

**Towards an understanding of the causes of
physiological needle blight in *Pinus radiata***

Gould N, Bulman LS, Dick MA

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Gould N
Plant and Food Research, Ruakura

Bulman LS
Scion, Rotorua

Dick MA
Scion, Rotorua

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The New Zealand Institute for Plant and Food Research Ltd
Private Bag 92 169, Auckland 1142, New Zealand

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Research Scientist/Researcher
Date:

Group Leader, Quality Systems
Date:

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Executive Summary

Towards an understanding of the causes of physiological needle blight in *Pinus radiata*

Report to New Zealand Forest Health Research Collaborative, Project 2007-02
Gould N, Bulman LS, Dick MA December 2008

Objective

The objective of this work was to investigate the hypothesis that needle water stress is a factor in the onset of physiological needle blight (PNB) in *Pinus radiata*. Experiments were carried out simulating the effect of environmental changes throughout winter and early spring on the regulation of water uptake in radiata pine needles.

Two trials were designed to maximise water potential gradients between needles and roots, allowing an investigation into the capacity of radiata pine to regulate needle water potential when under temperature and humidity stresses.

Key Results

Monitoring of climatic conditions within a radiata pine plantation with a history of PNB highlighted both a large reduction in relative humidity (RH) and an increase in soil temperature over a two-week period towards the end of September and beginning of October 2007.

In laboratory-based experiments, reducing relative humidity from 90+ to 52% reduced needle water content, increased the water potential difference between the roots and needles and resulted in 40% death rate of needles after 10 days at the lower relative humidity. Reducing the RH from initial 65% or 58% to 52% did not adversely affect the plants.

Soil temperature had no effect on needle water potential or needle water content.

Application of Results

These results so far are consistent with the hypothesis that control of needle water content in radiata pine can be lost following large changes in relative humidity, such as those that may exist in the radiata pine plantations late winter to early spring. A popular article outlining our understanding of PNB in New Zealand plantations has been prepared for publication and can be found in the Appendix. .

Further Work

Further monitoring of PNB-susceptible trees is required to build a complete picture of the relationship between forest climatic conditions and tree water status. It is suggested that needle water content is measured in conjunction with needle and root water potentials in the field.

For further information, please contact:

Nick Gould
The New Zealand Institute for Plant & Food Research Ltd
Private Bag 3123,
Waikato Mail Centre
Hamilton 3240
NEW ZEALAND
Tel: +64-7-959 4430
Fax: +64-7-959 4431
Email ngould@hortresearch.co.nz

Introduction

Episodes of physiological needle blight (PNB) have been occurring in *Pinus radiata* plantations in late winter/early spring since the early 1980s. It is generally characterised by the death of needles during early spring in trees aged from about 15 years. Needles turn red/brown but remain firmly attached to the tree after needle death. The trees remain alive and new growth occurs in the months following the needle blight. Trees which have previously suffered from PNB can be identified in subsequent years by relative crown thinning. Needle blight outbreaks appear to be associated with high mid-winter rainfall and non porous soils, and are more prevalent within gullies and on ridges, leading to suggestions that high air humidity and wet soils may contribute to the onset of PNB (Bulman 2002).

Given these observations, this work set out to investigate the hypothesis that PNB is caused by a breakdown in regulation of needle water content, driven by soil water availability and relative air humidity. Climate data were collected from plantations that had shown previous PNB symptoms. Climatic conditions were then replicated in the laboratory with the aim of recreating the conditions thought to contribute to the onset of PNB.

Two trials were designed to maximise water potential gradients between needles and roots, allowing an investigation into the capacity of radiata pine to regulate needle water potential when under temperature and humidity stresses.

The work described in this report is run in parallel with the FBRC FRST-funded work (Gould, 2008) where shoot and root water potential were measured in the field and correlated to soil water content, soil and air temperature and relative humidity.

Material and Methods

Two experiments were carried out to assess the effect of localised changes in forest climatic conditions which mark the change from winter to spring and how the tree water relations responded.

Climatic data collection

In conjunction with FRST-funded monitoring of tree water relations (Gould 2008), climate monitoring was carried out at the Hikurangi Forest Farms Ltd plantation on Waimanu Road, Gisborne, that had previously been prone to outbreaks of PNB. Daily air temperature, air humidity, and soil temperature were measured continuously within the plantation from 4 July 2007 to 14 November 2007. Two sites were selected for monitoring. Site 1 (grid ref: 2957366 6279868) was selected as it contained *P. radiata* planted in 1983, which showed crown thinning consistent with trees that had previously suffered PNB. Site 2 (grid ref: 2956823 6280830) was selected as it contained healthy trees planted in 1983 that appeared to be free from any previous crown thinning.

Plant material

Pinus radiata seedlings (GF Plus™; Olsen Seeds, PF Olsen and Company Ltd. N.Z.) were propagated in vermiculite in a controlled environment room at 22°C with a 16:8 h light/dark cycle ($140 \mu\text{mol m}^{-2} \text{s}^{-1}$). After four weeks, the seedlings were potted into 0.5 L pots containing bark-based potting mix with Osmocote Plus® controlled release fertiliser (Daltons, New Zealand). Experiments were carried out on seedlings three months after germination in a controlled environment room.

The effect of soil temperature on needle water relations

Twelve-week-old radiata pine seedlings were grown for 82 days at a soil temperature of 4, 9, 17 or 21°C. After 82 days, soil temperature was changed to 21°C for all plants. After 10 days at 21°C, root and shoot water potential, and needle water content were then measured. The seedlings were kept well watered throughout the treatment periods.

The effect of relative humidity on needle water relations

Twelve-week-old radiata pine seedlings were grown at a relative humidity of 99, 65, or 58% for 82 days. All the plants were then transferred to a relative humidity of 52% for 10 days to investigate the ability of needles to control water uptake under changing humidity conditions. Root and shoot water potential and needle water content were measured before and after the transfer to 52% relative humidity. The seedlings were kept well watered throughout the treatment periods.

Root and needle water potentials

Needle water potentials were measured at midday using a pressure chamber (Scholander et al. 1965), on apparently healthy-looking needles from approximately mid height on the seedling. Root water potentials were estimated from needle water potentials measured following a 24-h dark period, assuming equilibrium of water throughout the seedling over 24 h. Eight seedlings were sampled for each treatment.

Needle Water content

Water content was calculated as percentage weight loss following drying at 40°C for 48 h from sixteen seedlings per treatment.

Data analysis

All data were subjected to ANOVA using Genstat, 9th edition [(PC/Windows XP) Copyright 2006, Lawes Agricultural Trust (Rothamsted Experimental Station)]. The angular transformation was used to transform the percentage water content of the needles before analysis, to meet the assumptions of ANOVA.

Results

Climate data

Soil and air temperature and air humidity were monitored at two sites throughout the 2007 winter. Average daily soil temperature was quite stable from July until the end of September at both sites, being slightly higher at site 1 than site 2 during this period. Soil temperature increased at both sites from the end of September (Figure 1). Maximum daily relative humidity (RH) was fairly constant at both sites from July to October, ranging from approximately 65 to 95% at site 1 and 70 to 100% at site 2 (Figure 2). Daily minimum humidity from July until the end of September ranged from 40 to 95% at site 1, and 40 to 100% at site 2. At the end of September, both sites saw a large reduction in daily minimum humidity over a two-week period, humidity falling 75-80% during this time. In addition, this period at the end of September and the beginning of October saw the largest differences in daily minimum and maximum humidity, ranging from 20% to 85% RH over 24 hours on 7 October at site 2. Humidity at site 1 ranged from 20 to 84% over the same period (Figure 2). Daily maximum and minimum air temperatures (Figure 3) were very similar at both sites 1 and 2. The lowest recorded temperature at both sites was 3°C and the highest was 20°C. There was a general increase in maximum daily temperature from July through to October. There were no cases of PNB observed at either site in 2007.

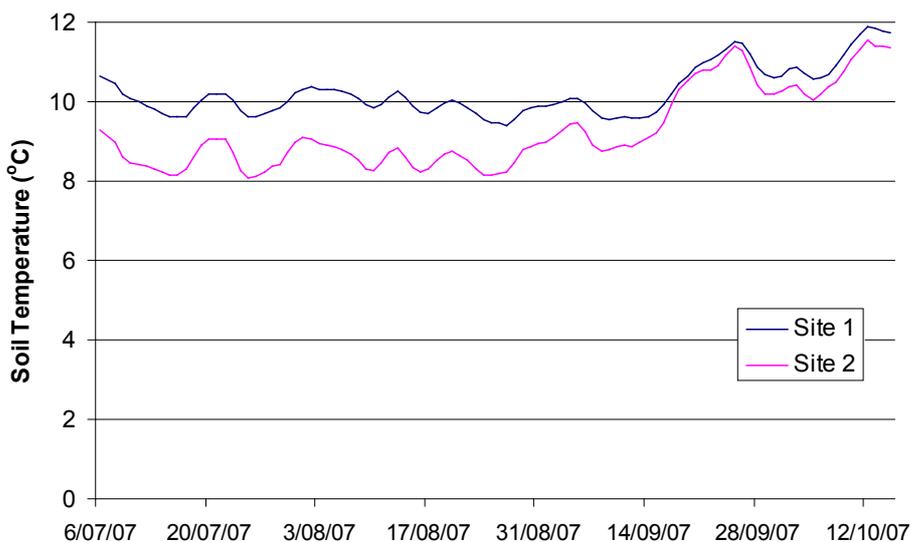
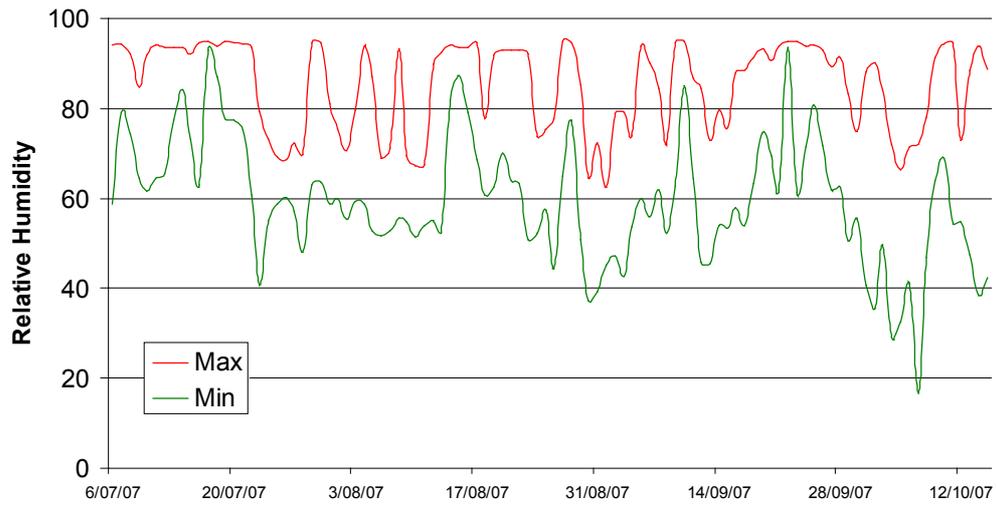


Figure 1. Average daily soil temperature measured at *Pinus radiata* trial sites 1 and 2 between 6 July 2007 and 15 October 2007.

A)



B)

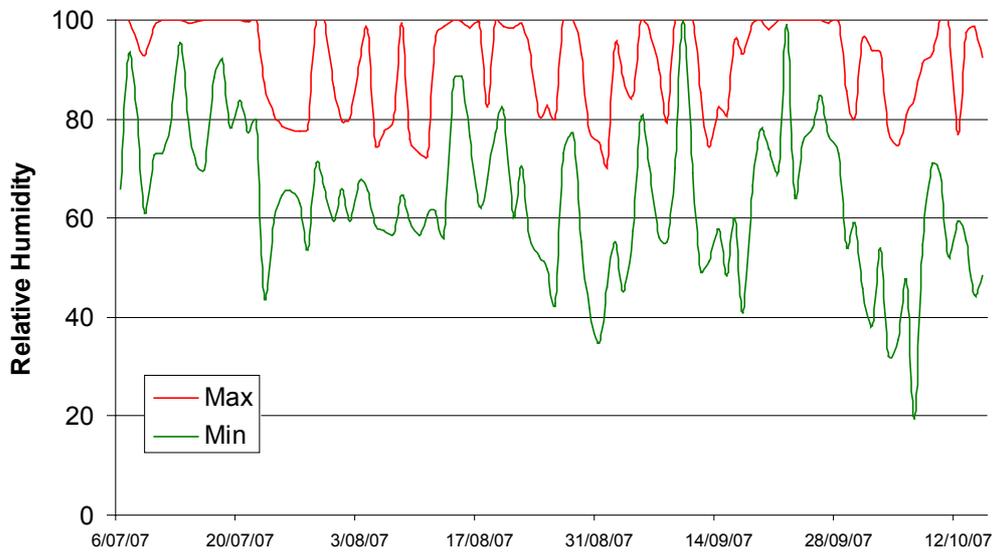


Figure 2. Daily maximum and minimum relative humidity (%) at *Pinus radiata* trial sites A) 1 and B) 2, recorded between 6 July 2007 and 15 October 2007.

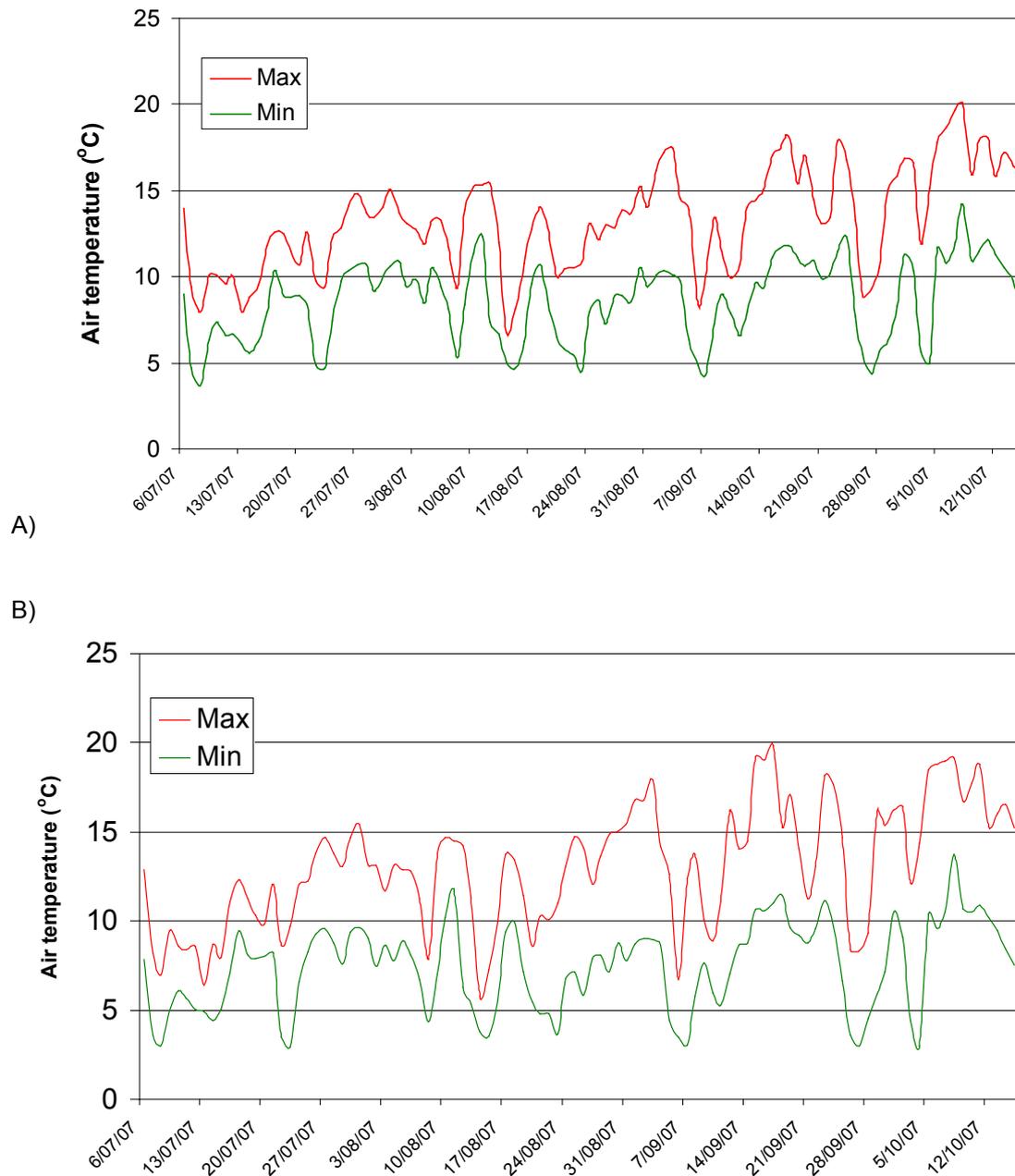


Figure 3. Daily maximum and minimum air temperature at *Pinus radiata* trial sites A) 1 and B) 2, recorded between 6 July 2007 and 15 October 2007.

The effect of RH and root temperature on needle water relations

Growing seedlings for 82 days at 99, 65 or 58% RH had no effect upon root or needle water potential, needle water content or growth rates in any of the treatments ($P>0.05$). However, following a further 10 days of growth at 52% relative humidity, the plants previously grown at 99% RH showed reduced water content and reduced needle water potential compared with both pre RH reduction and the other two treatments (Figure 4; $P<0.05$). The reduction in humidity to 52% had no effect on needle water potential or needle water content in the seedlings grown initially at 65 or 58% RH (Figure 4; $P>0.05$). Approximately 40% of the seedlings transferred from 99% to 52% RH died following the reduction in relative humidity. Of those which survived, many had needles that were drooping and turning chlorotic (Figure 5). A reduction in humidity did not affect the apparent health of the seedlings grown initially at 65 or 58% RH; all these plants survived the reduction in RH and remained healthy looking (Figure 5). There was no difference in root water potential between any of the treatments either before or after the

reduction to 52% RH (Figure 6; $P>0.05$). Root temperature had no effect on root or needle water potential or needle water content, either pre- or post-root warming to 21°C ($P>0.05$; data not shown).

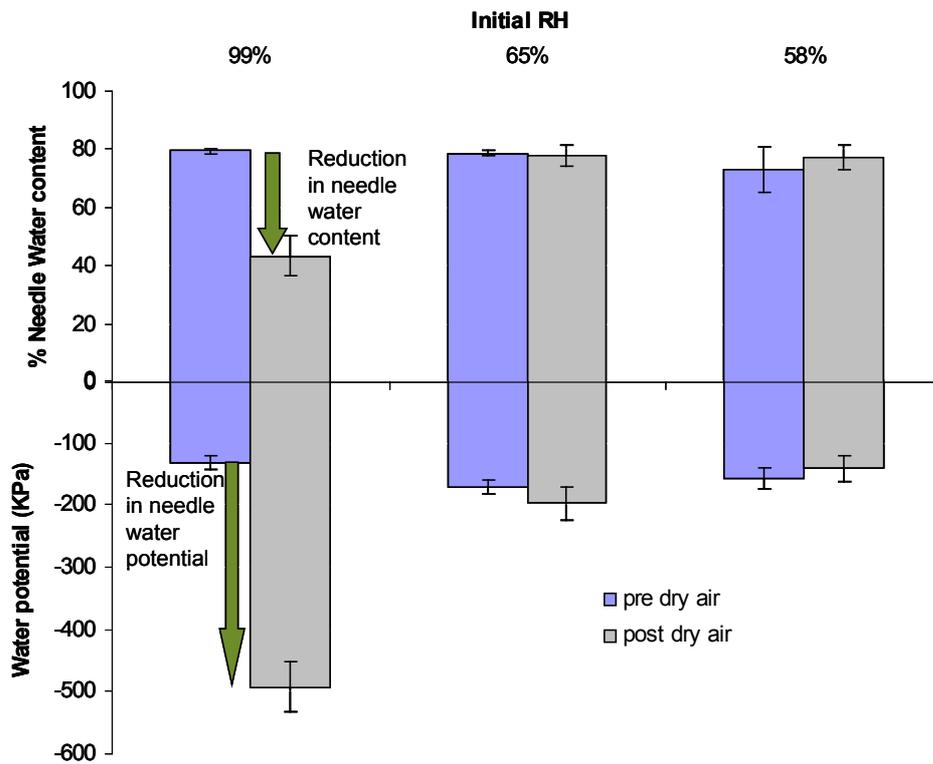


Figure 4. Needle water potential and percent needle water content of radiata pine seedlings grown for approximately 12 weeks at 99, 65 or 58% relative humidity (RH) then transferred to 52% RH for a further 10 days. Measurements were carried out before the transfer to 52% RH (pre dry air) and after 10 days at 52% RH (post dry air). Standard errors shown, $n=16$ for percent needle water content and $n=8$ for water potential measurements.

A) 99% to 52% RH



B) 65% to 52% RH



C) 58% to 52% RH



Figure 5. Examples of *Pinus radiata* seedlings grown for 82 days at A) 99% relative humidity (RH), B) 65% RH, or C) 58% RH and then transferred to 52% RH for 10 days.

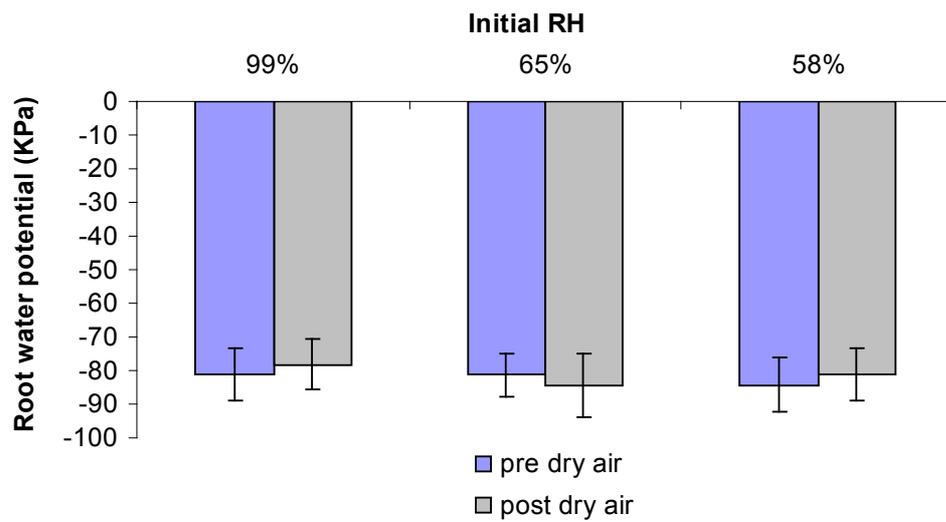


Figure 6. Root water potential of radiata pine seedlings grown for approximately 12 weeks at 99, 65 or 58% relative humidity (RH) then transferred to 52% RH for a further 10 days. Measurements were carried out before the transfer to 52% RH (pre dry air) and after 10 days at 52% RH (post dry air). Standard errors shown, n=8.

Discussion

Symptoms of physiological needle blight occur within New Zealand radiata pine plantations in late winter to early spring and may be associated with high winter water availability and high canopy humidity (Bulman 2002; Dick & Bulman 2005). Furthermore, continuous misting of trees over an eight-week period resulted in needle death even in the presence of a broad-spectrum fungicide (Dick & Bulman 2004). This project has thus focused upon the relationship between local climatic conditions and tree water relations throughout winter and early spring.

The monitoring of air and soil temperature and relative humidity within a radiata pine plantation in Gisborne highlighted a period at the end of September and beginning of October where changes occurred in both soil temperature and relative humidity. As root temperature can affect the hydraulic conductance of the roots (Norisada et al. 2005) and changes in humidity levels of the air surrounding the needles directly affect the needle-to-air vapour pressure difference, which drives water loss from the needle, the effects of root temperature and relative humidity on needle water relations were selected for further investigation using potted trials.

When grown at high RH (99%), all plants survived the initial three-month treatment period, but when transferred to a lower relative humidity, 30-40% of the plants which had been initially grown at high RH showed complete needle death. Of the remaining plants which survived the transfer from high to low RH, the percentage water content of the needles was reduced by approximately 40% and the needle water potential by 150% compared with that of the other plants which had initially been grown at lower RH. An increase in needle water loss had the effect of increasing the water potential difference between the roots and needles (a measure of the 'pull' of water from roots to needles), from approximately 10 KPa in the plants maintained at low RH to almost 400 KPa in plants transferred from high to low RH.

The large reduction in water potential difference between the roots and needles of the high RH-treated plants (and thus greater pull of water from roots to needles) is thought to be in response to a high needle water loss as the needles struggle to adapt to their new drier environment.

It is proposed that the plants grown for three months at high humidity adapt their water relations according to their environment. Evaporative water loss from the needles is low at high RH, so excessive water loss from the needle is not an issue in these plants at this time. However, given a sudden reduction in RH, which can occur in spring, the plant is unable to adapt quickly enough and water is pulled from the needles into the atmosphere. If the force exerted upon the water is too great, it may cause the water vessels within the plant to collapse or cavitate, thereby reducing water flow to the desiccating needles. Measurements of hydraulic conductivity through the branches of radiata pine have shown that the point of attachment of the needle to the branch has one of the highest points of resistance to water flow (Gould 2006). It is this junction which is most vulnerable to cavitations and hence it is only the needle not the rest of the branch which experiences reduced water flow.

These results are consistent with the hypothesis that sudden reductions in relative humidity, (such as those that may exist in the radiata plantations in late winter to early spring), can dramatically affect the

regulation of needle water content. The most likely cause of needle death in the seedlings used in these experiments is the loss of hydraulic conductivity from the roots to the needles.

Conclusion

These results so far are consistent with the hypothesis that control of needle water content in radiata pine can be lost following changes in relative humidity, such as those that may exist in the radiata pine plantations in late winter to early spring.

Recommendations

Further monitoring of PNB susceptibility in the field is required to build a complete picture of the relationship between forest climatic conditions and tree water status.

Needle water content should be measured in conjunction with needle and root water potentials of PNB-affected trees, as this would tie this laboratory-based study and previous field studies on radiata pine water relations together.

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Appendix

Physiological Needle Blight – an update

Nick Gould (The New Zealand Institute For Plant & Food Research Limited), Lindsay Bulman and Margaret Dick (Scion)

What is it?

Physiological needle blight (or PNB) occurs within New Zealand radiata pine plantations. Symptoms appear on trees from late winter to early spring where needles either fade to a pale green then brown colour, or develop brown, yellowish or khaki bands before entirely changing colour. Despite drooping, the needles generally remain quite strongly attached to the tree; indeed, the brown needles can be seen still attached to the tree when the spring flush of new unaffected green needles are emerging from the end of the branches. Although PNB can occur in trees as young as 10 years old, it is more often found in trees of 15 years and older. Symptoms first appear on the lower part of the tree but may spread to the whole crown. It is often not identified until a large area is affected, creating a quite striking change in colour across a hillside, down a gully or along a ridge top (Figure 1). The dead needles fall by mid summer, leaving the current season crop of needles unaffected. Sequential severe occurrences in a single stand are not common, but if this occurs the crown may remain thin for a number of years.



Figure 1. Aerial view of a radiata pine plantation showing Physiological needle blight (PNB) symptoms.

Where is it?

Reports of PNB within New Zealand radiata pine plantations have been made since the 1970s. Occurrences have been irregular over this period, both in time and location, but monitoring by

scientists from Scion Forest Biosecurity and Protection over the past 10 years appears to show incidences are more likely in the Northland and East Cape regions, with outbreaks also reported in the West Coast, Taranaki, Auckland, and Central North Island regions.

Initial records of occurrences and severity of PNB, particularly in the early days of the 1970s and 1980s, are unclear, because early cases of PNB were confused with fungal diseases caused by *Dothistroma* or *Cyclaneusma*, or conversely cases of the fungal infections were reported as PNB. Indeed, PNB was first referred to as either ‘atypical *Cyclaneusma*’ or ‘*Strasseria*-related defoliation’ indicating the fungi thought to be important in the needle disease at the time. Subsequent tests on needle tissue over the past 25 years have shown that a wide range of fungi is found in trees with the disorder, and these vary temporally and between locations. Most are opportunist fungi colonising the already dying needle. Some are common endophytes that may be capable of some pathogenic activity when the trees are stressed by environmental conditions. The cause of PNB is now thought to be physiological. Scion have produced a leaflet highlighting the difference between *Dothistroma*, *Cyclaneusma*, PNB, and other abiotic symptoms (Figure 2). It is available from rose.obrien@scionresearch.com.



Figure 2. ‘Needle Diseases of Radiata Pine’ leaflet produced by Scion.

What causes PNB?

The precise cause of PNB is still unknown. The sporadic nature of the disease makes it extremely difficult to predict which locations, let alone which trees, will be affected, and thus pre-symptom needle physiological measurements are hard to obtain. It has been reported that PNB is associated with high winter water availability and high canopy humidity. Aerial surveys of affected areas in East Cape and Northland carried out in spring have highlighted the occurrence of the blight more commonly on ridges and gullies, and associations have been drawn with PNB incidence and higher than average rainfall and temperature during mid winter. There is also evidence to suggest that the needle blight is associated with areas of non-

porous soils. Subsequent research has thus focused upon the relationship between local climatic conditions and tree water relations throughout winter and early spring.

Experimental investigations

Initial investigations as reported in *Tree Grower* in 2005 looked at replicating the effects of prolonged winter rain and mists on needle retention. Continuous misting of trees over an eight-week period resulted in needle death even in the presence of a broad-spectrum fungicide. Furthermore, work supported by the Forest Health Collaborative indicates that PNB may be caused by a sudden and prolonged reduction in humidity at the start of spring, following an extended wet period through winter. This hypothesis was supported by recent pot trials in which plants were placed for three months in either high humidity (up to 99% relative humidity (RH)), medium ($\approx 65\%$ RH) or low ($\approx 58\%$ RH) and then switched to a RH of 52% for ten days. All plants survived the initial three-month treatment period, but when transferred to the lower humidity, 40% of the plants that had been initially grown at high RH showed complete needle death. The change in humidity had no effect on needle health in the other two treatments. The transfer from high to low humidity reduced the percentage water content of healthy needles by 40% and the needle water potential by 150% compared with the other plants that had initially been grown at either medium or low RH. An increase in needle water loss had the effect of increasing the water potential difference between the roots and needles (a measure of the ‘pull’ of water from roots to needles), from 10KPa in the plants maintained at low RH, to 400KPa in plants transferred from high to low RH (Figure 3).

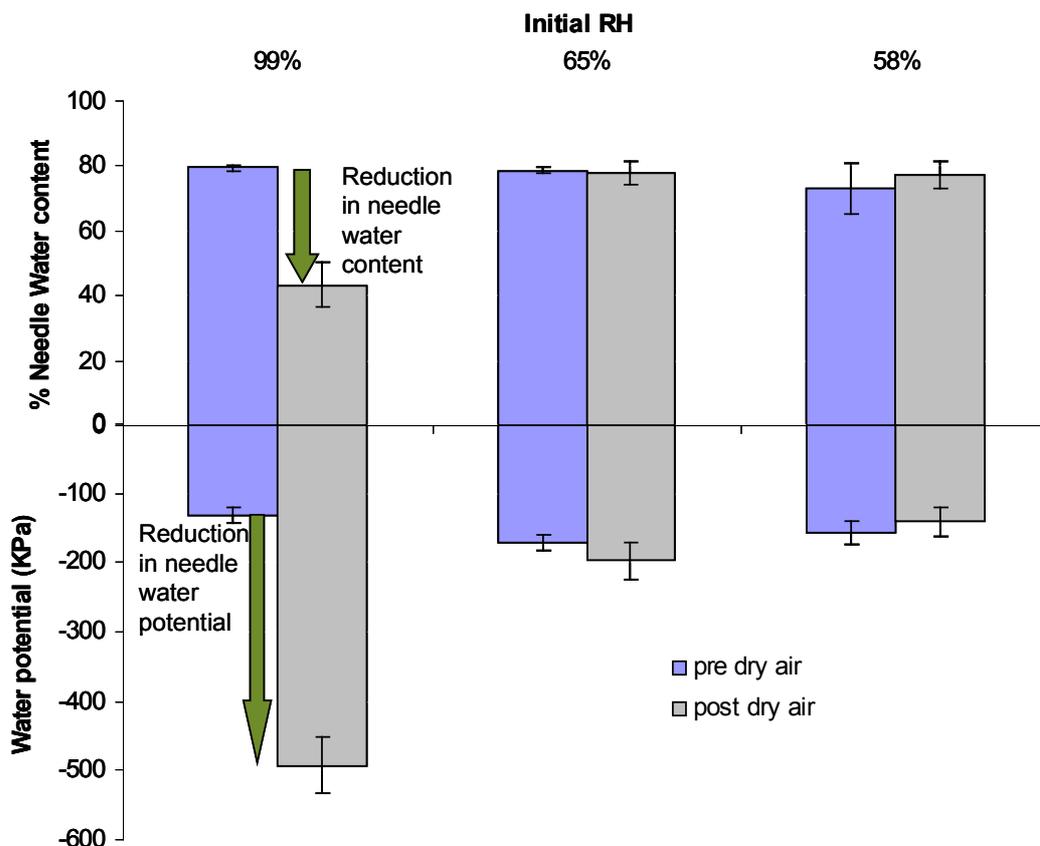


Figure 3. Needle water content and water potential of pine seedlings grown for three months at 99, 65 or 58% relative humidity (pre dry air, purple bars). The measurements were then repeated on the seedlings following a further 10 days of all seedlings being at 52% relative

humidity (post dry area, grey bars). Mean \pm standard error shown. Plants grown at 99% humidity and then transferred to 52% humidity were unable to control needle water content.

The large reduction in water potential difference between the roots and needles of the high RH-treated plants (and thus greater pull of water from roots to needles) is thought to be in response to a high needle water loss as the needles struggle to adapt to their new, drier environment. Providing the plants survived the first 10 days of lower humidity, needle water content returned to normal as the plants were able once again to control their water loss.

It is proposed that the plants grown for three months at high humidity adapt their water relations according to their environment. Evaporative water loss from the needles is low in high RH, so excessive water loss from the needle is not an issue in these plants at this time. But given a sudden reduction in RH, which can occur in spring, the plant is unable to adapt quickly enough and water is pulled from the needles into the atmosphere. If the force exerted upon the water is too great, it may cause the water vessels within the plant to collapse or cavitate, thereby reducing water flow to the desiccating needles.

Measurements of hydraulic conductivity through the branches of radiata pine have shown that the point of attachment of the needle to the branch has one of the highest points of resistance to water flow. It is this junction that is most vulnerable to cavitations, and hence it is only the needle and not the rest of the branch that experiences reduced water flow.

Why is tree age important?

PNB generally occurs in trees older than 15 years. Branches from 16-year-old trees have been shown to have higher resistance to water flow at the needle branch interface than branches from six-year-old trees. Following the argument above, this would make the older branches more vulnerable to cavitations and water vessel collapse. Indeed earlier work by Domec and Gartner (*Trees*, 2001) has shown that mature Douglas fir trees are more susceptible to cavitations than younger trees.

Should we worry about PNB?

The occurrences of PNB have been very sporadic to date. Where it does occur, despite the tree crown being rather thin, the spring flush of new needles soon establishes the green foliage of the tree. So is PNB a long-term problem about which we should be concerned?

1. Although PNB normally occurs in radiata pine stands of 15 years and over, it has occurred in younger stands and been mistaken for *Dothistroma* needle blight and sprayed unnecessarily.
2. Whole stands can be affected and give the appearance of large-scale tree death.
3. Where it does occur, the crown remains thin for a couple of years following needle death. Whilst one episode of PNB is unlikely to affect tree growth rate markedly, more than one occurrence of PNB in the same stand within the space of a few years will reduce the productivity of the stand.

Identification of PNB by foresters within their plantations is difficult because it is easily confused with other pine diseases. The ability to monitor severity of the blight over time will enable a true assessment of the scale of the problem. The 'Needle Diseases of Radiata Pine' leaflet is available to assist with disease identification.

Summary

PNB appears to be a physiological disorder, related to wet winter conditions. Experiments have shown that large reductions in relative humidity following sustained periods of high humidity can result in the loss of needle water content control, leading to desiccation and ultimately death of radiata pine needles. The desiccation may be enhanced by the breakdown of the water column feeding the needles. Older trees may be more vulnerable to such an effect than younger trees, because of higher resistances in the flow of water to the needles. More information is required to build up a true picture of its frequency and severity within New Zealand, so any possible occurrences should be reported to Scion.

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