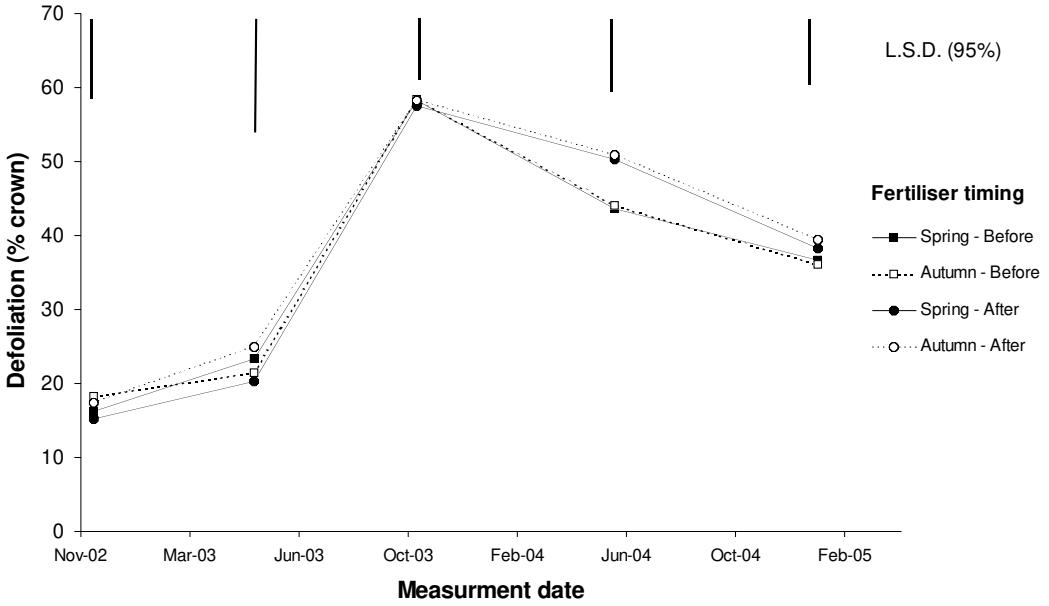

Figure 4.3. Time series of average defoliation (total) between November 2002 and January 2005 of trees in each of four fertiliser timing treatments.

Height growth

There were initially no differences in tree height among the three blocks. However, by the second measurement (May 2003) tree heights were significantly lower ($P<0.01$) in Block III than the other two blocks, a difference that persisted for the duration of the trial. Average (plot) tree height did not differ significantly among the four fertiliser timing treatments at any time over the duration of the trial (Figure 4.4).

At the time of the final remeasurement in May 2005, trees in the unfertilised plots adjoining the fertiliser timing trial were 13-21% shorter than trees in the fertilised plots. However, these differences in tree height were not statistically significant.

Diameter growth

Paralleling the differences in height growth among the three blocks, the average DBH of trees in block III were smaller than the other two blocks for the duration of the trial. This block effect intensified over the course of the trial so that by the last two remeasurments the differences in DBH between block III and the other two blocks were statistically significant ($P<0.01$ or less). Average DBH did not differ significantly among the fertiliser timing treatments at any time over the duration of the trial (Figure 4.5).

Trees in the unfertilised plots adjacent to the fertiliser timing trial had 20-30% smaller stem diameter than trees in the fertilised plots at the final remeasurement in May 2005. Overall these differences in diameter were not significant although in individual treatment contrasts, trees in the unfertilised plots had significantly ($P<0.05$) smaller diameters than trees that were fertilised in the autumn 2003.

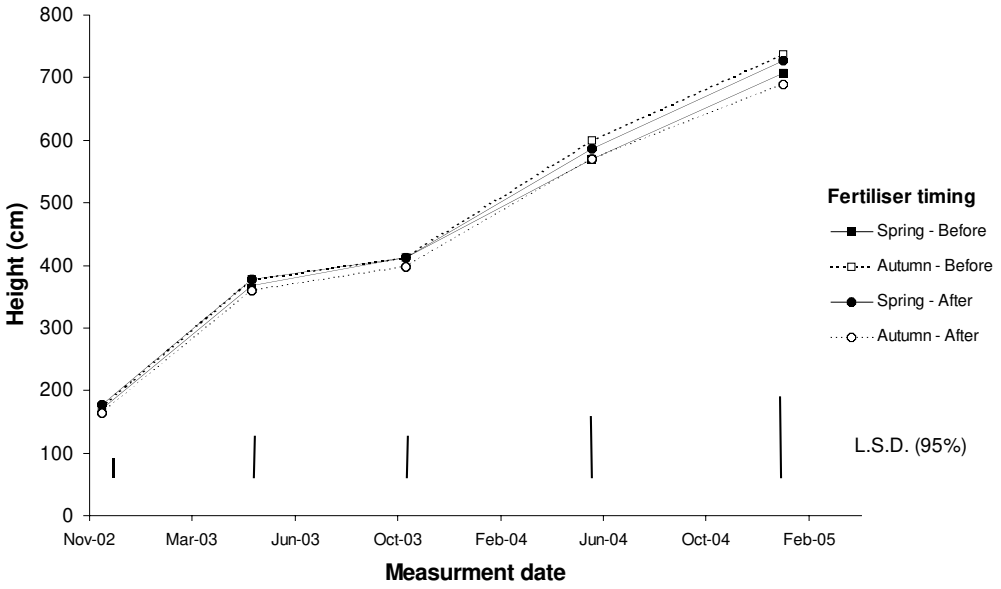

Figure 4.4. Time series of average height between November 2002 and January 2005 of trees in each of four fertiliser timing treatments.

Phase change to adult foliage

Trees in block II began phase change earlier than the other two blocks and had a significantly higher ($P<0.01$) proportion of trees beginning to produce adult foliage at the May 2003 remeasurement. However, by the November 2003 remeasurement differences in the proportion of trees producing adult foliage among blocks were not significant. There were no differences among fertiliser timing treatments in the timing of the phase change to adult foliage (Figure 4.6).

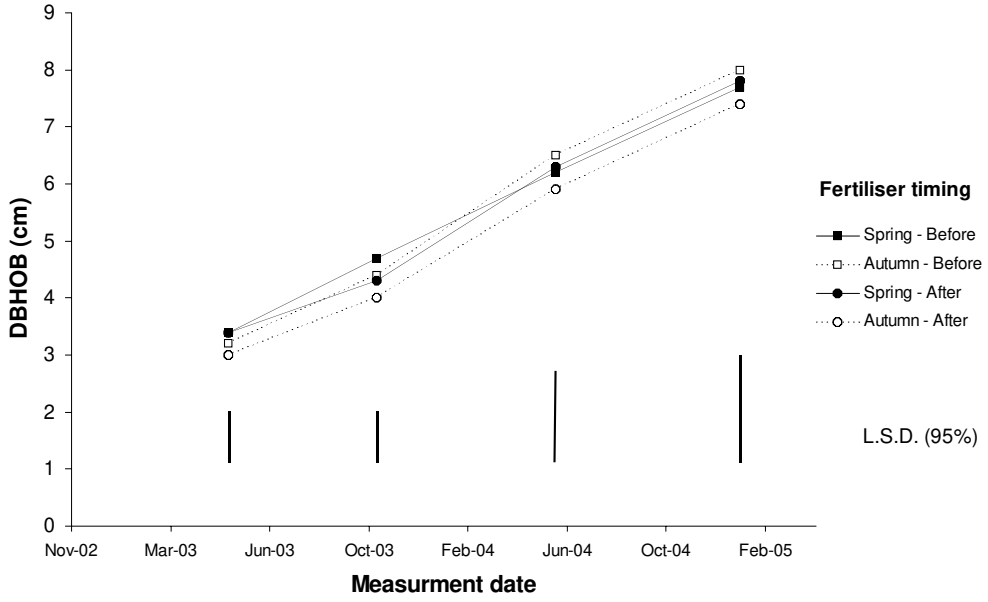

Figure 4.5. Time series of average tree DBH between November 2002 and January 2005 for trees in each of four fertiliser timing treatments.

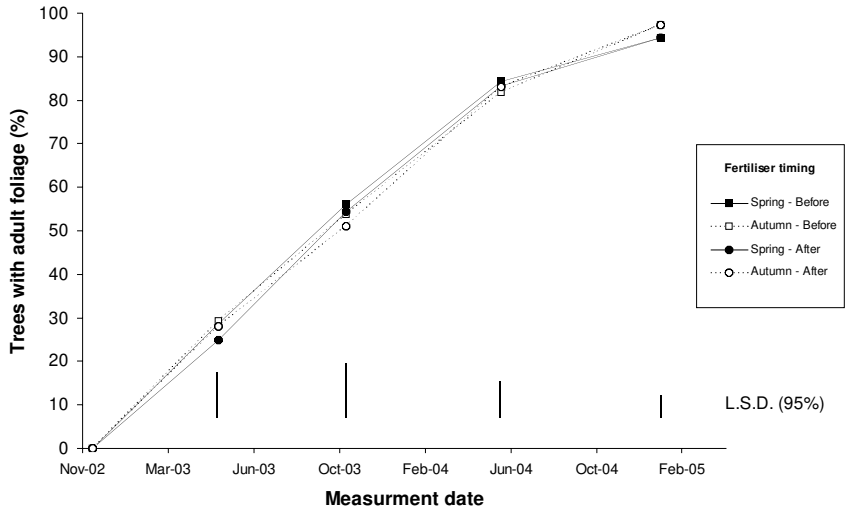

Figure 4.6. Time series of the average proportion of trees that had commenced phase change to adult foliage for each of four fertiliser timing treatments between November 2002 and January 2005.

Foliar and soil nutrient levels

There were no significant differences in levels of nitrogen or phosphorus among treatments or blocks in the leaf samples collected in June 2004. The foliar levels of both elements in the samples (Table 4.2) were within the normal range of concentrations for *E. globulus*.

Soil phosphorus levels sampled at the final remeasurement in May 2005 did not differ significantly among fertiliser treatments and the unfertilised plots had comparable levels of phosphorus as the fertilised plots (Table 4.3). By contrast, soil nitrogen levels did differ significantly among treatments ($F_{4,10} = 7.39$, MSE = 0.00735, Pr = 0.0049). Levels of soil nitrogen were highest in plots that were fertilised most recently (autumn 2004) and declined with increasing time since initial fertiliser application (Table 4.3). Levels of soil nitrogen in the two earliest applications (spring 2002 and autumn 2003) were not significantly different from the unfertilised soil in the plots adjoining the fertiliser timing trial.

Table 4.2. Average leaf tissue nitrogen and phosphorus concentrations of (a) fertiliser timing treatments and (b) blocks. Values within treatments or blocks that have different subscripts are significantly different at the 5%-level.

Factor	Nitrogen	Phosphorus
Fertiliser timing: (standard error)	(0.07)	(0.0068)
Spring 2002 (pre-MLD)	1.74_a	0.115_a
Autumn 2003 (pre-MLD)	1.82_a	0.113_a
Spring 2003 (post-MLD)	1.80_a	0.119_a
Autumn 2004 (post-MLD)	1.90_a	0.125
Block: (standard error)	(0.06)	(0.0059)
A	1.79_a	0.115_a
B	1.90_a	0.125_a
C	1.76_a	0.115_a

Table 4.3. Average soil nitrogen and phosphorus concentrations of (a) fertiliser timing treatments and (b) blocks. Values within treatments or blocks that have different subscripts are significantly different at the 5%-level.

Factor	Nitrogen	Phosphorus
Fertiliser timing: (95% LSD)	(0.156)	(0.032)
Unfertilised	0.156_{a}	0.012_{a}
Spring 2002 (pre-MLD)	0.116_{a}	0.034_{a}
Autumn 2003 (pre-MLD)	$0.230_{a,b}$	0.026_{a}
Spring 2003 (post-MLD)	0.346_{b}	0.024_{a}
Autumn 2004 (post-MLD)	$0.441_{b,c}$	0.032_{a}
Block: (95% LSD)	(0.207)	(0.019)
A	0.292_{a}	$0.024_{a,b}$
B	0.209_{a}	0.012_{a}
C	0.273_{a}	0.038_{b}

Discussion

Fertilisation with N and P prior to the 2003 MLD epidemic resulted in those trees suffering higher levels of the blighting-type infection that causes top-down defoliation. This could be explained simply as the consequence of fertilised trees producing more new season's foliage that is susceptible to the blighting-type infection injury. Counteracting the heightened susceptibility to blighting-type infection, however, is evidence that the trees fertilised before the MLD epidemic recovered lost foliage more quickly than trees receiving delayed fertilisation. This characterises one of the observed visual differences in the response between windrow and bay trees exposed to epidemic MLD. However, early fertilisation failed to prevent the senescence of older, spotted leaves in the lower crown after the mild 2003 MLD epidemic nor did it hasten the transition from juvenile to adult foliage, two of the other visual differences that are readily observed between windrow and bay trees (Wardlaw 2002).

Although trees receiving an application of nitrogen and phosphorus before the 2003 MLD epidemic had different upper-crown foliar responses than trees that were unfertilised at the time of the epidemic, this failed to alter the trajectories of height and diameter growth. This is not surprising given the epidemic at its peak in winter-spring 2003 caused less than 20% top-down defoliation. Top-down defoliation of this magnitude or less has been reported as having minimal impact on the growth of plantation eucalypts (Candy *et al.* 1992, Elek 1997, Smith *et al.* 2005). Carnegie and Ades (2003) reported significant growth effects from MLD in a 3-year-old *E. globulus* plantation that suffered bottom-up defoliation following the premature senescence of spotted leaves. The level of bottom-up defoliation from the 2003 MLD epidemic measured in this study was intermediate between the sprayed and unsprayed treatments reported by Carnegie and Ades (2003). However, because fertilisation prior to the MLD epidemic had no effect on the severity of bottom-up defoliation, no growth effects of the bottom-up defoliation could be resolved.

Pinkard (2003) showed that pre-canopy closure plantations of *E. globulus* growing on high productivity sites could tolerate the bottom-up removal of up to 40% of the leaf area, an amount comparable with the bottom-up defoliation measured in this trial.

However, Pinkard (2003) found that tolerance to leaf removal declined as site productivity worsened. Soil evaluation of the trial site prior to plantation establishment assessed the site as high productivity provided later-age fertilisation with nitrogen and phosphorus was done (Forestry Tasmania records). The lack of growth response, mid-trial (at age 3 years), in the two early fertiliser treatments (spring 2002 and autumn 2003) compared with the two treatments that received fertiliser later suggests that nutrients were not limiting at that time. This suggests that, mid-trial, the site was still performing as a high productivity site and the, as yet, unfertilised trees should have tolerated the degree of bottom-up defoliation measured with negligible impact on growth.

The trial aimed to reproduce the greater resilience to MLD shown by trees planted in windrows. By the end of the trial (age 4.5 years) there was a measurable, although not statistically significant, growth response to the four fertiliser treatments compared with the unfertilised plots. However, even the fertilised trees failed to match the growth of trees planted in the nearby windrows of burnt logging debris. It is probable that windrow trees have supra-optimal nutrition based on their very much greater growth and the form characteristics of large branches and abundant forking of the main stem. By contrast, the fertilised trees in this trial have merely adequate nutrition based on the levels of N and P measured by foliar analysis. While the trial provides evidence that pre-epidemic N and P fertilisation does assist post-epidemic recovery it does not mimic the windrow effect. It remains to be seen whether pre-epidemic N and P fertilisation at current operational rates will be sufficient to minimise growth impacts following a severe epidemic of MLD.

5. Photosynthesis of *Eucalyptus globulus* Labill. with Mycosphaerella leaf disease

Libby Pinkard and Caroline Mohammed

Introduction

Mycosphaerella leaf disease (MLD) is a major cause of foliage damage in temperate eucalypt plantations around the world (Ahumada et al. 2003; Dick and Dobbie 2001; Hunter et al. 2004; Mohammed et al. 2003; Tejedor 2004). Symptoms include discoloration and development of necrotic lesions on leaves, and, in severe cases, premature leaf senescence (Milgate et al. 2001a). The most common species causing damage in eucalypt plantations in Australia are *Mycosphaerella nubilosa* and *M. cryptica* (Milgate et al. 2001a; Mohammed et al. 2003), although in at least one plantation a suite of up to seven *Mycosphaerella* species has been detected on foliage (Smith et al. under review b). Severe damage is predominantly observed on juvenile foliage, making MLD a problem mainly of young eucalypt plantations. Significant short-term reductions in growth have been reported in young plantations suffering from MLD (Carnegie et al. 1997; Lundquist and Purnell 1987; Tejedor 2004).

Mycosphaerella species are hemibiotrophs. While necrotrophs generally have a limited effect on host physiology because they kill host cells before invading them, biotrophic pathogens can cause substantial modification to host physiology (Guest and Brown 1997). This can be directly via secretion of chemicals by the pathogen, or indirectly via pathogen-induced host responses. One of the major effects of foliar biotrophic pathogens is on photosynthetic processes. In general, infection by biotrophic pathogens results in a decrease in photosynthesis (Le May et al. 2005; Lopes and Berger 2001; Robert et al. 2004; Roloff et al. 2004), although increases in photosynthesis are sometimes observed in the early stages of infection (Buchanan et al. 1981; Scholes 1992). Photosynthetic reductions may be proportional to the decrease in green leaf tissue, or may be less or greater than would be expected if the reduction were related to disease severity alone (Bassanezi et al. 2001; Habershaw 1979; Shtienberg 1992), suggesting either some degree of photosynthetic upregulation, or an inhibitory effect of the pathogen in asymptomatic host tissue. The degree of inhibition of photosynthesis may be indicative of the aggression of the pathogen (Guest and Brown 1997). While infection commonly results in increases in dark respiration, photorespiration may be suppressed (Farrar 1992). Changes in rates of photosynthesis in diseased plants have been attributed to factors such as: changes in mesophyll (Lopes and Berger 2001) or stomatal resistance to CO_2 (McGrath and Pennypacker 1990; Meyer and Genty 1998b); changes in the rates of biochemical reactions of photosynthesis (Buchanan et al. 1981; Meyer and Genty 1998a); changes in the number or structure of chloroplasts (Buchanan et al. 1981; Shtienberg 1992); a build-up of carbohydrates in the leaf tissues surrounding sites of infection (Scholes 1992); or the secretion by the pathogen of phytotoxic chemicals into asymptomatic tissue (Guest and Brown 1997).

We undertook a study investigating the effects of MLD on photosynthetic processes in *E. globulus*. Our hypotheses were that (1) photosynthetic rates would be reduced by MLD as a result of changes in the rates of biochemical reactions of photosynthesis, and (2) that this reduction would be proportional to the reduction in green leaf tissue. Measurements were made at two field sites with natural infection and on pot-grown plants.

Materials and methods

Plant material

Sites

Experiments were conducted at two *E. globulus* plantations in Tasmania, Australia: Weilangta in the south east and Christmas Hills in the north west. Site details are given in Table 5.1. Trees at Weilangta were slightly older and larger than those at Christmas Hills, and had approximately 50% adult foliage whereas those at Christmas Hills had none at the time of measurement.

Both sites were experiencing infection by *Mycosphaerella* spp. A study at Christmas Hills using molecular detection methods determined that a suite of at least five *Mycosphaerella* species were present on the foliage (Smith et al. under review b), including *M. nubilosa* and *M. cryptica* which are considered to be responsible for much of the *Mycosphaerella* damage in *E. globulus* plantations in Australia (Park and Keane 1982). Conventional taxanomic procedures were used to identify the *Mycospharella* species present at Weilangta, and it was concluded that the pathogen responsible for most *Mycosphaerella* damage at this site was *M. nubilosa*.

Pot experiment

E. globulus seedlings were planted into 30 cm diameter pots containing a low-phosphorus potting mix, and grown in the open for four months. In April, a third of the seedlings were inoculated with *Mycosphaerella* spp.. The inoculation process involved placing the seedlings into a glasshouse with mean monthly minimum and maximum temperatures of 1 °C and 20 °C respectively and mean relative humidity of 74%. The plants were saturated with drippers twice daily. Fresh *E. globulus* leaves with mature *Mycosphaerella* pseudothecia were soaked overnight in water and hung from a string 10 cm above the seedling crowns. Molecular detection indicated that the *Mycosphaerella* species present on the foliage were a suite of *M. cryptica*, *M. nubilosa*, *M. vespa*, *M. grandis* and *M. tasmaniensis* (Glen et al. 2005). An overhead misting system was used to produce a fine mist for between three and seven minutes, six times per day, for seven days. During this time two portable fans were used to help reduce relative humidity. Plants were removed from the glasshouse after seven days, and symptoms of infection were apparent within eight weeks.

All plants (inoculated and uninoculated) were watered twice daily using drip irrigation, and fertilised with a slow release complete fertiliser. At the start of the experiment, seedlings had a mean height of 0.81 m, a mean diameter (at 5 cm height) of 0.88 cm and a mean leaf area of 0.30 m^2.

Experimental design

Field experiment

Sample trees were randomly selected from a 0.1 ha area at each site. There were eight sample trees at Weilangta and 15 at Christmas Hills. Mean tree height (m), diameter over bark at 1.3 m height (DBHOB, cm), and height to the junction of juvenile and adult foliage were measured. Disease was assessed at each site using a visual assessment method similar to the Crown Damage Index (Stone et al. 2003), where the incidence (i) and severity (s) of necrosis (i_n, s_n), discoloration (i_d, s_d) and insect

damage(i_{def}, s_{def}) were scored on a linear scale using visual standards. Infection at Weilangta was classed as bottom-up, where infection was concentrated in the entire volume of the lower (juvenile) crown. At Christmas Hills, infection was throughout the crown. There was negligible insect attack at either site.

Table 5.1. Site details for the two field sites used in the study.

	Weilangta	**Christmas Hills**
Grid reference	42°44'S 147°50'E	40°58'S 145°0' E
Soil type	Krasnozem	Podsol
Geology	Jurassic dolerite	Cambrian sandstone
Altitude (m asl)	340	100
Mean annual rainfall (mm)[¥]	694	1106
Mean maximum temperature (°C)[¥]	18.6	16.9
Mean minimum temperature (°C)[¥]	8.4	7.8
Stems per hectare	1100	1100
Age at start of expt (yrs)	3	2.5
Mean tree height (m)	6.9	3.8
Mean tree diameter (cm)	6.7	3.9
Predicted peak mean annual increment, MAI* (m^3 ha^{-1})	30	28
% crown with adult foliage	45	0
Height to first green branch (cm)	20	5
Infection pattern	Bottom-up	Bottom-up and out-in[#]

[¥] From closest Bureau of Meteorology station
* MAI estimated using the model of (Battaglia et al. 1999)
[#] bottom-up refers to infection that starts at the bottom of the crown and affects foliage moving up the crown. Out-in refers to infection that starts in the outer foliage and works its way in, resulting in an infection cone

Pot experiment

Two treatments were applied in the pot experiment: untreated control, and *Mycosphaerella* infection. There were five seedlings per treatment. Seedlings with similar levels of *Mycosphaerella* (approximately 25% of tissue affected) were selected from the 'infection' treatment for measurements.

Gas exchange

Light-saturated CO_2 uptake, A_{max}

At Weilangta, 12 leaves per tree, with a range of levels of tissue necrosis (0 – 90%), were selected for gas-exchange measurements. A CIRAS infra red gas analyser (PP Systems, UK) was used to measure light-saturated CO_2 uptake in late summer, at 360 ppm CO_2, a leaf temperature of between 16.1 and 18° C and a photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1}. The 2.5 cm^2 leaf chamber was placed onto tissue that had no obvious signs of *Mycosphaerella* infection. Each leaf was labelled following measurement and collected for leaf area analysis.

At Christmas Hills, five leaves per tree with a range of levels of tissue necrosis (0 – 80%) were used for gas exchange measurements. The CIRAS was used to measurelight-saturated CO_2 uptake in early summer, under the same light and CO_2 conditions as at Weilangta. Leaf temperature varied between 20 and 25° C.

Measurements were made at three positions per leaf with varying levels of tissue necrosis and discoloration. The locations of each measurement were marked on the leaf. The leaves were then collected for leaf area analysis.

Leaf analysis

Leaf analysis involved scanning whole leaves with a STD 1600+ scanner (Regent Instruments, USA) in 'flatbed' mode. Area analysis was done on the images using Winfolia software (Regent Instruments USA). Area was calibrated using standards of known area. For the Christmas Hills samples, separate images were produced for damage to whole leaves (I_l) and for damage at the individual gas exchange locations on each leaf (I_s). Colour classes were defined in Winfolia to differentiate between healthy, necrotic and discoloured tissue, and each image was tested using a feature of the software to ensure that the colour classes accurately described the actual damage shown on the image. Colour analysis was performed on the I_l and I_s images, to give estimates of total image area and area of healthy, necrotic and discoloured tissue.

Photosynthetic responses to varying p[CO_2]

The response of CO_2 assimilation (A) to varying intercellular p[CO_2] (C_i) was measured on three pot-grown plants per treatment using a Portable Photosynthesis System (LI-6400, Licor, Lincoln, NB, USA). Measurements were started at a p[CO_2] of 400 ppm, and the p[CO_2] was progressively reduced to 0 before being progressively increased to 2000. A PPFD of 1500 μmol μmol m^{-2} $s^{-1}$was used and leaf temperature was maintained at 20° C. Measurements were made on one healthy leaf per plant for the control, and on asymptomatic tissue of one infected leaf per plant for the infection treatment. Leaves were selected from the top one third of the crown and between two and four leaf pairs from the branch apex.

Data analysis

A whole tree infection score was calculated using:

$$I_t = \left(\frac{i_n * s_n}{100}\right) + \left(\frac{i_d * s_d}{100}\right)$$

where i_n and s_n are incidence and severity of necrosis, and i_d and s_d are incidence and severity of discoloration. A whole leaf infection score (I_l) and a leaf section infection score (I_s) were calculated using the above equation, but at a leaf or leaf-section level. Defoliation at the sites was minimal and was not included. Trees were than classed into four classes of tree-level infection: 1, I_t<10; 2, I_t=11-20; 3, I_t=21-30; 4, I_t>30. The percentage of tissue that was necrotic, discoloured or necrotic + discoloured was calculated for each I_l or I_s.

Group regression analysis provides regression equations under different models where groups are present in the data. It tests the hypotheses that (1) the regression lines have common slopes allowing for the possibility that they have different intercepts, and (2) that the same line applies to all groups (McPherson 1990). The following relationships were explored:

- A_{max} and leaf-level damage (I_t as groups) for both sites
- A_{max} and leaf level damage (site as group)
- A_{max} and damage (I_l and I_s as groups), Christmas Hills only

- A_{max} and G_s (stomatal conductance)
- C_i/C_a (the ratio of intercellular to ambient CO_2) and leaf level damage (I_t as group)

It was not possible to separate out the effects of necrosis and discoloration, so all analyses were done for total unhealthy tissue. Data were found to be normally distributed in all cases.

Differences between I_t and I_l classes in C_i/C_a, and mean leaf size were determined using analysis of variance.
A non-rectangular hyperbolic function was used to describe the shape of the A/C_i curve of each seedling (Sands 1995). This was used to estimate the photosynthetic utilisation of CO_2 (carboxylation efficiency, C_e), the capacity for ribulose bisphosphate (RuBP) regeneration (V_j) and total daytime respiration (leaf dark respiration plus photorespiration), r_d (Caemmerer and Farquhar 1981; Farquhar et al. 1982). The potential electron transport rate (J_{max}) and maximum rate of Rubisco activity (V_{cmax}) were calculated using the equations of (Medlyn et al. 2002) and (Bernacchi et al. 2001). Following (Wullschleger 1993) and (Medlyn et al. 2002) J_{max} and V_{cmax} were fitted over the entire *A/Ci* curve using non-linear regression. The CO_2 compensation point (Γ) was taken as the value of C_i where net assimilation was zero (Larcher 1975). Differences between treatments in A/C_i parameters were determined using analysis of variance.

Mean leaf size for each level of I_t was determined using analysis of variance. Genstat (GENSTAT Committee 1989) was used for all analyses.

Results

Field experiment

The A_{max} of leaves with <5% damage averaged 15.8 μmol m^{-2} s^{-1} at Weilangta and 16.4 μmol m^{-2} s^{-1} at Christmas Hills. These values are similar to values reported for healthy *E. globulus* foliage in other studies (Pinkard 2003). There was a highly significant ($P < 0.001$, R^2=0.60) negative relationship between A_{max} and leaf-level damage (I_l) (Figure 5.1). There were no significant differences between the slope and intercept of the relationship at any level of I_t at either Weilangta and Christmas Hills, except for I_t class 2, where the intercept was greater than the other I_t classes at Christmas Hills but not Weilangta (Table 5.2, Figure 5.2A).

Table 5.2. The constant (a) and slope (b) of the linear relationships between light saturated CO_2 uptake (A_{max}) (μmol m^{-2} s^{-1}) and percentage leaf infection (I_l) at two sites, Weilangta and Christmas Hills. Infection level 1 = < 10% infection; 2 = 10 – 20% infection; 3 = 20 – 30% infection; 4 = >30 % infection, where infection was scored as incidence (proportion of crown affected) x severity (severity of infection in affected crown). *Indicates a significant difference between severity classes

Site variation in A_{max} x I_l	**Severity of tree infection, I_t**								***P***	**R^2**
	1		**2**		**3**		**4**			
	a	**b**	**a**	**b**	**a**	**b**	**a**	**b**		
Weilangta	14.24	-0.15	14.24	-0.15	14.24	-0.15	-	-	<0.001	0.60
Christmas Hills	13.94	-0.16	16.95*	-0.16	15.59	-0.16	15.42	-0.16	<0.001	0.60

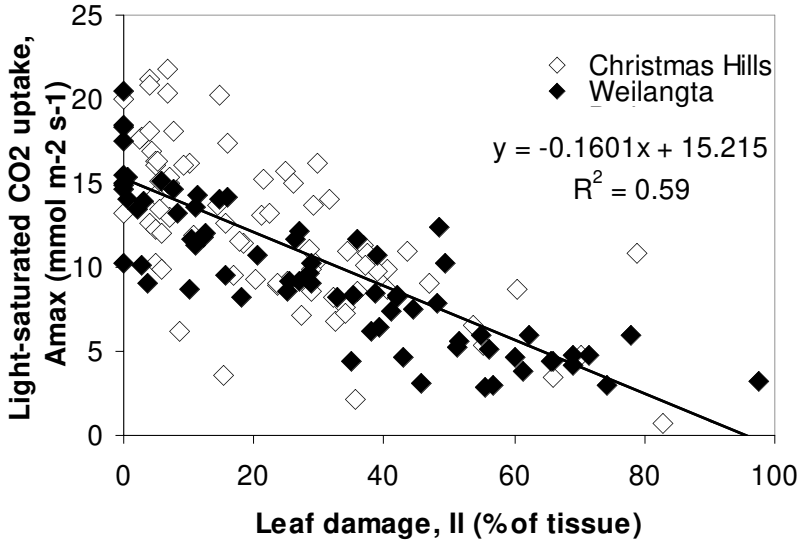

Figure 5.1. Relationship between light-saturated net CO_2 uptake and the percentage of damage on the leaf, at Weilangta and Christmas Hills.

Group regression analysis indicated that leaf-level (I_l) rather than leaf section-level (I_s) damage assessment was adequate for exploring the A_{max}/damage relationship. The sample type (I_l or I_s) had no significant effect on the relationship (data not presented).

There was a negative linear relationship between G_s and I_l ($P < 0.001$, R^2=0.49) (Figure 5.2B). The intercept of the relationship was significantly greater at an I_t of 3 than at lower levels of I_t (Table 5.3), indicating greater G_s for a given level of leaf-level damage when tree-level damage was more severe.

The positive linear relationship between A_{max} and G_s (Table 5.3, Figure 5.2C) ($P<0.001$, R^2=0.59) was also affected by I_t. The relationship between A_{max} and G_s flattened as I_t increased. This was more pronounced when I_t was 3 ($P<0.05$), and caused by the presence of higher values of G_s at this I_t level. These high values of G_s were not necessarily associated with higher levels of I_l (Figure 5.2B). There was also a positive relationship between C_i/Ca and I_l that was unaffected by I_t (Table 5.3, Figure 5.2D).

At lower levels of leaf damage (I_l), the reduction in photosynthetic rate relative to that of asymptomatic leaves was greater than might be expected if the photosynthetic reduction was proportional to the percentage of the leaf with visible *Mycosphaerella* damage (Figure 5.3). *Mycosphaerella* influenced mean leaf size at both sites (Table 5.4). There was a linear decline in mean leaf size as I_t increased.

Pot experiment

The relationship between *A* and *Ci* was considerably altered by the presence of MLD (Figure 5.4). Leaves with MLD had a significantly lower V_{cmax}, J_{max}, r_d, C_e and V_j than control leaves ($P < 0.05$) (Table 5.5). However Γ and L_s were unaffected by MLD.

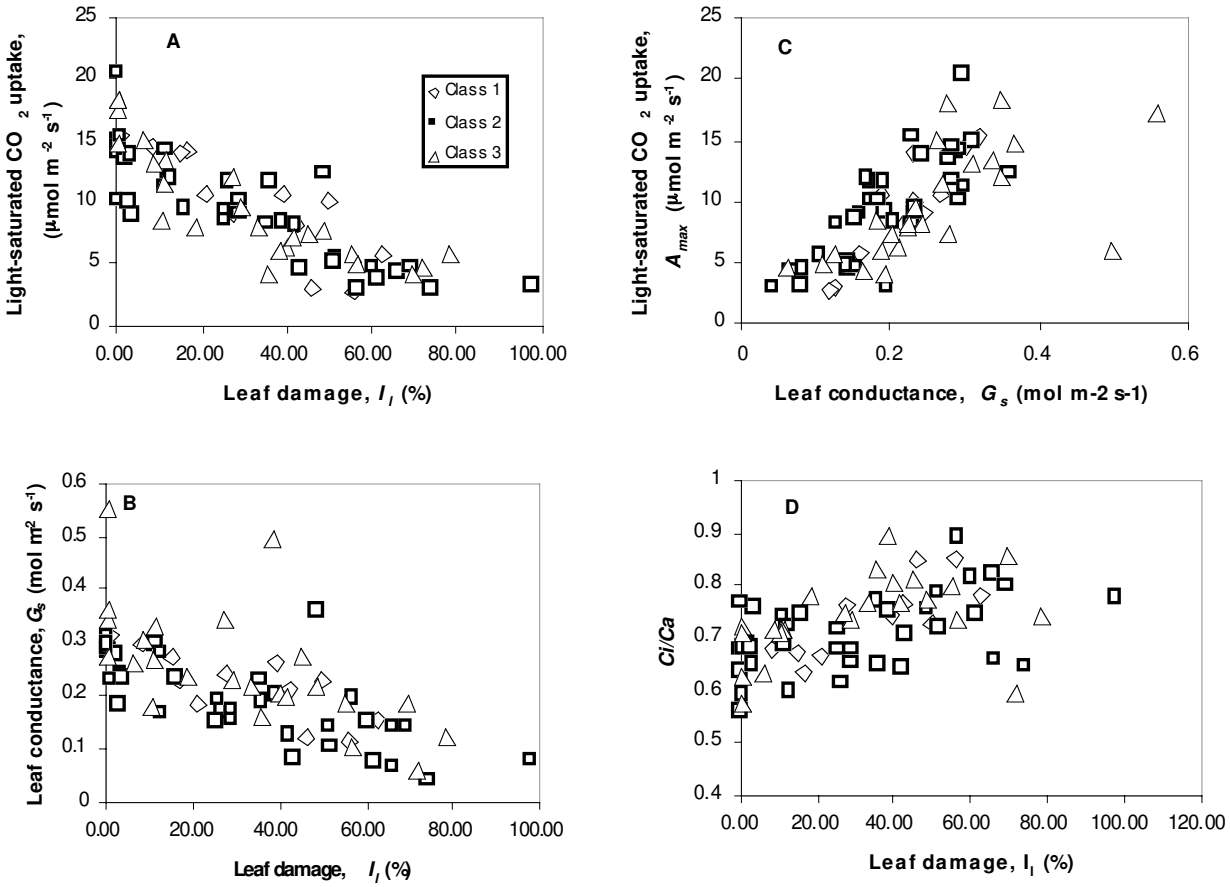

Figure 5.2. Relationships between (A) light-saturated CO_2 uptake (A_{max}) and percentage leaf damage (I_l); (B) leaf conductance (G_s) and percentage leaf damage (I_l); (C) light-saturated CO_2 uptake (A_{max}) and leaf conductance (G_s), and (D) *Ci/Ca* and percentage leaf damage (I_l)for *E. globulus* trees with three classes of *Mycosphaerella* infection (I_t) at Weilangta (See Table 3).

Table 5.3. The constant (a) and slope (b) of the linear relationships between leaf conductance (G_s) (mol m^{-2} s^{-1}) and I_l, A_{max} (µmol m^{-2} s^{-1}) and G_s, and C_i/C_a and I_l, for three intensities of whole tree infection (I_t), measured at Weilangta. Infection level 1 = < 10% infection; 2 = 10 – 20% infection; 3 = 20 – 30% infection, where infection was scored as incidence (proportion of crown affected) x severity (severity of infection in affected crown).

Relationship		**Severity of tree infection, I_t**						***P***	**R^2**
		1		**2**		**3**			
Y	**X**	**a**	**b**	**a**	**b**	**a**	**b**		
G_s	I_l	0.29	-0.003	0.27	-0.003	0.33*	-0.003	<0.001	0.49
A_{max}	G_s	-3.43	60.6	1.14	42.2	3.34*	25.4*	<0.001	0.55
Ci/Ca	I_l	0.68	0.001	0.68	0.001	0.68	0.001	<0.001	0.22

* indicates a significant difference from I_t = 1

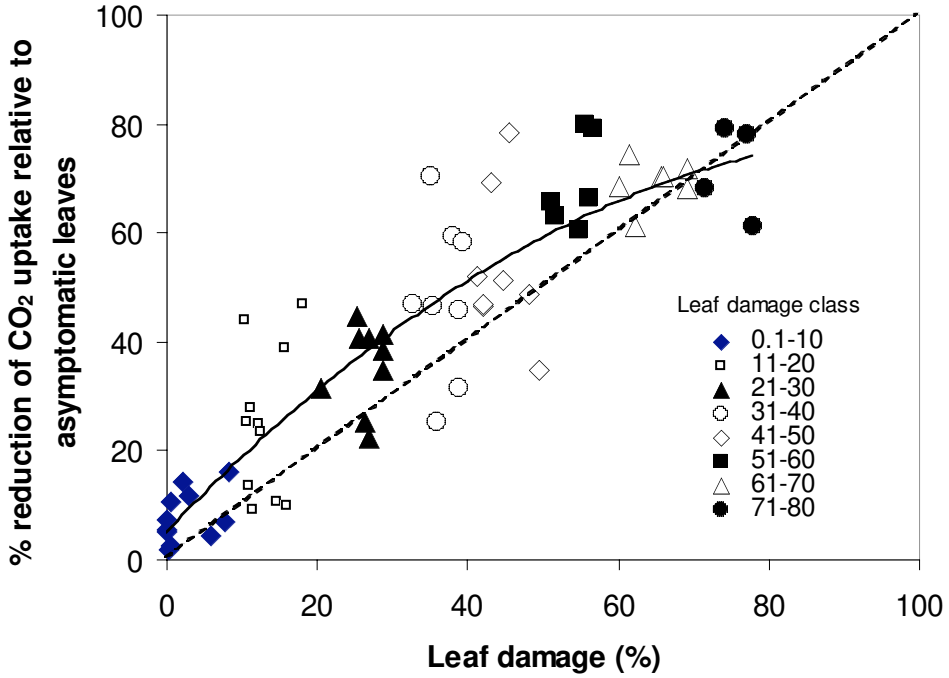

Figure 5.3. The relationship between percentage leaf damage and the reduction in CO_2 uptake relative to CO_2 uptake measured on asymptomatic leaves at Weilangta. The hatched line is the 1: 1 line. The solid line indicates the line of best fit for the data ($P < 0.001$, $R^2 = 0.75$, $y=-0.007x^2+1.44x+4.84$).

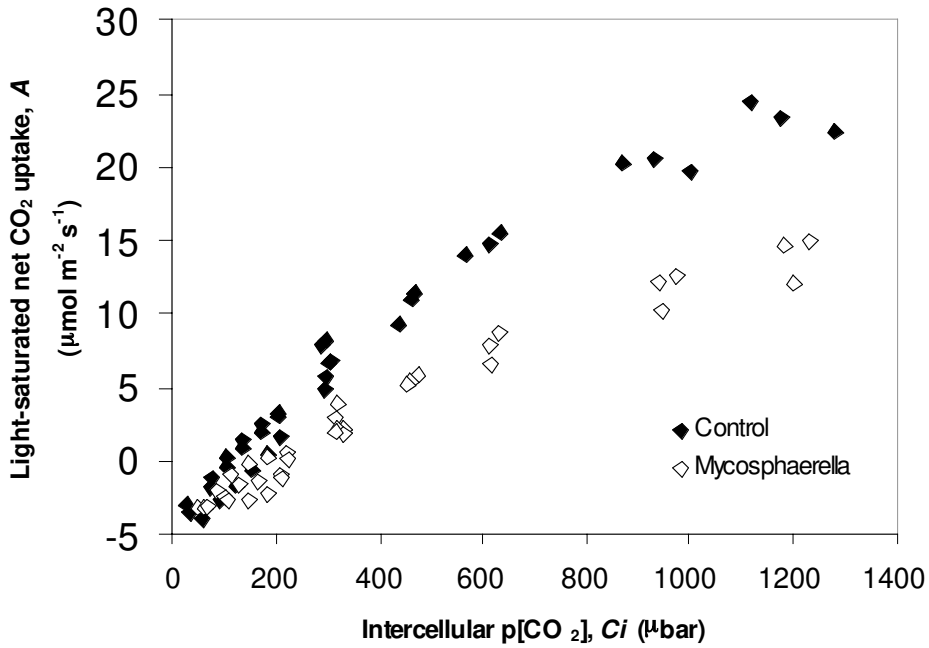

Figure 5.4. Relationship between light-saturated CO_2 uptake (A) and intercellular p[CO_2], Ci, of control seedlings and seedlings infected with *Mycosphaerella* leaf blight.

Table 5.4. Mean size of leaves infected with *Mycosphaerella* on trees suffering from a range of levels of infection (I_t) at Weilangta and Christmas Hills. SE gives the least squares standard error. Infection level 1 = < 10% infection; 2 = 10 – 20% infection; 3 = 20 – 30% infection; 4 = >30 % infection, where infection was scored as incidence (proportion of crown affected) x severity (severity of infection in affected crown).

Severity of infection per tree, I_t	**Mean leaf size (cm^2)**	
	Weilangta	**Christmas Hills**
1	11.3	12.7
2	10.6	9.4
3	9.0	8.1
4	-	6.6
SE	0.4	1.4

Table 5.5. Carboxylation efficiency (*Ce*, μmol CO_2 m^{-2} s^{-1} μbar^{-1}), RuBP-limited rate of CO_2 assimilation, *Vj* (μmol m^{-2} s^{-1}), total daytime respiration, r_d (μmol m^{-2} s^{-1}), the CO_2 compensation point (Γ) (μbar), maximal rubisco carboxylation rate (V_{cmax}, (μmol m^{-2} s^{-1}), and potential electron transport rate (J_{max}, μmol m^{-2} s^{-1}) of leaves from healthy *E. globulus* seedlings, and leaves infected with MLD. Different letters denote significant differences between treatments ($P < 0.05$).

Treatment	C_e	V_j	r_d	Γ	V_{cmax}	J_{max}	Ls
Control	0.045^a	38.3^a	5.07^a	120.0^a	32.3^a	67.0^b	0.303^a
Infected	0.030^b	26.7^b	4.35^b	168.0^a	24.5^b	55.8^a	0.197^a

Discussion

We have demonstrated that MLD substantially reduces light-saturated photosynthesis (A_{max}) in *E. globulus*. The linear pattern of reduction with increasing proportion of diseased leaf area is similar to that reported for both necrotrophs and biotrophs (eg. (Le May et al. 2005; Robert et al. 2004), although exponential reductions also have been observed (eg. Roloff et al. 2004; Shtienberg 1992). That the effect of MLD was similar at different sites and irrespective of tree-level infection suggests a general leaf-level response of *E. globulus* to MLD. The relationship between A_{max} and the percentage leaf damage may also be influenced by nutrition and water availability (Ayres 1984; Balachandran et al. 1994; Olesen et al. 2003), and further experimentation is required to determine the effects of such environmental stresses on the A_{max} /leaf damage relationship in *E. globulus*.

Investigation of the responses of *A* to changing *Ci* provides insight into factors that may be limiting photosynthesis at a cellular level. Our results suggest that the reductions in photosynthesis associated with MLD were related to reduced Rubisco activity and changes in the capacity for RuBP regeneration (von Caemmerer 2000). Reduced Rubisco activity also has been highlighted as a cause of reductions in photosynthesis associated with rust or anthracnose infection in beans (Bassanezi et al. 2002). The shape of the *A/Ci* curve did not suggest phosphate limitation (von Caemmerer 2000), suggesting that a build-up of carbohydrate was not the cause of the reductions in A_{max} (Scholes 1992). Wong and Thrower (Wong and Thrower 1978) also concluded that carbohydrate accumulation was not the cause of decreased photosynthesis in bean leaves with anthracnose. The slight increase in *Ci/Ca* with increasing severity of MLD per leaf suggests that reductions in A_{max} were a function of changes in mesophyll resistance to CO_2 uptake (Bassanezi et al. 2002; Farquhar and Sharkey 1982; Meyer et al. 2001). The reduction in C_e, considered by some authors to approximate mesophyll

conductance (von Caemmerer 2000), that we observed in diseased leaves, and the intense activity that occurs in the mesophyll during barrier zone formation (Smith et al. Under review-a), support this conclusion. There was no evidence of changes in stomatal limitation as a result of MLD that may have affected A_{max}.

The reduction in A_{max} related to leaf disease may theoretically be either proportional, proportionally greater or proportionally smaller than the corresponding reduction of green leaf area due to disease (Meyer et al. 2001; Shtienberg 1992). In the MLD/*E. globulus* system, the reduction in A_{max} was greater than would be expected for the visible symptoms of the disease, suggesting that asymptomatic tissue also is affected by MLD. While *E. globulus* produces a barrier zone in response to MLD (Smith et al. Under review-a), the development of the barrier zone is relatively slow, with hypertrophic changes to existing cells and limited cell division of the single adaxial palisade layer. Deposition of lignin and suberin only occurs late in lesion development, which may be too slow to contain hyphal development into new leaf tissue. In addition, in the field there can be numerous infection events on a single leaf, through sporulation from older lesions. As it takes 6 – 8 weeks for necrotic lesions to develop in the field, it is possible to have younger lesions with only minimal visual symptoms on the same leaf as older lesions. Both of these factors make it likely that asymptomatic tissue of leaves measured in the field experiment contained *Mycosphaerella* hyphae. It is possible that plant defence responses to hyphal development may explain the changes in biochemical processes noted above in response to MLD (Guest and Brown 1997). For example, Smith et al. (under review) reported significantly higher anthocyanin levels in the early (pre-visual) stages of MLD lesion development on *E. globulus*.

The relationship between G_s and percentage leaf damage was similar to that observed in other pathosystems (Roloff et al. 2004; Shtienberg 1992). Tree-level damage associated with MLD affected leaf-level stomatal responses in a way that suggested that, in the absence of water stress, trees with MLD would have a lower tree conductance than healthy trees. Conductance however increased at both high levels of tree and leaf infection, suggesting that stomatal control is less effective in trees more affected by MLD. The plantations used in this experiment were not water-stressed. Further clarification is required to identify stomatal responses to MLD under water-limited conditions.

Following a defoliation event, trees change patterns of biomass production to optimise the ratio of crown to roots (Cannell 1985). Production of smaller leaves is observed following severe defoliation events (Pinkard and Beadle 1999) and is an indication that trees are experiencing a shortage of assimilate for growth (Valentine 1985). The 20% reduction in leaf size observed following severe MLD infection suggests a shortage of assimilate that could be expected to reduce stem growth of *E. globulus* at least in the short term (Carnegie and Ades 2002; Tejedor 2004).

Severe epidemics of MLD have only been considered to be a problem by forest managers relatively recently in Australia, coinciding with the rapid expansion of the *Eucalyptus globulus* plantation estate (Inventory 2003). Consequently there are no long-term growth data available with which to quantify the long-term effects of MLD on plantation productivity. It is unknown whether short-term reductions in growth translate into rotation-length reductions in stem volume. In other situations where no long-term growth data are available, productivity models such as CABALA (Battaglia

et al. 2004) and 3PG (Landsberg and Waring 1997) have been used to model long-term effects of environmental conditions or silvicultural treatment on eucalypt growth. There is considerable potential to use such models for predicting the effects on growth of forest health problems such as MLD, but further model development is required to account for changes in physiological, and particularly photosynthetic, processes associated with disease. The results of our experiments can be used for this type of model development.

6. Influence of defoliation and nitrogen application on photosynthetic processes of young *Eucalyptus globulus* Labill.

Libby Pinkard and Caroline Mohammed

Introduction

Previous studies have determined that the level of growth loss associated with defoliation of *E. globulus* is related to frequency (Wills et al. 2004), severity, pattern (Collett and Neumann 2002; Pinkard 2003) or timing (Pinkard and L. 2000) of defoliation. It has been observed that the effect of defoliation on stem growth is more severe on lower productivity sites (Pinkard and Beadle 1998a), which suggests that environmental constraints on productivity can influence responses to defoliation (Prins and Verkaar 1992).

In many species, reductions in stem growth following defoliation events are proportionally less than the reduction in leaf area (Hoogesteger and Karlsson 1992; Kulman 1971; Langstrom and Hellqvist 1991; Lavigne et al. 2001; Reich et al. 1993). Photosynthetic upregulation and changes in patterns of biomass partitioning allow such species to compensate to some degree for loss of leaf area (Lavigne et al. 2001; Pinkard and Beadle 2000). Photosynthetic upregulation has been attributed to the reduction in source:sink ratio that occurs with defoliation, whereby a greater demand for carbohydrate to rebuild defoliated crowns results in an increase in the rate of the photosynthetic carbon reduction cycle (Geiger 1987; Stitt et al. 1990; Wareing and Patrick 1975). It has been demonstrated that photosynthetic upregulation occurs in *E. globulus* following pruning-related defoliation, and that the increase in photosynthetic rate is greater with more severe levels of pruning (Pinkard 2003). The influence of pattern, frequency and timing of defoliation on photosynthetic processes in *E. globulus* is unknown.

Photosynthetic responses to defoliation are likely to be influenced by environmental factors. For example, a number of studies of herbivory have demonstrated that photosynthetic upregulation only occurs when nitrogen (N) is added to defoliated plants, or under conditions of adequate water supply (Prins and Verkaar 1992). Of the possible management strategies available to limit the effects of defoliation on *E. globulus* stem growth, N application is more feasible than irrigation in most situations. There is evidence that stem growth of *E. globulus* following insect defoliation is increased by application of N (Chapter 2), suggesting that N availability can influence physiological, and possibly photosynthetic, processes in that species.

We undertook a field study to examine the hypothesis that the effects of defoliation on stem growth of young plantation-grown *E. globulus* could be compensated for by improving N availability. The effects of pattern, frequency and severity of simulated insect defoliation on photosynthetic processes, and the role of N application in changing these, was examined. Because of the difficulty in controlling natural levels of defoliation in the field, artificial defoliation was used.

Methods

Site details

The experiment was established in south-eastern Tasmania at Barnback (43°02' 80''S 146° 46' 40''E). The site, formerly a wet sclerophyll eucalypt forest, was cleared, windrowed and burnt before being ripped and mounded. Mean annual maximum and minimum temperatures and rainfall recorded at the closest Australian Bureau of Meteorology site (~ 20 km away) are 17.0°, 5.8° C and 755 mm, respectively. It has gradational soils over Triassic mudstone with predominantly clay loam topsoils overlying light or light medium clays. *E. globulus* seedlings were planted in September 2003 at 1100 stems ha^{-1}, and were fertilised with 24.7 g nitrogen (N) and 11.3 g phosphorus (P) (as diammonium phosphate) per seedling approximately six weeks after planting. At the start of the experiment, in March 2004, seedlings had a mean height and diameter (15 cm) of 0.92 m and 1.66 cm, respectively. Soils analysis prior to the start of the experiment indicated adequate levels of N and P (Chapter 2).

Treatments and experimental design

The experiment was conducted in six plots of 30 x 20 m, each with an internal measurement plot of 25 x 12 m (approximately 30 trees). Treatments applied in the experiment included two fertiliser treatments applied at the plot level, and six defoliation treatments applied within plots. The site was divided into three blocks of two plots, to account for slight variation in slope and aspect across the site. Each fertiliser treatment was represented once per block. There were three replicates of each defoliation treatment per plot. Treatments were randomly applied within blocks and plots.

Table 6.1. Description of the defoliation treatments included in the experiment. All treatments were applied in unfertilised and fertilised (300 kg ha^{-1} N) plots.

Treatment No.	Treatment	Description
1	Control	No defoliation
2	Autumn 25%	25% leaf area removed from throughout the crown at the start of the experiment (autumn 04)
3	Autumn 38%	35% leaf area removed from throughout the crown at the start of the experiment (autumn 04)
4	Spring out-in (O-I)	All leaves removed from the upper 50% of crown length in spring 04, plus apical leaves from the remaining crown
5	Spring bottom-up (B-U)	All leaves removed from the lower 50% of crown length in spring 04, except apical leaves
6	Autumn-Spring-Autumn	Autumn 25% + spring bottom-up + a further bottom up defoliation in the following autumn as per the spring defoliation

The fertiliser treatments involved either no application of N, or application of N at 300 kg ha^{-1}. The N was applied as urea in two doses. 100 kg ha^{-1} was applied in March 2004, prior to the application of defoliation treatments, as a surface application in a 15 – 80 cm circle around each tree. A further 200 kg ha^{-1} was applied in August 2004 prior to a second defoliation. This fertiliser was hand-broadcast across the plots. Phosphorus (as triple superphosphate) was applied across all treatments, at a rate of 100 kg P ha^{-1}.

This was applied at the same time as the first N application, into a spade slit on either side of the stem and around 15 cm from the stem, to a depth of 5 cm.

Defoliation did not involve disbudding. It was done using hand snippers. The defoliation treatments applied in the experiment are given in Table 6.1. The autumn 2004 defoliation involved removing either 50% of the lamina of every second leaf (25% total leaf area removed) or 50% of the lamina of three out of four leaves (38% total leaf area removed). The spring 2004 defoliation involved removing leaf area from 50% of the crown length, by either removing all leaf area from the upper 50% of the crown length plus apical growth from the remaining crown (out-in defoliation), or removing all leaf area from the lower 50% of the crown length, excluding apical growth (bottom-up defoliation). The autumn 2005 defoliation involved again removing all leaf area from the lower 50% of crown length, excluding apical growth.

Insects were controlled on the site by spraying Dominex (active ingredient alpha-cypermetherin) at a rate of 100 g l^{-1} in December 2004. No other insect or browsing control was required.

Measurements

Stem growth

Height and diameter of trees in all plots were measured prior to application of treatments, and then at 3-montly intervals. Diameter at 15 cm height was measured 3, 6 and 9 months after the start of the experiment. Diameter at 1.3 m height (diameter at breast height, DBH) was measured 9, 12 and 16 months after the start of the experiment.

Gas exchange and leaf traits

One block was selected for examination of gas exchange and leaf traits. Within this block light-saturated net CO_2 uptake (A_{max}) (μmol m^{-2} s^{-1}) was measured on three trees per defoliation and nitrogen treatment at the start of the experiment, and 2, 6, 15, 19, 26, 41, 46 and 52 weeks following treatment application. The measurements were made on three healthy, entire leaves per tree, selected from the top one third of the crown and between three and five leaf pairs from the branch tip. An open-flow gas analysis system (CIRAS, PP Systems, Herts, UK) was used to determine light- A_{max} and leaf conductance, G_s (mol m^{-2} s^{-1}). The leaves were enclosed in a leaf chamber (area 2.5 cm^2) fitted with a light source that provided a photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1}. The p[CO_2] was maintained at 370 ppm. Readings were made when G_s stabilised.

The response of CO_2 assimilation (A) to varying PPFD was measured in January 2005. Measurements started at a PPFD of 1200 μmol m^{-2} s^{-1}. The PPFD was reduced in eight steps to 0 using neutral density filters.

Leaves used for gas exchange measurements were collected. An estimate of total chlorophyll content was made 0, 2, 6, 19, 26 and 41 weeks into the experiment, using a Minolta SPAD-502, which gives an estimated chlorophyll content based on leaf absorbance in the wavelengths 650 and 940 nm. There is a strong relationship in *E. globulus* between measured chlorophyll content and chlorophyll content estimated with the SPAD (Pinkard et al. 2006). Three measurements were made per leaf immediately after leaf collection, and averaged.

The leaf area:weight ratio (specific leaf area, SLA (kg m^{-2})) of gas exchange leaves was determined 0, 2, 15, 19 and 52 weeks into the experiment. Leaf area was measured using a planimeter (Delta-T Devices, Hoddeston, Herts, UK), and leaves were dried at 65° C until constant mass.

Light attenuation

In May 2004, three months after the start of the experiment, light attenuation in the crowns of trees grown with or without N application was measured. Tree crowns were divided into equal thirds based on height. Quantum sensors (Licor) were placed into the upper, mid and lower crowns, on the northern side of four trees per treatment, at mid-radius. A single sensor was placed into the open from which continuous data were collected. Data were recorded from these sensors with a Campbell CR10X automatic logger (Campbell Scientific, Logan, UT) that was programmed to record every 5 s and store means every 5 minutes, over a period of 6 days.

Four months after the spring 2004 defoliation, quantum sensors were placed into the crowns of a single, representative tree of the control, out-in or bottom-up defoliation treatments. These trees had been grown with no added N. Tree crowns were divided into thirds based on tree height. Quantum sensors were placed into the vetical centre of the upper, mid and lower crown zones, on the northern and southern side, and at approximately one third and two thirds crown radius. A single sensor was placed into the open. Data were recorded as above, over a period of 9 days.

Biomass

At the start of the experiment six trees, covering a range of sizes, were harvested from an area immediately adjacent to the experimental site for analysis of above-ground biomass. The trees were removed flush with the ground, placed into plastic bags, and transported to the laboratory where they were kept at 4° C until processed (maximum 5 days). Stem basal diameter and length were measured, and stem, branches and leaves were separated. A stratified random sample of 10 leaves per tree was randomly taken for analysis of SLA. Leaf, branch and stem material was then dried at 65° C until constant mass.

A second biomass harvest was done in spring 2004, immediately before the spring defoliation treatments were applied. Four unfertilised trees and four trees fertilised with 300 kg ha^{-1} N were harvested. They were all undefoliated, and covered the range of diameters present at the site. The harvesting method was as described above. The diameters of all branches per tree were measured 3 cm from the branch base, and a sample of five branches per tree was removed. These branches covered the range of diameters present on the tree. The diameter of these branches was measured, and all leaves were removed. Ten leaves per tree were randomly selected and placed into plastic bags for analysis of SLA. All leaf and branch material was processed as described above.

As part of each harvest, a sample of the stem was collected for determination of basic density. The bark was removed and green volume determined. Samples were dried at 65° C for 48 hours and weighed.

Data analysis

The relationship between diameter at 15 cm height and DBH was determined for the nine month measurement using linear regression. The equation describing the relationship was used to estimate diameter at 15 cm height for the remaining measurement times. Diameter and height increment were calculated for each measurement time. Repeated measures analysis of variance with initial height or diameter as covariates, was used to determine differences between treatments in height and diameter increment.

Total chlorophyll content (*TChl*) (μg mm^{-2}) was calculated from the SPAD-derived chlorophyll index (*CI*) using the following equation (Pinkard et al. 2006):

$$LnTChl = -8.79 + 2.08 * Ln(CI)$$

The ratio of A_{max}*:TChl* was calculated. Analysis of variance also was used to explore differences between treatments in A_{max}, G_s, *TChl, SLA* and A_{max}*:TChl*.

A non-rectangular hyperbolic function was used to describe the shape of each light response curve (Sands 1995). The parameters derived from the curves gave estimates of apparent quantum yield ( ) (mol mol^{-1}), maximum CO_2 uptake (A_{max}) and dark respiration (R_d) (μmol m^{-2} s^{-1}). Analysis of variance was used to determine differences between treatments in these parameters.

Leaf dry mass was converted to area using the SLA. Following the first harvest, the relationships between stem diameter and leaf area, and leaf, stem, branch and total above ground dry mass were explored using linear regression analysis. The relationships were used to estimate the leaf area of each tree at the start of the experiment. Following the second harvest, the relationship between log-transformed branch diameter and leaf or branch dry mass was examined using group regression analysis with N treatment as the group. The equations derived from this analysis were used to calculate leaf and wood dry mass of every branch on every harvested tree. Total leaf dry mass per tree was determined, and converted to leaf area using the SLA. Conic stem volume was calculated, and converted to mass using the basic density (wood green volume/dry mass) of 455 kg m^{-3} (calculated from the stem samples). A second regression analysis was undertaken between stem diameter (5 cm height) and tree leaf area, leaf dry mass, branch dry mass and stem dry mass. The resulting equations were used to estimate the leaf area and leaf, branch and stem dry mass of each undefoliated tree in the experiment. Analysis of variance was used to examine differences in these variables related to N treatment and the Autumn 04 defoliation treatments.

Light intercepted in the three crown zones (upper, mid and lower) was averaged for the four trees per treatment in the first measurement, and for 2 – 4 quantum sensors per crown zone in the second measurement. A trendline was fitted to data from each crown zone per treatment, for a typical day during each measurement period.

Results

Values of A_{max} recorded at the site ranged up to 25 μmol m^{-2} s^{-1} (Figure 6.1C). The lower value observed at week 26 coincided with very dry conditions, and the reduction in *Gs* measured at the time supports the conclusion that trees were experiencing water stress at this time (Figure 6.1D).
Over the course of the experiment a small increase in *TChl* was observed (Figure 6.1E), but decreases in SLA that occurred (Figure 6.1F) may have affected estimates of *TChl* made using the SPAD (Thompson et al. 1996).

Effect of N in the absence of defoliation

Tree responses to application of N in the absence of defoliation had the following features:

- a 6% increase in diameter increment (Figure 6.1A, $P < 0.05$), but no change in height increment (Figure 6.1B)
- up to 16% higher A_{max} in the spring/summer following fertiliser application (Figure 6.1C, $P < 0.05$), but no consistent trend in *Gs* (Figure 6.1D) and no change in the relationship between A_{max} and *Gs* (Figure 6.2)
- an increase in *TChl* of up to 12% in the spring/summer following fertiliser application (Figure 6.1E, $P < 0.05$), but no consistent trend in the relationship between A_{max} and *TChl* (data not presented)
- a trend towards increasing apparent quantum yield ( ) and dark respiration (R_d) (Table 6.2)
- 16% lower SLA at the end of the experiment ($P < 0.05$)
- a 130% increase in branch dry mass ($P < 0.001$) and 30% less stem dry mass ($P = 0.08$) six months after fertiliser application (Table 6.3)
- less incident light measured in the mid and lower crown (Figure 6.3).

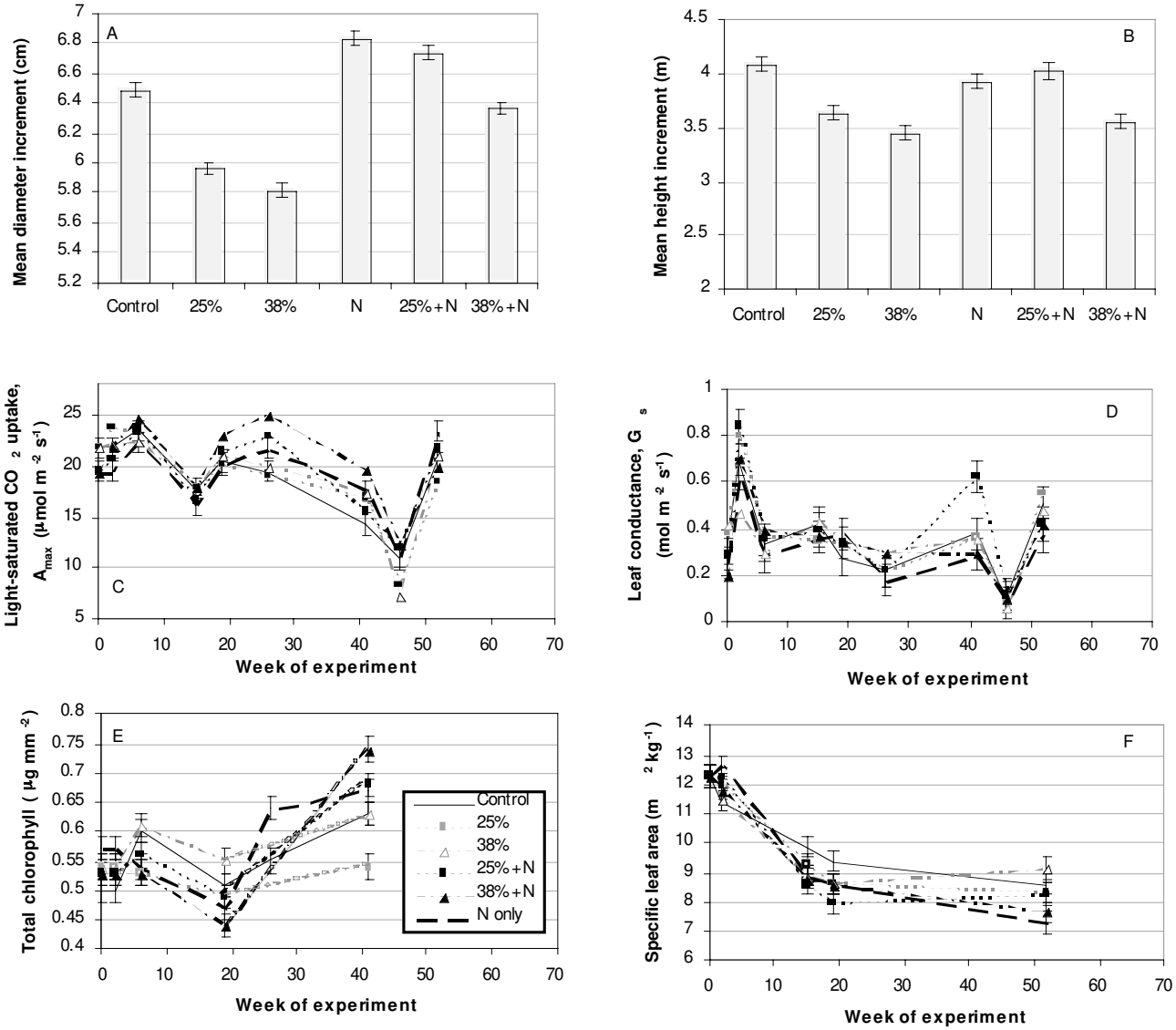

Figure 6.1. Effect of severity of defoliation and fertilising with 300 kg ha^{-1} N on (A) diameter increment (cm), (B) height increment (m), (C) light-saturated CO_2 uptake (A_{max}) (µmol m^{-2} s^{-1}), (D) leaf conductance (G_s) (mol m^{-2} s^{-1}), (E) chlorophyll content (µg mm^{-2}) and (F) specific leaf area (m^2 kg^{-1}), of field-grown *E. globulus*. Error bars indicate standard errors ($P < 0.05$). The defoliation treatments involved removing either 25% or 38% of the total leaf area by removing leaves from throughout the crown when trees were 9 months old.

Table 6.2. Parameters derived from the light response curves taken 30 weeks into the experiment, for *E. globulus* trees growing in plots that were either unfertilised or had been fertilised with 300 kg ha^{-1} N. The trees were either undefoliated, or had been subjected to three defoliation events (autumn-spring-autumn), as per Table 1. A_{max} is light-saturated net CO_2 uptake; is apparent quantum yield; R_d is dark respiration. There were no significant differences between treatments ($P = 0.05$).

Nitrogen treatment	**Defoliation treatment**	A_{max}		R_d
0	0	16.19	0.056	1.19
0	3 Def	17.62	0.101	1.88
300	0	18.52	0.070	1.28
300	3 Def	14.83	0.101	1.38

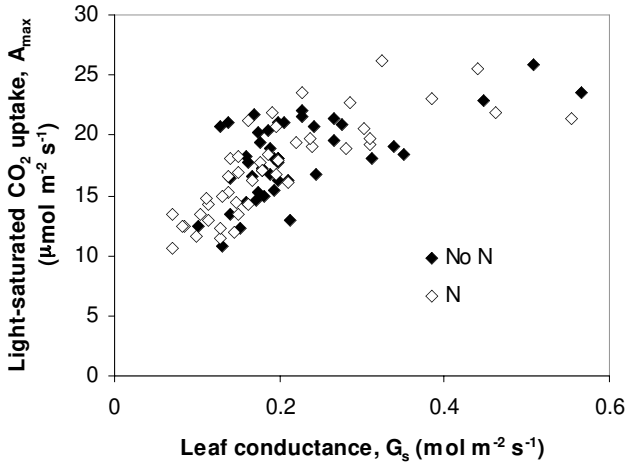

Figure 6.2. Relationship between light-saturated CO_2 uptake (A_{max}) and leaf conductance (G_s) of *E. globulus* trees grown with or without the application of 300 kg ha^{-1} N. Data are for a single, representative, measurement time (week 26 of the experiment).

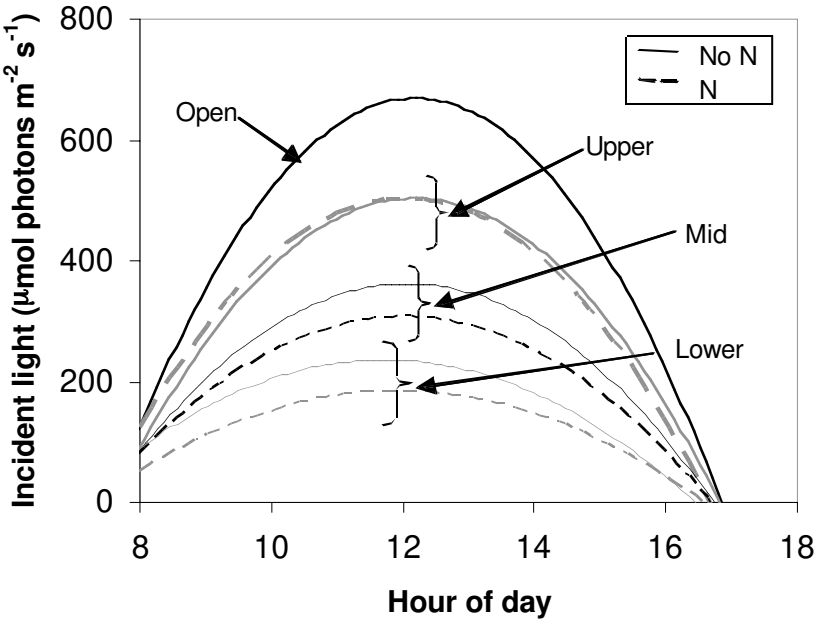

Figure 6.3. Incident light (µmol photons m^{-2} s^{-1}) measured in the open and in the upper, mid or lower crowns of *E. globulus*. Values are averaged for four trees, for a typical day in autumn 2004, three months after fertiliser application.

Table 6.3. Above-ground biomass of fertilised and unfertilised *E. globulus* immediately before and six months after application of 300 kg ha^{-1} N. Leaf, branch, stem and total above-ground dry mass (DM) and leaf area were estimated for fertilised and unfertilised trees using allometric relationships developed from biomass harvests.

TMT	Initial					After 6 months				
	Leaf		Branch	Stem	Total	Leaf		Branch	Stem	Total
	DM (kg)	Area (m^2)	DM (g)	DM (kg)	DM (kg)	DM (kg)	Area (m^2)	DM (g)	DM (kg)	DM (kg)
N	0.07[a]	0.85[a]	0.03[a]	0.09[a]	0.19[a]	0.20[a]	1.86[a]	0.35[a]	0.35[a]	0.95[a]
No N	0.08[a]	0.28[a]	0.04[a]	0.10[a]	0.22[a]	0.21[a]	1.92[a]	0.15[b]	0.46[a]	1.03[a]

Effects of severity of defoliation

In the absence of fertiliser, both 25% and 38% defoliation significantly reduced diameter and height increment (Figure 6.1A, B), and the more severe the defoliation, the greater the reduction in increment ($P < 0.05$). There was no photosynthetic response to these treatments (Figure 6.1C). While 38% defoliation did not affect *TChl*, trees in the 25% defoliation treatment had 14% less *TChl* in leaves at the end of the experiment (Figure 6.1E, $P < 0.05$). These defoliation treatments had no significant effect on the relationship between A_{max} and *Gs* (data not presented), A_{max} and *TChl* (data not presented), or on SLA (Figure 6.1F).

When fertiliser was applied to these defoliation treatments, the following was observed:

- substantial increases in diameter and height increment following both defoliation treatments compared with the control (Figure 6.1A, B). Diameter increment of trees in the 25%+N treatment was greater than that of trees in the control ($P < 0.05$), and both diameter and height increment were at least at a level similar to that measured in the N only treatment
- increases in A_{max} in response to defoliation compared to the control in the spring/summer following defoliation. However A_{max} was only significantly greater than that measured in the N only treatment when defoliation was 38% ($P < 0.05$), suggesting that the increases in A_{max} measured in the 25%+N treatment were a result of N application rather than defoliation
- significantly lower SLA of trees in the 38%+N treatment compared to the control at the end of the experiment ($P < 0.05$), although the values were similar to that of trees in the N only treatment (Figure 6.1F). The SLA of trees in the 38%+N treatment was 18% lower than that of trees in the 38% (no fertiliser) treatment.

Effects of pattern of defoliation

Out-in rather than bottom-up defoliation reduced stem growth of unfertilised trees (Figure 6.4A, B, $P < 0.05$). A_{max} increased in response to both defoliation patterns (Figure 6.4C), and the increase was observed both 6 and 14 weeks after defoliation (Figure 6.4C). Although there was no consistent effect of pattern of defoliation on *Gs*, substantial increases in *Gs* were observed in both defoliation treatments at week 41, and the increase was greater in out-in than bottom-up defoliated trees (Figure 6.4D). *TChl* of trees in the out-in treatment was significantly greater than that of control trees at the end of the experiment (Figure 6.4E), but there were no consistent trends in the A_{max}:*TChl* relationship (data not presented) or in SLA (Figure 6.4F). Pattern of defoliation did not affect patterns of incident light measured in the upper or mid crown (Figure 6.5), but bottom-up defoliation resulted in more incident light in the lower crown.

Application of fertiliser had the following effects on responses to out-in or bottom-up defoliation:

- greater diameter increment than control trees, and a similar height increment to that of trees in the N only treatment, irrespective of pattern of defoliation (Figure 6.4A, B)
- proportionately greater diameter and height increment of trees in out-in+N than bottom-up+N defoliation treatments

- similar levels and pattern of increases in A_{max} as were observed in unfertilised, defoliated trees (Figure 6.4C)
- lower *Gs* in defoliated trees in the peak observed at week 41 of the experiment (Figure 6.4D)
- significant increases in *TChl* compared to the control, with higher *TChl* measured in response to bottom-up+N than out-in+N defoliation at the end of the experiment (Figure 6.4E). However only bottom-up+N defoliation resulted in greater *TChl* than was measured in the N only treatment.

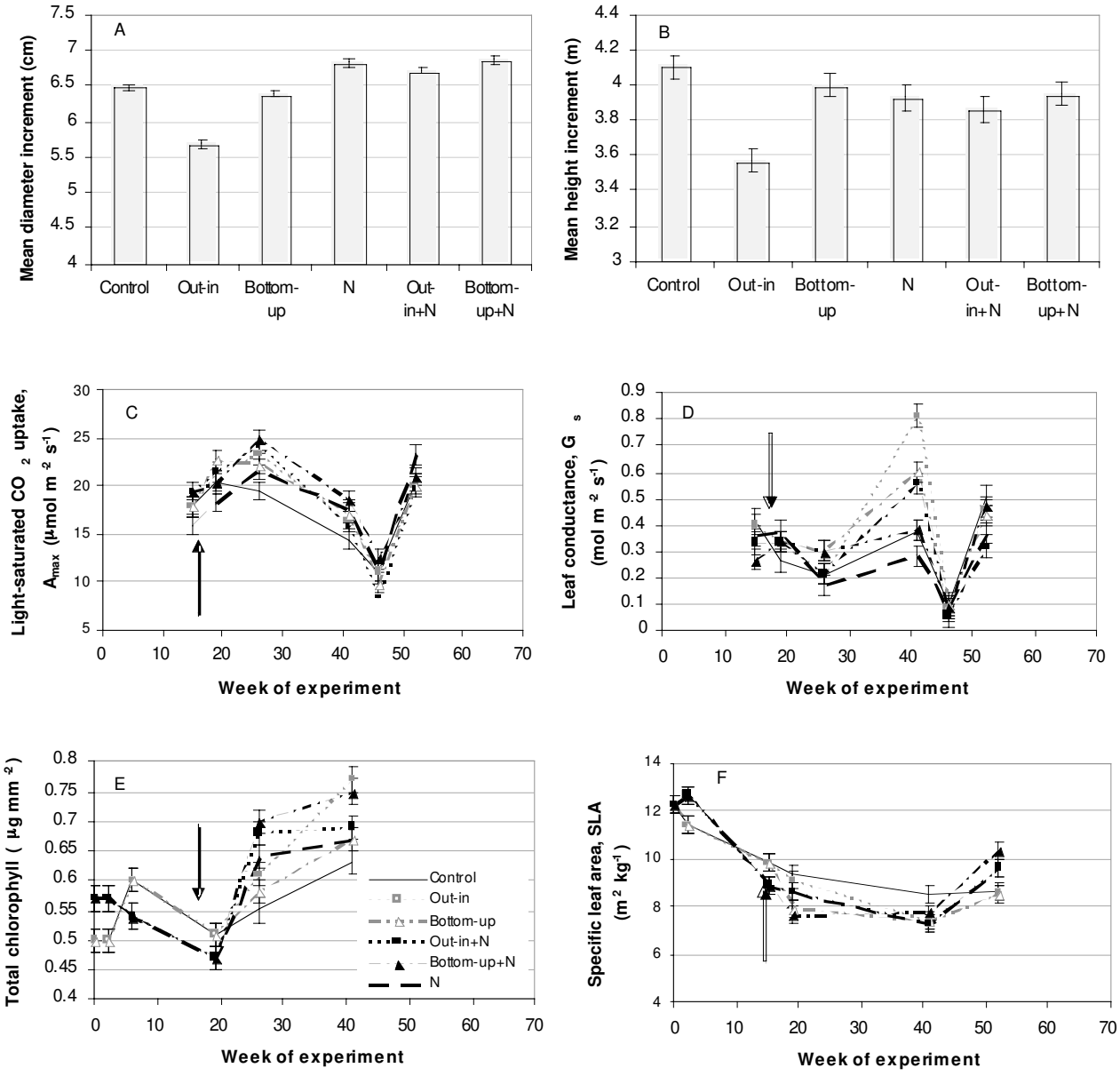

Figure 6.4. Effect of pattern of defoliation and fertilising with 300 kg ha^{-1} N on (A) diameter increment (cm), (B) height increment (m), (C) light-saturated CO_2 uptake (A_{max}) (µmol m^{-2} s^{-1}), (D) leaf conductance (G_s) (mol m^{-2} s^{-1}), (E) chlorophyll content (µg mm^{-2}) and (F) specific leaf area (m^2 kg^{-1}), of field-grown *E. globulus*. Error bars indicate standard errors ($P < 0.05$). The defoliation treatments involved removing leaves from 50% of the crown length in either an out-in pattern (remove all leaves from upper 50% of crown length plus all apical foliage from remaining crown) or a bottom-up pattern (remove all leaves from the lower 50% of crown length but not apical foliage). Arrows indicate when the defoliation treatments were applied.

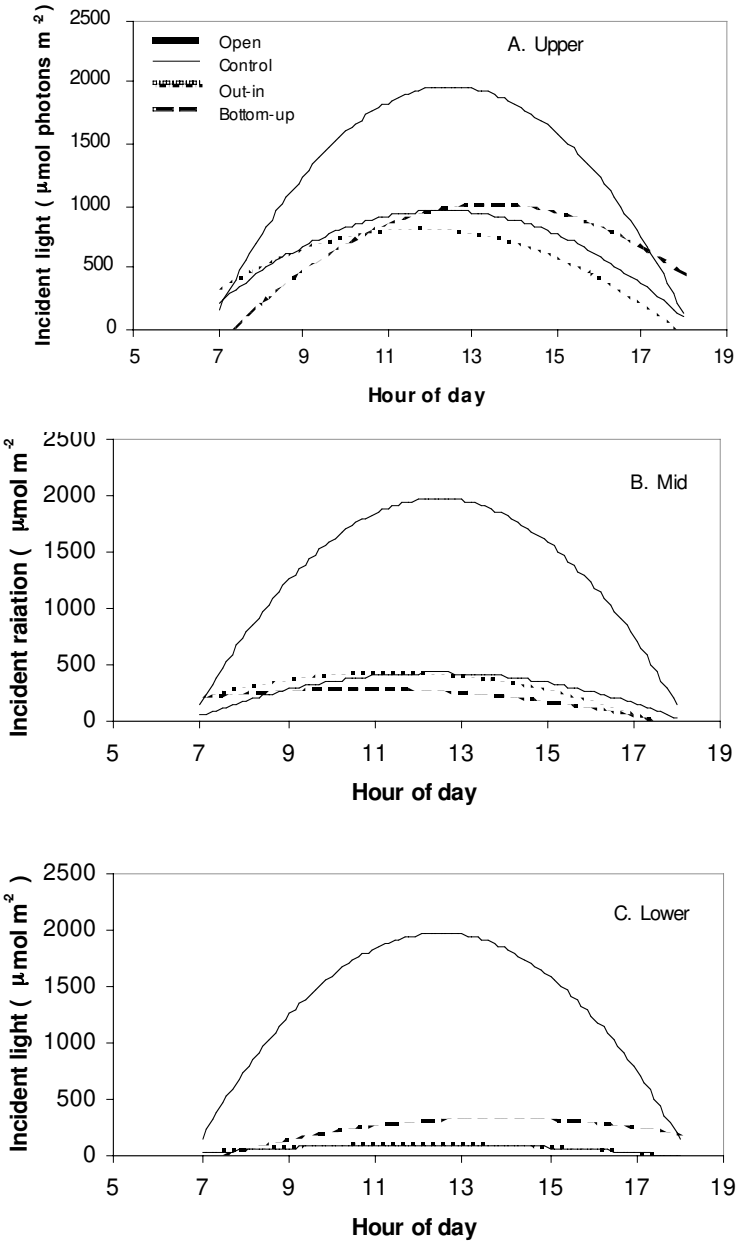

Figure 6.5. Diurnal pattern of incident light measured on a typical day in January 05, four months after application of out-in or bottom-up defoliation to *E. globulus*. Values are given for the (A) upper, (B) mid and (C) lower crown of defoliated and control trees, and for above the crown.

Effects of defoliation frequency

In the absence of fertiliser, 3 defoliation events reduced diameter and height increment more than did 1 defoliation event (Figure 6.6A, B). An increase in A_{max} was only observed in response to the 3 defoliation treatment, and then only at one measurement time (6 weeks after the second defoliation event) (Figure 6.6C). Although not statistically significant, there was a 96% increase in and a 58% increase in R_d in the 3-defoliation treatment compared with the control (Table 6.2). The only increase in *Gs* compared with the control occurred at week 41 of the experiment, in the 3-defoliation treatment (Figure 6.6D). Both the 1- and 3-defoliation treatments reduced *TChl*, and values were 15% lower than the control at the end of the experiment (Figure 6.6E). SLA was not significantly affected by frequency of defoliation (Figure 6.6F).

Fertilising with N changed the effects of frequency of defoliation in the following ways:

- diameter increment in the 1-defoliation+N treatment was similar to that of trees in the N only treatment, and greater than that of the control (Figure 6.6A)
- height increment in the 1-defoliation+N treatment was similar to that of the control, whereas height increment in the 3-defoliation+N treatment was significantly less than the control at the end of the experiment (Figure 6.6B, $P < 0.05$). There was a proportionally greater increase in diameter and height increment of defoliated compared to undefoliated plants in response to N application, and particularly in response to the 1-defoliation+N treatment
- A_{max} was up to 26% greater than that of the control in the spring/summer, and the increase was observed earlier in the 3-defoliation+N than in the 1-defoliation+N treatment (Figure 6.6C, $P < 0.05$)
- Only the 3 defoliation+N treatment resulted in A_{max} greater than that observed in the N only treatment
- R_d was increased to a lesser extent in the 3-defoliation+N treatment than in the 3-defoliation (no N) treatment compared to the control (Table 6.2)
- *TChl* increased substantially for both the 1-and 3-defoliation+N treatments compared to defoliated, unfertilised trees, but was not significantly different from the control or the N only treatments at the end of the experiment (Figure 6.6E)
- SLA of defoliated trees was significantly greater than that of trees in the N only treatment at the end of the experiment, irrespective of the frequency of defoliation.

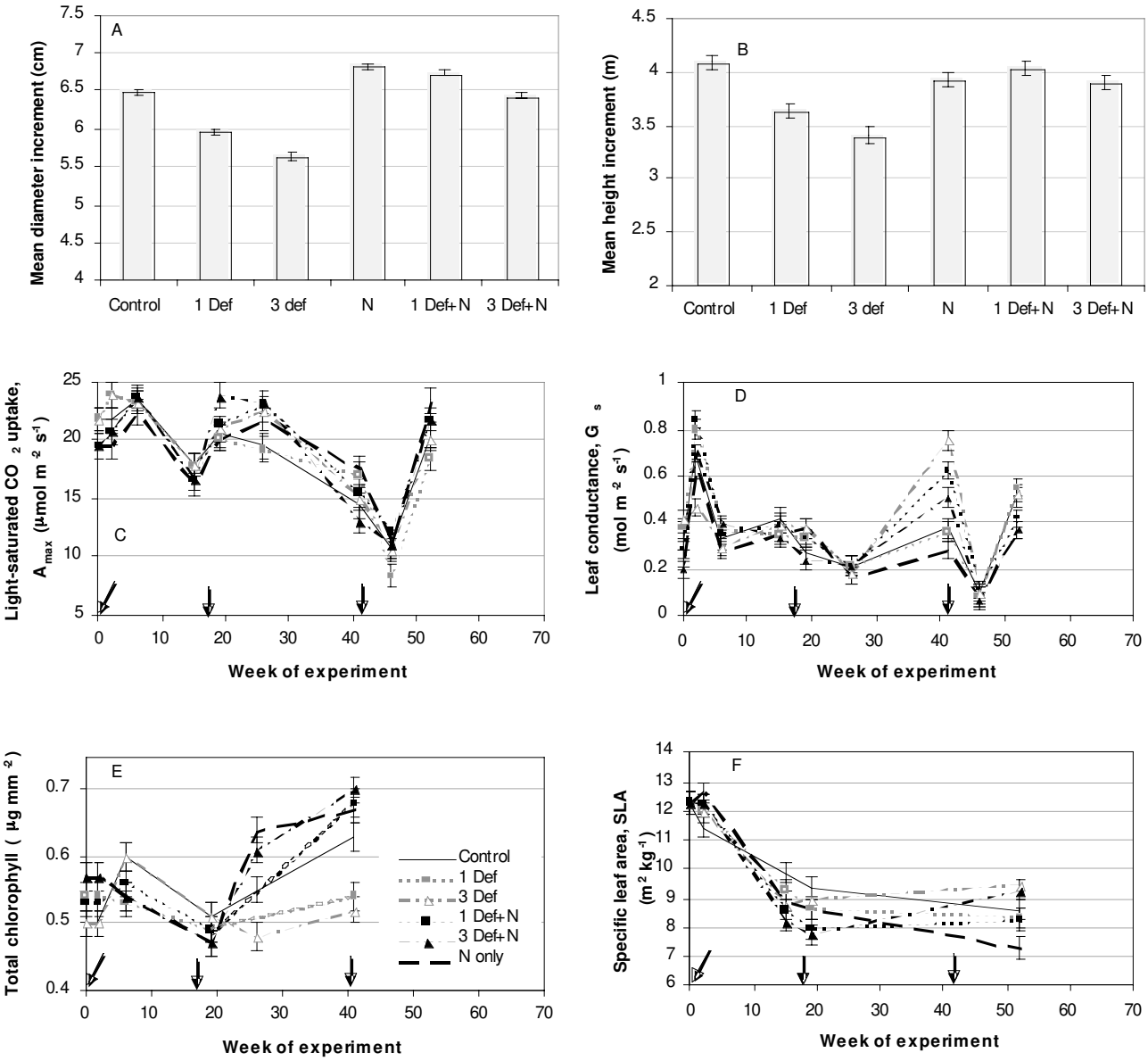

Figure 6.6. Effect of frequency of defoliation, with or without fertiliser (300 kg ha^{-1} N applied at the start of the experiment) on (A) diameter increment (cm), (B) height increment (m), (C) light-saturated CO_2 uptake (A_{max}) (µmol m^{-2} s^{-1}), (D) leaf conductance (G_s) (mol m^{-2} s^{-1}), (E) chlorophyll content (µg mm^{-2}) and (F) specific leaf area (m^2 kg^{-1}), of field-grown *E. globulus*. Error bars indicate standard errors ($P < 0.05$). Arrows indicate when defoliation occurred. The defoliation treatments involved either a single defoliation in autumn (start of experiment) to remove 25% of leaf area from throughout the crown, or 25% defoliation in autumn followed by removal of 50% of leaf area in the following spring (bottom-up pattern), followed by a further bottom-up defoliation in the following autumn.

Discussion

What drives the photosynthetic response to defoliation?

There is a strong relationship in many plant species between photosynthetic rate and leaf N content (Evans 1989). This has also been observed in *E. globulus* (Sheriff and Nambiar 1991; Medhurst and Beadle 2005). However there can be plasticity in foliar N content in eucalypts, related to the dilution of foliar nutrients in actively growing

crowns (Medhurst and Beadle 2005) or storage of excess N in leaves (Close et al. 2004) that can result in poor correlations between A_{max} and foliar N content. Our results suggest that, in undefoliated trees, A_{max} was related, at least in part, to foliar N content via *TChl*, but different mechanisms were influencing the increases in A_{max} that we observed following defoliation.

It is widely acknowledged that changes in the source:sink ratio drive increases in photosynthetic rate observed following a defoliation event (Neales and Incoll 1968; Wareing and Patrick 1975; Reich *et al.* 1993; Layne and Flore 1995; Lavigne *et al.* 2001). The responses that we observed following spring defoliation suggested that source:sink ratio was important in determining photosynthetic rate. In a source-limited system, more frequent defoliation events would be expected to prolong the period of source limitation. As would be expected with this scenario, we found that more frequent defoliations reduced stem growth more than a single defoliation event, despite the earlier and more prolonged increases in A_{max} that were observed following three than one defoliation. Similarly, more severe defoliation would be expected to result in a greater increase in A_{max} than less severe defoliation (Pinkard 2003), although there will always be a maximum rate of photosynthesis, despite increase in source limitation, above which no increase will be observed, that is related to limitations in the rates of biochemical reactions of photosynthesis (Sharkey 1985; von Caemmerer 2000). However there was no increase in A_{max} in response to either 25% or 38% defoliation unless N had been applied, and we conclude that N was limiting upregulation in unfertilised trees. In fertilised plots, trees followed the expected pattern, with A_{max} increasing more in response to 38% than 25% defoliation.

The role of N in determining photosynthetic upregulation following defoliation has been highlighted in other studies (Prins and Verkaar 1992; Suzuki and Takano 2004). This effect, however, was only observed in our experiment in response to the first defoliation, which occurred in autumn. Leaf chlorophyll content did not explain the result, although total N content and partitioning to photosynthetic compounds such as Rubisco, not measured in this experiment, may have been important.

The delay in photosynthetic upregulation following the autumn defoliation until the following spring explains in part the long-term effect of these defoliation treatments on stem growth. Insect defoliation of *E. globulus* occurs throughout the growing season including autumn, and the result suggests that managers should be more concerned with autumn than spring defoliation events. The physiological reasons for the delay in photosynthetic upregulation following autumn defoliation are unclear and warrant further investigation. We hypothesise that the result can be explained in terms of source:sink relationships. In Australia, a reduction in PAR occurs as the seasons change from autumn to winter. Leaf development in *E. globulus* peaks at times of peak light intensity (Battaglia et al. 2004). Hence in spring, as light intensity increases, there is a requirement for more leaf area. A_{max} increases to allow production of more leaves. When plants are defoliated at this time, the tree must produce even more leaves to compensate for the loss, and hence A_{max} increases more. Conversely, in autumn, allocation to leaves declines and so the effects of a defoliation event may not produce the same photosynthetic effect as is observed in spring.

While A_{max} was higher in spring in fertilised than unfertilised trees, the growth response to fertilisation was only small. The decrease in SLA that was observed in response to N

application may have increased light absorption in the upper crowns, explaining the higher levels of incident light measured in the mid and lower crowns of unfertilised than fertilised trees. Hence the crowns of fertilised trees may have been no more productive than those of unfertilised trees despite the increase in leaf-level A_{max}.

Does N application help with compensation following defoliation?

It has been demonstrated in a number of studies that alleviation of environmental stresses can help plants compensate for defoliation (Prins and Verkaar 1992; Otronen and Rosenlund 2001; Suzuki and Takano 2004). Our results indicate that application of N prior to defoliation can allow defoliated trees to maintain stem growth, particularly diameter increment, at a level similar to that of undefoliated, unfertilised trees, and in many instances at a level similar to undefoliated and fertilised trees. This can not be explained simply in terms of photosynthetic upregulation. For example, a similar A_{max} was measured in response to both out-in and bottom-up defoliation irrespective of fertiliser treatment, but the proportionate increase in diameter and height increment associated with out-in defoliation following fertilising was much greater than that observed following bottom-up defoliation. Our results indicate that, while total above-ground biomass production did not change in response to N application 6 months after treatment, partitioning between branches and stems did change. Defoliation also is known to change patterns of biomass allocation (Cannell 1985). For example *Eucalyptus nitens* (Deane and Maiden) Maiden pruned to remove 50% of leaf area allocated more biomass to leaves, particularly in the upper crowns (Pinkard and Beadle 1999). Patterns of biomass partitioning following defoliation were not examined in this experiment, but the diameter increment measured for defoliated plants suggested that trees that had been fertilised and defoliated produced more leaf area than unfertilised, defoliated trees (Shinozaki *et al.* 1964; Valentine 1985). We hypothesise that the extra N from fertiliser, and increased carbohydrate produced by increased A_{max}, were used to rebuild tree crowns, thereby increasing the potential productivity of the crowns. N application offers a potential management option for forest growers following defoliation events.

7. Photosynthesis and nitrogen allocation in *Eucalyptus globulus* Labill. seedlings subjected to artificial defoliation or infection with *Phaeophleosopora eucalypti*

Libby Pinkard, ZiHong Xu, M. Paul and Caroline Mohammed

Introduction

Pest attack (insect, pathogen, mammal) occurs to some extent in most *Eucalyptus globulus* Labill. plantations. Loss of photosynthetic tissue associated with pest attack in *E. globulus* can result in reductions in stem growth (Carnegie and Ades 2002; Elek 1997; Jordon et al. 2002; Rapley 2005). A small number of studies have determined that the degree of recovery in growth that occurs following pest attack depends on the severity, timing, frequency and pattern of attack (Carnegie and Ades 2002; Collett and Neumann 2002; Elek 1997; Smith 2005; Chapter 3). All of these factors influence the physiological processes that enable recovery, such as enhanced CO_2 assimilation (Lavigne et al. 2001; Pinkard 2003; Reich et al. 1993) and changes in patterns of biomass partitioning to favour leaf development (Cannell and Dewar 1994; Pinkard and Beadle 1999). Foliar nitrogen (N) concentration drives production (Hollinger 1996; Le Roux et al. 1999; McMurtrie 1991) and hence N dynamics within the tree also influence recovery. While there is some understanding of N dynamics in *E. globulus* (Saur et al. 2000), the effects of pest attack on N dynamics in this species have not been examined.

Loss of leaf area following pest attack may reduce N available for remobilisation. Millard et al. (2001) found that defoliation had a different effect on N remobilisation in deciduous and evergreen species, because deciduous species stored N in perennial organs whereas evergreen species stored N in leaves. However this is not universal. (Cerasoli et al. 2004) determined that N reserves located in stem and roots of cork oak (*Quercus suber* L.), an evergreen species, were important in recovery following defoliation. *E. globulus*, an evergreen species, has the capacity to store N in leaves under conditions of high N availability (Close et al. 2004), and N remobilisation from older to newer leaf tissue occurs throughout the spring and summer growing season (Saur et al. 2000). Millard et al. (2001) concluded that stem growth reductions following defoliation of *Pinus sylvestris* L. were partly related to removal of N stored in the needles, but it is unknown whether this is the case in *E. globulus*.

Root uptake as well as remobilisation of N can be important in determining recovery after defoliation (Cerasoli et al. 2004; Millard et al. 2001). In *E. globulus*, fertilising with N improves crown recovery following defoliation, and can increase stem growth to a level similar to undefoliated trees (Chapters 2, 3), suggesting that N uptake is also important in this species in determining responses to defoliation. There is some evidence that N loading prior to defoliation improves growth responses following defoliation more than applying N after defoliation (Chapter 3), highlighting the importance of timing of N application.

Attack by insects or mammal browsers has a different effect on total leaf area per tree than infection by a foliar pathogen. Browsing reduces leaf area directly. Foliar infection may or may not result in defoliation via premature leaf senescence, but probably results in one or more of tissue necrosis, production of secondary metabolites, or changes in patterns of cell division, as the host attempts to prevent the spread of the pathogen (Ayres 1992; Edwards 1992). While changes in the root:shoot ratio that occur in response to

both defoliation and infection may influence N uptake (Paul 1992), little is known of the differential effects of infection and defoliation on patterns of biomasss allocation and N retranslocation or uptake in tree species, and virtually nothing in *E. globulus*.

We undertook a glasshouse study with *E. globulus* defoliated to remove 60% of leaf area, or infected with the foliar pathogen *Phaeophleospora eucalypti,* to examine the effects of pest attack on biomass and N allocation. Our hypotheses were that (1) defoliation and infection will produce different patterns of resource allocation and photosynthetic responses; and (2) these patterns will be influenced by N availability. Our aims were to firstly quantify the differences in responses between modes of attack, secondly to determine the relative importance of N remobilisation and uptake, and thirdly to identify the best timing of N fertiliser application in terms of optimising growth following pest attack.

Materials and methods

Plant material

Eucalyptus globulus seedlings grown from open-pollinated seed orchard seeds were planted into 15 cm diameter plastic pots filled with potting mix, and grown for two months. The roots were then carefully washed and the plants were repotted into washed river sand and grown for a further two months after which the experiment commenced.

All plants were watered to saturation three times per day, and fertilised every second day with a complete nutrient solution containing 2 mM nitrogen (N) as NH_4NO_3. The pots were flushed out with water prior to each fertiliser application. The plants were grown outside until the start of the experiment, when they were moved into a glasshouse with a day/night temperature of 20° C and relative humidity of 50%. At the start of the experiment average, diameter (at 5 cm height) and height were 1.38 cm and 0.88 m, respectively. Seedling attributes are given in Table 7.2.

Treatments and experimental design

The treatments applied in the experiment are outlined in Table 7.1. Two crown-level treatments were applied and compared to a control:

- Defoliation, where 60% of leaf area was removed by snipping off two complete leaves and half of a third leaf out of every four leaves
- Inoculation with *Phaeophleospora eucalypti*, where a conidical suspension of ~200 000 conidia mm^{-3} was produced from cultures of *P. eucalypti*, and sprayed onto plants using a fine mist spray setting. Plants were sprayed to saturation on both sides of the leaves. Plastic bags were placed over the plants for a total of 9 days, but removed for a one hour period every three days to avoid development of foliar oedema. Plants were kept in a shadehouse during the inoculation process.

Plastic bags were also placed over defoliated and control seedlings as per the inoculation treatment. Ammonium sulphate with 10% ^{15}N excess was applied to seedlings to examine the effect of timing of N application on N distribution within the crown:

1. 2 weeks before defoliation, or before the development of visible infection (2 weeks after inoculation)

2. 2 weeks after defoliation, or after the development of visible infection (6 weeks after inoculation)
3. As three applications of one third of the full dose (as per treatments 1 and 2, and a third 4 weeks after defoliation/inoculation)
4. No ^{15}N (Table 7.1)

These N treatments were applied to 4 - 5 seedlings per crown damage treatment (4 for inoculated plants, 5 for defoliated and control plants). The N was applied as a 100 ppm solution in 1 l water. The application was spread over three days (330 ml day^{-1}).

Immediately before the application of the N treatments, plants were placed in trays that fed into bags to collect all runoff following watering. Runoff was redirected back into the pots. Complete nutrient solution minus any N was applied to pots at approximately fortnightly intervals.

Table 7.1. List of treatments applied in the experiment

Treatment	Description
Control	^{15}N applied as a single pulse at the start of the experiment; no defoliation or infection treatments
D1	^{15}N applied as a single pulse two weeks before removal of 60% of leaf area from throughout the crown
D2	^{15}N applied as a single pulse two weeks after removal of 60% of leaf area from throughout the crown
DC	^{15}N applied in three doses (1. two weeks before defoliation; 2. at defoliation; 3. two weeks after defoliation); defoliation as per the above treatments
I1	^{15}N applied as a single pulse two weeks after inoculation (ie. before visible symptom development)
I2	^{15}N applied as a single pulse six weeks after inoculation (ie. after visible symptom development).
IC	^{15}N applied in three doses two, four and six weeks after inoculation
No ^{15}N	No ^{15}N, no defoliation, no infection

Table 7.2. Mean seedling diameter, height, dry mass and leaf area at the start of the experiment, and for each of the treatments at the end of the experiment. * denotes significantly different from the control (C) at the end of the experiment (*=P<0.05, **=P<0.01). The treatment codes are defined in Table 7.1.

Variable	Initial	End of experiment						
		C	D1	D2	DC	I1	I2	IC
Diameter (cm)	1.48	1.6	1.5	1.3*	1.4	1.5	1.2*	1.3
Height (m)	0.9	1.2	1.1	1.1	1.1	1.3	1.0**	1.3
Total dry mass (g)	103.3	222.9	223.2	112.2**	143.7**	190.2	139.0**	146.2**
Leaf DM (g)	29.5	21.8	19.5	13.7**	19.2	30.1**	25.7	30.1**
Senescent leaf DM (g)	0.0	7.3	3.4*	0.6*	0.8*	4.3	1.9*	5.6
Branch DM (g)	13.0	15.8	15.6	6.5*	14.0	12.7	8.4*	16.2
Stem DM (g)	25.7	37.8	35.2	26.7*	23.3*	37.8	22.0*	37.5
Root DM (g)	34.1	138.3	149.5	64.7*	86.4*	117.7	61.8*	89.3*
Leaf area (m^2)	0.3	2.1	1.9	1.3	2.1	2.4	2.7	2.3
Shoot:root	2.01	0.56	0.58	0.66	0.61	0.50	0.83*	0.67*
Leaf area removed (m^2) #	0.19							

defoliated treatments only

Measurements

Growth

Stem height and diameter at 5 cm above the soil were measured at the start and end of the experiment. At the start of the experiment, the ends of all branches and the stem were marked. Branches and stem were re-marked 2 and 5 weeks into the experiment to track growth.

Gas exchange

The response of CO_2 assimilation (A) to varying intercellular p[CO_2] (C_i) was measured on three plants per treatment using a Portable Photosynthesis System (LI-6400, Licor, Lincoln, NB, USA) with a leaf temperature of 20°C and a photon flux density (PFD) of 2000 μmol m^{-2} s^{-1}. Measurements were started at a p[CO_2] of 400 ppm, and the p[CO_2] was progressively reduced to 0 before being progressively increased to 2000. Measurements were made on one healthy leaf per plant for the control, and on asymptomatic tissue of one entire leaf per plant for the crown damage treatments. In the infection treatments, measurements were also made on asymptomatic tissue of one leaf with symptoms of disease per tree. Leaves were selected from the top one third of the crown and between two and four leaf pairs from the branch apex.

The response of A to varying PFD was measured on the same plants using the Portable Photosynthesis System with a leaf temperature of 20° C and a p[CO_2] of 400 ppm. Measurements started at a PFD of 2000, and the PFD was progressively reduced to 0 before returning to 2000 μmol m^{-2} s^{-1}.

Biomass production and patterns of allocation

At the start of the experiment, five plants were harvested for biomass analysis. The sand was washed from the roots of each plant, and root, stem, branch and leaf material was separated. Ten leaves per plant were collected for analysis of fresh area: dry mass, specific leaf area (SLA). The area of these leaves was determined using a planimeter (Delta-T Devices, Hoddeston, Herts, UK), and the dry mass was measured after drying at 65° C for three days. All remaining plant material was dried at 65° C and weighed.

The remaining plants were harvested at the end of the experiment. The sand was washed from the roots, and the stem was separated from the roots. The seedling crowns were divided into four vertical zones. The first three of these related to seedling height at the start of the experiment (V1=bottom third; V2=mid third; V3=top third; Vnew=new growth). The fourth zone (Vnew) included all new vertical growth subsequent to the start of the experiment (Figure 7.1). Within the vertical zones, the plant material was divided into 4 horizontal zones: tissue that existed at the start of the experiment (H1), and tissue that had developed between 1-2 (H2), 2-5 (H3) and 5-9 (H4) weeks after the start of the experiment. Leaf branch and stem material was separated for each zone. Five leaves per plant were collected from crown zones V3(H2) and Vnew(H3) for analysis of SLA. All plant material was dried at 65° C for 3 days before weighing.

Chemical analyses

Samples were ground to a fine powder with a ring grinder prior to determination of ^{15}N. Sample $^{15}N/^{14}N$ ratios were measured on a Europa Scientific Roboprep CN

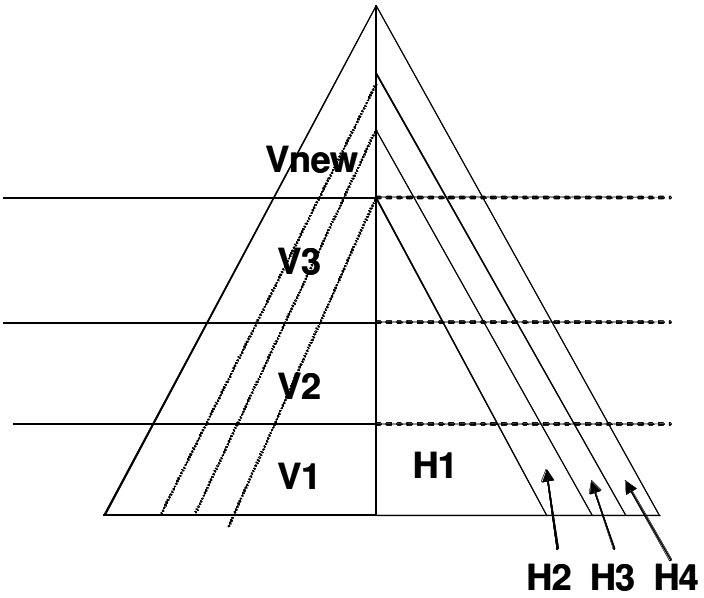

Figure 7.1. Vertical (V) and horizontal (H) crown zones used for biomass and N analyses of *E. globulus* seedlings. The top of V3 indicates the top of the crown at the start of the experiment; Vnew is new vertical growth during the experiment. H1 indicates plant tissue present at the start of the experiment, while H2 – 4 indicate plant tissue developed in the horizontal plane 0-2, 2-5 and 5-9 weeks respectively after the start of the experiment.

(7001)/ Tracerman System (9001) at Griffith University, Brisbane, Australia. ^{15}N was calculated as:

$$\delta^{15}N = \left[\frac{R_{sample}}{R_{s\tan dard}} - 1\right] \times 1000^{0}/_{00}$$

where R_{sample} is the sample $^{15}N/^{14}N$ ratio and $R_{standard}$ is the $^{15}N/^{14}N$ ratio of standard nitrogen gas (N_2). The accuracy and precision of the analyses of sample ^{15}N were checked by making repeated measurements of ^{15}N and using a standard in each batch of the samples.

Data analysis

Differences between treatments in height and diameter were determined with analysis of variance, using initial height or diameter as a covariate.

A non-rectangular hyperbolic function was used to describe the shape of the *A/Ci* and light response curves of each seedling (Sands 1995). In the *A/Ci* analysis, this was used to estimate the photosynthetic utilization of CO_2 (carboxylation efficiency, C_e), the capacity for ribulose bisphosphate (RuBP) regeneration (V_j) and total daytime respiration (leaf dark respiration plus photorespiration), R_p (von Caemmerer and Farquhar 1981; Farquhar et al. 1982). The potential electron transport rate (J_{max}) and maximum rate of Rubisco activity (V_{cmax}) were calculated using the equations of Medlyn et al. (2002) and Bernacchi et al. (2001). Following Wullschleger (1993) and Medlyn et al. (2002) J_{max} and V_{cmax} were fitted over the entire *A/Ci* curve using non-linear regression.

The light response curve analysis was used to calculate apparent quantum yield ( ), leaf dark respiration (R_d), and maximum CO_2 assimilation rate (A_{max}). The CO_2 compensation point (Γ) was taken as the value of C_i where net assimilation was zero (Larcher 1975). The effects of treatment on A/C_i parameters were examined using analysis of variance.

Total root, stem, branch and leaf dry mass was calculated for each seedling. Specific leaf area (SLA) was used to estimate leaf area. Analysis of variance was used to determine the effects of treatment, crownzone or the interzction of treatment and cronwzone on the various biomass components and leaf area.

Calculations adapted from Deleens et al. (1994) were used to determine patterns of ^{15}N enrichment and partitioning. The ^{15}N atom % (A%) was calculated as:

$$A\% = 100\frac{^{15}N}{^{14}N + ^{15}N}$$

The proportion of newly-acquired N relative to total N of the component (relative specific allocation, RSA) was calculated as:

$$RSA\% = 100\frac{A\%_{pulse} - A\%_{control}}{A\%_{labeled.solution} - A\%_{Unlabeled.solution}}$$

The $A\%_{unlabeled}$ solution was assumed to be 0.366%. A mean value of 0.37% was calculated from unlabeled samples and used as the value of $A\%_{control}$. The size of the unlabeled background pool of N ($N_{Background}$) was calculated as:

$$N_{Background} = (1 - RSA_{component})xDM_{component}xN_{mass}$$

where $DM_{component}$ and N_{mass} are dry mass and N concentration of that component, respectively. The mass of N taken up from the labeled nutrient solution (N_{Pulse}) was calculated as:

$$N_{Pulse} = RSA_{component}xDM_{component}xN_{mass}$$

Partitioning of pulse and background N among the different components was calculated as:

$$\%P_{pulse.N} = 100\frac{PulseN_{component}}{PulseN_{seedling}}, \text{ and}$$

$$\%P_{background.N} = 100\frac{BackgroundN_{component}}{BackgroundN_{seedling}}$$

N_{Pulse}, $N_{Background}$, $\%P_{pulseN}$ and $\%P_{backgroundN}$ were determined for each tree component (V1 – Vnew, H1 - H4) as well as for the entire stem, branches, roots, leaves and senescent leaves. Analysis of variance was used to explore the effects of treatment, crownzone or the interaction of treatment and cronwzone on RSA, N_{Pulse}, $N_{Background}$, $\%P_{pulseN}$ and $\%P_{backgroundN}$ for entire plant components and for the V and H zones.

Results

Stem growth

When taken over all treatments, seedlings grew an average of 0.14 m in height and 0.03 cm in diameter at 5 cm above the potted sand surface over the 9 weeks of the experiment. Treatments D2 and I2 reduced mean diameter at the end of the experiment compared to the control ($P < 0.05$), and treatment I2 also reduced mean height at the end of the experiment ($P < 0.01$) (Table 7.2).

Gas exchange

RuBP regeneration capacity (V_j) and J_{max} of symptomatic tissue of infected seedlings were 40% and 23%, respectively, lower than that of the control (Table 7.3) ($P < 0.001$). A_{max} of infected plants was also 40% lower, while that of defoliated, or asymptomatic tissue of infected, seedlings was approximately 20% greater, than the control ($P = 0.1$). Although the differences were not statistically significant, symptomatic leaves of infected seedlings had 30% lower V_{cmax}, 42% lower C_e, 38% lower R_d, and 51% lower R_p than the control. Asymptomatic leaves of infected plants did not exhibit these reductions.

Table 7.3. Carboxylation efficiency (C_e, μmol CO2 m^{-2} s^{-2} μbar^{-1}), RuBP regeneration capacity (V_j, μmol m^{-2} s^{-1})), potential electron transport rate (J_{max}, μmol m-2 s-1)), maximal rubisco carboxylation rate (Vc_{max}, μmol m^{-2} s^{-1}), dark respiration (R_d, μmol m^{-2} s^{-1}); total daytime respiration (R_p, μmol m^{-2} s^{-1}) and apparent quantum yield (, mol mol^{-1}) of healthy *E. globulus* seedlings, or seedlings that were defoliated to remove 60% of leaf area or inoculated with *P. eucalypti*. Values are given for both asymptomatic and symptomatic leaf issue of inoculated plants. *indicates significantly different from the control.

Treatment	C_e	V_j	J_{max}	Vc_{max}	A_{max}	R_d	R_p	
Control	0.057	16.39	57.7	25.3	13.9	0.79	2.47	0.058
Defoliated	0.066	17.78	65.9	28.2	16.5	0.75	2.50	0.051
Infected (asymptomatic)	0.064	17.51	63.4	27.6	17.4	0.75	2.46	0.057
Infected (symptomatic)	0.033	11.15*	44.3*	18.0	8.3	0.49	1.21	0.052

Biomass

Averaged over all seedlings, root biomass ranged between 50% and 60% of total biomass, while stem, branch and leaf biomass ranged between 18% and 25%, 8% and 12% and 10% and 25%, respectively (Table 7.2). Seedlings that had been infected with *P. eucalypti* had proportionally greater biomass allocated to leaves (treatments I1, I2, IC), and less to roots (treatments I2 and IC), than did control seedlings (Table 7.2, $P < 0.05$). There were no other significant differences between treatments in the proportional allocation of dry mass between plant components.

Applying a single dose of N before treatment (D1, I1) resulted in no loss of total dry mass per plant following treatment application (Table 7.2). Splitting the N into 3 doses applied at fortnightly intervals resulted in an approximately 35% decrease in total plant dry mass (DC, IC) compared to the control, whereas applying a single dose of N after treatment application reduced total plant dry mass by 50% (D2) and 38% (I2).

Mean leaf area per plant was 0.32 m^2 at the start of the experiment (Table 7.2). This only increased over the experimental period in one treatment (I2) ($P < 0.05$). The apparent decrease in leaf area of control plants over the period of the experiment can be explained in terms of leaf senescence, which occurred mainly in the control, D1, I1, I2 and IC treatments. While plants in the defoliation treatments had between 9% and 40% less leaf area than the control plants at the end of the experiment, the differences were not statistically significant ($P > 0.05$). The defoliation treatment where leaf area recovered the most was D1, which involved applying N prior to defoliation. Leaf area recovery was least in treatment D2, where N was applied following defoliation.

Leaves produced following treatment had greater area per unit dry mass (SLA) than did leaves of control seedlings (Table 7.4), particularly for crown positions Vnew and H3. The increase in SLA ranged between 27 and 76% for defoliation treatments, and between 17 and 47% for infection treatments ($P < 0.05$).

Table 7.4. Ratio of fresh area: dry mass per leaf (specific leaf area, SLA) (m^2 kg^{-1}) of *E. globulus* seedlings either defoliated or infected with *P. euclypti*. The treatments are as per Table 7.2. * denotes significantly different from the control treatment ($P < 0.05$). Sampling locations were from the upper third of the initial crown length (V3) and from newly-formed crown (Vnew, H3) (Figure 7.1).

Treatment	**Crown position**	
	V3	**Vnew, H3**
Control	10.56	15.36
D1	10.52	19.59*
D2	10.32	20.80*
DC	9.10	26.98*
I1	10.37	22.57*
I2	11.19	17.96
IC	11.00	21.02*

The decreased diameter growth observed in response to treatments D2 and I2, and the lower height growth of seedlings in treatment I2 compared to the control (Table 7.2), is consistent with the reductions in stem dry mass that were observed in response to treatments, especially in zone V1 (Figure 7.2).

In general, defoliation and infection resulted in less dry mass in senescent leaves and roots (D2, DC, I2, IC) compared with the control (Table 7.2). Patterns of biomass partitioning to branch and leaf dry mass also were affected by treatment. Control seedlings had the greatest branch dry mass in the lower crowns, but most new branch dry mass developed in the upper crowns (V3, Vnew) in the first two weeks of the experiment (H2) (Figure 7.2A). Virtually no new branch biomass developed in zone V1. Branch dry mass was reduced compared to the control in the lower crowns of treatments D2 (V1, V2) and DC (V1), but only in zone V2 of infected seedlings (I2, IC).

Initial leaf dry mass of control seedlings was greatest in zone V2 (Figure 7.2B). Most new leaf material was produced in zone Vnew, with lesser new dry mass in zone V3, and virtually none in zone V1. Unsurprisingly, defoliation treatments generally resulted in less leaf area in zone H1 than the control. Leaf dry mass was less than that of the control in zones V2 and Vnew (D1, D2) and V3 and Vnew (DC). In general, infected

seedlings had more leaf dry mass than control seedlings in zones, V1 (I1, I2), V2 (I2) and V3 (I1, I2, IC).

Stem dry mass of control seedlings was concentrated in the lower crown (V1, V2) (Figure 7.2C). Defoliation reduced stem dry mass in zones V1 and V2 (D1, D2, DC) and zone V3 (DC), whereas infected plants only had less stem dry mass in zone V1 (I2, IC).

Nitrogen partitioning

Application of the D1 and DC treatments at the start of the experiment removed 0.002and 0.0003 g N_{Pulse}, respectively, and the D1, D2 and DC treatments removed 0.014, 0.003 and 0.006 g of $N_{Background}$ respectively. At the end of the experiment, seedlings contained between 0.65 and 1.58 g N not derived from the nutrient pulse ($N_{Background}$) (Table 7.5). While $N_{Background}$ values were between 37% and 59% lower in treated than control plants, the differences were not statistically significant ($P > 0.05$). N_{Pulse} ranged between 0.10 and 0.29 g plant $^{-1}$ (Table 7.5). All treatments significantly reduced N_{Pulse} plant^{-1} ($P < 0.001$). Applying the N_{Pulse} before treatment (D1, I1) reduced N_{Pulse} to a lesser extent than did splitting the N dose between three applications (DC, IC), or applying the N after treatment (D2, I2). Plants in the infection treatments had more N_{Pulse} plant^{-1} than did plants in defoliation treatments, although $N_{background}$ did not differ between infected and defoliated treatments.

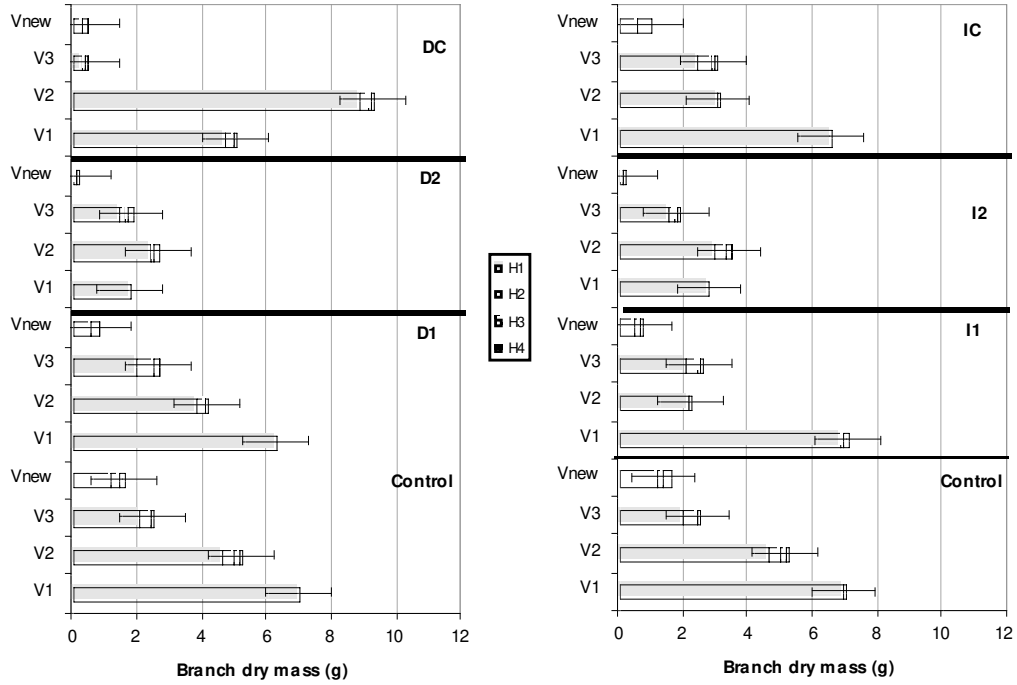

Figure 7.2A. Mean branch dry mass in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

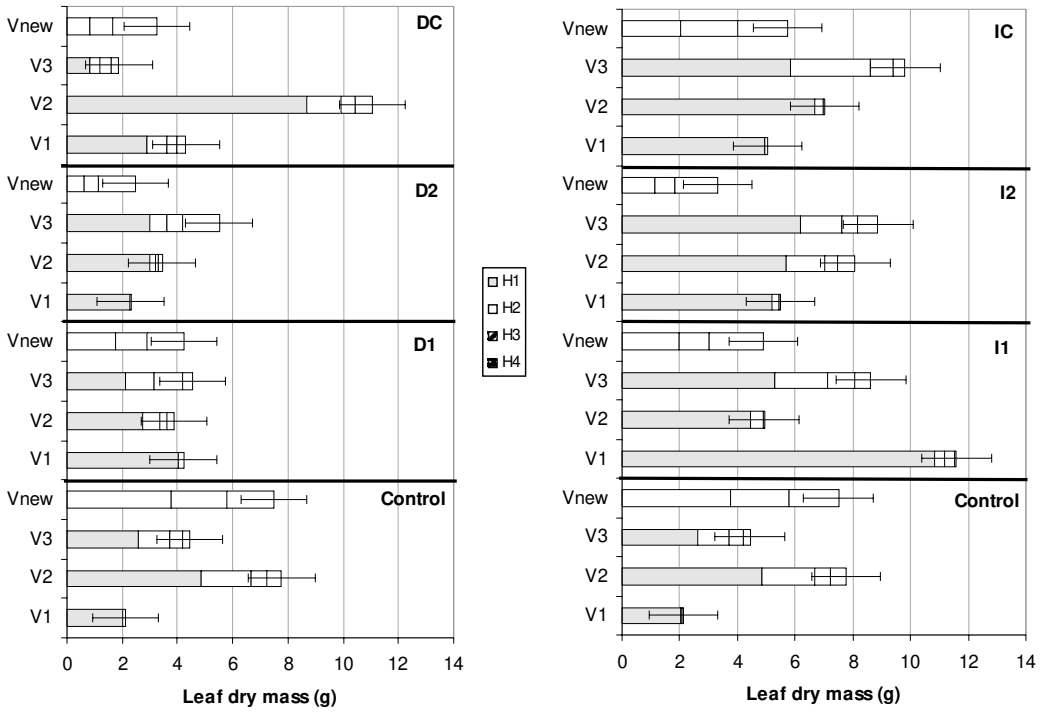

Figure 7.2B. Mean leaf dry mass in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

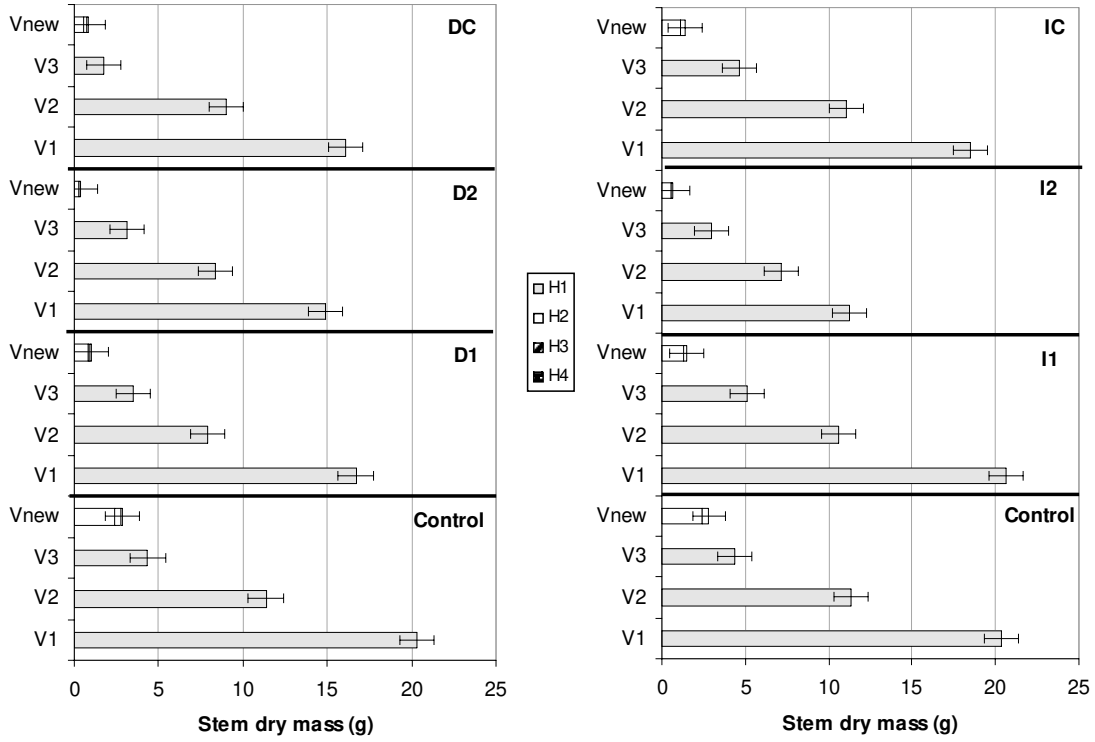

Figure 7.2C. Mean stem dry mass in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected E. globulus seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

The decrease in $\%P_{BackgroundN}$ in roots observed in treatments D2, I1, I2 and IC, and the increase in $\%P_{BackgroundN}$ in leaves of these treatments compared to the control ($P < 0.05$) indicates that there was translocation of N from the roots to the leaves in these treatments. A significant increase in N_{Pulse} in leaves also was observed in infected plants ($P < 0.0001$), and defoliated plants had lower $N_{Background}$ in stems than did control plants.

Nitrogen uptake, as indicated by N_{Pulse}, was significantly less to the roots, stem and senescent leaves of plants in all treatments compared to the control. The $\%P_{PulseN}$ did not vary in plant components except in the leaves where treatments partitioned between 54 and 120% more N_{Pulse} to leaves than did control plants ($P < 0.001$) (Table 7.5).

Effects of defoliation on patterns of N partitioning

Of particular interest in this experiment was the pattern of N partitioning within the crown following treatment. The effects of defoliation on N partitioning varied with the timing of N application with respect to defoliation. Treatment D1 resulted in less N_{Pulse}

Table 7.5. Effect of treatment (as defined in Table 1) on N_{pulse} (g), $N_{background}$ (g) and partitioning of pulse ($\%P_{pulseN}$) and background ($\%P_{backgroundN}$) N to either branch, root, stem, leaves or senescent leaves of *E. globulus* seedlings. * indicates significant differences from the same component of the Control (*=P < 0.05); **=P < 0.01; ***=P < 0.0001).

Treatment	Component	N_{pulse} (g)	$N_{background}$ (g)	$\%P_{PulseN}$	$\%P_{BackgroundN}$
Control	Branch	0.016	0.06	5.02	4.19
	Root	0.199	1.12	50.60	62.4
	Stem	0.025	0.12	8.13	9.20
	Leaves	0.05	0.24	15.60	14.5
	Sen lvs	0.006	0.04**	1.84	1.70
	Total	0.296	1.58	81.19	
D1	Branch	0.012	0.04	7.17	4.44
	Root	0.092*	0.64	51.60	62.3
	Stem	0.017**	0.07*	11.06	7.37
	Leaves	0.043	0.20	25.50***	18.6
	Sen lvs	0.001**	0.01**	0.73	1.52
	Total	0.165**	0.96	96.06	
D2	Branch	0.007	0.03	6.81	3.86
	Root	0.048*	0.34	41.50	48.2*
	Stem	0.016**	0.06*	14.94	9.18
	Leaves	0.033*	0.22	24.10***	33.8*
	Sen lvs	0.0001**	0.002**	0.14	0.40
	Total	0.104**	0.65	87.47	
DC	Branch	0.014	0.04	9.98	5.77
	Root	0.058*	0.40	38.50	51.8
	Stem	0.013**	0.05*	9.27	6.44
	Leaves	0.048	0.21	34.40***	29.3
	Sen lvs	0.0006**	0.005**	0.38	0.62
	Total	0.133**	0.71	92.53	
I1	Branch	0.012	0.04	4.69	4.15
	Root	0.078*	0.51	40.80	48.2*
	Stem	0.025	0.09	13.36	9.34
	Leaves	0.065	0.33***	32.60***	31.2*
	Sen lvs	0.003**	0.03**	2.10	2.96
	Total	0.183**	1.00	93.55	
I2	Branch	0.008	0.03	5.46	3.69
	Root	0.066*	0.33	42.70	40.8*
	Stem	0.015**	0.05*	9.96	6.90
	Leaves	0.055	0.31***	32.60***	33.3*
	Sen lvs	0.001**	0.009**	0.51	1.11
	Total	0.145**	0.73	91.23	
IC	Branch	0.009	0.04	6.11	4.59
	Root	0.072*	0.43	46.20	44.0*
	Stem	0.018**	0.09	11.78	8.91
	Leaves	0.048	0.35***	31.00***	35.6*
	Sen lvs	0.0009**	0.03**	0.62	2.74
	Total	0.148**	0.94	95.71	

in branches in the lower crown (V1, V2), but more in the upper crown (V3) compared to the control (Figure 7.3A). This treatment had no significant effect on leaf N_{Pulse} (Figure 7.3B), but reduced stem N_{Pulse} in zone V1 (Figure 7.3C). In general, there was less N_{Pulse} allocated to wood in the lower crown and more in the upper crown, than in the control. This is confirmed with the RSA which increased for branches in zone V3 (H2-4) (Figure 7.4A) and for stems in Vnew (H2) (Figure 7.4C) compared to the control.

In contrast, treatment D2 seemed to reduce N_{Pulse} throughout the crown rather than just in the lower crown. There was less N_{Pulse} in branches in zones V1, V2 and Vnew (Figure 7.3A), and less N_{Pulse} in leaves (Figure 7.3B) and stems (Figure 7.3C) in V2 and Vnew. However RSA to branches (Figure 7.4A) increased in V3 in response to this treatment, primarily in the newly formed tissue. In addition, RSA to leaves (Figure 7.4B) in this treatment was less than that of the control in the upper crown (V3, Vnew), particularly in newly-formed tissue.

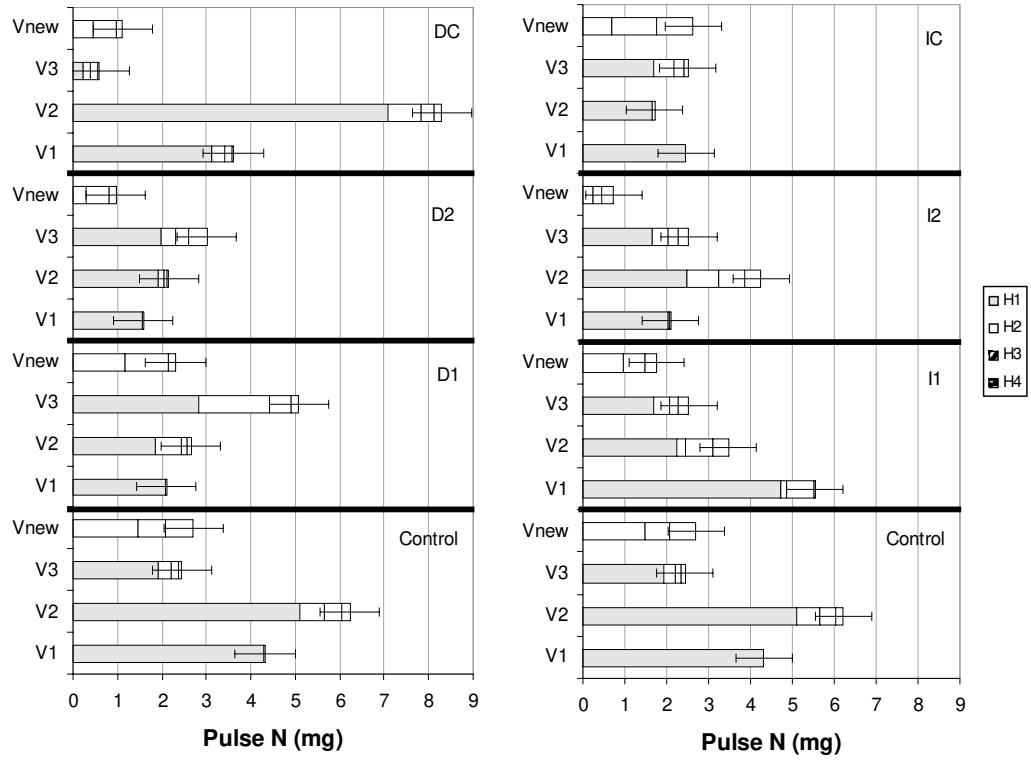

Figure 7.3A. Mean branch N_{Pulse} in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

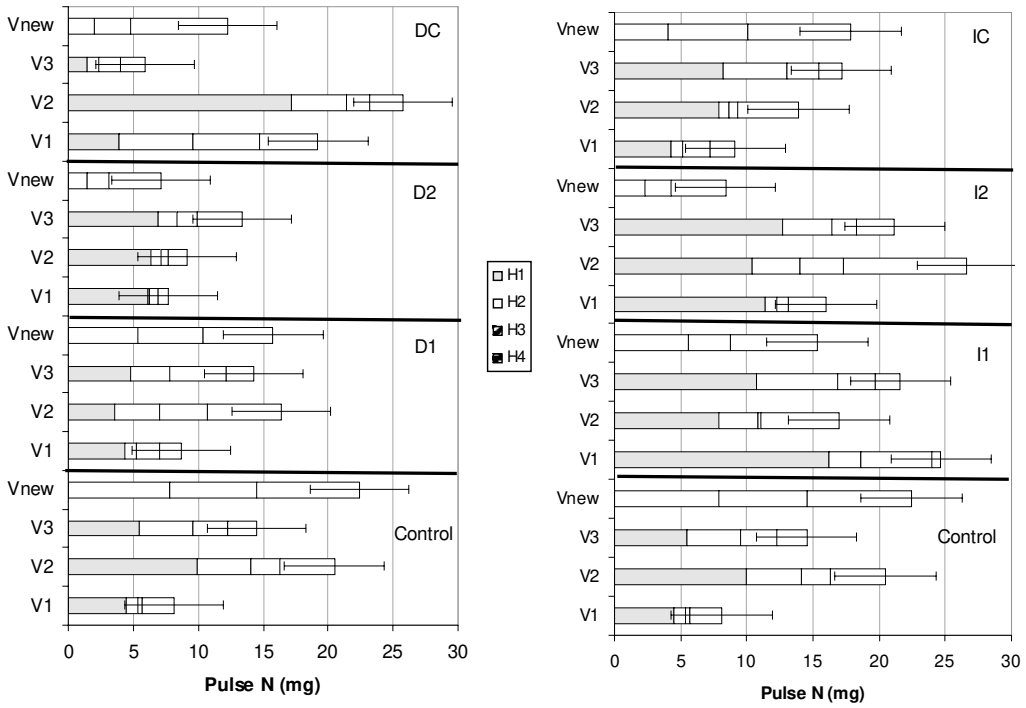

Figure 7.3B. Mean leaf N_{Pulse} in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

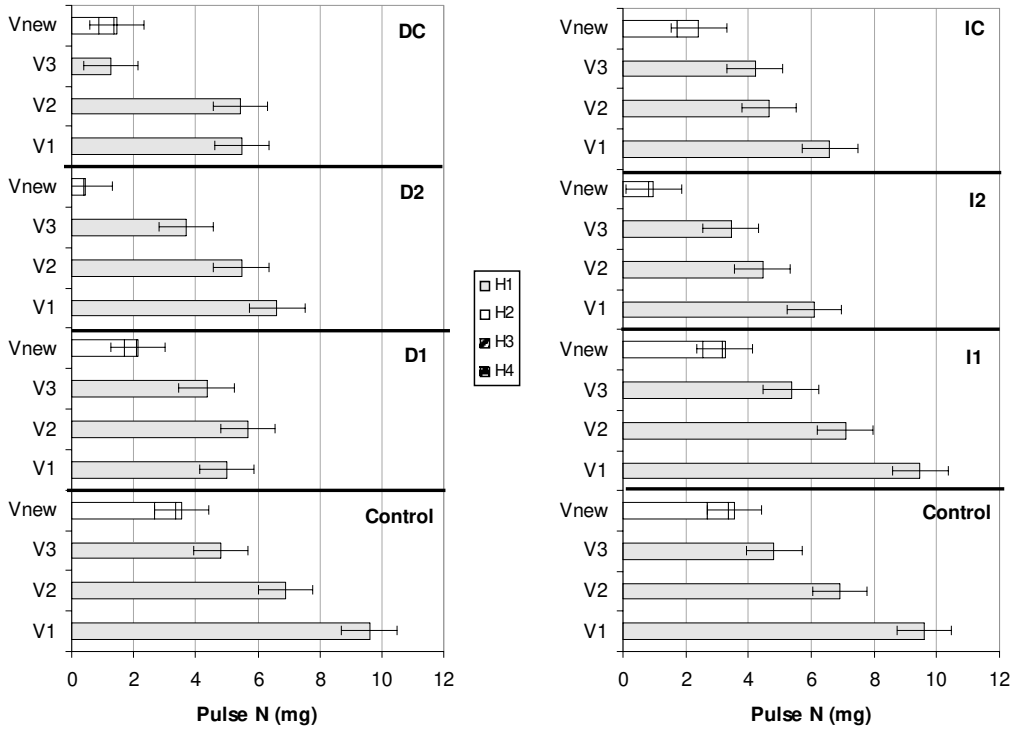

Figure 7.3C. Mean stem N_{Pulse} in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

The effects of treatment DC on N_{Pulse} differed again from the other defoliation treatments. N_{Pulse} in branches increased in zone V2, but decreased in Vnew compared to the control (Figure 7.3A), although the RSA to branches increased in zones V3 and Vnew in this treatment (Figure 7.4A). There was also less N_{Pulse} in leaves in the upper crown (V3, Vnew) in this treatment (Figure 7.3B), and less in stems throughout the crown (V1, V3, Vnew) (Figure 7.3C). RSA to leaves increased in the upper crown (V3, Vnew) (Figure 7.4B), and to stems increased in Vnew (Figure 7.4C).

Compared to the control, there was less $N_{Background}$ in leaves in zones V3 (D1, DC) and Vnew (D1, D2, DC) (Figure 7.5B); less $N_{Background}$ in branches in V1 (D1, D2, DC) and V2 (D1, D2) (Figure 7.5A); and less $N_{Background}$ in stems in zone V1 (Figure 7.5C).

Effects of infection on patterns of N partitioning

Infection had a different effect on patterns of N partitioning compared to defoliation. Treatment I1 resulted in an increase in N_{Pulse} in branches (via increases in new tissue) (Figure 7.3A) and leaves (increases in initial and new tissue) (Figure 7.3B) in the lower crown (V1), and an increase in RSA to branches (Figure 7.4A) in V1 and V3 (new tissue) and to leaves (Figure 7.4B) in V1 (H3) and V3 (H4).

Treatment I2 had a similar effect to D2 in that effects were observed throughout the crown. N_{Pulse} in branches was less in all crown positions (Figure 7.3A), although RSA to branches increased in V1, V2 and V3 (primarily in tissue formed in H2) (Figure 7.4A). While there was more N_{Pulse} in leaves in zone V1 of this treatment compared to the control (Figure 3B), there was less in Vnew. There was less N_{Pulse} in stems throughout the crown (V1, V2, Vnew) of this treatment (Figure 7.3C). RSA to

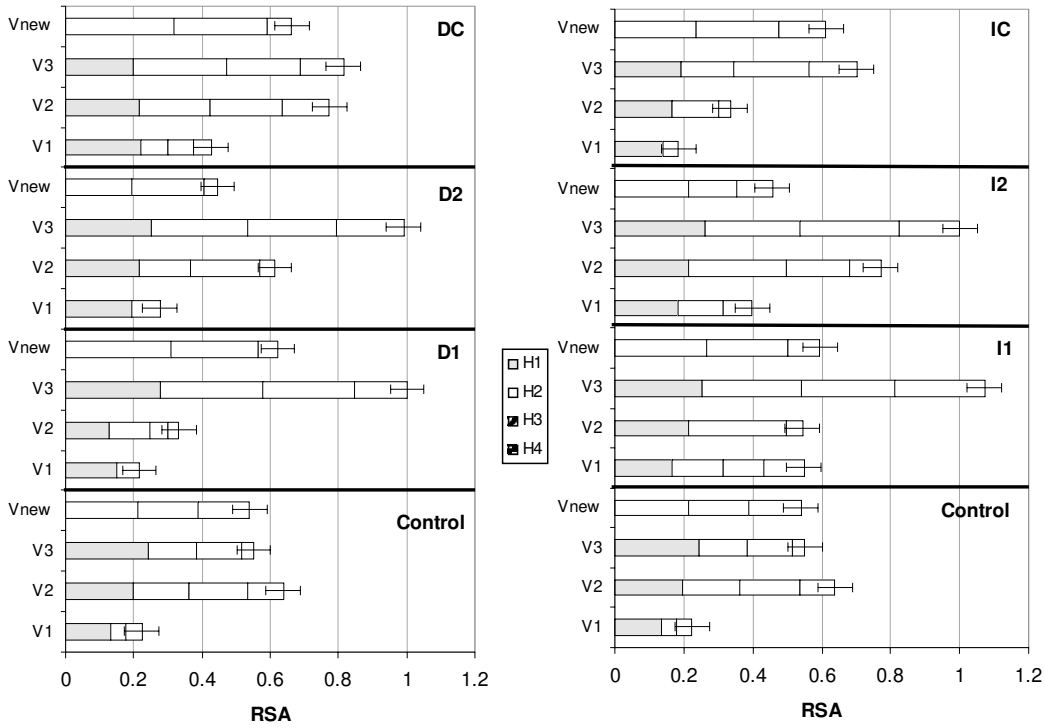

Figure 7.4A. Mean branch RSA in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

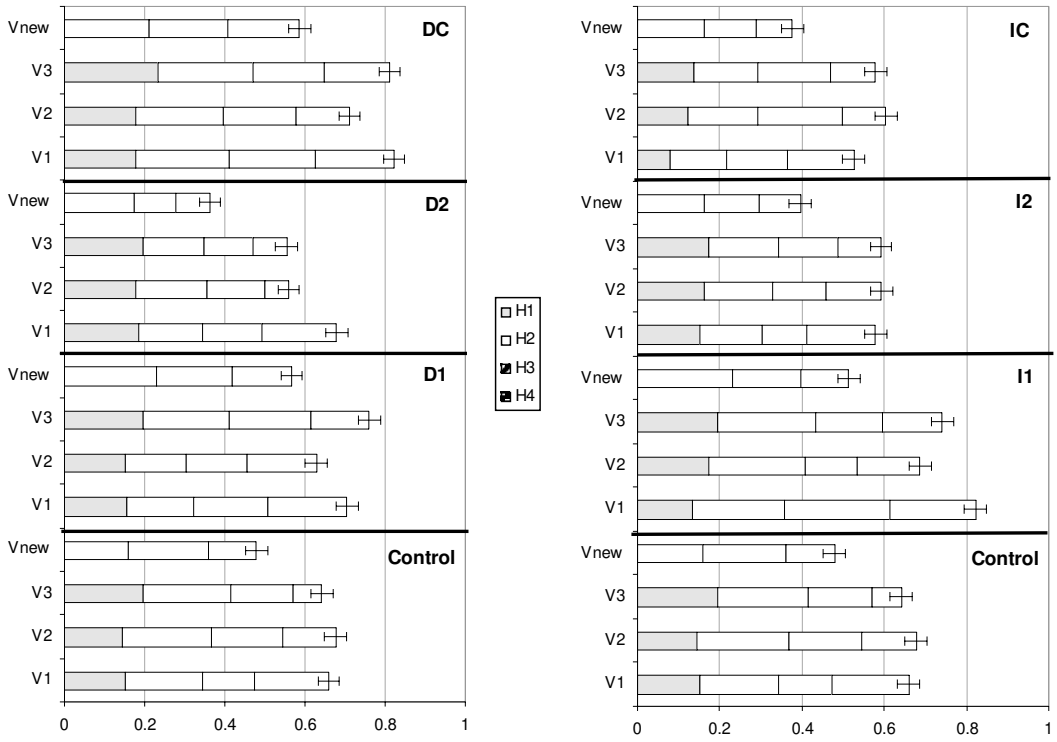

Figure 7.4B. Mean leaf RSA in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

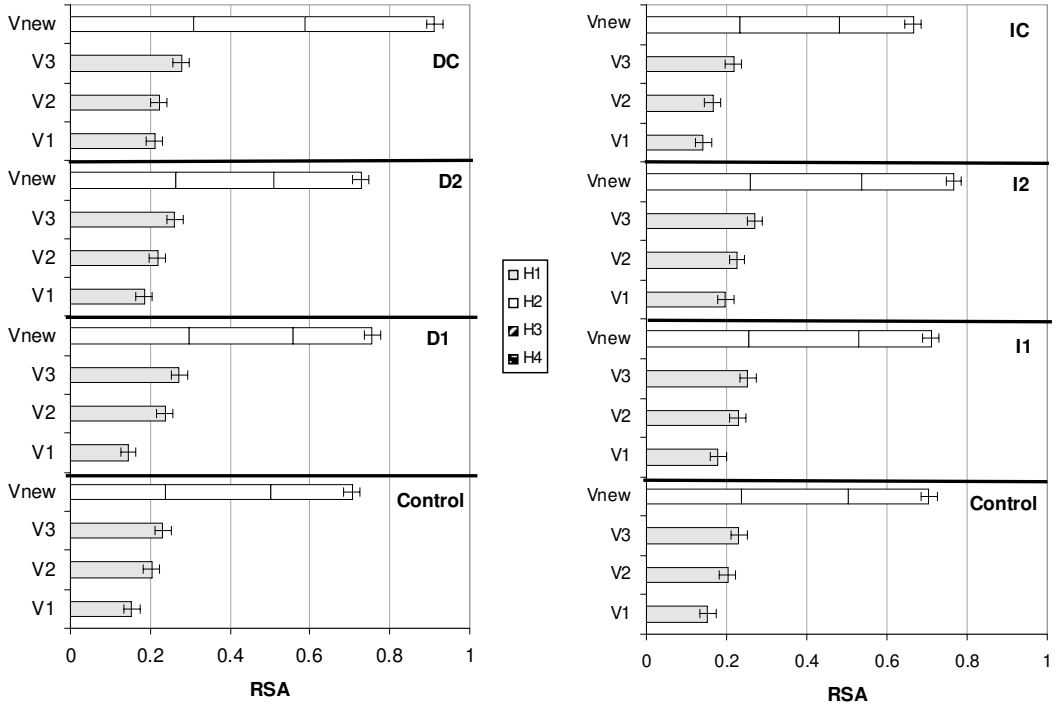

Figure 7.4C. Mean stem RSA in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

leaves decreased throughout the crown (V1, V2, Vnew) (Figure 7.4B), and RSA to stems increased in Vnew, in this treatment (Figure 7.4C),

Seedlings in treatment IC had less N_{Pulse} in branches (Figure 7.3A) and stems (Figure 7.3C) in the lower crown (V1, V2), for all tissue ages. There was no effect however on leaf N_{Pulse}. RSA to branches (Figure 7.4A) increased in V3, but RSA to leaves (Figure 7.4B) decreased throughout the crown (V1, V2, V3, Vnew) compared to the control. The RSA to stems also decreased in Vnew (Figure 7.4C).

Greater $N_{Background}$ was observed in leaves compared to the control in zones V1, V2 and V3 (I1, I2, IC), although less was observed in Vnew (I1, I2) (Figure 7.5B). Branches had less $N_{Background}$ than the control in zones V1 (I2, IC) and V2 (I1, I2, IC) (Figure 7.5A), while there was less $N_{background}$ in the stems in zone V1 of all infection treatments (Figure 7.5C).

Total pulse ^{15}N recovery in seedlings

The total N_{Pulse} recovery averaged 81.2% in whole seedlings in the control after 9 weeks of N application while 18.8% was lost or retained in the sand culture (Table 7.5). This compared with 87.5-96.1% of the N_{Pulse} recovered in seedlings with the defoliation and infection treatments and 3.9-12.5% lost or retained in the sand culture. There were no significant differences between treatments in total N_{Pulse} recovery ($P > 0.05$).

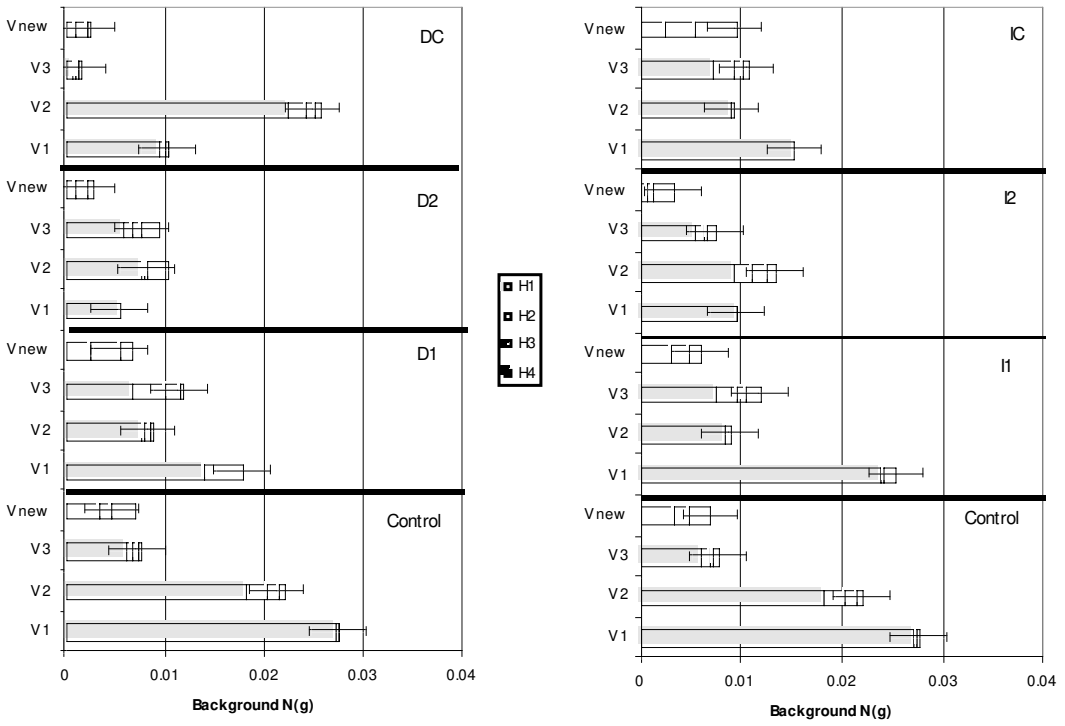

Figure 7.5A. Mean $N_{Background}$ in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

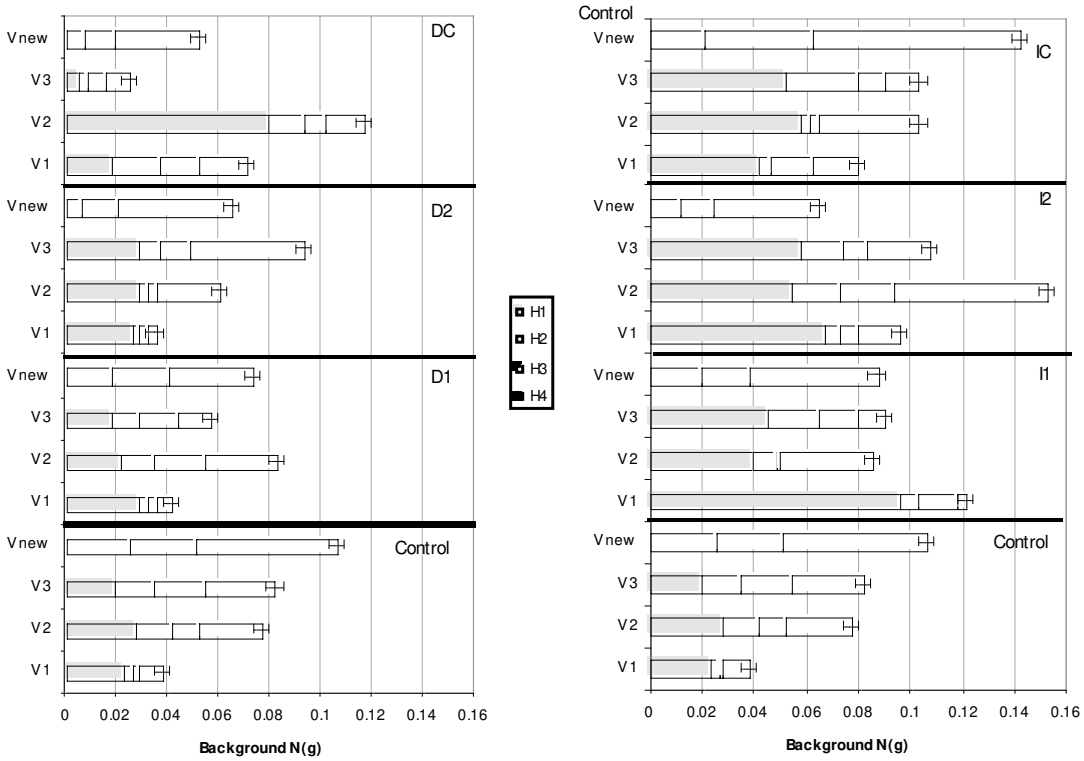

Figure 7.5B. Mean leaf $N_{Background}$ in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected E. globulus seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

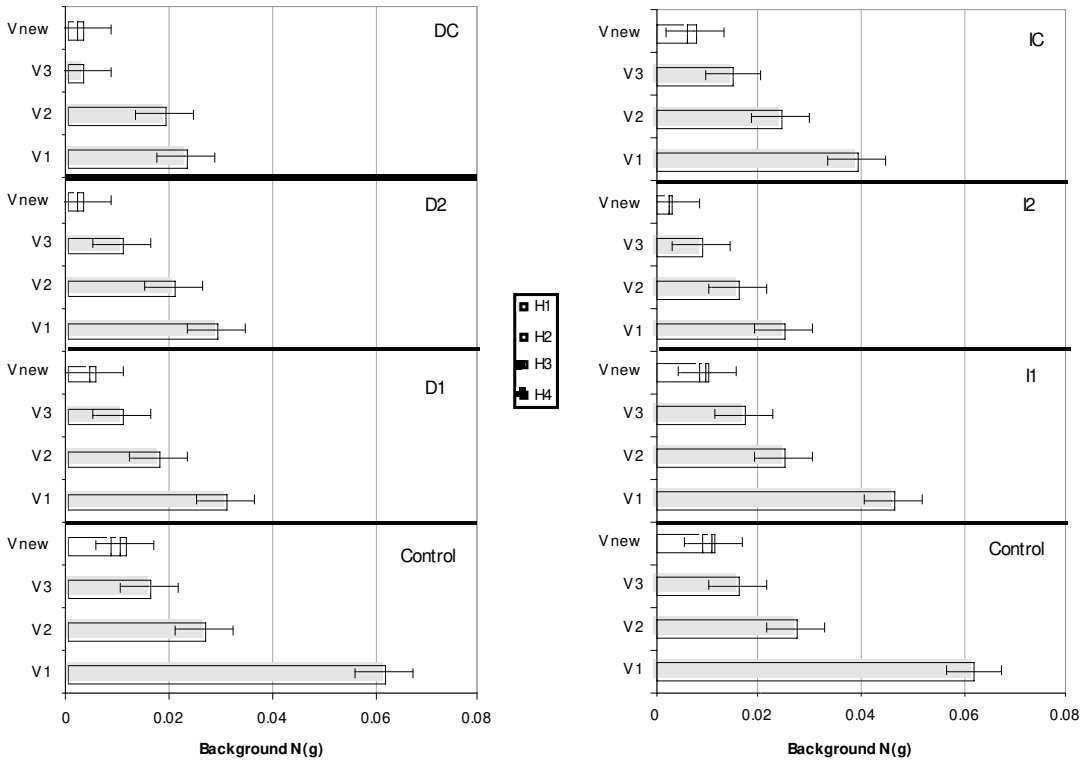

Figure 7.5C. Mean stem $N_{Background}$ in the 4 vertical and 4 horizontal crown positions of control, defoliated or infected *E. globulus* seedlings grown with 4 nitrogen fertiliser treatments (Table 7.1), 9 weeks after the start of the experiment.

Discussion

Defoliation and infection by foliar pathogens affect most plantations to some extent (Christensen and Gibson 1964; Dallot et al. 2003; Hunter et al. 2004; Lundquist 1987; Mohammed et al. 2003; Waggoner and Berger 1987). While defoliation reduces leaf area, infection can result in leaf loss as well as reductions in the photosynthetic capacity of remaining foliage affected by the disease (Buchanan et al. 1981; Shtienberg 1992; Waggoner and Berger 1987). We have demonstrated that defoliation and infection of *E. globulus* seedlings produce different patterns of resource allocation and photosynthetic responses. This is critical information when attempting to model productivity in response to different forms of pest attack. In addition, understanding the influence of N availability and timing of N application with respect to pest attack on the tree's responses to pest attack allows us to start developing appropriate management strategies for minimising the effects of pest attack on plantation productivity. Our experiment was conducted on young seedlings, and while we cannot conclude that similar changes in patterns of resource allocation and photosynthesis would occur for larger trees, there is evidence from other studies that similar changes in biomass partitioning (Pinkard and Beadle 1999) and photosynthetic processes (Pinkard 2003; Pinkard and Mohammed 2006) occur following defoliation or infection in 1 – 3-year-old eucalypts as we observed. Further research is, however, required to confirm this in larger trees growing under field conditions.

At a canopy level, pest attack can affect both light interception and canopy light utilisation efficiency (CLUE) (Rossing et al. 1992). Light interception is affected directly by changes in leaf area. Changes in CLUE are determined by photosynthetic

responses to pest attack, and by resource availability/patterns of resource allocation. We found that both infected and defoliated *E. globulus* seedlings maintained or increased leaf dry mass and leaf area compared with the control plants, suggesting that maintaining levels of light interception in response to pest attack is a priority in this species. This was achieved at the expense of total dry mass per plant, and specifically root, stem and branch dry mass. These are commonly-observed responses to pest attack (Cerasoli et al. 2004a; Clancy et al. 1995; Prins and Verkaar 1992). The decrease in the root:shoot ratio that we observed also is an expected response to infection or defoliation which is associated with reduced translocation of carbohydrate to the roots (Paul 1992). Hence pest attack has implications for resource storage in the roots, which could affect the plant's capacity to compensate for future stresses such as drought or further pest attack (Paul 1992).

Leaf senescence was observed in all treatments, possibly related to inadequate nutrient levels over the period of the experiment. The lower levels of leaf senescence that we observed in response to defoliation and infection are consistent with the delayed leaf senescence reported in other defoliation studies (Hodgkinson 1974; Thomas and Stoddart 1980).

While changes in patterns of dry mass allocation to leaves and wood were consistent between treatments, patterns of allocation within the crown differed between defoliated and infected plants. For example, while stem dry mass decreased throughout the crown of defoliated plants, it only decreased in the lower stem of infected plants compared to the control. Defoliated plants had less leaf dry mass in the mid and upper crown, but infected plants responded by maintaining more leaf dry mass in the lower crown. This suggests that different modes of attack, which can have similar effects on total dry mass, may affect patterns of allocation within the crowns in different ways than have implications for light interception (Rossing et al. 1992). The portion of the crown affected by infection or defoliation is also likely to influence patterns of allocation (Chapter 3).

Canopy light use efficiency is largely driven by photosynthesis (Jarvis and Leverenz 1983; Rossing et al. 1992). A common response to defoliation in many species is to increase rates of photosynthesis (Heichel and Turner 1983; Hoogesteger and Karlsson 1992; Lavigne et al. 2001), whereas a downregulation of photosynthesis often occurs in leaf tissue affected by foliar pathogens (Buchanan et al. 1981; Roloff et al. 2004; Shtienberg 1992). The 11 – 20% increases in C_e, J_{max}, Vc_{max} and A_{max} observed following defoliation are in accordance with the findings of other studies (Lavigne et al. 2001; Layne and Flore 1995; Pinkard and Beadle 1998b; Reich et al. 1993), and can be expected to have increased CLUE to some extent. The potential reduction in CLUE of infected plants, associated with the downregulation of photosynthetic processes that we observed, may have been offset by the increases in leaf dry mass and the upregulation of photosynthesis of asymptomatic leaves. The reductions that we observed in photosynthetic parameters of infected compared to control plants suggests a general downregulation of the Calvin cycle, possibly limited by rates of RuBP regeneration (Scholes 1992). The changes in J_{max} may have been a consequence of the reduced activity of the Calvin cycle rather than a direct effect of the pathogen. The lack of change in apparent quantum yield supports this (Scholes 1992). This is one of the first studies of the photosynthetic responses of *E. globulus* to *P. eucalypti*, and the responses were similar to those reported for *E. globulus* with Mycosphaerella leaf disease, also a

leaf spot disease causing tissue necrosis and discoloration (Pinkard and Mohammed 2006).

Because of the link between photosynthesis and N (Evans 1989; Hollinger 1996), N allocation within the crown also may influence CLUE. In common with other studies, we found that both N remobilisation and uptake occurred following defoliation and infection (Millard et al. 2001; Cerasoli et al 2004). The reduction in total N uptake that we observed in response to treatment is consistent with the reductions in root dry mass that occurred (Clancy et al. 1995). There was remobilisation of N from the roots to the leaves of infected and defoliated plants, and new N entering the plant also was preferentially partitioned to leaves, with less N_{pulse} in root and stem material of treated than control plants. This strategy, while it may optimise CLUE, leaves plants vulnerable to N depletion in the event of further pest attack. Our results indicated that allocation (RSA) was preferentially to newly-formed leaf material, that may be most susceptible to insect or pathogen attack. Following defoliation, N was allocated preferentially to leaves and branches in the upper crown, whereas in infected plants allocation increased in the lower and mid-crowns where leaf dry mass also increased. N remobilisation was more important following infection than defoliation, with increased $N_{Background}$ in leaves in most crown positions, and decreases in stem and branch $N_{Background}$ in the lower crown.

The link between plant growth and resource availability is well-established (Jarvis and Leverenz 1983). Tree vigour can be improved by reducing resource limitation, and there is evidence that pest attack has less effect on growth and biomass production when plants are growing vigorously (Prins and Verkaar 1992; Stone and Birk 2001). Consistent with this, our results demonstrated that loading plants with N prior to pest attack allowed plants to maintain whole plant dry mass at a level similar to that of control plants. An important aspect of this may be that root dry mass and the root:shoot ratio were not affected by infection or defoliation in plants where N loading occurred prior to treatment.

In many crop productivity models, processes are defined at the plant rather than the organ level, although subsystems (water, carbon, nutrients) may be defined (Rossing et al. 1992). Injury at the plant level is classified according to the subsystems affected and the processes affected within each subsystem (Rossing et al. 1992). Our results provide valuable insights into the effects of infection and defoliation on subsystem processes in *E. globulus*, that will allow the development of productivity models that encompass pest attack, although the need for confirmatory experiments with larger trees is recognised.

8 Modelling light interception, photosynthetic and growth impacts of defoliation

Michael Battaglia and Peter Sands

Introduction

Little work has been done to model the impacts of leaf damage or defoliation on tree growth. At the leaf scale the process of infection and plant response is well known (Ayres 1981, Manter *et al.* 2003). However how this scales to whole canopy production (but see Béasse *et al.* 2000, Bethenod *et* al 2005, Le May 2005) and on to tree performance in subsequent years is more problematic and less studied. Some attempts have been made to model the impacts on subsequent production of the simpler intervention of green pruning (Nygren *et al.* 1996, Génard *et al.* 1998, Pinkard *et al.* 1999, Balandier *et al.* 2000). These studies collectively show us that if we are to predict the impacts of given levels of leaf area damage or infection on subsequent growth through to rotation length we need consideration of:

1. The relationship between leaf area necrosis, leaf damage and leaf loss and reduction or up-regulation in leaf level gas exchange and changes in respiration rates;
2. How patterns of necrosis cause shading of foliage and what is the subsequent loss of crown light interception by the healthy foliage;
3. What affect on canopy production does the loss of leaf area from different areas in the crown cause,
4. Changes in patterns of biomass allocation subsequent to loss of effective leaf area,
5. Remobilisation of nutrient and carbohydrate reserves from storage pool,
6. The pattern, rate and timing of refoliation and, if it occurs, photosynthetic recovery.

Few models of the models routinely used for forest management are suitable for exploring the combined effect of these changes. Array models such as MAESTRA (Wang and Jarvis 1990, Medlyn 1997) are well suited to calculating the effect on light interception and could be modified to predict net canopy production however unless linked to allocation and growth models they are not useful for asking forestry management, risk analysis and impact questions. More easily applied forest management models such as 3PG (Landsberg and Waring 1997) do not have the sophistication within the submodules of allocation, light interception or nutrition to deal with the problem. SimWal (Balandier *et al.* 2000) requires detailed branch and root architectural information and the carbon balance model of Génard *et al.*(1998) is a single season model and makes highly simplified assumptions such as fixed photosynthetic rates not suitable for plantation crops growing through highly variable conditions of water stress, frost and marked seasonality in temperature.

The process-based model CABALA (Battaglia *et al.* 2004) has many of the components at suitable scale and degree of generality necessary to model the impacts of loss of effective leaf area on forest production. Carbon-water- nitrogen cycles are linked so that simultaneous impacts of leaf area loss on biomass allocation, transpiration and water use and nitrogen reallocation can be assessed. While the model allows for different spacing arrangements, in its published form it makes assumptions of uniform

leaf area density and a fixed attenuation pattern of photosynthetic capacity throughout the crown. This chapter details modification to the model to extend the light interception and photosynthetic sub-modules of CABALA to better suit them to assessing the impacts of leaf area damage and loss. It also describes the method used to regrow leaf area in the crown.

The Model

Assumptions

Assumptions additional to those inherent to CABALA necessary to model loss of effective leaf area are listed below.

1. *A stand is composed of identical individual trees and all trees in the stand are equally affected by defoliation or decline in photosynthetic capacity.* This assumption is necessary because of CABALA current structure in which light interception and allocation are calculated for the 'typical' tree and aggregated to the stand level. Future developments should see this condition relaxed. However, as the model stands the current assumptions may lead to errors where infection or damage is highly variable from tree to tree (In situations where damage changes gradationally or in patches in may be reasonable to model each as a separate stand).
2. *Each tree is an ellipsoid. When trees merge within rows they are treated for light interception purposes as a hedgerow.* At this stage tree or hedgerow units do not interact or shade each other. This will mean a slight overestimation of production early and late in the days. Evidence from comparison with daily estimates from Maestra suggests that these errors are not significant (Battaglia *et al.* 2004).
3. *The tree is broken up into two radial and three vertical crown zones.* Within each crown zone leaf properties (leaf area distribution and extinction coefficient) are identical (these assumptions are not necessary to the model structure but this is how they are programmed in the interface).
4. *Leaves are either removed or remain on the tree for their normal longevity with reduced photosynthetic capacity. No damage occurs to the capacity of trees to produce new leaves (no bud or branch loss).* This means that browsing or infection that leads to bud removal or if branch material dies then the model is likely to under-predict the impact.
5. *Tree leaf area is added to leaf zone proportional to the volumetric enlargement of that zone – so when trees are expanding laterally as well as vertically leaf area will be added to all leaf zones, but once closure is within and between rows leaf area is only added to the top of trees.*
6. *Maximum photosynthetic rate at any point in the crown is proportional to time average irradiance.* The maximum rate of photosynthesis attenuates through the crown with the leaf area along the average sun pathlength. The maximum rate at the top of the crown is determined by factors such as nitrogen, frost and temperature (see Battaglia *et al.* 2004 for details). The coupled conductance and assimilation model applied in CABALA is solved for each point within the crown.
7. *Defoliation does not affect the foliage longevity.*

Light interception

The irradiance of a beam of sunlight illuminating a tree crown depends on its direction but is constant across the beam. But since different portions of the beam have different path-lengths in the crown, transmittance will vary across the beam. So, crown transmission, and hence the total radiation absorbed by the crown, is obtained by integrating across the beam. Analytical formula exist for the amount of light absorbed by the crown and the cross-sectional area of the intercepted beam only if leaf characteristics are independent of position in the crown, and the crown has a simple shape (e.g. vertical ellipsoid with circular cross-section, or hedgerow with elliptical cross-section). Otherwise, a numerical algorithm is required.

The algorithm developed here performs these integrations for general distributions of leaf properties and more general crown shapes. It examines rays of light aimed at a rectangular grid normal to the beam direction, and determines which rays intersect the crown. For each of these it determines the attenuation of irradiance with distance travelled in the crown, and by summing over those rays that intersect the crown it determines the amount of light absorbed by the crown and the cross-sectional area of the transmitted beam.

The crown transmission τ_C is the fraction of radiation incident on the crown that is subsequently transmitted, and is given by

$$\tau_c = \frac{1}{A_B} \iint e^{-\int k(s)\rho_F(s)ds} dA ,$$

where the integrals are over all rays that intersect the crown, and along that portion of a ray inside the crown. The intercepted radiation is then

$$E_c = (1-\tau_c) A_B I_0 ,$$

where I_0 is the beam irradiance (W m^{-2}) and A_B (m^2) is the cross sectional area of that portion of the beam that intersects the crown. The algorithm for numerically integrating $_C$ is described in Appendix 1.

Crown and beam geometry

Crown geometry and leaf characteristics

The crown is assumed to be an ellipsoid with distinct axes, one of which is vertical, i.e. rotational symmetry is *not* assumed. The crown is specified by its horizontal diameters D_{Cx} and D_{Cy}, crown depth H_C, and the compass bearing ψ_y of the D_{Cy}–axis. A hedgerow with elliptical cross-section can be modelled by setting the D_{Cy} very much larger than D_{Cx}, and the algorithm then calculates interception by a unit length of the hedgerow.

The crown is further characterised by the leaf area density ρ_F (m^2 m^{-3}), i.e. single-sided leaf area per unit volume in the crown, and light extinction coefficient k. Both ρ_F and k may vary with position, and are specified either through user-supplied functions giving their spatial variation within the crown, or by segmenting the crown both vertically and radially. In the latter case the crown is divided into vertical layers, within which are radial zones. The vertical layers have horizontal boundaries, and the radial zones are elliptical cylinders centred on the stem and with axes in the same directions as the axes

of the crown. In this implementation we define an inner and an outer zone and three vertical zones (upper, mid and lower) and for each zone ρ_F and k are assumed constant.

Crown zones

When the crown is divided into zones the following conventions and definitions provide a flexible description of the distribution of leaf properties in the crown.

- There are n_v vertical layers bounded by horizontal surfaces, and n_r radial zones bounded by ellipsoidal cylinders. Vertical layers are numbered downwards, radial zones outwards.
- The j^{th} vertical layer is specified by the depth of its bottom surface expressed as a fraction h_j of the crown depth, measured from the top.
- The diameters of the i^{th} radial zone in the j^{th} vertical layer are the fractions r_{xij} and r_{yij} of the diameters D_{Cx} and D_{Cy} of the crown.
- Crown characteristics are given by the values ρ_{Fij} and k_{ij} of ρ_F and k in the ij^{th} zone.

It follows that the bottom of the j^{th} vertical layer is a distance $h_j H_C$ from the top of the crown, and the actual diameters of the radial zones are $r_{xij}D_{Cx}$ and $r_{yij}D_{Cy}$. Note that if $r_{xij} = r_{yij}$ the radial zones are of similar shape to the cross section of the crown, and if r_{xij} or r_{yij} is very large (>>1), the radial zone approximates a rectangular box. Care should be taken when assigning the r_{xij} and r_{yij} within a given layer to make sure that zones do description of the light interception model is given in Appendix 1.
Photosynthesis

Algorithmically canopy photosynthesis is calculated in a similar way to light interception. Using the light interception algorithm given above the light intensity is calculated on the same coordinate system.

At grid points within the tree the potential maximum rate of photosynthesis in non-light limited situations, P_x (μmol [CO2] m^{-2} [leaf] s^{-1}) is assumed to be affected by the long term light environment at that point within the crown, such maximum photosynthetic rate at that point with in the crown, P, is given by:

$$P = P_x \frac{2\hat{I}}{1+\hat{I}+\sqrt{(1+\hat{I})^2 - 4\theta\hat{I}}};$$

where $\hat{I} = \alpha \bar{I}_S / P_x$, $_S$ (μmol PAR m^{-2} s^{-1}) is the 30 midday average light intensity after it has traversed distance s (m) through the tree crown from the point of ray incidence to the grid point, α ($molCO_2$ mol^{-1}PAR) and θ are parameters of the single leaf light-response curve.

The potential maximum light limited photosynthetic rate at any point in time is then calculated using this base photosynthetic rate and the illuminance at that point in the crown. This value is then used to solve the coupled conductance-photosynthesis model (as per Battaglia *et al.* 2004 equations 1.15-1.21). The resultant photosynthetic rate is applied to all leaf area within that portion of the crown for the hour of the simulation and hourly photosynthesis summed to daily totals.

Photosynthetic up-regulation

After a defoliation event photosynthesis remains up-regulated until the crown leaf area is restored to the pre-defoliation leaf area. The assumption is that until this equilibrium is restored source-sink relationships remain out of balance. Up-regulation is affected by multiplying P_{max} by a user defined quantity. Once leaf area reaches the pre-defoliation leaf area photosynthetic up-regulation is switched off. (Discussion in chapter 6 pertains to this point).

Restoration of leaf area

Before canopy closure leaf area is assumed to be restored from the outer zone. Following canopy closure leaf area is assumed to be added to the top of the tree. The photosynthetic properties of the crown zone are calculated from the weighted average of the new foliage, assumed to be fully functional, and the photosynthetic properties of the old foliage.

The Interface

The model is implemented in two formats.

Spreadsheet implementation

The first implements only the light interception and photosynthetic production models without connection to the CABALA model. The algorithm is implemented in an Excel spreadsheet (`CrownInterception.xls`) that enables the effects of various defoliation patterns on the diurnal and seasonal patterns of light interception and daily photosynthetic production to be examined (Figure 8.1). This is a simple, easy to use application for quick impact assessment. It allows a relative ranking of potential defoliation episodes but does not allow for dynamic tree responses such as refoliation. Similarly, because it does not integrate estimates of assimilation with other trees processes such as respiration and biomass allocation it can not be used to assess rotation length impacts.

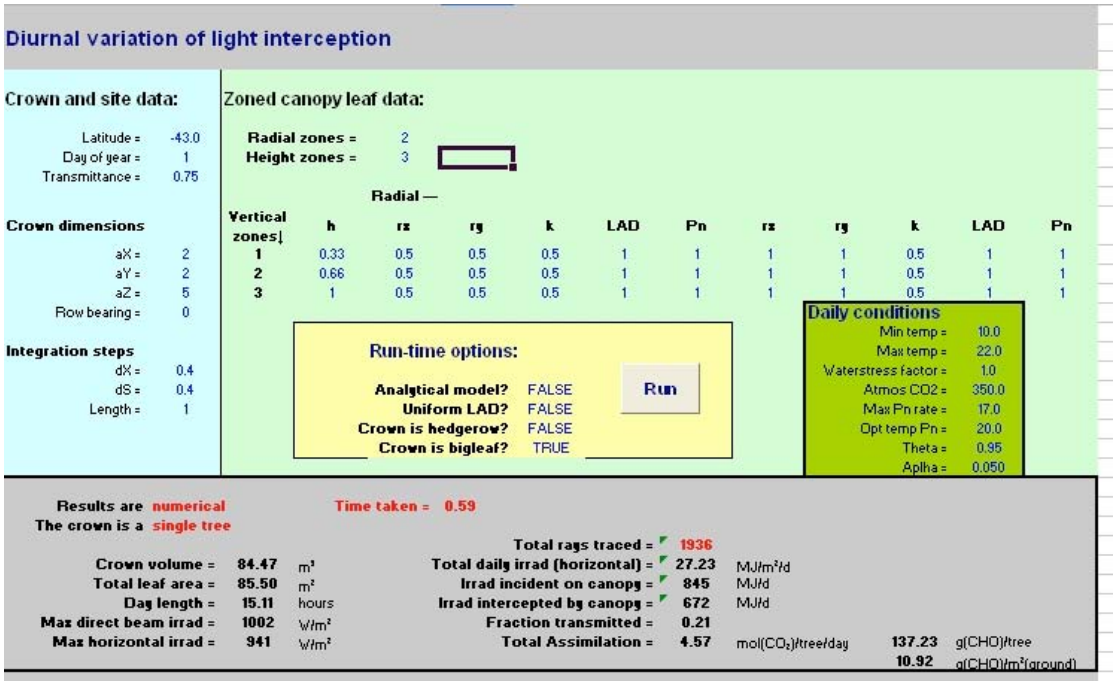

Figure 8.1. Screen capture of spreadsheet implementation of photosynthetic and light interception program.

CABALA VB.NET implementation

Full implementation of the above specified algorithms and integration with CABALA has been achieved by making defoliation and leaf pathology an event in the Regime Events Explorer (Figure 8.2). In this interface option the user can specify whether the event is loss of leaf area alone (defoliation or browsing) or involves leaf necrosis or loss of photosynthetic activity (so that not only can pathogen infection be simulated but also events such as frost damage).

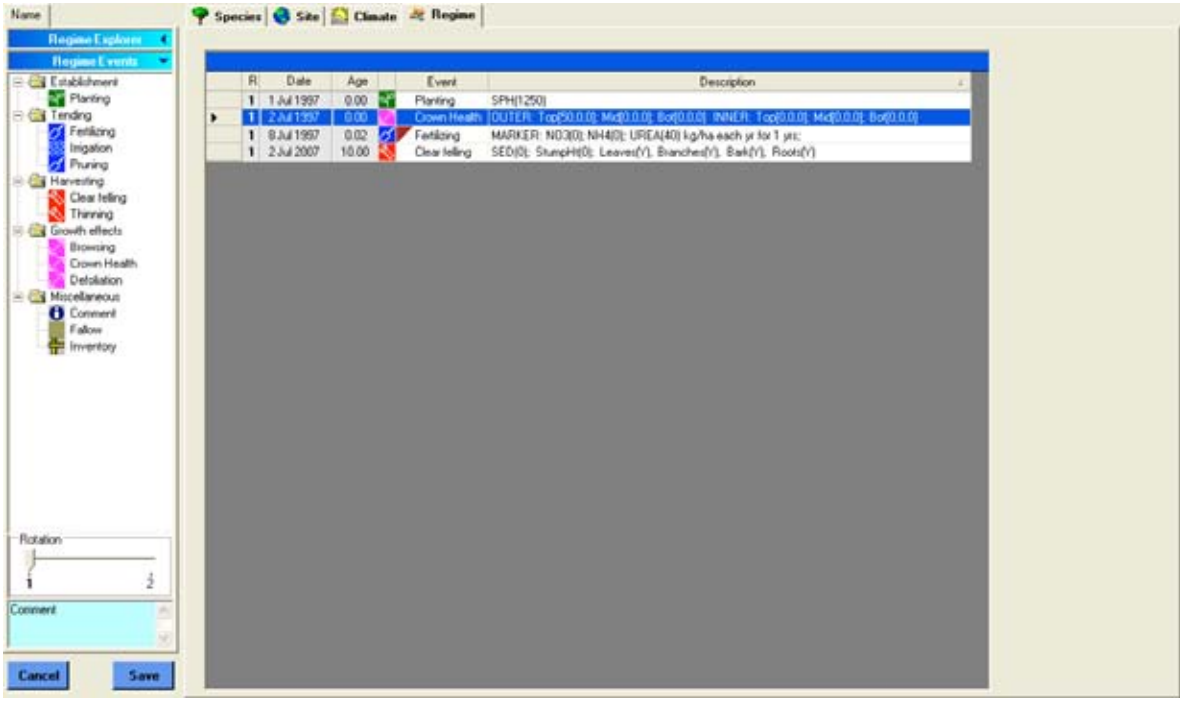

Figure 8.2. Screen capture of the Regime Event Explorer in CABALA showing the 'Growth Effects'.

The user selects the particular effect from the Regime Events Explorer and enters the event effects on the event dialogue box (Figure 8.3).

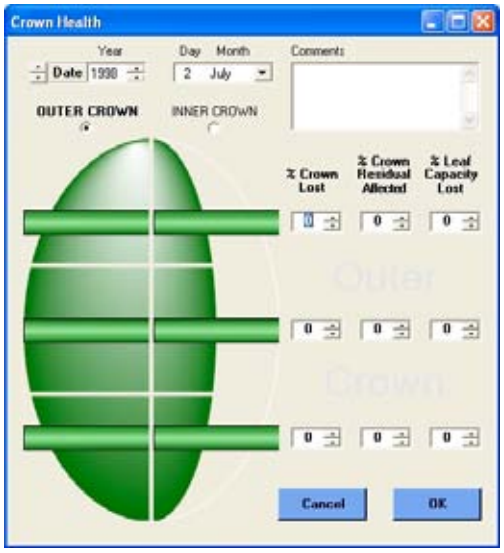

Figure 8.3. Screen capture of the Crown Health dialogue box showing the way that the user characterises and crown health event.

Example predictions and results

In this section we apply the tools developed above to explore the productivity responses identified in the field component of this project (Chapter 3). For simplicity we demonstrate the system for only two treatments: 50% crown removal from below and 50% crown removal from throughout the crown.

We can use the spreadsheet tool to examine the significance of the up-regulation of photosynthesis in mitigating the effect of defoliation. We take two days, one mid-summer and one mid-winter and compare the intercepted radiation and the total assimilate produced under the various scenarios.

Assumptions

For the spreadsheet calculations the following assumptions were made:

1. Trees had a crown length of 3m and a crown diameter of 2m.
2. Leaf area density was 1 m^2m^{-3} resulting in a leaf area index of 2 m^2m^{-2}.
3. Daily maximum temperature was assumed to be 22 ^oC and daily minimum temperate 10 ^oC with sinusoidal variation; no water stress was imposed.
4. Mid-summer production was simulated on 1 January with a total irradiance of 27.3 MJ m^{-2} d^{-1} (Fig. 8.4) and mid winter on 1 July with a total irradiance of 4.5 MJ m^{-2} d^{-1}.

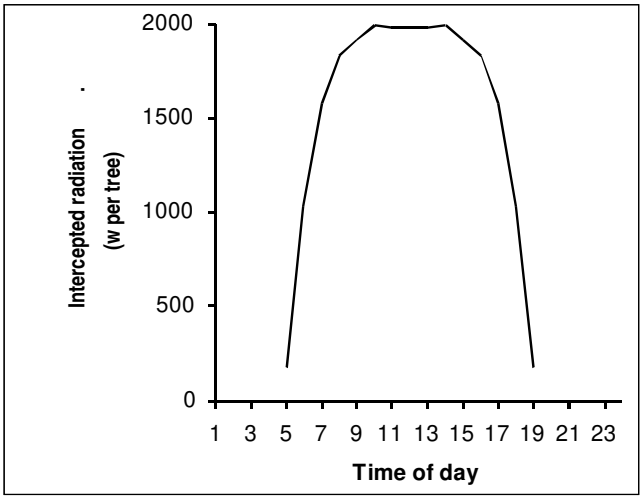

Figure 8.4. Simulated radiation interception a tree with semi-axes 1m by 1m in the horizontal plane and 1.5m the vertical plane, leaf area density 1m^2m^{-3}, leaf area index 2 m^2m^{-2} on a clear day midsummer.

Defoliation results in a greater reduction in intercepted radiation than in total assimilation (Table 8.1). While 50% defoliation is predicted to result in a 40-50% reduction in light interception the effect on total assimilation was less at 30-40%. For a tree of the small size and comparatively low leaf area index as used in the simulation there was little difference between bottom-up or foliage loss from through-out the crown, though total radiation interception was less when total crown length was reduced by bottom-up defoliation compared with reduction in leaf area density with defoliation from throughout the crown (Fig. 8.5).

Table 8.1 Simulated values of intercepted radiation and total assimilation per tree under different defoliation scenarios mid-winter and mid-summer.

Treatment	Mid-summer intercepted radiation (MJ/d)	Mid-winter intercepted radiation (MJ/d)	Total assimilation mid-summer (g[CH_2O]/tree)	Total assimilation mid-winter (g[CH_2O]/tree
No defoliation	78	30	28.3	8.3
50% removal throughout, no up-regulation	48	18	19.0	5.8
50% removal throughout, 26% up-regulation	48	18	23.0	6.1
50% removal bottom-up, no up-regulation	46	16	17.9	5.5
50% removal bottom-up, 26% up-regulation	46	16	21.7	5.7

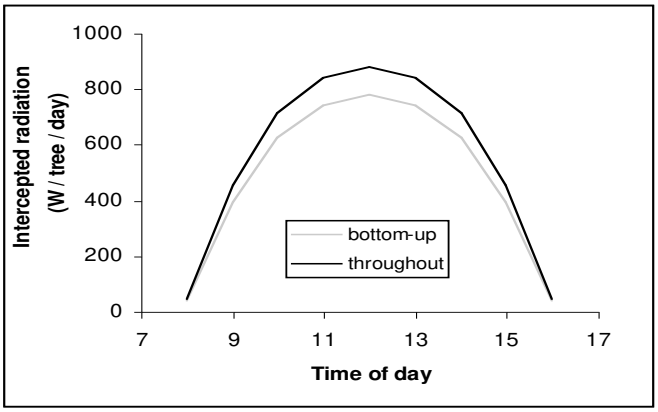

Figure 8.5. Simulated radiation interception a tree with semi-axes 1m by 1m in the horizontal plane and 1.5m the vertical plane, leaf area density $1m^2m^{-3}$, leaf area index 2 m^2m^{-2} on a clear day midsummer with 50% foliage removal from below and 50% foliage removal from through out the crown.

Up-regulation of photosynthesis by 26% (the level observed in the field) came close to removing the gross assimilation difference between defoliated and un-defoliated trees. When the impact of reduced respiration is taken into account analysis with the full CABALA model show that, as we observe in the field, no impact on growth is anticipated.

Appendix 1: Mathematical details of light interception

Introduction

The irradiance of a beam of sunlight illuminating a tree crown depends on its direction but is constant across the beam. However, different portions of the beam have different path-lengths in the crown, so transmittance will vary across the beam and total radiation absorbed by the crown is obtained by integrating across the beam. Analytical formula exist for the amount of light absorbed by the crown and the cross-sectional area of the intercepted beam only if leaf characteristics are independent of position in the crown, and the crown has a simple shape (e.g. vertical ellipsoid with circular cross-section, or hedgerow with elliptical cross-section).

The algorithm developed here performs these integrations for general distributions of leaf properties and more general crown shapes. It examines rays of light aimed at a rectangular grid normal to the beam direction, and determines which intersect the crown. For each of these it determines the attenuation of irradiance with distance travelled in the crown, and by summing over those rays that intersect the crown it determines the amount of light absorbed by the crown and the cross-sectional area of the transmitted beam.

Definition of crown transmittance

Let the irradiance of the direct beam be I_0 (W m^{-2}). Consider a single ray in this beam and let $I(s)$ be its irradiance after penetrating a distance s (m) along the beam. The fraction of light absorbed by the crown depends on the leaf area $l(s)$ accumulated along the beam and projected normal to the beam, and

$$I(s) = \mathrm{e}^{-l(s)}\, I_0 . \tag{1}$$

Let ρ_F (m^2 m^{-3}) be the leaf area density and k the light extinction coefficient (i.e. the average of the cosine of the angle between the normal to a leaf and the beam direction). In general, both ρ_F and k vary with position in the crown and hence are functions of s, and

$$l(s) = \int_{s_+}^{s_-} k(s)\rho_F(s)ds , \tag{2}$$

where s_+ and s_- are the values of s for the entry and exit points of the ray. Now consider a full beam, and let A_B (m^2) be the cross-sectional area of that portion that intercepts the crown. The radiation E_c absorbed by the crown is the difference between the amount E_0 (= I_0A_B) intercepted by the crown and the amount E_t that is transmitted (and calculated by integrating $I(s)$ across the beam). It follows that

$$E_c = (1-\tau_c)A_B I_0 , \tag{3}$$

where the crown transmittance τ_c is given by

$$\tau_c = \frac{1}{A_B}\iint \mathrm{e}^{-\int_{s_+}^{s_-} k(s)\rho_F(s)ds}\, dA , \tag{4}$$

and integration over dA is over that portion of the beam that intersects the crown.

Crown and beam geometry

Consider a crown that is a general ellipsoid with one axis vertical. Let crown depth be H_C and the two horizontal diameters D_{Cx} and D_{Cy}. The orientation of the crown is specified by the compass bearing ψ_y from true North of the D_{Cy} axis. If the crown is a hedgerow, assume the hedgerow has compass bearing ψ_y and set D_{Cy} very large.

The leaf area density ρ_F and light extinction coefficient k can be specified as a function of position in the crown either as an explicit function of position, or as a lookup-table as described in the body of this report.

The direction of a beam of light is specified by the elevation α_S of the sun above the horizon and its azimuth ψ_S as a compass bearing from true North.

Mathematical description of crown and beam

A rectangular coordinate system (x, y, z) is established with origin at the centre of the ellipsoid, the z–axis vertical, the x– and y–axes parallel to the other axes of the ellipsoid, and the y–axis oriented with compass bearing ψ_y. If the semi-axes of the ellipsoid are a_x (= D_{Cx}/2), a_y (= D_{Cy}/2) and a_z (= H_C/2), the equation of the crown is

$$\left(\frac{x}{a_x}\right)^2+\left(\frac{y}{a_y}\right)^2+\left(\frac{z}{a_z}\right)^2=1\,. \tag{5}$$

If the crown is a hedgerow, a_y is very large.

Relative to this coordinate system, a beam of light is specified by its elevation θ, measured positive above the horizontal plane (i.e. $z = 0$), and its azimuth ϕ, measured from the x–axis and positive towards the y–axis. In terms of the solar direction (α_S, ψ_S)

$$\theta=\alpha_S,\ \ \phi=\frac{\pi}{2}+\psi_y-\psi_S\,. \tag{6}$$

The direction cosines (γ_x, γ_y, γ_z) of the beam are given by

$$\gamma_x=\cos\theta\cos\phi,\ \ \gamma_y=\cos\theta\sin\phi,\ \ \gamma_z=\sin\theta\,, \tag{7}$$

and the equation of a single ray aimed at a point P_0 with coordinates (x_0, y_0, z_0) is

$$x=x_0+s\gamma_x,\ \ y=y_0+s\gamma_y,\ \ z=z_0+s\gamma_z\,, \tag{8}$$

where s is distance along the ray from P_0.

Intersection of a ray with the crown

The points of intersection with the crown of the ray through P_0 are found by substituting Eq (8) for (x, y, z) into Eq (5) for the crown. The quadratic equation

$$as^2 + 2bs + c = 0 \tag{9}$$

is found for s, where

$$\begin{aligned} a &= \left(\gamma_x / a_x\right)^2 + \left(\gamma_y / a_y\right)^2 + \left(\gamma_z / a_z\right)^2 \\ b &= \left(\gamma_x x_0 / a_x{}^2\right) + \left(\gamma_y y_0 / a_y{}^2\right) + \left(\gamma_z z_0 / a_z{}^2\right) \\ c &= \left(x_0 / a_x\right)^2 + \left(y_0 / a_y\right)^2 + \left(z_0 / a_z\right)^2 - 1 \end{aligned} \tag{10}$$

and its solutions are

$$s_+ = \frac{-b + \sqrt{b^2 - ac}}{a}, \quad s_- = \frac{-b - \sqrt{b^2 - ac}}{a}. \tag{11}$$

The condition for the ray to intersect the crown is that these solutions are real, i.e.

$$b^2 - ac \geq 0. \tag{12}$$

The path length of the ray within the crown is then

$$\Delta = \frac{2}{a}\sqrt{b^2 - ac}. \tag{13}$$

Scanning a beam

Crown transmittance τ_C is given in Eq (4) as an integral over all rays that intersect the crown. In this algorithm, this is computed by summing over a discrete set of rays that intersect the crown. Rays are aimed at a square grid of grid-size δ_x normal to the beam direction. Each ray then samples the same area ($\delta_x{}^2$) of the beam cross-section A_B and hence all rays contribute to τ_C with equal weight. The scanning process proceeds as follows.

A second coordinate system (x_S, y_S, z_S) is established with origin at the centre of the ellipsoid, x_S–axis directed along the beam and z_S–axis in the vertical plane defined by the crown centre and the sun. The grid for selecting rays is the $x_S = 0$ plane in this system, and the point where a ray intersects this grid is taken as the reference point P_0 used in Eq (8). The grid is scanned in each of the four quadrants, starting from near the origin and scanning along a row parallel to the y_S–axis. When Eq (12) is not satisfied, that scan is terminated and the same row is scanned in the opposite direction. In this case, when a ray fails to intersect the crown, a new row further from the origin is scanned. This is repeated until no rays in a new row intersect the crown. Finally the whole process is repeated on the other side of the crown centre.

When calculating $l(s)$ for each ray, k and ρ_F are specified relative to the coordinate system associated with the crown so it is necessary to transfer from the beam-based (x_S, y_S, z_S), to the crown-based (x, y, z). By considering the necessary rotations of the coordinate systems it can be shown that

$$\begin{aligned} x &= -y_S \sin\phi - z_S \sin\theta \cos\phi \\ y &= y_S \cos\phi - z_S \sin\theta \sin\phi \\ z &= z_S \cos\theta \end{aligned} \tag{14}$$

where x_S was 0 by definition. This transformation is applied to each ray in the above scan to obtain the coordinates (x_0, y_0, z_0) of the point P_0 in the crown-based coordinate system.

Integration along a ray

The contribution by a single ray to crown transmittance involves the integral $l(s)$ given by Eq (2) and appearing in the exponential function in the integral over rays in Eq (4). This is the integral of $k\rho_F$ along that portion of the ray that lies within the crown, and is determined numerically by stepping along the ray.

The coordinates (x_0, y_0, z_0) of the point P_0 are determined by applying the transformation in Eq (14), and Eqs (11) are used to find the points of intersection of the ray with the crown. The algorithm then steps along the ray from its point of entry to its point of exit by increasing s in steps of δ_s. At each step it applies Eqs (8) to determine the coordinates of each point, and evaluates $k(s)\rho_F(s)$ at that point. The integral for $l(s)$ is then determined by the trapezoidal or Simpson's methods. The integration interval δ_s must be sufficiently fine so that discrete variations in k and ρ_F are properly sampled.

If the crown is uniform, the integration is trivial and $l(s) = k\rho_F\Delta$, where Δ is the path length in the crown.

Integration over rays

Crown transmission τ_C is an integral over all rays intersecting the crown, and calculated as a sum over the contribution by each ray, weighted by the fraction of the cross-sectional area of the beam associated with that ray.

The cross-sectional area A_B is simply the number N_r of rays transmitted times the area associated with each ray, i.e. $A_B = N_r\delta_x^2$. The shadow area A_S is obtained by projecting this onto the horizontal plane. Thus

$$A_S = A_B / \sin\alpha_S \,. \tag{15}$$

Total leaf area L_C (m^2) within the crown and crown volume V_C (m^2) are given by

$$\begin{aligned} L_c &= \iint \left(\int_{s_+}^{s_-} \rho_F(s)\,ds \right) dA \\ V_c &= \iint \left(\int_{s_+}^{s_-} ds \right) dA \end{aligned} \tag{16}$$

and can be computed in a similar manner as a sum of contributions by individual rays. These quantities do not depend on beam direction so a vertical beam (i.e. $\alpha_S = 90°$) is used, and the contribution of each ray is weighted by the cross-sectional area (δ_x^2) associated with it.

9. Chlorophyll and nitrogen determination for plantation-grown *Eucalyptus nitens* and *E. globulus* using a non-destructive meter

Libby Pinkard, Vin Patel and Caroline Mohammed

Introduction

Foliar chlorophyll content is a good indicator of plant stress and hence of the potential for plant carbon dioxide uptake and growth (Evans 1989). Foliar nitrogen (N) concentration is less useful as a stress indicator because nitrogenous compounds such as amino acids can increase under stress without any change in total N (Warren et al. 2000), but it is a good indicator of N deficiency. Monitoring chlorophyll and N content may assist in managing nutrition and forest health for optimal growth.

Destructive methods of chlorophyll and N determination are accurate but time-consuming and expensive to perform. However portable non-destructive meters are used successfully with many species for estimating foliar chlorophyll or N (Abdelhamid et al. 2003; Castelli et al. 1996; Loh et al. 2002; Schaper and Chacko 1991), and allow measurement of chlorophyll or N on the same leaf over time (Yamamoto et al. 2002). These meters calculate a chlorophyll index (CI) based on reflectance or absorbance at particular wavelengths, and need to be calibrated to give an estimate of actual chlorophyll or N content. Calibrations have not been developed for *E. nitens* or *E. globulus*.

The relationship between CI and chlorophyll or N can be affected by species, genetic differences within species (Chapman and Barreto 1997; Kim et al. 2002; Sandoval-Villa et al. 2002), specific leaf weight and hence leaf age and ontogeny (Thompson et al. 1996) and growing conditions (Barraclough and Kyte 2001; Campbell et al. 1990). For many species, site or experiment-specific calibration curves may be required for accurate determination of chlorophyll or N from CI.

In this paper, we investigated whether the Minolta SPAD-502, a hand-held absorbance meter, could be used to estimate chlorophyll or N content of *E. globulus* and *E. nitens* leaves. Our objectives were to determine (1) how accurately the SPAD estimated total chlorophyll, chlorophyll A and chlorophyll B; (2) whether the relationship differed between *E. nitens* or *E. globulus*, between sites within species, or between field and pot-grown plants; and (3) now accurately the SPAD estimated foliar N concentration.

Methods

Plant material

The experiment was conducted at two field sites, and with seedlings grown in pots. The first field site was at Christmas Hills in north-west Tasmania (40°29' S 146°0' E). Two and a half year old *E. globulus* and 1.5 year old *E. nitens* were growing adjacent to each other on a site experiencing infection by *Mycosphaerella* leaf disease (MLD) (suite of *M. nubilosa*, *M. cryptica*, *M. tasmaniensis*, *M. vespa* and *M. parva*, (Smith et al. 2005). This foliar pathogen causes leaf discoloration and necrosis of juvenile foliage, and can result in premature leaf senescence. Approximately 80% of *E. globulus* leaves were affected, with an average affected area per leaf of 15%. *E. nitens* crowns were less

affected (30% of leaves affected, with an average affected area per leaf of 5%). Mean tree height and diameter at 1.3 m height (diameter at breast height, DBH) were 3.8 m and 3.9 cm for *E. globulus* and 2.5 m and 2.7 cm for *E. nitens*. The second field site (Barnback, 52° 29' S 146° 46' E) was a healthy 2.5 year old *E. globulus* stand in south east Tasmania, with a mean tree height and DBH of 5.0 m and 4.9 cm. At both field sites foliage was predominantly juvenile as opposed to adult.

Sampling of potted plants was undertaken on four month old *E. nitens* and *E. globulus* seedlings grown in full sun in a glasshouse compound. *E. nitens* seedlings had a mean height and diameter of 0.48 m and 0.42 cm, and *E. globulus* had a mean height and diameter of 0.54 m and 0.49 cm at the time of sampling. The seedlings were watered to saturation twice daily and were provided with an at-planting application of Osmocote® complete fertiliser. All foliage was juvenile.

Foliar sampling

Thirty healthy (ie showing few symptoms of MLD or other damage) young fully expanded juvenile leaves were selected for sampling at each site and from the pot experiment. In all cases leaves of a similar age (3 – 5 fully expanded leaf pairs from the branch tip) and crown position (mid-crown) were selected to cover the range of colours present. At Barnback, a smaller quantity of apical foliage (10 leaves) also was sampled (first two leaf pairs present on branches from mid-crown).

A Minolota SPAD-502 chlorophyll meter was used to determine a chlorophyll index (CI). This measures absorption at 650 and 940 nm wavelengths to estimate chlorophyll levels. Leaves were removed and five measurements were immediately taken per leaf and averaged to provide a single CI per leaf. The SPAD sensor was placed randomly on leaf mesophyll tissue only, with veins avoided. Measurements were made at a photon flux density of ~ 800 μmol m^{-2} s^{-1}.

Immediately after measurement the leaves were placed into plastic bags and put into an esky full of ice. Within an hour of sampling they were frozen at -4°C and within a day moved to -20° C. Chlorophyll was extracted within two weeks of sample collection, using the acetone method. Chlorophyll suspensions were kept on ice in the dark between processing steps. Samples were centrifuged at 2500 RPM for 10 min, and absorbance was measured on a UV-visible recording spectrophotometer at 645 and 663 nm. Chlorophyll content was determined using the method of Arnon (1949).

Leaf tissue from the Christmas Hills site was dried at 65° C to constant weight and ground in a hammer mill. Samples were prepared for nitrogen (N) analysis using the single acid-hydrogen peroxide technique (Lowther 1980), and total N (mg g^{-1}) was measured using a continuous flow colorimetric autoanalyser (McLeod 1992).

Data analysis

Data were log-transformed to allow liner regression to be performed. Group regression analysis was used (McPherson 1990) to test the effects of:

1. species (*E. nitens* and *E. globulus*) on the relationship between total chlorophyll (TChl) and CI

2. site (same age of *E. globulus* at two sites) on the relationship between TChl and CI
3. growing conditions (pots versus field) on the relationship between chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) or TChl and CI
4. species (*E. nitens* and *E. globulus*) on the relationship between foliar N concentration and CI.

Group regression analysis provides estimated regression equations under different models where groups are present in the data. The procedure tests the hypotheses that: (1) the regression lines have common slopes allowing for the possibility that they have different intercepts, and (2) that the same line applies to all groups. The root mean square error (RMSE) and a back-transformation correction factor (Sprugel 1983) were calculated for each regression.

The following equations were used to predict TChl from CI for the data collected from Barnback:

- *E. globulus* and *E. nitens*: Table 3
- paper birch: (TChl (mg cm^{-2}) = 5.52E-04+4.04E-04*CI+1.25E-05*$SPAD^2$) (Richardson et al. 2002)
- mango: (TChl (mg m^{-2}) = -5.4 + 11.1*CI) (Schaper and Chacko 1991)
- rice: (TChl (mg m^{-2}) = 1.034 + 0.308* CI + 0.11* CI^2)((Monje and Bugbee 1992))
- apple: (TChl (µg cm^{-2}) = -83.0 + 2.37* CI) (Campbell et al. 1990).

All values were converted to mg m^{-2}.

The ratio of TChl:CI was calculated. Differences between treatments in CI, chlorophyll, N and TChl:CI were determined using analysis of variance.

Results

Chlorophyll and CI

There was a strong relationship between foliar chlorophyll content and CI, with coefficients of determination ranging between 0.85 and 0.96 ($P<0.001$) and low RMSE's. The relationship was stronger for *E. globulus* than *E. nitens*, and was weaker for Chl *b* than for Chl *a* or TChl (Table 9.1). In both species and in field and pot-grown plants most of the chlorophyll present was Chl *a* (Figure 9.1), and the relationship between chlorophyll content and CI was similar for total chlorophyll and Chl *a*. The relationship was much flatter for Chl *b*.

Differences between pot and field grown plants

Both *E. nitens* and *E. globulus* had substantially lower mean Chl *a*, Chl *b* and TChl, and less chlorophyll per unit CI, in pot-grown than field-grown plants ($P < 0.01$) (Table 9.1). Mean CI was greater in field than pot-grown plants ($P < 0.05$), which was probably related to the greater range of values of CI observed in the field (Figure 9.1). Both the slope and intercept of the relationships between chlorophyll and CI were affected by plant type (pot versus field grown plant) for both *E. nitens* and *E. globulus* (Table 9.1, Figure 9.1). In general, differences between plant types in the chlorophyll/CI relationships were less for Chl *a* than for Chl *b* or TChl. Both the intercept and slope of

the relationships between Chl *b* or TChl and CI were less in field-grown than pot-grown plants for *E. nitens*, but greater in field than pot-grown plants for *E. globulus*.

Table 9.1. Slope (a) and intercept (b) of the relationship between Chl *a*, Chl *b* or TChl and CI, for *E. nitens* and *E. globulus* grown in either pots or the field. The relationship takes the form of: $LnChl = a + b * \ln(CI)$. RMSE is the root mean squares error; CF is the back-transformation correction factor; Chl:CI is chlorophyll per unit CI. * indicates significant differences between pot and field (P<0.01).

Species	Chl type	Sample type	a	b	R^2	Pr>F	RMSE	CF	Mean CI	Mean Chl	Chl:CI
E. nitens	*a*	Pot	-5.74	1.13	0.89	<0.001	0.002	1.03	31.7	0.17	0.005
		Field	-5.28*	1.13					45.0*	0.38*	0.008*
	b	Pot	-8.17	1.16	0.85	<0.001	0.003	1.04	31.7	0.07	0.002
		Field	-8.55*	0.97*					45.0*	0.11*	0.002
	Total	Pot	-6.38	1.41	0.88	<0.001	0.003	1.03	31.7	0.24	0.007
		Field	-4.45*	0.98*					45.0*	0.49*	0.010*
E. globulus	*a*	Pot	-7.16	1.58	0.96	<0.001	0.001	1.01	29.2	0.17	0.005
		Field	-7.16	1.58					49.6*	0.44	0.008*
	b	Pot	-7.84	1.44	0.94	<0.001	0.002	1.02	29.2	0.06	0.002
		Field	-12.01*	2.56*					49.6*	0.16	0.003*
	Total	Pot	-6.49	1.46	0.96	<0.001	0.001	1.01	29.2	0.22	0.007
		Field	-8.79*	2.08*					49.6*	0.60	0.011*

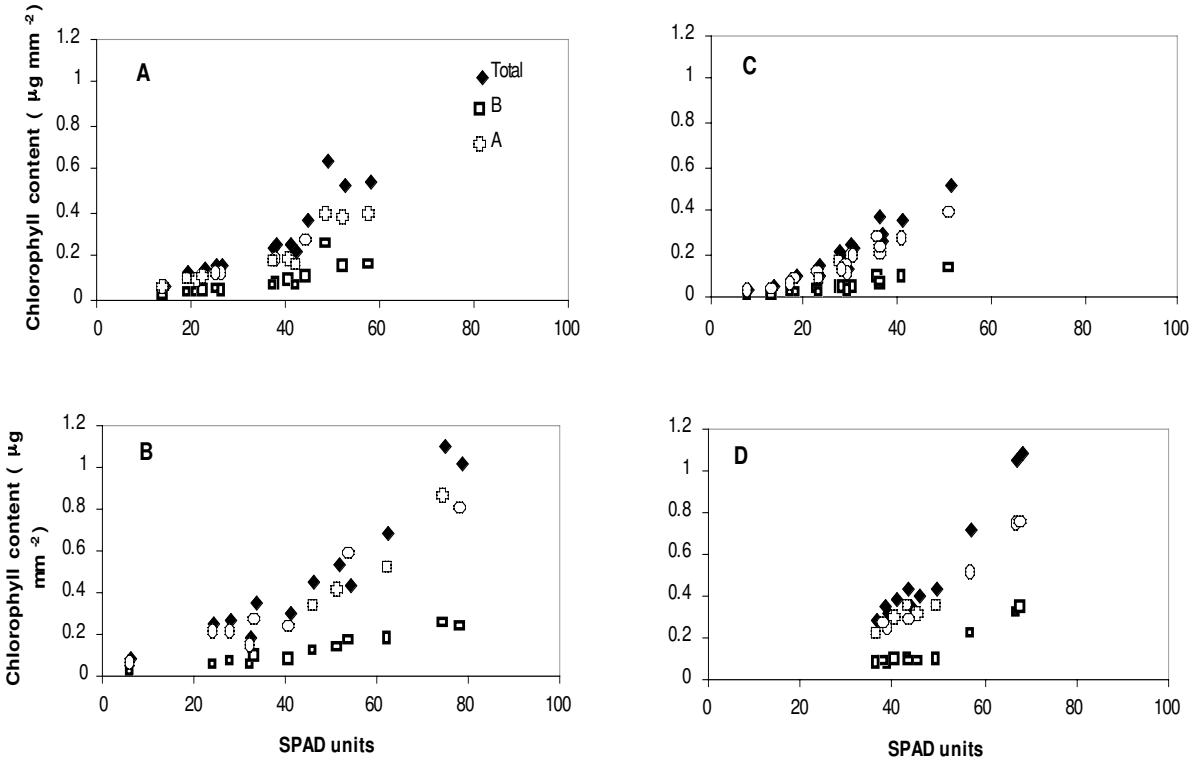

Figure 9.1. Relationship between TChl, Chl *a* or Chl *b* (µg mm^{-2}) and CI for *E. nitens* (A, B) and *E. globulus* (C, D) growing in pots (A, C) or in the field (B, D) at Christmas Hills.

Differences between species

The same relationship between TChl and CI applied to both *E. nitens* and *E. globulus* grown in pots (R^2=0.92, P < 0.001), but at the field site at Christmas Hills the relationship for *E. globulus* had a lower intercept and greater slope than the relationship for *E. nitens* (Table 9.2, Figure 9.2) (R^2=0.88, P < 0.001). Differences between species were most obvious at CI's greater than 50 (Figure 9.2). In pot-grown plants the CI was <60, whereas in the field CI ranged up to almost 80 (Figure 9.2).

Table 9.2. Differences between species, grown in either pots or the field, in the relationship between Tchl and CI. The relationship takes the form of $LnChl = a + b * \ln(CI)$. RMSE is the root mean square error, and CF is the back-transformation correction factor. * indicates significant differences between species (P < 0.05)

Sample type	Species	a	b	R^2	Pr>F	RMSE	CF	Mean CI	Mean Chl
Pot	*E. nitens*	-6.41	1.43	0.92	<0.001	0.001	1.02	34.2	0.24
	E. globulus	-6.41	1.43					29.2	0.22
Field	*E. nitens*	-4.50	0.98	0.88	<0.001	0.002	1.02	45.0	0.49
	E. globulus	-8.79*	2.09*					49.6	0.59

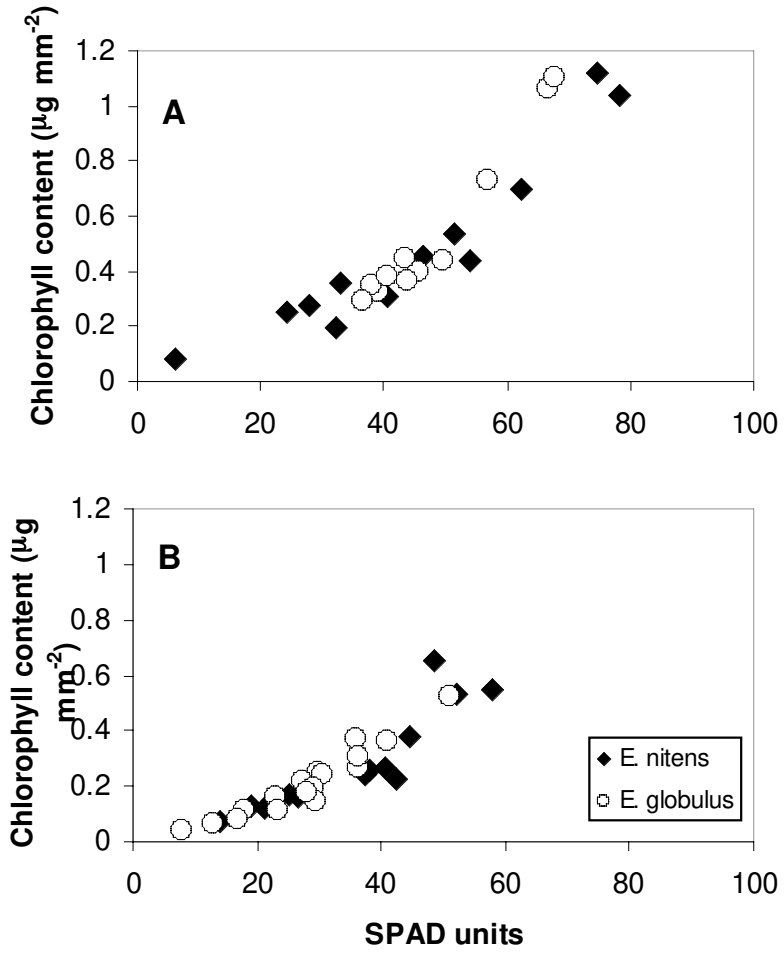

Figure 9.2. Relationship between TChl (µg mm^{-2}) and CI for *E. nitens* and *E. globulus* growing (A) at Christmas Hills and (B) in pots.

Variation with site

The relationship between TChl and CI for *E. globulus* was the same at Christmas Hills and Barnback, despite mean CI and TChl values being lower at Barnback than at Christmas Hills (Table 9.3, Figure 9.3) ($R^2 = 0.96$, $P < 0.001$).

Table 9.3. The effect of site on the relationship between Tchl and CI, for 2.5 year old *E. globulus*. Trees at CH (Christmas Hills) were suffering from infection by the foliar pathogen *Mycosphaerella nubilosa*, whereas trees at BB (Barnback) were healthy. The relationship takes the form of $LnChl = a + b * \ln(CI)$. RMSE is the root mean square error, and CF is the back-transformation correction factor. * indicates significant differences (P<0.05) between sites.

Site	a	b	R^2	Pr>F	RMSE	CF	Mean SPAD	Mean Chl
CH	-8.22	1.95	0.96	<0.001	0.0006	1.01	49.6	0.59
BB	-8.22	1.95					40.6*	0.39*

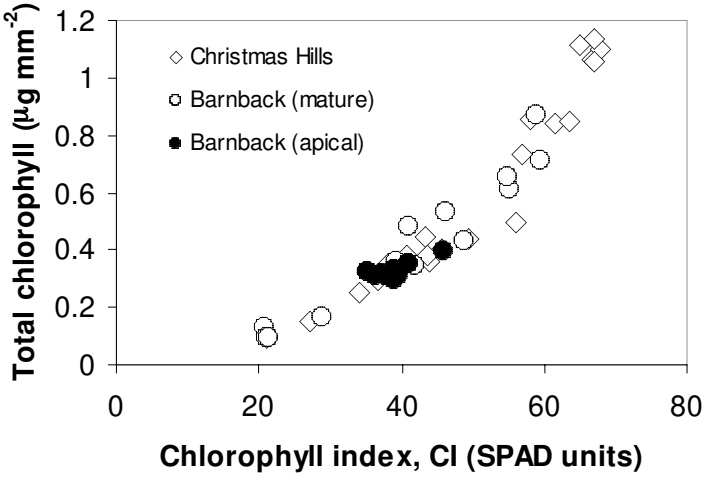

Figure 9.3. Relationship between total chlorophyll (µg mm^{-2}) and CI for *E. globulus* growing at two sites, Christmas Hills and Barnback, in Tasmania. Trees at Christmas Hills were experiencing infection from the foliar pathogen *Mycosphaerella nubilosa*, whereas trees at Barnback were healthy. The chlorophyll-CI relationship is shown for both apical and mature foliage at one site, Barnback.

Effects of foliage type

Only a small number of apical leaves were sampled at Barnback. However, the relationship between TChl and CI appeared to be the same as for mature juvenile foliage (Figure 9.3).

Is the TChl-CI relationship species-specific?

The relationship developed between TChl and CI for *E. globulus* growing at Christmas Hills provided good estimates of values observed at Barnback (Figure 9.4). However the *E. nitens* relationship from Christmas Hills did not. Values predicted using equations developed for paperbirch (Richardson et al. 2002), mango (Schaper and Chacko 1991), rice (Monje and Bugbee 1992) and apple (Campbell et al. 1990) suggested that the Tchl-CI relationship is species-specific.

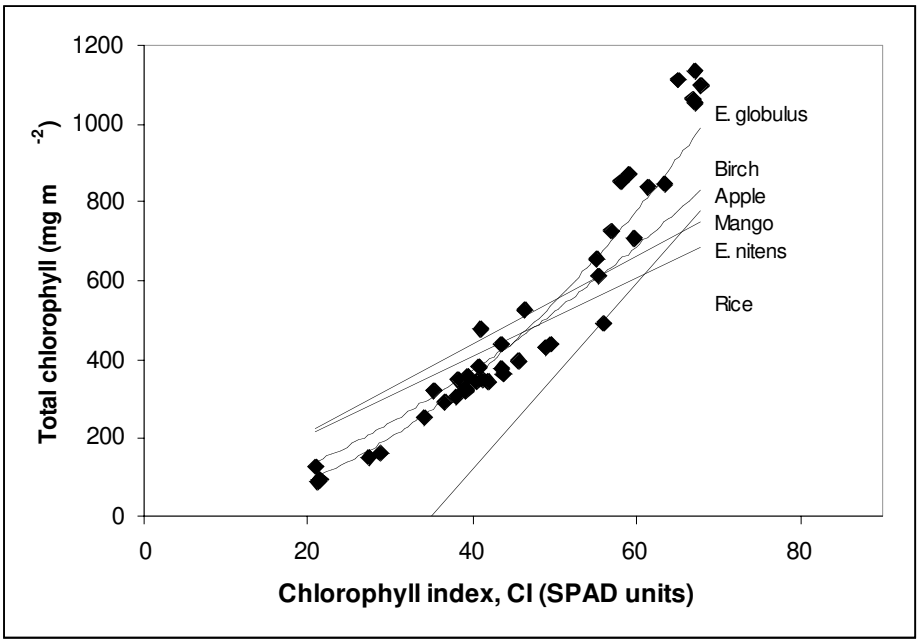

Figure 9.4. Relationship between TChl (μg mm^{-2}) and CI for *E. globulus* growing at the Barnback site. Lines are predicted values for *E. globulus* and *E. nitens* (Table 3), paper birch (TChl (mg cm^{-2}) = 5.52E-04+4.04E-04*CI+1.25E-05*CI^2) (Richardson et al. 2002), mango (TChl (mg m^{-2}) = -5.4 + 11.1*CI) (Schaper and Chacko 1991), rice (TChl (mg m^{-2}) = 1.034 + 0.308*CI + 0.11*CI^2)((Monje and Bugbee 1992)) and apple (TChl (μg cm^{-2}) = -83.0 + 2.37*CI) (Campbell et al. 1990).

Foliar N and CI

There were no significant differences between species in foliar N content or CI (P = 0.05) (Table 9.4). The relationship between foliar N and CI was significant (P = 0.002), but the coefficient of determination was not particularly high (R^2=0.47). The relationship differed between *E. nitens* and *E. globulus* (Table 9.4), and there was a smaller range of CI's observed in *E. globulus* than *E. nitens* (Figure 9.5).

Table 9..4. Effect of species on the relationship between foliar nitrogen (N) and CI, for the Christmas Hills field site. The relationship takes the form of $LnN = a + b * \ln(CI)$. RMSE is the root mean square error, and CF is the back-transformation correction factor. *indicates significant differences between species (P < 0.05).

Species	a	b	R^2	Pr>F	RMSE	CF	Mean CI	Mean N (ppm)
E. globulus	5.11	-0.28	0.47	0.002	0.003	1.03	49.6	55.0
E. nitens	2.14*	0.52*					45.0	60.8

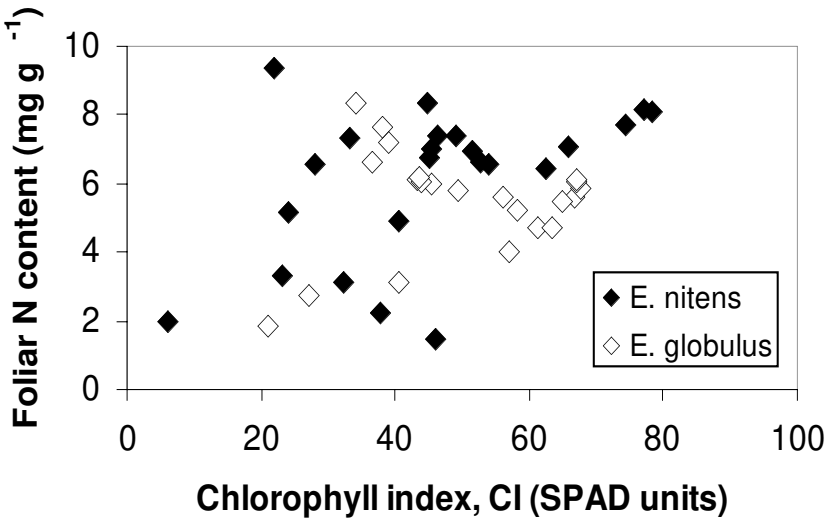

Figure 9.5. Relationship between foliar nitrogen (N) and CI for *E. nitens* and *E. globulus* growing at Christmas Hills.

Discussion

The Minolta SPAD-502 provided good estimates of chlorophyll in both species tested in this experiment, with coefficients of determination greater than 0.88. The relationship between chlorophyll and CI in many species is linear (Barraclough and Kyte 2001; Campbell et al. 1990; Cate and Perkins 2003; Schaper and Chacko 1991). The curvilinear nature of the relationship for *E. globulus* and *E. nitens* suggested that the SPAD overestimated chlorophyll at high CI. This also was observed for rice, wheat and soybean (Monje and Bugbee 1992), and paper birch (Richardson et al. 2002). It highlights the importance of sampling the extremes when developing calibration curves.

The differences between *E. globulus* and *E. nitens* in the relationship between chlorophyll and CI observed in this experiment may have been a function of the differences in age between the *E. globulus* (2.5 years) and *E. nitens* (1.5 years), presumably related to differences in leaf age. However, care was taken to select leaves of similar age for measurement. Alternatively, the species differences could have been related to species differences in specific leaf area (ratio of fresh area:dry mass, SLA). *E. nitens* has a lower SLA than *E. globulus* (e.g. 6.2 and 8.3 kg m^{-2} for *E. nitens* and *E. globulus*, respectively, averaged from three sites of similar age per species. Unpublished data). (Thompson et al. 1996) found a strong correlation between CI and specific leaf weight, the inverse of SLA, in soybean. The SPAD-502 measures absorbance by the leaf of two wavelengths of light (660 and 940 nm). SLA can be expected to affect this, which could account for the higher CI for a given chlorophyll concentration measured in *E. nitens* than *E. globulus*. Similarly, the level of irradiance at the time of measuring CI can influence CI (Hoel and Solhaug 1998). Care was taken in this experiment to measure CI under similar light conditions.

Fitting equations from other species to *E. globulus* data made it clear that the chlorophyll-CI relationship is species specific. This is likely to be related to differences between species in SLA and leaf optical properties (Monje and Bugbee 1992; Thompson et al. 1996).

The differences between *E. nitens* and *E. globulus* in the chlorophyll-CI relationship were most obvious at CI's greater than 50. At this level, chlorophyll content was relatively high and leaves appeared dark green and healthy. At lower CI's, it may be possible to apply the same calibration equation to both *E. globulus* and *E. nitens*. However, an understanding of the optimal foliar chlorophyll content for optimal photosynthesis is required before this can be recommended.

Environmental factors are known to affect leaf morphology, which in turn affects foliar optical properties, and can be expected to influence CI (Monje and Bugbee 1992). While the chlorophyll-CI relationship was not affected by site for *E. globulus*, the sites tested in this experiment may not have been representative of the extremes at which *E. globulus* is planted. Further testing from warmer and dryer sites, and from sites with nutrient limitations, is required before it can be concluded categorically that a single calibration can be used for the species.

The flatter relationship observed for pot than field-grown plants was also reported for apple (Campbell et al. 1990). (Campbell et al. 1990) concluded that the disparity between field and glasshouse plants was related to lower SLA in field than pot-grown plants. This was also observed in the above experiment (6.9 vs 8.9 kg m^{-2} for field and pot-grown *E. globulus*, respectively (data not presented)).

The relationship between foliar N content and CI was not strong, although strong relationships have been observed in other species. It has been observed that *Eucalyptus* often stores N at levels greater than the physiological requirements for photosynthesis (Close et al. 2004), so a consistent relationship between CI and foliar N may not be expected. While foliar N content may have been affected by the presence of MLD on the sample trees, this does not adequately explain the poor relationship between foliar N and CI in *E. nitens*, because *E. nitens* had very low levels of MLD.

Conclusions

The Minolta SPAD-502 is suitable for determining foliar chlorophyll content of *E. globulus* and *E. nitens* as a 'generic' indicator of eucalypt stress, but caution is required for it to be used directly to detect N deficiency in young crowns. It has potential as a management tool for optimising fertiliser regimes in plantations of these species. Its usefulness in plantation management would be greatly enhanced if a threshold CI could be identified, below which production will be curtailed and remedial actions such as fertilising may be warranted. More research is required to determine the relationship between foliar N and chlorophyll content for *E. globulus* and *E. nitens*.

While we observed good correlation between chlorophyll and CI, it is unlikely that the calibration equations will allow accurate identification of small changes in chlorophyll content. In instances where differences in chlorophyll content are likely to be small, extraction methods are preferable to the use of hand-held meters such as the SPAD.

10. Working with *Mycosphaerella* in inoculation studies

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Introduction

While over 30 species of *Mycosphaerella* have been found on the leaves of eucalypts (Maxwell 2004)), the species considered to cause most damage in temperate eucalypt plantations are *Mycospharella nubilosa* and *M. cryptica* (Carnegie et al. 1997; Dick 1982; Park 1984), although it is probable that in most cases a suite of *Mycosphaerella* species occurs together (Glen et al. 2005). The disease caused by these species results in tissue necrosis and discoloration, and in severe cases causes tissue blighting and premature leaf senescence (Park 1988b). These symptoms, if severe enough, can reduce stem growth and affect wood quality (Carnegie and Ades 2002; Lundquist and Purnell 1987; Smith 2005), with potential reductions in merchantable volume at harvest.

In order to study the eucalypt-*Mycosphaerella* host-pathogen system in detail, controlled experiments are highly desirable, where the effects of a single *Mycosphaerella* species can be examined and different pattens and severities of infection can be induced. This requires inoculation of potted plants, preferably using spores or conidia from known isolates. There has been only limited success with inoculation of eucalypts with *M. nubilosa* and *M. cryptica*. The following reviews methods that have been used successfully to produce ascospores in culture and to inoculate eucalypts, and summarises a series of experiments that we undertook investigating methods of ascospore production and inoculation with *Eucalyptus globulus*.

Production of ascospores *in vitro*

While *in vitro* sexual reproduction can be induced with certain *Mycosphaerella* species (eg. Kennedy et al. 1999), this does not seem to be the case with *M. nubilosa* and *M. cryptica*. *M. cryptica* does produce conidia in culture (Crous 1998). (Ganapathi 1979) also succeeded in inducing condial ascospore production from "*M. nubilosa"* cultures but these isolates were probably misidentified, and were in fact *M. cryptica*. He found that his cultures of grew best at 25° C on Emerson yeast phosphate soluble starch media, although it also grew well on Asparagine agar medium, V8 juice agar and surface sterilised eucalypt leaves. Best sporulation occurred at 5° and 10° C on Emerson yeast phosphate soluble starch agar, although he also found that different strains also sporulated readily between 10 - 25° C. Sexual reproductive structures, however, were not produced. The conidia produced germinated readily. Light type (white fluorescent, black light, no light) had no effect on sporulation.

Ascospores of *M. brassicicola* were readily produced when cultures were grown on a SLD:agar-amended filtrate from 100 h fresh weight of senescent Brassica leaves (brussel sprout) homogenised in 140 ml of distilled water (Kennedy et al. 1999). The media was maintained at its natural pH of 6.5 – 7.5. Plates were incubated under either cool white fluorescent/black light or warm white fluorescent/black light, with a photoperiod of 16 hours. Temperature was maintained at 17° C. Pseudothecia developed within 14 days, and ascospore discharge occurred readily. These researchers

also attempted to use the same technique with cultures of *M. nubilosa* and *M. cryptica,* and got pseudothecial initials with *M. nubilosa.*

We undertook a study to identify methods of inducing sporulation of *M. cryptica* and *M. nubilosa in vitro*. *E. globulus* leaves with lesions containing mature pseudothecia were soaked in distilled water for 12 hours. A single lesion was then placed onto sterilised paper towel inside a laminar flow cabinet. An MEA plate was placed on top of the lesion (facing down) and left for one hour. Single spores were then transferred to new plates (three spores per plate). The plates were positioned 40 cm below lights. Two previously isolated *M. nubilosa* cultures and two *M. cryptica* were also subject to treatments as controls (see Table 10.1). The following treatments were applied:

- Light conditions
    - Two white lights adjacent to two black lights (continuous light)
    - One black light between two white lights (12/12 photoperiod)
- Photoperiod
    - Continuous light
    - 12 hour photoperiod
- Temperature
    - ambient temperature (24±2° C)
- Media
    - Two standard media (either 2% MEA (pH 6.5) or Emersons yeast starch agar (pH 6.5)
    - Media rich to nutrient poor media
        - First month 2% MEA (pH 6.5)
        - Second month water agar or either water agar + 10 ml of eucalypt extract 1 EEA (prepared from 30 g fresh weight eucalypt foliage homogenised in 140 ml distilled water and filter sterilised), or water agar + 15 ml of eucalypt extract 2 EEB (prepared from 20 g dry weight eucalypt foliage homogenised in 140 ml distilled water, centrifuged and the supernatant filter sterilised)

Results

Conidia were formed fairly quickly from spores freshly subcultured from lesions – on very small colonies, irrespective of treatment. It is probable that these were *M. cryptica*, and molecular detection methods will be used to clarify this. These conidia germinated readily when plated out. Spores were not produced on old cultures.

Inducing ascospore discharge from mature leaf lesions

A number of studies have examined methods for inducing ascospore discharge from mature leaf lesions of eucalypts (Beresford 1978; Carnegie et al. 1994; Milgate et al. 2001b; Park 1988a; Park and Keane 1987). All highlight the importance of soaking lesions in water to induce discharge. (Ganapathi 1979) found that 30 minutes soaking was sufficient. (Beresford 1978) soaked leaves for one hour, and (Park 1984) soaked leaves for one to two hours. Wetting and drying cycles also may be effective (Beresford 1978). (Park 1984) found that ascospores could be released for up to seven months following repeated wetting and drying cycles.

These authors found that, once leaves had been soaked, ascospores could be collected by inverting water agar plates over the lesions (Ganapathi 1979), by placing lesions between glass slides (Beresford 1978), or by covering with a layer of cellophane (Park 1984).

Table 10.1. Treatments and results of the ascospore experiment.

ID N$^{o.}$	1st Media	Photoperiod	2nd Media	Result (N$^{o.}$ of single spore cultures with conidia)
1-4	EYA	12	Stay on EYA	3 (N^{o} 4) eg 3 out 12
33-36	EYA	continuous	Stay on EYA	6 (N^{o} s 33 and 35)
5-8	MA	12	Stay on MA	
9-12	MA	continuous	Stay on MA	
13-16	MA	12	Change to WA	3 (N^{o} s 14a, 15a, 13c)
17-20	MA	continuous	Change to WA	1 (18a)
21-24	MA	12	Change to EEA	1 (22a)
25-28	MA	continuous	Change to EEA	2 (26 a and c)
29-32	MA	12	Change to EEB	2 (30 b and c)
37-40	MA	continuous	Change to EEB	3 (39)
41	MA-EYA	12	Stay on EYA	
42	MA-EYA	12	Stay on EYA	
43	MA-EYA	Continuous	Stay on EYA	
44	MA-EYA	Continuous	Stay on EYA	
45	MA-EYA	Continuous	Stay on EYA	
46 spare	MA	Continuous	flexible	
47 spare	MA	12	flexible	
48 spare	EYA	12	flexible	
49 spare	EYA	Continuous	flexible	
50 spare	MA	12	flexible	
51 spare	MA	12	flexible	
52 spare	MA	Continuous	flexible	
53 spare	MA	Continuous	flexible	
54 spare	MA	Continuous	flexible	Multiple spores
55 spare	MA	12	flexible	Multiple spores
56-57	EYA	Continuous	Stay on EYA	
58-59	EYA	12	Stay on EYA	
60-61	MA	Continuous	Stay on MA	
62-63	MA	12	Stay on MA	
64-65	EYA	Continuous	Stay on EYA	
66-67	EYA	12	Stay on EYA	
68-69	MA	Continuous	Stay on MA	
70-71	MA	12	Stay on MA	

We undertook a study to examine methods of inducing ascospore discharge from lesions on *E. globulus* leaves. Lesions with mature pseudothecia were selected and soaked in distilled water for between 15 minutes and 12 hours. Lesions were placed:

- onto sterilised paper towel with glass slides placed over the top (with or without weight applied to the stack)
- between two glass plates (with or without weight applied to the stack)

and left for between 15 minutes and one hour. The spores were then washed off the glass plates using distilled water containing Tween 20 (40 ml Tween 20/100 ml of distilled water v/v).

Results

- Soaking lesions for 30 minutes was sufficient to induce ascospore discharge
- The highest levels of discharge occurred when lesions were placed onto paper towel with a glass plate and a weight put on top

Inoculating *E. globulus* with *Mycosphaerella*

In inoculation studies using ascospores, two methods have been used. The first involves clipping pre-soaked lesions onto healthy leaves (Ganapathi 1979). The second involves preparing an ascospore solution and spraying in on to the plant (Park 1984). Maintaining the foliage at high relative humidity post-inoculation is considered to be important. (Ganapathi 1979) found that covering plants in plastic bags for seven days was sufficient for infection to occur in *Eucalyptus delegatensis*, whereas (Park 1984) recommended leaving bags on for five days, but removing them for one to two hours on the third day to avoid development of foliar oedema (Warrington 1980). Park (1984) and Milgate (pers. comm.) also tested applying hyphal macerations to the foliage, but only had limited success.

The leaf surface to which ascospores are applied may influence the level of infection achieved. (Park 1984) found that *M. nubilosa* only infected *E. globulus* juvenile foliage when the spore solution was applied to the abaxial leaf surface. He determined that *M. nubilosa* enters the leaf via the stomata, and most stomata on *E. globulus* juvenile foliage are located on the abaxial surface. In contrast, *M. cryptica* could infect *E. obliqua* from either surface of juvenile and intermediate foliage, because it could penetrate directly through the cuticle.

The presence of leaf waxes may influence the success of inoculations. (Smith et al. 2005) found that *E. globulus* genotypes resistant to *Mycosphaerella* infection, while having a similar number of stomata per unit leaf area, had significantly less visible stomata due to the presence of leaf waxes.

We undertook a study to identify possible methods of inoculating *E. globulus* seedlings with *Mycosphaerella* ascospores, with emphasis on a method that could be used for inoculating large numbers of seedlings. The following methods were tested:

1. Press lesions to leaves for 15 minutes
2. Spray ascospore solution to both sides of the leaf
3. Macerate lesions, centrifuge and spray the supernatant onto the plants
4. Hang pre-soaked, infected *E. globulus* leaves with mature pseudothecia 20 cm above the crowns of *E. globulus* seedlings, and expose seedlings and leaves to periods of wetting and drying that might be experienced in a field situation

Methods 1 – 3 were also tested on dewaxed leaves. The dewaxing was done by gently wiping the leaf with methanol. In Methods 1 – 3, plastic bags were placed over the inoculated seedlings, for either six or nine days. The bags were removed for one hour every three days to avoid the development of foliar oedema. Leaf samples were taken after 3 days and stained with Toluidine blue, and then examined under the microscope for germinating spores.

Results

The time that plastic bags were left on the seedlings was critical. While spore germination was observed soon after inoculation in all treatments, the six-day bagging treatment did not result in infection, presumably because the conditions conducive to growth were not maintained for long enough. The nine-day treatment did result in infection.

All of the methods tested resulted in some level of infection. Method 4 was the most successful in terms of severity and incidence, but the method resulted in the development of foliar oedema as well as infection, because of the high relative humidity environment associated with the method. The success of methods 1 – 3 improved dramatically when leaves were dewaxed.

It was very difficult with methods 1 and 2 to induce high levels of infection in plants. The methods are of limited value for large-scale inoculation trials unless a more effective method of collecting large quantities of spores can be developed.

Method 3, with dewaxing, was the most successful method used, in terms of the levels of infection achieved, the ease of preparing spore solutions, and avoiding the development of foliar oedema. However, it is very difficult to determine spore loads in solution with this method, and it must be assumed that the solution contains multiple *Mycosphaerella* species (Glen et al. 2005).

11. Formation of environmental response lenticel-like structures (ERLS) on leaves of *Eucalyptus nitens* and *E. globulus* seedlings grown under glasshouse conditions

Libby Pinkard, Warwick Gill and Caroline Mohammed

Introduction

Intumescences, or non-pathogenic blister-like protuberant abnormal growths which develop on oedematous plant tissues, predominantly occur on leaves (Wolf & Lloyd, 1912). They may also arise on stems (Atkinson, 1893), roots (Hahn *et al*., 1920), flowers and fruits (Wolf & Lloyd, 1912). First described by Sorauer in 1886 (La Rue, 1933c), intumescences have since been referred to as excrescences (Hahn *et al*., 1920; La Rue, 1933c), neoplasms (Dodds & Matthews, 1966; Nilsen & Lersten, 1977), galls (Warrington, 1980), enations (Mitchell & Vojtik, 1967; Kirkham & Keeney, 1974; Warrington, 1980), genetic tumours (Jones & Burgess, 1977; Morrow & Tibbitts, 1988), leaf lesions (Petitte & Ormrod, 1986) and oedemata (Digat & Albouy, 1976). Deferring to history, we refer to these phenomena as intumescences.

Historically, a number of abiotic and biotic causal agents have been reported to trigger intumescence formation on a range of horticulturally and agriculturally important plant species (Table 12.1) including *Eucalyptus* spp. grown under both glasshouse conditions (La Rue, 1933c; Warrington, 1980) and in the field (Unpublished results; Dick, 1992). However, it is now widely agreed that this abnormality is due to physiological rather than pathogenic agents (Eisa & Dobrenz, 1971), and more specifically, the interaction of high temperature and high humidity where the rate of water absorption by plant tissues exceeds transpiration. Further environmental factors, such as the wavelength of light, may also exacerbate (Morrow & Tibbitts, 1988) or alleviate (Jones & Burgess, 1977) intumescence symptomotology.

The developmental process of intumescence formation remains unclear. Balge *et al*., (1969) and Eisa & Dobrenz (1971) described the rupturing of epidermal cells caused by cell enlargement (hypertrophy) of underlying water-soaked palisade cells, the walls of which have been softened by cellulase activity (Kawase, 1981). Others however, believe that intumescences are formed predominantly by cell division (hyperplasia) (La Rue, 1933c) or by a combination of hyperplasia and hypertrophy (Wolf & Lloyd, 1912) which may be cytokinin- and/or auxin-regulated (Morrow & Tibbitts, 1988).

It is likely that the presence of intumescences affects leaf function and particularly photosynthetic processes, although few detailed studies were found. Roloff *et al.* (2004) observed in blueberry cultivars that there was a significant reduction in photosynthetic rate as leaf area affected by oedema-like protruberences increased, but they did not study the likely mechanisms behind this response. The structural changes described above may affect light absorption and the amount of photosynthetic tissue in a given area of leaf, which in turn may influence rates of biochemical processes. As it has been observed with some species that intumescences are associated with veins (Douglas, 1907; La Rue, 1933a,b,c; Warrington, 1980), leaf water relations may be affected.

Table 12.1. Reported contributing agents to foliar intumescence development and afflicted species.

Contributing agent	Afflicted species	Reference
Mechanical injury	*Brassica oleracea capitata*	Wolf, 1918
	Brassica oleracea capitata	Harvey, 1918
	Hieracium venosum	La Rue, 1933c
Chemical injury	*Solanum tuberosum*	Sorauer, 1893 (in von Schrenk, 1905)
	Brassica oleracea botrytis	von Schrenk, 1905
	Solanum tuberosum	Douglas, 1907
	Brassica oleracea botrytis	Smith, 1917
	Ricinus communis	Smith, 1917
Nutrient status	*Solanum tuberosum*	Douglas, 1907
	Populus spp.	La Rue, 1933b
	Pelargonium hortorum	Metwally *et al*., 1970b
	Solanum tuberosum	Petitte & Ormrod, 1986
Hormones (including ethylene)	*Populus grandidentata*	La Rue, 1936
	Populus tremuloides	La Rue, 1936
	Solanum tuberosum	Kirkham & Keeney, 1974
	Solanum tuberosum	Petitte & Ormrod, 1986
	Lycopersicon hirsutum	Morrow & Tibbitts, 1988
Genetics	*Solanum melongena*	Eisa & Dobrenz, 1971
	Pelargonium hortorum	Metwally *et al*., 1970b
	Lycopersicon hirsutum	Morrow & Tibbitts, 1988
Insect injury	*Dianthus* sp.	Woods, 1900
	Populus tremula	Küster, 1903 (in von Schrenk, 1905)
Fungal infection	*Peperomia* sp.	Alfieri, 1969
	Eucalyptus regnans	Dick, 1992
Air quality & airborne factor(s)	*Populus* spp.	La Rue, 1933a,b
	Lycopersicon sp.	Mitchell & Vojtik, 1967
	Eucalyptus spp.	Warrington, 1980
	Lycopersicon spp.	Lang & Tibbitts, 1983
	Solanum tuberosum	Petitte & Ormrod, 1986
Light wavelength	*Hibiscus vitifolius*	Dale, 1901
	Solanum sp.	Nilsen, 1971
	Lycopersicon esculentum	Nilsen & Lersten, 1977
	Lycopersicon spp.	Lang & Tibbitts, 1983
	Lycopersicon hirsutum	Morrow & Tibbitts, 1988
Light intensity	*Lycopersicon* sp.	Atkinson, 1893
	Hibiscus vitifolius	Dale, 1900
	Populus tremula	Küster, 1903 (in La Rue, 1933b)

Table 12.1 continued

Contributing agent	Afflicted species	Reference
	Vitis vinifera	Viala & Pacottet, 1904 (in von Schrenk, 1905)
	Solanum tuberosum	Douglas, 1907
	Pelargonium hortorum	Balge *et al*., 1969
	Pelargonium hortorum	Metwally *et al*., 1970b
	Pelargonium sp.	Digat & Albouy, 1976
Temperature	*Lycopersicon* sp.	Atkinson, 1893
	Ficus elastica	Sorauer, 1899 (in von Schrenk, 1905)
	Impatiens fulva	Sorauer, 1899 (in von Schrenk, 1905)
	Hibiscus vitifolius	Dale, 1900
	Solanum sp.	Douglas, 1907
	Philodendron hastatum	Munnecke & Chandler, 1957
	Pelargonium hortorum	Balge *et al*., 1969
	Solanum melongena	Eisa & Dobrenz, 1971
	Brassica oleracea capitata	Harvey, 1918
Excess moisture	*Ficus elastica*	Sorauer, 1899 (in von Schrenk, 1905)
	Impatiens fulva	Sorauer, 1899 (in von Schrenk, 1905)
	Lycopersicon sp.	Atkinson, 1893
	Hibiscus vitifolius	Dale, 1900, 1901
	Ruellia Formosa	Steiner, 1905 (in La Rue, 1933b)
	Aphelandra porteana	Steiner, 1905 (in La Rue, 1933b)
	Solanum tuberosum	Douglas, 1907
	Populus spp.	La Rue, 1933a,b
	Pelargonium hortorum	Balge *et al*., 1969
	Pelargonium hortorum	Metwally *et al*., 1970b
	Solanum melongena	Eisa & Dobrenz, 1971
	Pelargonium sp.	Digat & Albouy, 1976
	Eucalyptus spp.	Warrington, 1980
	Lycopersicon sp.	Boulard *et al*., 1989
Causal factor undefined	*Eucalyptus coccifera*	Sorauer, 1886 (in La Rue, 1933c)
	Manihot spp.	Wolf & Lloyd, 1912
	Eucalyptus spp.	La Rue, 1933c
	Thurberia thespesioides	La Rue, 1933c
	Mitchella repens	La Rue, 1933c
	Ipomoea batatas	Wetzstein & Frett, 1984

Eucalyptus globulus Labill., and to a lesser extent *Eucalyptus nitens* Deane and Maiden (Maiden), are important plantation species in temperate zones around the world. Foliar pathogens can be a serious problem in many areas where the species are grown (Dick & Dobbie, 2001; Ahumada *et al*., 2003; Hunter *et al*., 2004). Inoculating the target species with foliar pathogens in pot experiments to study the pathogens' effects on plant physiology and growth requires a high relative humidity environment (often in a glasshouse) during the inoculation process. Such an environment also results in the formation of intumescences. We undertook a study to examine the histology of intumescences that develop on *E. globulus* and *E. nitens* foliage under glasshouse conditions with high relative humidity, and to quantify the effects of these intumescences on photosynthetic processes.

Materials and methods

Plant material

Eucalyptus globulus and *E. nitens* seedlings were planted into 1 L pots filled with low phosphorus potting mix in August 2003. They were transplanted into 5 L plant bags in March 2004. Seedlings were grown outside and watered daily to saturation. A slow release fertiliser (Osmocote) was used to maintain adequate nutrition. At the start of the experimental work *E. globulus* seedlings had attained an average height (H) and diameter (D) (at 5 cm height) of 0.88 m and 1.08 cm respectively, and *E. nitens* had attained an average H and D of 0.79 m and 1.09 cm, respectively.

Treatments

In each species six seedlings were placed in a glasshouse for five days, and six seedlings of each species were left outside as controls. The glasshouse had a relative humidity of approximately 80% with little air movement. Intumescence formation was induced within this period on all seedlings placed in the glasshouse. These seedlings were then removed from the glasshouse and grown as per the control seedlings. Measurements commenced one month after this. At the start of measurements, intumescences were well-developed and extensive on *E. globulus* (approximately 30% of tissue affected per leaf, 80% of leaves per tree affected), but much less frequent and less well-developed in *E. nitens* (approximately 10% of tissue affected per leaf, 40% of leaves affected per tree). This was assessed subjectively using a visual guide

Physiology

Three control and three intumescent plants of each species were selected for physiological measurements. The photosynthetic response (A) to varying intercellular p[CO_2] (C_i) was determined for fully-expanded leaves from control plants, and for asymptomatic and symptomatic fully expanded leaves of intumescent plants. All leaves sampled were juvenile, as no adult foliage was present. The leaves were selected from the top one third of the seedling crown, and between three and five leaf pairs from the branch apex. Intumescent *E. globulus* leaves selected for measurement had around 30% of tissue affected by intumescences; *E. nitens* sample leaves had around 10% of tissue affected by intumescences. Measurements were made with a Licor 6400, with a leaf temperature of 20°C and a photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1}. Measurements were made at 400 ppm CO_2. The p[CO_2] was then reduced stepwise to 0, and increased stepwise to 1200 ppm. A total of 11 steps were used (0, 50, 100, 150, 200, 400, 600, 800, 1200, 1500 ppm). Leaves were left to equilibrate for a maximum of 3 min at each new p[CO_2], which was sufficient for stomatal conductance to stabilise.

The maximum rate of light-saturated photosynthesis (A_{max}) was also measured on each leaf, at a leaf temperature of 20°C, a p[CO_2] of 370 ppm and a PPFD of 1500 μmol m^{-2} s^{-1}.

Leaves used for the gas exchange study were collected and weighed immediately. Leaf area was measured using a planimeter (Delta-T Devices, Cambridge, UK), and leaves were dried at 65°C for 3 d before re-weighing.

Intumescence symptomotology

Three branchlets with approximately five intumescent leaves per branchlet were removed from intumescent plants. The stems were immediately placed into an aqueous solution of 1% (w/v) Toluidine Blue O (TBO) and recut with the cut end immersed in the solution. The branchlets were left for 12 hr, after which leaves were removed and photographed with a Nikon Coolpix 990 digital camera.

Histology

Ten leaves from five control plants, and from asymptomatic (no intumescences) and symptomatic (in *E. globulus*, ~30% of tissue affected, with well-developed intumescences; in *E. nitens* ~10% of tissue affected) leaves of five intumescent plants were collected. These were fully expanded (mature) juvenile leaves, selected from the top one third of the plant, and between three and five leaf pairs from the branch apex. Within 3 hr of harvest, tissue samples approximately 2 mm × 4 mm were excised from the laminar margin and immersed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 (Sörensen, 1909). The samples were fixed in this solution under vacuum for 15 hr at 4ºC. Following two buffer washes (each for 20 min), the samples were dehydrated in an ascending acetone series in 20% increments and taken to 3 changes of 100% acetone (each for 30 min), finishing with two changes (each of 20 min) of propylene oxide. The leaf blocks were slowly infiltrated with Spurr's resin of medium hardness (Spurr, 1969).

Polymerised blocks were hand-trimmed with a razor blade and semi-thick sections (4-5 µm thick) were cut with a glass knife fitted to a Reichert Om U2 ultramicrotome. The sections were transferred to a drop of sterile distilled water on a clean glass microscope slide and gently heat-fixed to the glass. The slides were then immersed in 1% (w/v) TBO in 1% (w/v) sodium borate solution for 30 s, rinsed in distilled water, decolourised in 70% ethanol for 30 s, rinsed again in distilled water and air dried. The sections were mounted in Euparal (Australian Entomological Supplies, NSW, Australia) beneath a coverslip and cured on a cool to moderate hotplate.

Data analysis

The ratio of leaf fresh area:dry mass (specific leaf area, SLA) was determined. Relative water content (RWC) of the leaves was calculated as:

$$RWC = \frac{M_f - M_d}{M_f}$$

where M_f is leaf fresh mass and M_d is dry mass. Analysis of variance was used to determine differences between treatments in SLA and RWC.

A non-rectangular hyperbolic function was used to describe the shape of the A/C_i curve of each leaf (Sands, 1995). This was used to estimate the photosynthetic utilisation of CO_2 (carboxylation efficiency, C_e), the capacity for ribulose bisphosphate (RuBP) regeneration (V_j) and daytime respiration, r_d (Farquhar *et al*., 1980; von Caemmerer & Farquhar, 1981). The potential electron transport rate (J_{max}), and maximum rate of ribulose bisphosphate carboxylase (rubisco) activity (V_{cmax}) were calculated using the equations of Medlyn *et al*. (2002) and Bernacchi *et al*. (2001). Following Wullschleger (1993) and Medlyn *et al*. (2002) J_{max} and V_{cmax} were fitted over the entire A/C_i curve

using non-linear regression. The CO_2 compensation point (Γ) was taken as the value of C_i where net assimilation was zero (Larcher, 1975). Stomatal limitation (l_s) was calculated using the equation of Farquhar & Sharkey (1982). Analysis of variance was used to assess differences between treatments in these parameters.

Results

Physiology

The presence of intumescences reduced A_{max} by 23% in *E. globulus* compared to the control (Table 12.2) ($P < 0.001$), but there were no significant differences between treatments in *E. nitens*. The A_{max} of healthy leaves on intumescent plants was similar to that of leaves of control plants.

The presence of intumescences affected the response of A to varying $p[CO_2]$ (Figure 12.1). There were no significant differences between treatments or species in

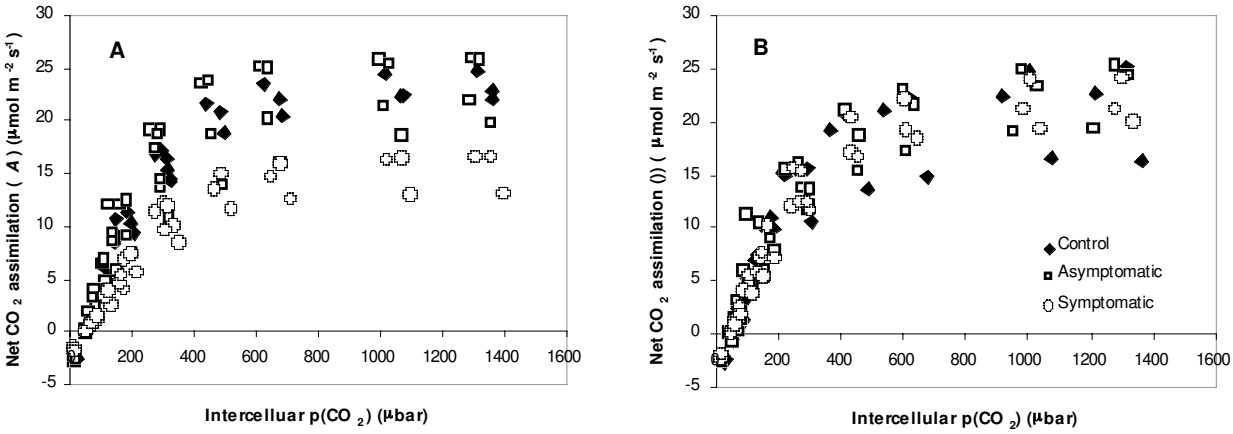

Figure 12.1. Relationship between net CO_2 assimilation rate (A) and intercellular $p[CO_2]$ (C_i) for (A) *E. globulus* and (B) *E. nitens* leaves from control plants, and from symptomatic and asymptomatic leaves of intumescent plants.

C_e ($P = 0.05$) (Table 12.2). RuBP regeneration rate (V_j) and J_{max} of intumescent leaves of *E. globulus* were 38 and 50%, respectively, less than those of either control leaves or of asymptomatic leaves of intumescent-affected plants (Table 12.2). In contrast, V_j and J_{max} were unaffected by the presence of intumescences in *E. nitens*. In both species, r_d of intumescent leaves was less than either healthy leaves of symptomatic plants or leaves of control plants. Stomatal limitation, l_s, was greater in *E. nitens* than *E. globulus* ($P < 0.001$), but was unaffected by treatment. The CO_2 compensation point, Γ, was unaffected by treatment or species.

Intumescent leaves had a small but significantly lower RWC than control leaves ($P < 0.05$) (Table 12.3). The SLA was affected by species but not treatment ($P < 0.001$), with *E. nitens* having a lower SLA than *E. globulus*.

Table 12.2. Carboxylation efficiency (C_e), light-saturated CO_2 uptake (A_{max}, μmol m^{-2} s^{-1}), ribulose bisphosphate (RuBP) regeneration capacity (V_j, μmol m^{-2} s^{-1}), maximum electron transport rate (J_{max}, μmol m^{-2} s^{-1}) and maximal rubisco carboxylation rate (V_{cmax}, μmol m^{-2} s^{-1}) total daytime respiration (leaf dark respiration plus photorespiration), (r_d, μmol m^{-2} s^{-1}), stomatal limitation (l_s), and the CO_2 compensation point (Γ, μbar), of leaves sampled from control seedlings, and asymptomatic and symptomatic leaves sampled from intumescent seedlings. Different letters within a column indicate significant differences ($P < 0.05$).

Species	Leaf status	C_e	A_{max}	V_j	J_{max}	V_{cmax}	r_d	l_s	Γ
E. globulus	Control	0.09^{a}	15.23^{ab}	28.79^{a}	140.2^{a}	41.1^{a}	3.72^{ab}	0.12^{b}	40.9^{a}
	Asymptomatic	0.11^{a}	17.33^{a}	30.55^{a}	151.7^{a}	44.5^{a}	4.63^{a}	0.16^{b}	43.8^{a}
	Symptomatic	0.05^{a}	10.70^{c}	18.09^{b}	88.1^{b}	25.8^{a}	2.33^{b}	0.14^{b}	50.0^{a}
E. nitens	Control	0.11^{a}	14.03^{b}	27.49^{a}	139.9^{a}	41.0^{a}	4.96^{ab}	0.21^{a}	46.1^{a}
	Asymptomatic	0.13^{a}	13.8^{b}	30.49^{a}	154.8^{a}	45.4^{a}	5.41^{a}	0.20^{a}	49.6^{a}
	Symptomatic	0.08^{a}	13.1^{b}	26.41^{a}	128.8^{a}	37.7^{a}	3.46^{b}	0.23^{a}	46.7^{a}

Table 12.3. Relative water content (RWC) and specific leaf area (SLA) of leaves sampled from healthy (control) seedlings and leaves affected with oedema. Different letters within a column indicate significant differences ($P < 0.05$).

Species	Leaf status	RWC	SLA
E. globulus	Control	0.67^{a}	125.2^{a}
	Oedema	0.65^{b}	137.0^{a}
E. nitens	Control	0.59^{c}	83.1^{b}
	Oedema	0.57^{d}	77.2^{b}

Intumescence symptomology

Control leaves appeared smooth and devoid of blemishes (Figure 12.2a). In mild cases of intumescence development, intumescences appeared as discreet, raised spherical bodies rising above the leaf lamina surface (Figure 12.2b). Staining the vascular system with TBO indicated that intumescences were associated with the leaf vascular tissues and did not develop in interveinal areas (Figure 12.2c) except where intumescence development was severe. A severe case of intumescence development (Figure 12.2d) resulted in the coalescence of neighbouring intumescences involving the majority of the leaf lamina and leaf epinasty.

Histology

Asymptomatic control leaves of *E. globulus* were characterised in longitudinal section by a lacunose mesophyll containing sparse spongy parenchyma (Figure 12.3a). The chlorophyllous isolateral palisade parenchyma was distributed solely along the adaxial surface and stomata were numerous throughout the abaxial epidermis. Intumescence development in *E. globulus*, (Figure 12.3b) was initiated by prolific periclinal and anticlinal division (hyperplasia) of spongy parenchyma cells (compare with control tissue Figure 12.3a).

The epidermal cells became circumferentially and longitudinally elongated (hypertrophy). The expanding tumorous mass exerted pressure on the overlying palisade parenchyma cells which in turn increased longitudinally (hypertrophy). The

afflicted palisade parenchyma became necrotic; the resultant green colouration following staining with TBO indicated the accumulation of polyphenolics within this tissue. In later stages of intumescence development (Figure 12.3c), the epidermis erupted and affected internal tissues lost integrity and collapsed, leaving large air spaces within the mesophyll and exposing the internal leaf structure to the atmosphere.

While the development of intumescences in *E. nitens* was similar to that described for *E. globulus*, there were some fundamental morphological differences. Most significantly, *E. nitens* was isobilateral, ie it formed palisade parenchyma on both the adaxial and abaxial surfaces of the leaf (Figure 12.3d). Furthermore, during intumescence development, a second upper (subtending) epidermis and lower (subtending) epidermis were seen to develop adjacent to intumescences, most often during the early stages (Figure 12.3e). As in *E. globulus*, a zone of prolific cell division initiated the formation of the intumescence and the patterns of necrosis of afflicted tissue were also the same except for the upper epidermis which, in *E. nitens*, rarely erupted (Figure 12.3f).

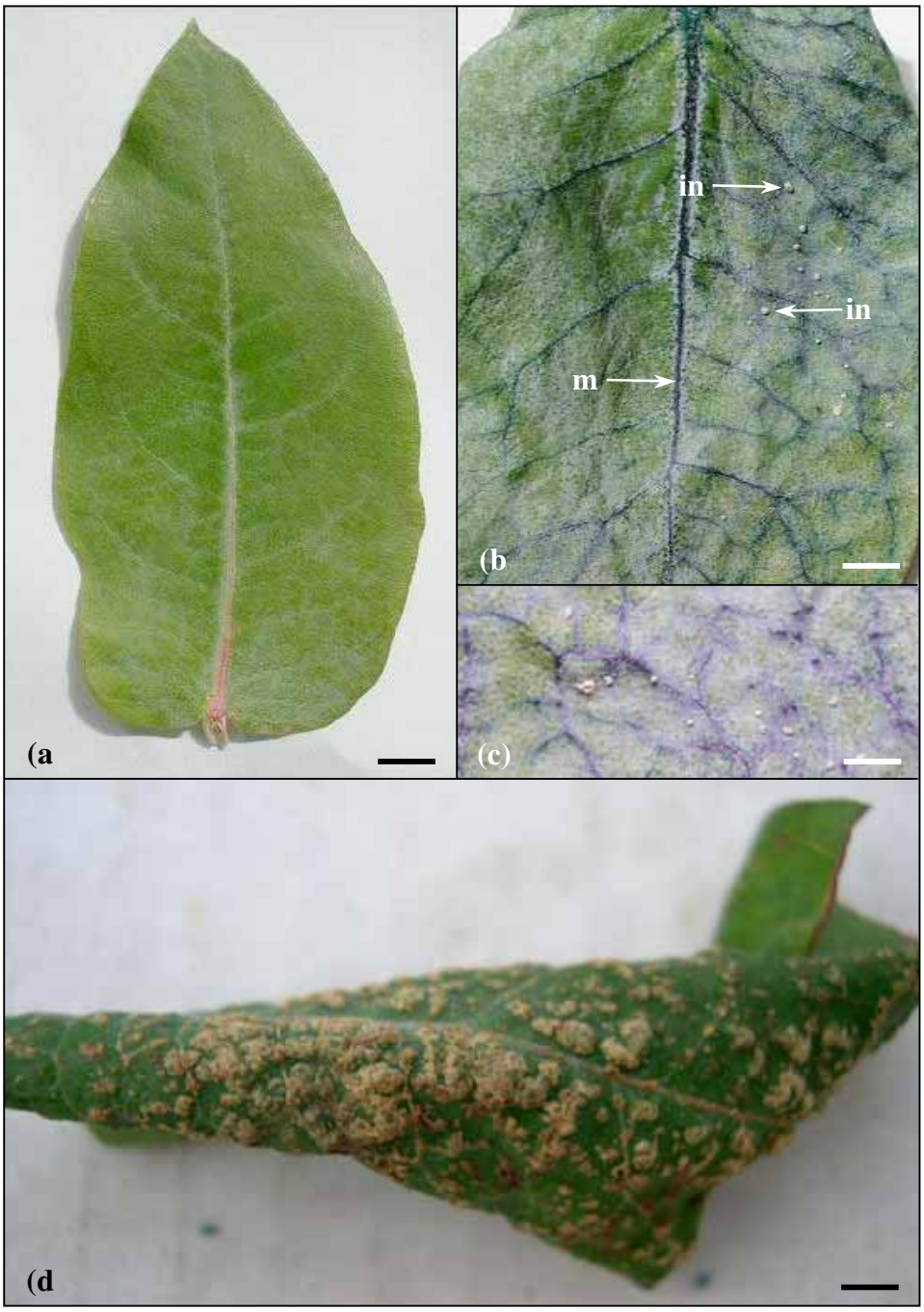

Figure 12.2. Gross external morphology of unaffected and oedema-afflicted leaves of *E. globulus* and *E. nitens* following venal staining. All bars = Xmm. (a) Adaxial surface of an asymptomatic *E. globulus* control leaf prior to staining. (b) Adaxial surface of an oedema-afflicted *E. globulus* leaf in early stages of oedema. Discrete intumescences (in) forming on one side of the lamina. Note prominent midrib (mr) and lower orders of venal branching. (c) Adaxial surface of an oedema-afflicted *E. globulus* leaf. The intumesences are associated with the vascular system and do not form in intervenal areas. (d) Severely oedema-afflicted *E. globulus* leaf demonstrating coalescing tumorous intumescences and extreme epinasty.

Abbreviations
Vascular bundle (vb); spongy parenchyma (sp); palisade parenchyma (pp); upper epidermis (ue); lower epidermis (le); oil gland (og); involved palisade (ip); zone of prolific cell division (cd); erumpent tissue (et); stoma (st); adaxial palisade (adp); abaxial palisade (abp); upper subtending epidermis (use); lower subtending epidermis (lse); developing intumescence (in); collapsed tissue (ct); sclerenchymatous fibre bundle (fb); bundle sheath (bs); xylem (xy); vascular cambium (vc); phloem (ph); tumorous tissue (tt).

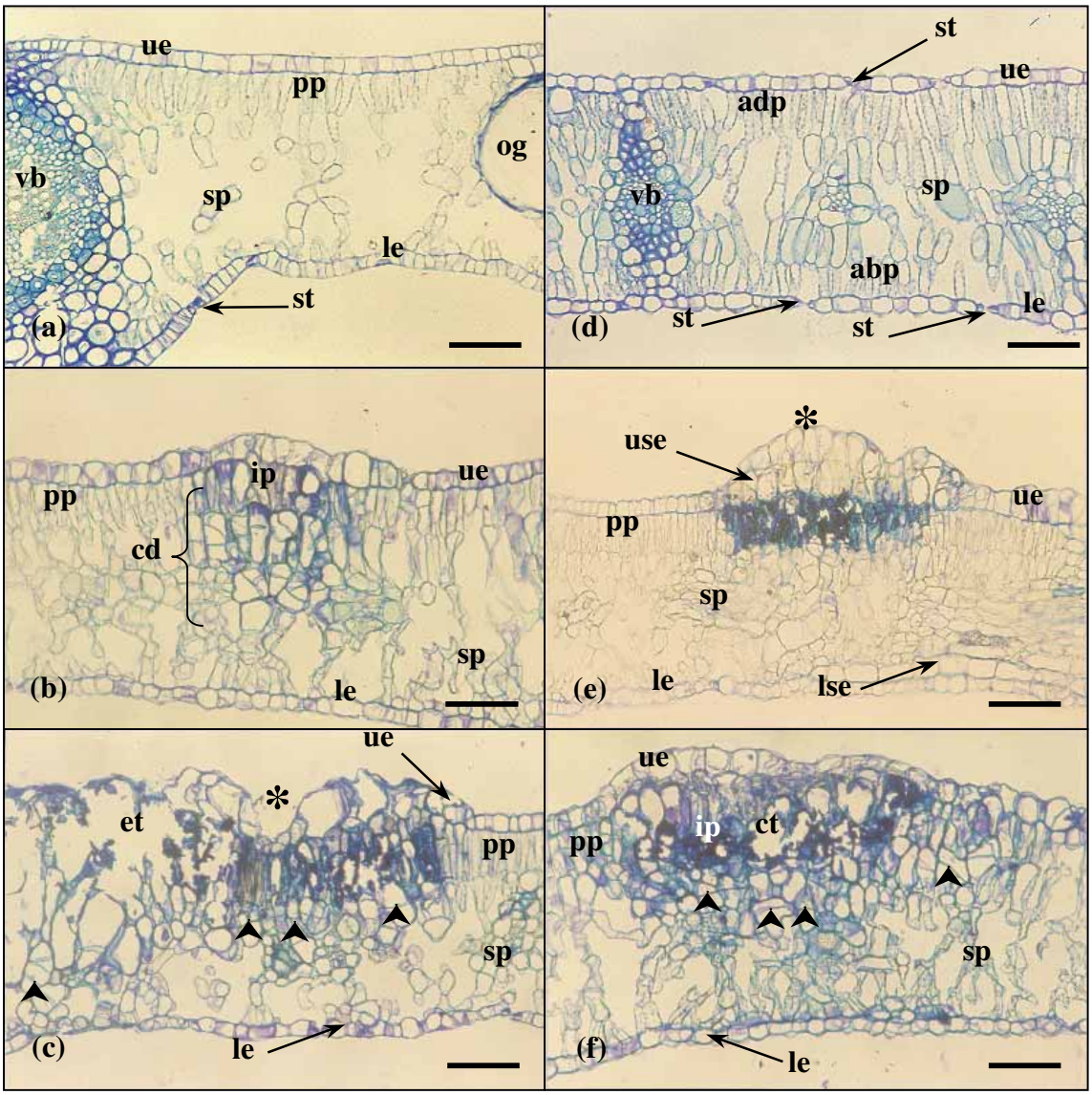

Figure 12.3 Internal morphology of asymptomatic control and oedema-afflicted leaves of both *E. globulus* and *E. nitens*. All transverse sections are of resin-embedded tissue stained with TBO. All bars = Xμm. (a) *E. globulus* asymptomatic control leaf. Note the paucity of spongy parenchyma and the dorsiventral distribution of the palisade parenchyma along the adaxial leaf surface. (b) *E. globulus* leaf in early stage of intumescence development. (c) *E. globulus* leaf in late stage of intumescence development. Note the area of erumpent tissue protruding through degraded epidermis and collapsed epidermis (*). The arrowheads indicate zones of prolific cell division. (d) *E. nitens* asymptomatic control leaf. Note the isolateral distribution of palisade parenchyma on both the adaxial and abaxial leaf surfaces (compare *E. globulus*, Fig. 3a). (e) *E. nitens* leaf in early stage of intumescence development. *E. nitens* commonly produces a double epidermis on both the adaxial (use) and abaxial (lse) surfaces. Note the epidermis is domed and is resistant to breakage (*). (f) *E. nitens* leaf in late stage of intumescence development. Even in advanced stages, the epidermis retains integrity and does not break. Note the zone of periclinal cell division directly below the intumescence (arrowheads). As in *E. globulus*, the tissue within mature intumescences on *E. nitens* leaves collapses, leaving a substantial space.

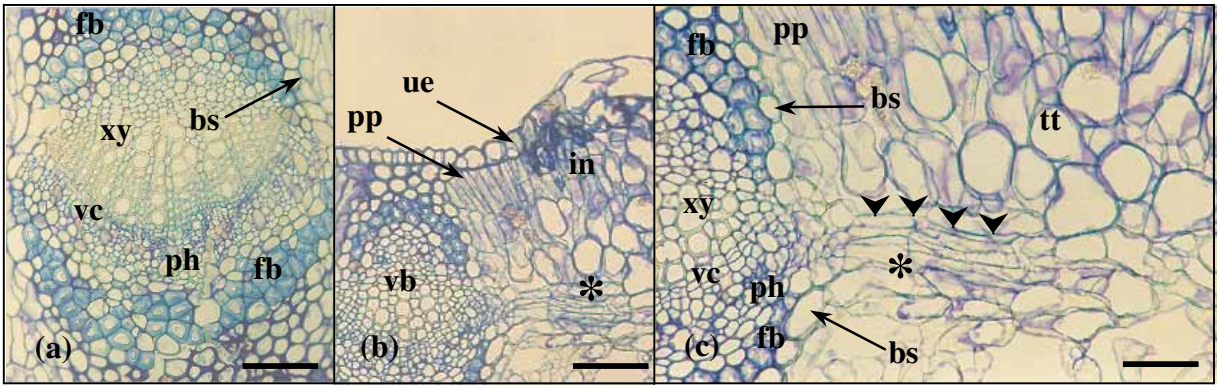

Figure 12.4 Relationship of intumescence to leaf vascular tissue within oedema-afflicted *E. globulus* leaves. (a) Vascular bundle from an asymptomatic *E. globulus* control leaf. Bar = Yμm. (b) Detail of oedema-affected *E. globulus* leaf demonstrating the formation of a developing intumescence (in) above a branch of vascular tissue (*). Bar = Zμm. (c) Enlargement of a section of Fig. 4b. The branch of vascular tissue, the upper limit of which is indicated by the arrowheads, is contiguous with the bundle sheath (*) and lies adjacent to the lower limit of the developing intumescence. Bar = Vμm.

Within asymptomatic control *E. globulus* leaves, the vascular bundle appeared quite 'normal' (Figure 12.4a). Within intumescent leaves, however, an interaction between the intumescence and vascular tissue was often observed (Figure 12.4b,c). The palisade parenchyma caught between the vascular bundle and the intumescence was compressed but morphologically unaltered. However, beneath the intumescence, the vascular tissue appeared to be contiguous with the bundle sheath region of the vascular bundle (Figure 12.4c).

Discussion

Oedema is a non-pathogenic, physiological disorder affecting the leaves of many plant species. Often, the only visible evidence for this disorder is intumescences, which are raised blister-like protrusions extending above the afflicted leaf lamina. Here, we present for the first time, evidence that intumescences which develop on eucalypts are actually lenticels or lenticel-like structures formed in response to environmental pressure. We refer to these in the following as environmental response lenticel-like structures (ERLS).

Lenticels, by definition, are raised pores on the stems and roots of woody plants that allow gas exchange between the atmosphere and internal tissues (Esau, 1965). In stems, parenchyma cells undergo division from which a phellogen becomes established. Growth of complementary or filling tissue from the phellogen pushes the overlying cells outward, rupturing the epidermis (Esau, 1965) thus allowing aeration of the underlying tissues. The intumescences observed in *E. globulus* and *E. nitens* leaves in this study resemble lenticels in their simplest form (Esau, 1965), both morphologically and developmentally. Parenchyma cells below the palisade layer undergo dedifferentiation and prolific anticlinal and periclinal division to form a lateral meristem. The intumescence protrudes above the plane of the leaf lamina as a consequence of the production of complementary tissue outward from the meristematic zone (hyperplasia) while the extant palisade mesophyll parenchyma cells enlarge both radially and longitudinally (hypertrophy). The subsequent rupture of the expanded

epidermis exposes the internal tissues to the ambient atmosphere and facilitates aeration of the leaf tissues, which is indicated by the significant reduction of the leaf water content of afflicted *E. globulus* leaves compared to controls.

In 1995, Neish *et al*. reported the occurrence of 'leaf margin lenticels' on four species of *Eucalyptus* bearing denticulate leaf margins. Although morphologically resembling lenticels, these structures were thought to function as herbivore deterrents, as discrete meristems formed beneath oil glands and, through hyperplasia, forced the glands and their unpalatable contents to the leaf margin. The tissues within these structures were tightly packed and not aerenchymous, thus negating the sole defined function of lenticels, aeration. Consequently, these structures cannot be considered lenticels.

The ERLS we have described on *Eucalyptus* are morphologically distinct from intumescences reported from non-eucalypt species such as *Hibiscus vitifolius* (Dale, 1900), *Brassica* sp. (von Schrenk, 1905), *Solanum* sp. (Douglas, 1907), *Manihot* spp. (Wolf & Lloyd, 1912), *Brassica oleracea capitata* (Wolf, 1918) *Populus* spp. (La Rue, 1933a), *Pelargonium hortorum* (Balge *et al*., 1969; Metwally *et al*., 1970a,b) and *Lycopersicon* spp. (Lang *et al*., 1983) which are all formed from hypertrophic cellular modifications. Although intumescences investigated from *Ipomoea batatas* (Wetzstein & Frett, 1984) and *Solanum melongena* (Eisa & Dobrenz, 1971) are formed from both hypertrophy and hyperplasia of the palisade and/or mesophyll parenchyma as are ERLS on *E. globulus* and *E. nitens*, they remain anatomically dissimilar. La Rue (1933c) and Warrington (1980) reported development of blister-like galls on leaves of several eucalypts grown in a controlled environment room. However, neither author recognised intumescences on *Eucalyptus* species as, or noted resemblance to, lenticels or lenticel-like structures, although we believe that the structures they observed on eucalypts probably were ERLS.

Despite morphological and anatomical expression of intumescences differing between crops (Eisa & Dobrenz, 1971), species (Lang *et al*., 1983) and cultivars (Petitte & Ormrod, 1986) and through a wide variety of published causative agents (Table 1), high humidity inside controlled climate growth chambers is a common factor linking intumescence development. Warrington (1980) found that at high relative humidity and day-night temperatures between 25ºC and 15ºC, severe symptoms occurred on young expanding leaves of all five *Eucalyptus* species examined. At reduced relative humidity, symptoms developed on three species, while at low relative humidity, mild symptoms occurred on a single species. He concluded that intumescence development could be prevented in *Eucalyptus* by growing them under a medium to low relative humidity regime. Our observation that *E. globulus* is more susceptible than *E. nitens* to ERLS development at high relative humidity concurs with Warrington's reported differential response and suggests that a low relative humidity environment is more critical for growing *E. globulus* under glasshouse conditions than it is for *E. nitens*. Observed differences in leaf cellular structure may explain this. We found that isobilateral *E. nitens* often develops a double epidermis in association with ERLS whereas *E. globulus* does not. The inner epidermal layer may firstly account for the reduced epidermal rupturing (and hence necrotization) observed, and secondly, act as a water-holding tissue thereby reducing the need for ERLS in the within the afflicted tissue.

We have found no definitive reports of the physiological stimuli regulating intumescence formation. However, sap removed from intumescent leaves of *Pelargonium* has been shown to incite intumescence formation when introduced into the leaf parenchyma of healthy plants (Digat & Albouy, 1976). Furthermore, La Rue (1936) provided evidence for the role of a translocatable agent in intumescence development by observing the formation of intumescences following injection of healthy poplar leaf petioles with extracts of intumescences excised from oedematous plants. Subsequent applications of both crude and purified auxin solutions elicited intumescences, suggesting the stimulatory mechanism to be hormonal. Phytohormones such as auxin and cytokinin (Morrow & Tibbitts, 1988) and ethylene (Kirkham & Keeney, 1974) have been implicated in intumescence development on leaf tissue (Petitte & Ormrod, 1986). In addition to leaves, ethylene, even in minute concentrations, has been shown to elicit intumescence formation in a variety of plant tissues (Doubt, 1917; Woffenden & Priestley, 1924; Wallace, 1926, 1927, 1928; Abeles, 1973; Kawase, 1981).

In an analogous situation to the observed ERLS development on *Eucalyptus* leaves exposed to high humidity, stems of woody plants experiencing waterlogged and flooded conditions develop hypertrophied lenticels on submerged tissue (Wheeler *et al*., 1979; Angeles *et al*., 1986; Savé & Serrano, 1986; Sena Gomes & Kozlowski, 1986; Topa & McLeod, 1986; Harrington, 1987; Yamamoto & Kozlowski, 1987; Larson *et al*., 1991; Terazawa & Kikuzawa, 1994; Osundina, 1998; Batzli & Dawson, 1999; Hebbar *et al*., 2001). These flood-induced lenticels, thought to facilitate exchange of dissolved gases (Hook *et al.*, 1970; Angeles *et al*., 1986; Osonubi & Osundina, 1987; Hebbar *et al*., 2001) and release accumulated toxins (Hook, 1984; Kozlowski, 1986), are formed by increased phellogen activity and concomitant hypertrophy (Angeles *et al*., 1986; Kozlowski & Pallardy, 2002). They are induced by an accumulation of ethylene in waterlogged tissues (Blake & Reid, 1981; Newsome *et al*., 1982; Tang & Kozlowski, 1982; Yamamoto & Kozlowski, 1987), most significantly, leaves (Sena Gomes & Kozlowski, 1988). Given the morphological, developmental and environmental parallels between ERLS described in this work and hypertrophied lenticels on waterlogged tissue, there is a strong likelihood that ERLS development, too, is regulated by accumulated endogenous ethylene. Moreover, waterlogged soil (REF) and emissions from fluorescent lighting (Wills & Patterson, 1970) have been identified as two sources of exogenous ethylene within controlled growth chambers.

La Rue (1936) demonstrated an association between applied auxin and intumescence initiation on poplar leaves. Both auxin (Wample & Reid, 1978) and ethylene (Blake & Reid, 1981; Tang & Kozlowski, 1982; Kozlowski, 1997) accumulate in tissues during waterlogging. They both promote cellulase production (Ridge & Osborne, 1969; Kawase, 1979) a pre-emptory step in lenticel formation, and both accelerate growth (Ridge & Osborne, 1969). Indeed, tissues enriched with auxin also accumulate ethylene (Burg & Burg, 1966; Chadwick & Burg, 1970) as auxin acts as a stimulant to ethylene production (Raven *et al*., 2003). However, the exact mechanism remains unclear and intumescence and ERLS formation may result from a complex synergy between a number of growth regulators, as suggested in lenticel development on waterlogged plant tissue (Ghouse & Yunus, 1974; Kramer & Kozlowski, 1979; Lledo *et al*., 1995).

As we have observed for ERLS in *E. globulus* and *E. nitens*, Douglas (1907), La Rue (1933a,b,c) and Warrington (1980) reported that intumescences on afflicted leaves

developed primarily along, or in close association with, the leaf vascular tissue. Conversely, other workers (Wolf & Lloyd, 1912; Eisa & Dobrenz, 1971; Wetzstein & Frett, 1984) have demonstrated that there is no preferential site for intumescence formation. It is unlikely that this distribution is due to hormonal influences as neither auxin nor ethylene are delivered by the vascular tissue (Raven *et al*., 2003) to the site of intumescence or ERLS development. It is more feasible that the distribution pattern is a function of soluble carbohydrate availability (Douglas, 1907; La Rue, 1933b) via the vascular system and is a reflection of the varying nutrient status of the tissues being tested (Douglas, 1907; Metwally et al., 1970b; Petitte & Ormrod, 1986).

We found few other studies of the effects of foliar intumescences on gas exchange characteristics. While Roloff *et al.* (2004) observed reductions in net CO_2 assimilation in blueberry plants with oedema-like structures on their leaves, they did not investigate likely causes of that reduction. In studies of lenticels, it has been demonstrated that lenticel development on roots or stems of plants subjected to waterlogging can result in increased photosynthetic rates in the foliage of affected plants compared to that of plants that do not produce lenticels (Parolin, 2001). In a system more analogous to the ERLS development we observed on leaves, the presence of lenticels on fruit has been found to reduce fruit photosynthesis (Blanke & Lenz, 1989). The presence of ERLS on *E. globulus* foliage dramatically reduced photosynthetic capacity. The necrotization of the palisade mesophyll cells, as well as the accumulation of polyphenols that was observed in *E. globulus,* is likely to have reduced the amount of light reaching photosynthetic tissue as well as interfered with biochemical processes and translocation of end-products of photosynthesis from the leaf. Cellular collapse, observed in severe cases of ERLS development, reduced photosynthesising tissue per unit area of leaf.

At the cellular level, CO_2 assimilation in C_3 plants occurs via the photosynthetic carbon reduction cycle, a series of biochemical reactions many of which are tightly regulated (Sharkey, 1985; Hall & Rao, 1992). Electron transport and the proton transfer that occurs in the chloroplasts as part of this, produces NADPH and ATP (von Caemmerer, 2000). NADPH and ATP are required for RuBP regeneration. At lower rates of electron transport, less NADPH and ATP are produced, thereby explaining the reduction in RuBP regeneration capacity that we observed. The reductions in J_{max} may have been related to less light penetration into affected leaf tissue, a slower rate of turnover of electrons (von Caemmerer, 2000), or reductions in transfer or mesophyll onductance as suggested by the trend towards a lower C_e in symptomatic than control tissue.

While there was no change in l_s in leaves with ERLS, the reduction in air spaces between cells that was observed during ERLS development in *E. globulus* may have increased mesophyll resistance to CO_2 uptake (Farquhar & von Caemmerer, 1982). The trend towards a lower C_e, sometimes referred to as mesophyll conductance (Farquhar & von Caemmerer, 1982) in leaves with ERLS, supports this hypothesis, and would have further affected CO_2 assimilation rates in the species.

That CO_2 assimilation of *E. nitens* leaves was unaffected by the presence of ERLS reflects the less severe nature of symptoms in the species compared to *E. globulus*. In particular, the ERLS density and degree of necrotization were much less than was observed in *E. globulus*. The results suggest that there may be a threshold level of ERLS development below which photosynthetic processes will not be affected.

However this is not supported by the results of Roloff *et al.* (2004), who measured a significant negative relationship between net assimilation and area of leaf affected by oedema-like structures.

Our study concluded that intumescences developing on *E. globulus* and *E. nitens* foliage under high relative humidity conditions were environmental response lenticel-like structures that probably formed to improve aeration of the leaf tissue. The effects of ERLS on CO_2 uptake of *E. globulus* leaves was primarily related to a reduction in electron transport rate. The reduction in CO_2 uptake rate associated with the presence of ERLS on *E. globulus* foliage can be expected to reduce net primary production of afflicted seedlings, and hence to compromise the results of experimental work. Studies with other species (Warrington 1980) suggest that formation of intumescences similar to the ERLS we observed on *E. globulus* and *E. nitens* can be avoided by keeping relative humidity levels below 60%, although this may not be sufficient for other eucalypt species (eg. *E. grandis*, unpublished results). It is recommended that glasshouse experiments involving these species be done under controlled humidity conditions.

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