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Lindsay Bulman, Rebecca Ganley and Margaret Dick

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NEEDLE DISEASES OF RADIATA PINE IN NEW ZEALAND

A review

Lindsay Bulman, Rebecca Ganley, Margaret Dick

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Summary - Dothistroma needle blight

Distribution

Dothistroma is thought to be native to central America and is widespread throughout the world. It can tolerate a very wide geographical and climatic range. Outbreaks have been observed in various parts of the world since the 1950s.

Biochemistry and Genetic Diversity

Dothistromin production of isolates collected from different parts of the world is highly variable. Isolates previously known as *D. pini* have recently been separated into two species, *D. pini* and *D. septosporum*, based on genetic variation. The New Zealand isolates tested so far belong to the species *D. septosporum*. A variety of molecular techniques are available to identify both *D. pini* and *D. septosporum.*

Infection process and epidemiology

Dothistroma needle blight is characterised by red bands on the needles. It causes defoliation of the tree and in rare extreme cases, death. In general, infections of *Dothistroma* are caused by asexual spores (conidia) of the fungus. Conidia germinate on the needle surface when temperature exceeds about 7^{o}C and the fungus then penetrates through the stomata to the mesophyll, killing the tissue. It subsequently sporulates by producing a fruit body that emerges through the needle surface. Fruit body development may be initiated by a build-up of dothistromin to levels where it is toxic to the fungus. Rain-splash is the primary mode of dispersal of conidia and length of survival and germination is dependent on the moisture and temperature the spores are exposed to. Conidia are also dispersed in mist and cloud and this is the mode for long distance dispersal. Optimum temperatures, prolonged needle wetness, high spore densities, and adequate light will result in high disease incidence and severity. In field conditions the time elapsed between germination of conidia landing on the needle surface and production of conidia-bearing fruit bodies can be as short as 4-5 weeks. Severe disease may be expected when rainfall is frequent and daily temperatures exceed 16-18^{o}C. The fungus can assume a latent phase and readily survive periods where the foliage is kept dry for 2 months or more. When foliage is remoistened fruit bodies may appear within 10 days. *Dothistroma* can be cultured in the laboratory but artificial inoculations with this fungus can be difficult. Host species vary markedly in susceptibility and there are conflicting reports on the susceptibility of some hosts. Much of the variation could be attributable to assessments being carried out under different conditions and provenance differences.

Dothistromin

The Dothistroma needle blight pathogen produces a toxin, dothistromin, which has been implicated in the development of Dothistroma needle blight in pine trees, although its role in disease development is unclear. There is evidence that host response, in the form of benzoic acid production, is responsible for lesion development.

Effect on host, social and environmental impact

Mortality associated with Dothistroma needle blight has been reported since the 1950s. Outbreaks of the disease during the early 2000s in north-western British Columbia were related to an increase in summer rainfall and have caused death of mature lodgepole pines. In New Zealand, mortality caused by Dothistroma needle blight is rare. Effect on growth is well documented and consistent. Height growth is less affected than diameter and volume growth. Growth losses are not measurable until over 25% of the crown is affected. There is a lag between the onset of infection and growth loss. In New Zealand, loss is in the order of $24 million per annum. Dothistromin is to be highly toxic and a mutagen but its health risk to forestry workers has been found to be low. There is no evidence to suggest that spraying is causing significant damage to the environment and no adverse environmental effects have been seen in the 40 years since spraying commenced in New Zealand.

Chemical Control

Spray trials showed that aerial application of fungicides provides good control of Dothistroma needle blight. Application rates have been reduced from 50 litres per hectare down to 5 litres per hectare and research is in progress to determine if it is possible to reduce this to 3 litres per hectare, and increase fungicidal persistence by improving the formulation.

Silvicultural Control

Pruning can suppress disease levels for at least one season. Pruning removes infected foliage and lowers the inoculum available to initiate new infection. Thinning increases air circulation, thus reducing the leaf wetness period and slowing infection. Aged cuttings show greater disease resistance than seedlings.

Fertilisers and soil

In Australia there appeared to be a positive relationship between S deficiencies and increased disease. In New Zealand no relationship with nutrition could be determined, and there was no reduction in disease after application with a broad spectrum fertiliser.

Resistance

Selection of resistant stock started in the mid-1960s and a *Dothistroma* resistant breed was developed in 1989. Adoption of this stock has taken place in some areas. *Dothistroma* resistance is moderately to highly heritable and gains from the *Dothistroma* resistant breed have been estimated to be in the order of 6-15%.

Age resistance is thought to be a result of the fungistatic effects of needle wax along with changes in stomatal structure and size with tree age.

Future research needs - Dothistroma needle blight

Distribution

- New research on disease distribution is of low priority and it is probably sufficient to keep a watching brief on developments. If fungal species distribution is considered important then research to determine the distribution of *Dothistroma* spp. (*D. pini*, *D. septosporum*, and others) could be carried out.

Genetic Diversity:

- Compare genetic change between archived specimens from herbaria with current isolates.
  - General patterns of genetic change
  - Changes in toxin biosynthetic genes
- Compare *D. pini* and *D. septosporum* isolates for variations in the genes involved in dothistromin production and changes in expression.
- Genetic/biochemical analyses to answer fundamental questions about variations in disease expression, i.e. is variation attributable to host and environment only.
- The impact of sexual reproduction on genetic diversity and subsequently, virulence and/or pathogenicity.
- Detection method for rapid border identification of *D. pini* and *D. septosporum*.
- Determine if *D. pini* and *D. septosporum* are sexually compatible, and if so, if this influences disease severity.

Epidemiology

- How important is dispersal of conidia by clouds – is it a biosecurity risk? How far are conidia dispersed – i.e. several hundred km or several thousand km?
- Determine if there are any variations in the infection process by *D. pini* and *D. septosporum*.
- The frequency, role and importance of endospores.
- Determine if stomata influence direction of germ tube growth.
- Inoculum levels in the field – effect of spray and silviculture on inoculum and following on from that, effect on disease level.
- Further investigate antagonism between phyllosphere fungi and *Dothistroma.*
- Compile weather data from regions where Dothistroma needle blight outbreaks have recently occurred and determine if relationships exist, or if other factors are responsible – i.e. increased planting of susceptible hosts, changes in silviculture. etc.

Effect on host

- The effect on growth is well documented. Economic impact assessments could be done every 5 years to determine trends in disease impact at the estate level.

Dothistromin, Social and Environmental Impact:

- Confirm whether dothistromin is required for pathogenicity/virulence and determine the significance of high dothistromin production with regard to disease.

- Determine the exact mode of action of dothistromin in natural lesions.
- There is quite a large variation in the dothistromin readings from samples collected by Forest Research Institute versus the Ministry of Health (all samples were analysed at Forest Research Institute). The Forest Research Institute samples have much higher levels of dothistromin. All of the skin and clothes readings were sampled by the Ministry of Health and these may be an under-estimation of what forest workers are exposed to.
- Determine whether long term exposure to dothistromin poses a health risk to forest workers and whether it contributes to the higher rate of cancer found in foresters in New Zealand (Kawachi *et al.* 2007). Clothing and skin tests were taken from 10^2 cm areas, how does this equate to the total clothing and skin exposed.
- Determine the health risk of high dothistromin producing isolates to New Zealand's forest workers and users, should other *Dothistroma* spp. be introduced.

Chemical control:

- Spray deposition needs to be tested when flying over rough surfaces in high wind.
- Actual droplet size used during spray applications needs to be determined.
- Spray formulations can be improved in order to improve persistence and reduce spray cost.
- The economics of spraying could be re-examined to allow for the effect of differing silvicultural regimes and end uses of the crop produced.

Silvicultural Control

- Economics of amending the silvicultural regime to fit with disease hazard. For instance, on high risk sites, would reduced spray costs and increased crop value from regular pruning and thinning outweigh the cost of silvicultural operations?
- Economics of planting alternative non-susceptible species on disease-prone sites.
- Assessing the relative performance of cuttings and seedlings, and aged propagated material, in disease-prone areas.

Fertilisers and soil

- There appears to be little benefit from carrying out more research on the effect of nutrition by fertiliser application on disease levels.
- Further investigation on the influence of soil types on disease incidence and severity may be warranted

Resistance

- Assessing the performance (field resistance, number of times sprayed) of the Dothistroma resistant breed when planted in large contiguous areas.
- Assessing the growth and form performance of the Dothistroma resistant breed compared with other stock when planted in large areas in locations conducive to disease development.
- Assessing the relative performance of cuttings and seedlings, and aged propagated material, in disease-prone areas.

Summary – Physiological disorders

Recognisable abiotic disorders

A variety of recognisable physiological disorders of pines exist. Salt damage is common in coastal forests of New Zealand, particularly those on the west coast. Common nutrient deficiencies in New Zealand are nitrogen, phosphorus, boron, and magnesium; with manganese, copper, zinc, potassium, iron and calcium deficiencies causing damage in localised areas. Herbicides, pollutants, and climatic effects can all lead to disease of pines.

Cryptic disorders

Cryptic foliar disorders are often caused by climatic events. Research efforts needed to determine the fundamental cause of many physiological disorders are often extensive and time consuming. "Red belt" in conifers is thought to be the result of rapid freezing and then thawing of needles. "Needle droop" of *P. resinosa* has been researched since the 1930s and poor root systems, prolonged drought, high air temperature and low RH, and relationships between soil type and water relations of trees have all been proposed as possible causes. Semi-mature needle blight of *P. strobus* in USA is also classified as a physiological needle blight of undetermined cause, although an unconfirmed study suggested fungal involvement. Water stress is another cause of physiological disease in pines. The role of non-pathogenic fungi in needle diseases has not been well investigated and in some cases is poorly understood.

Diagnosis of physiological disorders

Macroscopic response to physiological stresses can cause similar symptoms regardless of the cause. While generalisations can be made, the type of symptom caused by individual stress factors is often inconsistent.

Physiological needle blight of Pinus radiata in New Zealand

A disorder, now referred to as physiological needle blight, has been known in p. radiata plantations since the early 1980s. It results in outbreaks of severe defoliation over localised parts of plantations.

Future research needs – Physiological disorders

Recognisable abiotic disorders

- There are minimal research needs with regard to being able to readily identify the disorders described above.
- Other research needs are outside the scope of this review – for instance the economics of applying fertilizer to remedy nutrient deficiencies.

Cryptic disorders

- The role of non pathogenic fungi in PNB and other needle casts could be elucidated, but it is a complex issue and not something that can be accomplished readily and cheaply.
- Many of the other physiological disorders are a result of conditions experienced overseas, such as snow or drought, and will not occur in parts of New Zealand used for plantation forestry.

Diagnosis of physiological disorders

- None are justified.

Physiological needle blight of **Pinus radiata** *in New Zealand*

- Research to determine the cause of PNB is needed.
- Once the cause is confirmed, research into mitigation may be warranted.
- The economic impact of PNB should be determined.
- Mitigation could involve selecting resistant trees, reducing stress through silvicultural management, or planting non susceptible species.
- The influence of endophytic needle fungi should also be examined.

Summary - Cyclaneusma needle-cast

Cyclaneusma needle cast research is thoroughly reviewed in Bulman & Gadgil (2001) and it is not necessary to repeat that exercise here. Results from research on epidemiology, economic impact, and control were presented in that document, and are summarised below. Genetic resistance was not covered in Bulman & Gadgil (2001), but is discussed in Dungey *et al*. (2006). They found that narrow-sense and family-mean heritability estimates were in the range of 0.0-0.7. The majority of narrow-sense estimates were between 0.2 and 0.3, whereas the majority of family-mean estimates were between 0.6-0.7. On sites known to be susceptible to Cyclaneusma needle cast, heritabilities were low to moderate and genetic correlations between sites were moderate to high. It was concluded that there are good indications that breeding for this disease would be successful, which would significantly improve forest productivity in needle cast-prone areas.

Future Research Needs - Cyclaneusma needle-cast

- Genetic control – heritability determined from a trial set up to specifically examine resistance to Cyclaneusma needle-cast.
- Economics of delayed and selective thinning – is the increased cost from pruning every stem overcome by increased productivity?
- Disease mechanisms – what induces some trees to become susceptible while others remain unaffected by *Cyclaneusma*?

Summary – Fungal endophytes

Fungal endophytes are fungi that live within their hosts without causing disease symptoms and have been found in all plants examined. A mutualistic relationship between endophytes and the host plant has been proposed, with the host providing nutrients for the endophytes and in return the fungal endophytes providing beneficial functions, such as resistance, tolerance or enhanced growth, to the host. Identifying and characterising fungal endophytes present in conifer tissue is important for determining or inferring their ecological role within their host. However, despite the abundance of fungal endophytes that have been found, very little is known about the function of these symbionts.

Future Research Needs – Fungal endophytes

- Identification of fungal endophytes from conifer systems.
- Assessments of diversity and distribution of fungal endophytes across host ranges.
- Further understanding of how fungal endophytes are transmitted and how this relates to their role in host plants.
- Characterization of fungal endophytes and elucidation of their function(s) within their host.
- As so little is known about fungal endophytes in plantation trees the scope for future research needs is enormous.

Introduction

This review is funded by the Forest Biosecurity Research Council and written for the New Zealand forest industry. It summarises results of research in New Zealand on needle diseases of plantation pines. Overseas work is described if it has application to New Zealand forestry. The aim is to provide a reference point from which to identify future research needs. These needs are listed and summarised, with the aim of assisting development of a research strategy for foliar pathogens of *Pinus radiata*. Deliberately, future research needs are not prioritised because it was thought that industry should advise researchers on what research activities should be undertaken, rather than the reverse.

Dothistroma needle blight and Cyclaneusma needle-cast are the most economically important foliar diseases of pines in New Zealand. Outbreaks of Physiological needle blight have been recorded since the 1980s but its economic significance so far is likely to be small because consecutive annual outbreaks are rare. A considerable amount of research has been done in New Zealand over the last forty years on these diseases. The work on Cyclaneusma has been described in Forest Research Bulletin 222, but some of the Dothistroma work is not well known by industry and has been written up in unpublished reports that are difficult to access. For those reasons, this review focuses on Dothistroma needle blight. Research findings on Cyclaneusma needle cast can be readily accessed in Bulletin 222. Only gaps in knowledge of Cyclaneusma needle cast relevant to the New Zealand forestry sector will be discussed here, along with a brief summary of Bulletin 222.

A section on fungal endophytes has been included because although they are not pathogenic, they may play a role reducing in foliar disease on pines.

Acknowledgment

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Dothistroma Needle Blight

Lindsay Bulman and Rebecca Ganley

Nomenclature

The first known reference to the fungus now under the genus *Dothistroma* is made in Doroguine (1911) from Russia on *Pinus mugo*, as *Cytosporina septospora*. Morelet (1968) and Gremmen (1968), apparently independently, recognised the similarity between the Russian fungus and the fungus causing red band disease in USA and East Africa. Morelet requested type material from the Leningrad herbarium to be told the initial specimen was lost and the only available specimen was from material collected in 1914 from *P. sylvestris*. He concluded that the Russian fungus and the red band fungus from North America were identical and suggested the 1914 collection be designated the neotype (Morelet 1969). Gremmen (1968) reached the same conclusions when comparing Doroguine's description with a collection of material from Romania. Morelet (1969) proposed *Dothistroma septospora* (reclassified *septosporum*) in view of the combination of *Dothistroma pini* and *Cytosporina septospora.* Some authors regarded *Dothistroma septosporum* (Doroguine) Morelet as having priority over *Dothistroma pini* Hulbary.

The fungus was also found in herbarium material collected in USA in 1914 (Thyr & Shaw 1964). The binomial, *Dothistroma pini*, published by Hulbary (1941) was based on material collected in Illinois, USA. Until recently, this has been generally accepted by forest pathologists as the correct name for the imperfect (or conidial) stage of the needle-blight fungus.

Barnes *et al.* (2004) investigated the phylogenetic relationships between Dothistroma isolates from different countries. DNA from portions of the nuclear ribosomal internal transcribed spacer (ITS), -tubulin and elongation factor 1- genes were sequenced and analysed for isolates from 13 different countries representing five continents. Results showed that isolates of the pathogen encompass two divergent lineages representing distinct phylogenetic species. One phylogenetic species (proposed to be *D. septosporum*) was found worldwide, while the other (*D. pini*), was found only the North-Central U.S.A., although the geographical distribution from where samples were tested was limited. Interestingly, Thyr & Shaw (1964), well before the advent of molecular techniques, proposed two varieties of *D. pini* based on morphology of collections from western and central USA - *D. pini* var. *linearis* for the western USA, and var. *pini* for the central USA. Since then, Groenewald *et al*. (2007) have recorded *D. pini* in Ukraine and suggest that the distribution of *D. pini* is much wider than was believed at the time of Barnes *et al*. (2004).

The sexual stage of the fungus (*Mycosphaerella pini* Rostrup ex Monk) is not found in New Zealand.

For simplicity's sake, and because so much of the older literature referred to *D. pini*, we have not followed the phylogenetic lineages proposed by Barnes *et al.* (2004) in this review. Thus *D. pini* and *D. septosporum* are not distinguished here and we use *D. pini sensu lato*. This approach is not intended to convey disagreement with Barnes *et al.* (2004), it is used to avoid confusion.

Host susceptibility

Many host lists have been published over the years (Gilmour 1967d; Peterson 1967; Ito 1972; Osorio & Rack 1980; Ivory (1994), Brown *et al*. 2003; Bulman 2004; Bednarova 2006) and some have attempted susceptibility classification. It is unclear whether classification was based on experimental work or observation, but Gilmour & Noorderhaven (1969) published a list on degree of resistance that was based on a trial after three years' assessments (Table 1). In nearly all species 95-100% of test trees had some degree of infection. A five-year-old provenance trial of *P. contorta* in which 19 provenances were represented showed considerable variation in susceptibility. Susceptibility was found to increase with increase in the altitude from which the provenance originated. Some from the coastal region were completely uninfected, whereas one originating at 8,000 ft was 95% defoliated.

Table 1 – Species susceptibility based on a 3-year field trial in New Zealand

Degree of susceptibility	Species
Very high-high	*P. attenuata*, *P. radiata* (six provenances and three clones), *P. nigra* subsp. *laricio, P. ponderosa*, *P. muricata*, *P. pinaster*
Low	*P. contorta, P. elliottii, P. taeda, P. strobus, P. patula, P. lambertiana, P. monticola*
Very low	*P. arizonica, P. hartwegii, P. michoacana, P. pseudostrobus, P. ayacahuite, P. montezumae*

A broader list of host susceptibility is given in Bulman *et al*. (2004). That list is reproduced below.

Very Highly Susceptible
P. attenuata

Highly Susceptible (at all ages)
P. nigra subsp. *laricio, P. ponderosa* in the central North Island, *P. jeffreyi.*

Highly Susceptible (but exhibit a high degree of resistance with age) *P. radiata*-variable resistance after 15 years of age, depending on climate.

Moderately Susceptible
P. pinaster, P. canariensis, P. lambertiana
P. muricata (blue strain) variable, but usually more resistant than P. *radiata,* exhibits resistance with age probably earlier than *P. radiata.*

Slightly Susceptible
P. contorta, P. elliottii, P. hartwegii, P. monticola, P. nigra subsp. *nigra.*

Slightly Susceptible (usually infected only when growing near other highly infected species) *Larix decidua, Picea omorika, Picea sitchensis, Pseudotsuga menziesii.*

Very Slightly Susceptible
P. ayacahuite, P. coulteri, P. michoacana, P. montezumae, P. patula, P. pseudostrobus, P. sabiniana, P. serotina, P. strobus, P. sylvestris, P. taeda, P. torreyana.

Bednarova *et al*. (2006) provide a comprehensive host list for Dothistroma needle blight, including new host records on *P. aristata*, *P. leucodermis, P. rigida*, *P. rotundata, P. heldreichii* and *P. cembra* L. var. *sibirica*, along with *Picea pungens*, *Picea abies*, and *Picea schrenkiana*. The paper presents a total of 78 hosts. Following on from that, Groenewald *et al*. (2007) list *P. palassiana*; Lang & Karadzic (1987) list *P. cembra* and *P. koraiensis*; Kirisits & Cech (2006) record *P. mugo* subsp. *uncinata* as a host.

The host range for *Dothistroma* is certain to widen, as searches on uncommon hosts and in new locations have intensified during the mid 2000s.

Species susceptibility is difficult to determine as host is only one part of the disease triangle, with environment and pathogen forming the others. Therefore, a host that appears to be susceptible in one part of the world may appear to be only mildly susceptible or not susceptible in another part of the world where inoculum density and climate differ. For instance, Ito *et al.* 1972 lists *P. caribaea* as susceptible as *P. jeffereyi*, *P. ponderosa* and *P. contorta* when testing 2-year-old seedlings in Japan. Ford (1982) in Costa Rica recorded Dothistroma needle blight on several pines, particularly *Pinus caribaea* var. *hondurensis*. Ford noted that the most severely affected plantations appeared to be growing above the normal altitudinal range of *P. caribaea*, elsewhere *P. caribaea* was not particularly susceptible.

In order to adequately determine species susceptibility relative to geographic location, a series of provenance trials of same aged material would have to be replicated in different locations worldwide.

Distribution

Overseas Distribution of Dothistroma

Dothistroma has a worldwide distribution. It is present in most areas where pines grow and Fig. 1 provides an indication of the distribution. *Dothistroma* has been recorded in Africa; Europe; North America, central and South America; Australasia; and Asia. It exists in locations with widely differing climates, for instance Jamaica (Foster 1982), inland British Columbia, inner Mongolia, and Hawaii. It is thought to be native to the cloud forests in central America (Costa Rica, Honduras, Guatemala, Nicaragua) (Evans 1984), although Ivory (1994) suggested that *Dothistroma* was indigenous to pine forests in Nepal, and may be endemic in the Himalayas.

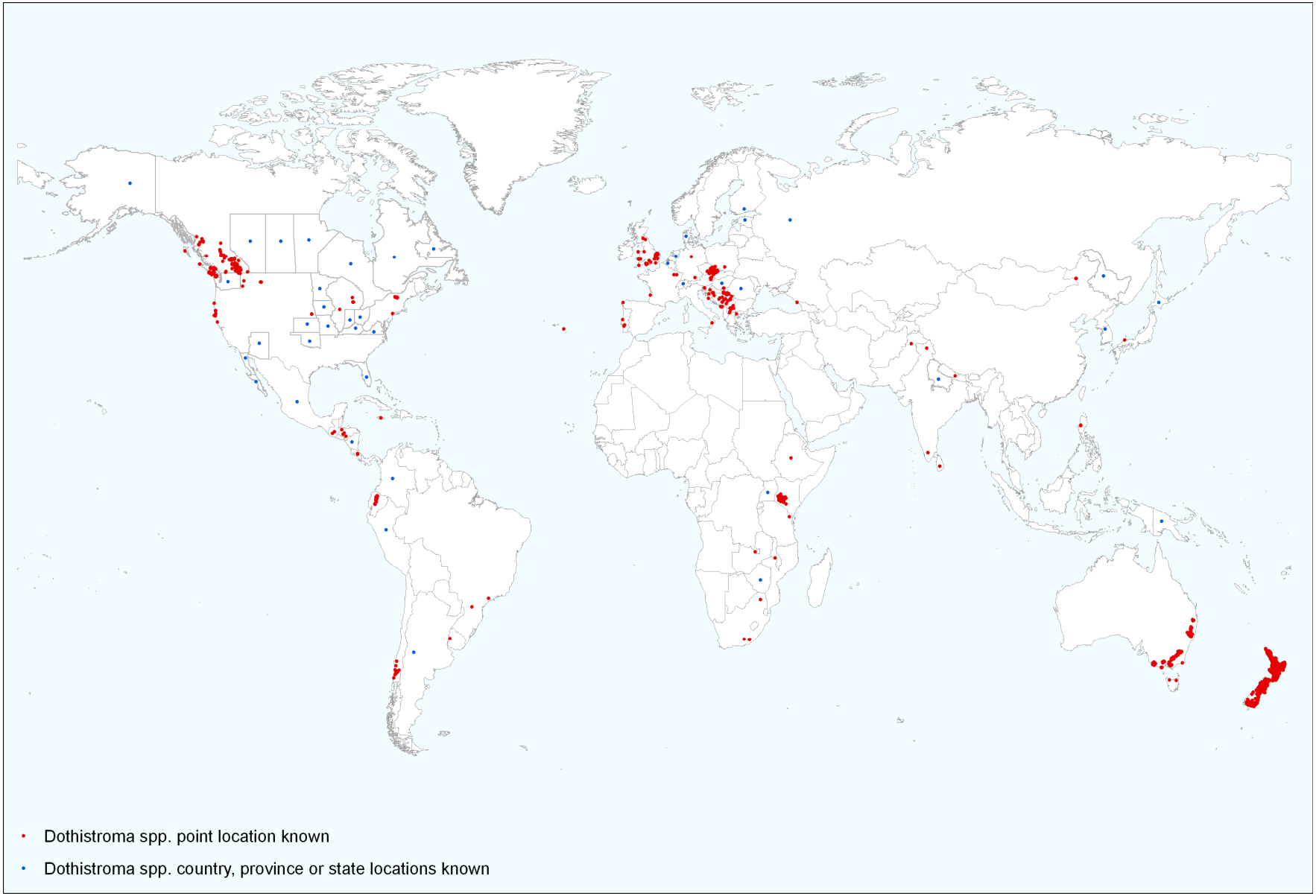

Fig. 1 – Locations of *Dothistroma* spp on pines

In Canada, Dothistroma needle blight was first noticed on Vancouver Island in 1963, where several species of exotic pines were seriously affected. The fungus was positively identified in 1964. Subsequent surveys revealed that the fungus was widespread throughout British Columbia on three native pines (*P. contorta, P. monticola, and P. ponderosa*). Dothistroma needle blight has been recorded in British Columbia, Saskatchewan, and Newfoundland (Peterson 1967b), Alberta (Reid *et al.*, 1999), Ontario and Quebec (Myren 1994).

Dothistroma pini was first described in USA in 1941. The description was based on material from *Pinus nigra* var. *austriaca* (=*P. nigra* subsp. *nigra*) in northern Illinois, but records of the fungus were also presented from Iowa, Ohio, and Oklahoma, the then known host species being *P. nigra* var. *austriaca* and *P. nigra* var. *calabrica* (=*P. nigra* subsp. *laricio*), *P. flexilis*, and *P. resinosa* (Hulbary 1941). The fungus is widespread in the USA, including Alaska, Washington, Oregon, California, Arizona, and many eastern and central states (Peterson 1967b). It has been recorded from Mexico (Gibson 1979).

In central America, Dothistroma needle blight has been recorded in Costa Rica, Ecuador, Honduras, Guatemala, and Nicaragua (Evans 1984). The first confirmed report in Chile was made in 1965, although the fungus was probably present on material collected in 1962 (Dubin 1965), followed by Uruguay in 1967 (Peterson 1969) and Brazil in 1968 (Figueiredo *et al* 1969). It has been reported from Argentina (Fresa 1968), Columbia (Gibson 1980), Peru (Ivory 1994), and Bolivia (1995 CABI Herb. IMI record No IMI 367865).

In Europe the fungus is widespread. *Dothistroma pini* is thought to have been first recorded on *Pinus mugo* in Russia in 1911 (as *Cytosporina septospora*, Doroguine 1911). Murray (1967) reported records on several pines in southern England (first record 1954), on *P. nigra* in Yugoslavia (1955), and on *P. pithyusa*, *P. pinaster* and *P. nigra* in Georgia (1964), with the most serious damage being in Georgia. It was first identified in Denmark on *P. sylvestris* (Munk 1957), France in 1967 (Morelet 1967), and in Poland in 1990 on *P. nigra* (Kowalski & Jankowiak 1998). Dothistroma needle blight has been recorded in Austria (Kirisits & Cech 2006), Belgium (Anon, 2008), Bulgaria (Zlatanov 1977), Croatia (Vidakovic *et al*. 1986), Czech Republic (Jankovsky *et al*. 2004), Estonia (Hanso & Drenkhan 2008), Hungary (Koltay 2001), Italy (Magnani 1977), Finland (J. Hantula, Finnish Forest Research Institute, pers. comm. 2008), Germany (Butin & Richter 1983), Greece (Evans 1984), Netherlands (Anon 2007), Portugal and Azores (Neves *et al*. 1986), Romania (Gremmen 1968), Russia (Doroguin 1911), Serbia (Karadžić 1989), Slovenia (Macek 1975), Slovakia (Kunca & Foffová 2000), Spain (Ana Magan 1975), Switzerland (CABI/EPPO 1990), and Ukraine (Groenewald *et al*. 2007).

In Africa, re-examination of herbarium material showed *Dothistroma* was present in Southern Rhodesia (Zimbabwe) as early as 1943 (Gibson *et al*. 1964). The first observation of disease was from Tanzania in 1957, and Dothistroma blight appeared in Kenya in late 1960 (Gibson *et al*. 1964). It has been recorded in Uganda, South Africa, Kenya, Tanzania, Ethiopia, Malawi, Zambia, Zimbabwe, and Swaziland (Ivory 1994).

The fungus is also known from parts of Asia and Oceania. Records include Japan (Ito *et al*. 1972); China (Wang DaoJun *et al*. 1998); North and South Korea (CABI/EPPO 1990); Pakistan (Zakaullah *et al*. 1987); India (Bakshi & Singh 1968); Nepal (Ivory 1970), Bhutan (Barnes *et al*. 2008), (Philippines and Sri Lanka (Ivory 1994); Brunei (Peregrine 1970), and Hawaii (Gardner & Hodges 1988). The first Australian report of Dothistroma needle blight was from the Barrington Tops State forest near Gloucester, NSW, in Nov. 1975. The disease was observed subsequently at 11 other sites

in NSW and ACT. With one exception all infections were on *Pinus radiata* (Edwards & Walker 1978). Dothistroma needle blight is now also present in Queensland, Victoria, and Tasmania.

In Africa, Chile, and New Zealand, there was a considerable lag between establishment of the fungus and its formal identification (Gibson 1974).

Distribution of Dothistroma in New Zealand

Dothistroma needle blight was first observed in New Zealand in 1962 as a chronic necrotic condition in young *Pinus radiata* plantations and in a trial plot of a *P. attenuata* x *P. radiata* hybrid planted in 1952. The location was in the Access Rd area at Lichfield which is near Tokoroa (Gilmour 1965a). At that time there was a brief but severe outbreak of Cyclaneusma needle-cast and it was only after that had subsided that the chronic condition became apparent. The causal agent was identified in 1964 by comparison with specimen material obtained from Kenya (Gilmour 1967a).

By the late 1960s *Dothistroma pini* had spread through most of the North Island. It was first discovered in Nelson in 1966, and had spread to Southland by the late 1970s. *Dothistroma pini* was first recorded in Canterbury in the early 1980s, and Otago Lakes and Mackenzie Country were the last regions to become infected in the late 1990s. It is now present in New Zealand wherever suitable hosts are grown (Fig 2).

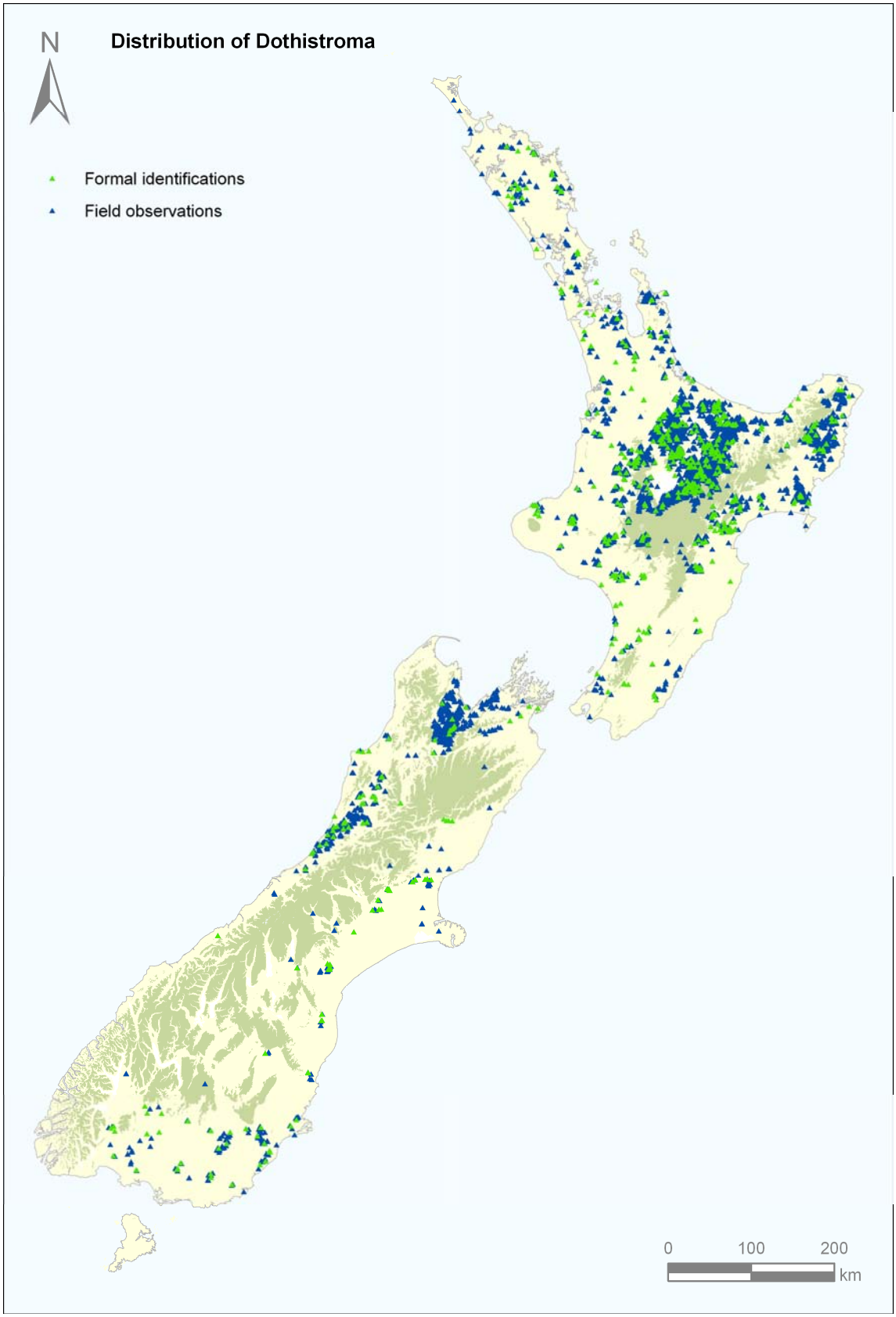

Fig. 2 – Distribution of Dothistroma in New Zealand (from Forest Health database records)

Regional risk in New Zealand

Although the fungus can now be found throughout the North Island, infection is generally low in forests north of Auckland, and in Hawke's Bay and Wairarapa. There is little or no discernible infection in coastal plantations on sand dunes. The central North Island, Waikato, and Taranaki are the most severely affected regions in the North Island. In the South Island Westland is the most severely affected region. Nelson, Otago, Southland, and Marlborough north of the Wairau River generally have light infection. There are a few scattered and localised infected areas in Canterbury. Figure 3 shows regional risk ratings, based on climate – regions with high rainfall and moderate temperatures will be at greatest risk, dry areas have the least risk. It should be noted that disease hazard may vary within the broad zones marked due to local topography or micro-site effects.

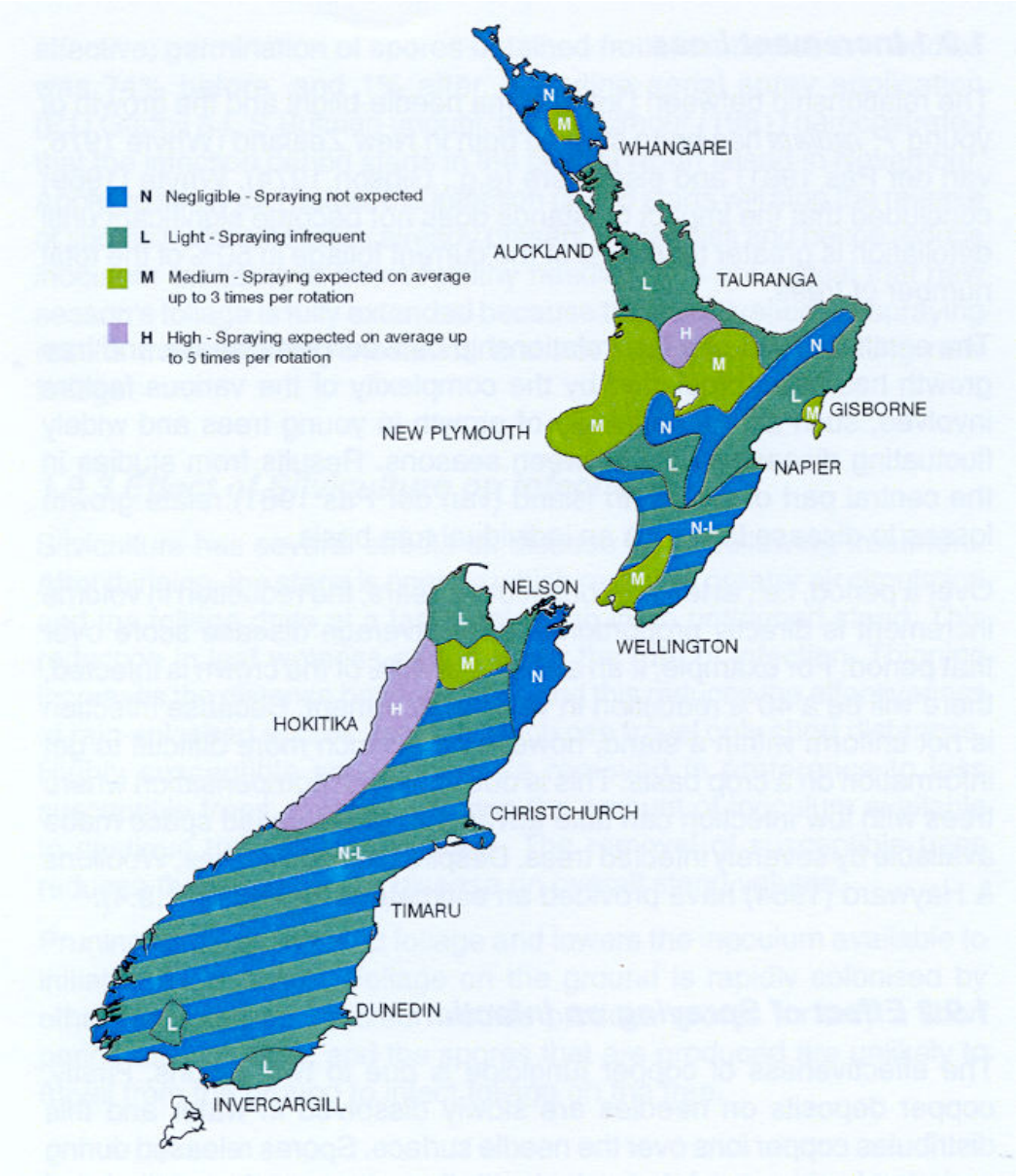

Fig 3 – Risk to susceptible *P. radiata* stands from Dothistroma needle blight in New Zealand.

Symptoms of Dothistroma needle blight

Dothistroma needle blight is characterised by well defined brick-red bands appear on green needles and persist long after the green needles have withered and become dull brown or grey. The red colour of the bands is due to the accumulation of the toxin dothistromin produced by the fungus. The red zone is distinctly marked off from the rest of the needle. Small black spots (fruiting bodies) erupt in the red infected band (Fig. 4).

The first symptoms are often found on the needles of branches near the ground. On current foliage of *P. radiata,* these symptoms usually first appear from mid- to late-summer (depending on weather) and become most obvious from about late June to early October. Some tree-to-tree variation in susceptibility to Dothistroma needle blight can usually be seen, but infection levels are generally fairly evenly distributed within a stand (Fig. 5), unless the terrain differs significantly. In this, the distribution of Dothistroma needle blight differs markedly from that of Cyclaneusma needle-cast. In stands affected by Cyclaneusma needle-cast individual diseased trees are scattered among healthy trees. Defoliation by Dothistroma can occur all the year round but is most apparent between September and October. Defoliation is least severe in early summer, as the newly flushed foliage is generally free of visible infection. On *P. nigra* and *P. ponderosa* symptoms of infection are at a maximum in November and early December. There can be some variation in this pattern in different parts of the country. The red bands are redder and the fruit bodies are often more pronounced on *P. nigra* compared with *P. radiata*.

Fig. 4 – Dothistroma symptoms on *P. radiata* needles – typical red banding and black fruit bodies

Fig. 5 – Dothistroma needle blight in a young *P. radiata* stand – note generally even distribution of disease among individual trees

Genetic diversity in Dothistroma

DNA sequencing of the ribosomal ITS region in a collection of *Dothistroma* isolates from a variety of countries showed that all isolates were identical, with the exception of some of the central USA strains which contained two nucleotide substitutions in the ITS region. Colony morphologies and growth rates were diverse, but all strains which sporulated showed a similar wide range of spore size. The morphological features examined did not support separation of the strains into the two groups shown by ITS sequences (Bradshaw *et al.* 2000). More recently, based on sequence data from portions of the nuclear ribosomal internal transcribed spacer (ITS), β-tubulin and elongation factor 1-α genes, Barnes *et al.* (2004) proposed the separation of these two strains of *Dothistroma* into two species: *D. pini* (restricted to the North-Central U.S.A.) and *D. septosporum* (worldwide distribution). Both species cause the disease known as Dothistroma needle blight or red band needle blight (Barnes *et al.* 2004) and both species produce dothistromin in culture (Bradshaw *et al.* 2000). Groenewald *et al*. (2007) recorded *D. pini* outside the North-Central U.S.A in Ukraine and they acknowledge that *D. pini* has a wider distribution than that proposed by Barnes *et al.* (2004)

Dothistroma pini and *D. septosporum* cannot be separated on morphology. A microsatellite-based DNA profiling system has been developed that can distinguish both within and between genetically diverse isolates of *D. pini* and *D. septosporum* (Ganley and Bradshaw 2001) and a PCR-restriction

fragment length polymorphism (RFLP) diagnostic protocol is available that distinguishes between *D. pini*, *D. septosporum*, and *D. rhabdoclinis* (the latter associated with *Rhabdocline pseudotsugae* on *Pseudotsuga menziesii*) (Barnes *et al.* 2004). The genetic diversity of the isolates of *Dothistroma* present in New Zealand has been investigated using randomly amplified polymorphic DNA (RAPDs). No genetic differences were detected and only one mating type has been identified (Bradshaw 2007a) suggesting that the entire population in New Zealand has been derived from a single introduction (Hirst *et al.* 1999). The strain of *Dothistroma* present in New Zealand belongs to the species now known as *D. septosporum*. The origin and global distribution of both *Dothistroma* species, based on molecular techniques, is currently being investigated by the Wingfield group at FABI in South Africa (Bradshaw 2007a).

Infection

Infection usually begins on needles on the main stem or on branches at the base of the crown and progresses upwards into the younger needles, but this depends on the season. Maximum infection occurs at the base of the tree and there is minimal attack at the top. The disease can cause a chronic state of defoliation and, in rare cases, tree mortality.

Groups of black fruit bodies (stromata) erupt through the needle epidermis on transverse red bands on infected needles. Conidia (spores produced asexually) are produced in a slimy, sticky mix on the exposed fruit bodies and are released when the needle surface becomes wet. Infected needles attached to the tree are the main source of inoculum.

The pathogen can last for two months in needle litter but has never been found to survive beyond four months, probably due to microbial competition (Gadgil 1970). However, in needles suspended above the forest floor, it has been found to survive for up to 6 months (Gadgil 1970). Diseased needles are rapidly colonised by saprophytes (Gibson *et al.* 1964) and microorganisms isolated from the phyllosphere of *P. radiata* were rarely found to be antagonistic to *Dothistroma* in culture nor have any significant effect on disease development (Ivory 1972).

Dispersal

Conidia are dispersed during rain or heavy mist (Rogerson 1951, Peterson 1967, Gibson 1972, Peterson 1973, Gibson *et al*. 1974, Peterson 1982, Rack 1986). The only report contrary to this has been the collection of conidia from *P. radiata* stands in California, which occurred during both wet and dry conditions (Cobb *et al.* 1968). In general, dispersal in the field is localised, most occurring within 50-100 m of the source, but some infection occurred at 300 m (Gibson *et al.* 1964). One report suggested dispersal will not extend beyond 1.5 m (Peterson 1973). Long distance dispersal is thought to occur by 'airborne' conidia, clouds or human transportation of infected material.

For airborne conidia, the original water droplet required for dispersal is thought to evaporate and the spore is able spread further distances in the air (Gibson *et al.* 1964). Likewise, water droplets in clouds are large enough to carry conidia and experiments have shown that spores can be carried in both clouds and heavy mists in the field (Gibson *et al.* 1964). Gilmour (1967a) presumed that during the early years of Dothistroma in New Zealand aerial dispersal of spores over distances of 100 miles or more took place. Furthermore, conidia suspended in water at 5^{o}C for 8 days will not germinate but will do so readily when returned to temperatures of 18-20^{o}C, showing the potential of this method for long range dispersal and establishment (Gibson *et al.* 1964). Insects have not been found

to be involved in the spread of *Dothistroma* conidia, although conidia have been found in bees' pollen baskets (Gibson *et al.* 1964).

In the early 1960s the disease was spread through the transport of infected seedlings. It is thought that the discovery of Dothistroma in Esk Forest in Hawke's Bay in late 1964 was due to planting contaminated seedlings imported from an infected area. In 1966, *P. contorta* seedlings infected by *Dothistroma pini* were exported from Kaingaroa nursery and planted in a shelterbelt at Mossburn in northern Southland. In April 1967, Dothistroma needle blight was detected there and the entire shelterbelt (about 2,000 trees) was burnt in June 1967. Dothistroma needle blight was not recorded in Southland again until 1979, at a different location.

Germination

The germination, survival and viability of *Dothistroma* conidia have been tested under a variety of conditions. Spore germination can occur between 5°C to 26°C but is optimal around 18-20°C (Gibson *et al.* 1964; Ivory 1967, 1972; Sheridan and Yen 1970; Gadgil 1971; Lasca *et al*. 1974), although Peterson (1967) suggested germination occurred between 12-28°C with an optimum at 24°C. Gilmour (1981) found that infection in the field occurred when temperatures were as low as 7°C, implying germination must have taken place at that temperature. A very low percentage of conidia germinate above 25°C or below 8°C (Gibson *et al.* 1964; Ivory 1967), although when returned to optimal conditions normal levels of germination can occur (Ivory 1967). Conidia germinated at 5°C but at that temperature only 1.3% germinated after 48 hours (Ivory 1972), although Sheridan and Yen (1970) found a slightly higher (about 5%) percentage of conidia germinated at that temperature. Germination is largely complete within 48 hours (Gadgil 1967; Ivory 1972). Water is required for germination of *Dothistroma* conidia (Gibson *et al.* 1964), and some studies suggested traces of nutrients in the water are needed (Gibson *et al.* 1964; Ivory 1967). Gibson *et al*. (1964) found that germination can not take place when the spores are dry, even in 100% humidity. Sheridan and Yen (1970) were able to get good (80-90%) germination at 98-100% RH. At 95%RH germination dropped to under 10% and at 76%RH a few conidia germinated but germ tubes didn't increase in length. Light is not required for germination (Gibson 1972). Laboratory studies have shown that conidia can survive up to 3 days without moisture, between 6-15 hours without water after a 15 hour water soak, and up to 4 hours without water in direct sunlight (Gibson *et al.* 1964; Peterson 1978). Spores stored in the dark at 15°C remain viable for longer periods of time than spores stored in light. However, storage can increase the length of time required for spore germination (Forest Research Institute 1971).

Needle penetration and infection

After germination, germ tubes grow over the surface of the needle and enter the needles through stomatal pores (Fig. 6), which takes approximately 3 days depending on the temperature and humidity levels (Peterson 1966; Gadgil 1967b; Ivory 1972; Muir & Cobb 2005).

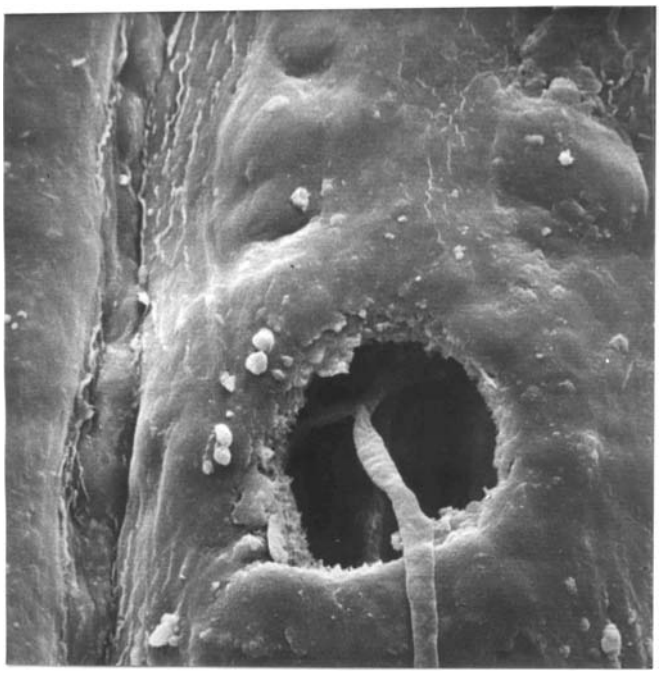

Fig. 6 – A germ tube penetrating a stoma

Conidia frequently produce more than one germ tube. There are conflicting reports over whether the stomata influence the direction of growth (Peterson 1978; Gadgil 1967b; Peterson 1969; Muir & Cobb 2005). It is possible that the variation in results could be due to the pine species studied or field versus laboratory experiments. In addition to needle penetration, fungal hyphae can grow over the needle surface for generally up to 10 days and produce secondary conidia (Gadgil 1967b; Peterson 1969). It is thought that secondary conidia do not contribute greatly to infection, because surface hyphae become less common after 10 days and by 45 days nearly all hyphae have disappeared (Gadgil 1967b). Endospores have been observed in cultures and were seen to germinate but their role in the life cycle of the fungus in unknown (Gibson 1972) and probably unimportant. Therefore inoculum in the form of secondary conidia is not being continuously produced. After the germ tubes enter the stomata, the hyphae branch and grow mainly in the mesophyll tissue, both inter- and intra-cellularly (Gadgil 1967b; Gibson 1972; Ivory 1972; Forest Research Institute 1976; Muir & Cobb 2005). The mesophyll tissue is killed in advance of the mycelium and the overall spread of the fungus is the needle is limited (Gadgil 1967b, Forest Research Institute 1976). Mycelium is abundant in the necrotic mesophyll tissue and this is where fruiting body development occurs. The fruit bodies (stroma) push their way though the needle epidermis and conidia are borne over the entire surface of the stroma. The cells adjacent to the infection also die but the accumulation of benzoic acid prevents colonisation of these cells by the pathogen (Franich *et al* 1986). The exact role of dothistromin in the infection process is still unclear and whether it is required for infection is still to be determined. It is thought that dothistromin levels build up to become toxic to the fungus and play a role in the initiation of fruiting body development (P. Gadgil pers. comm.).

Influence of climate and inoculum density on infection

In the field, the incidence and severity of infection by *Dothistroma* depends on a combination of factors including temperature, duration of needle-wetness period, the number of spores present and light intensity (Bulman 1993). Germination and successful infection can occur across a wide variety

of temperatures. In controlled conditions infection has been shown to occur at 4.4°C (Gadgil 1968). In field conditions a minimum of 7°C is needed (Gilmour 1981) but infection is favoured at ranges around 16-20°C when needles are continually moist for over 10 hours (Forest Research Institute Gadgil 1974; Gilmour 1981). Hocking & Etheridge (1966) found that infection first appeared on healthy seedlings exposed to high inoculum in the field after 4½ weeks. They found that the first marked increase in infection occurred when temperatures first exceeded 18°C. However, even a period of 20 minutes of continuous needle moisture is sufficient for infection to occur (Forest Research Institute 1978; Gadgil 1977). An absence of needle wetness has been shown to inhibit stromata formation rather than germination and penetration (Gadgil 1977). However, *Dothistroma* can survive in foliage over long dry periods. Gibson *et al.* (1964) showed that in the laboratory *Dothistroma* remained viable in dry needles stored at room temperature for 11 months, and at 30°C survived for 5 months.

Stromata appear sooner with high temperatures and a longer surface moisture period (Gadgil 1974) and under ideal conditions can form within 3 weeks of initial infection (Gadgil 1974, 1977; Gadgil & Holden 1976). Woods *et al*. (2005) demonstrated that an outbreak of Dothistroma needle blight in British Columbia during the late 1990s and early 2000s was a result of increased summer rainfall. . Figure 7 shows the relationship between severity of Dothistroma needle blight and summer rainfall from a forest in the central North Island. Disease was assessed in June or July, and is plotted against the preceding November to February rainfall.

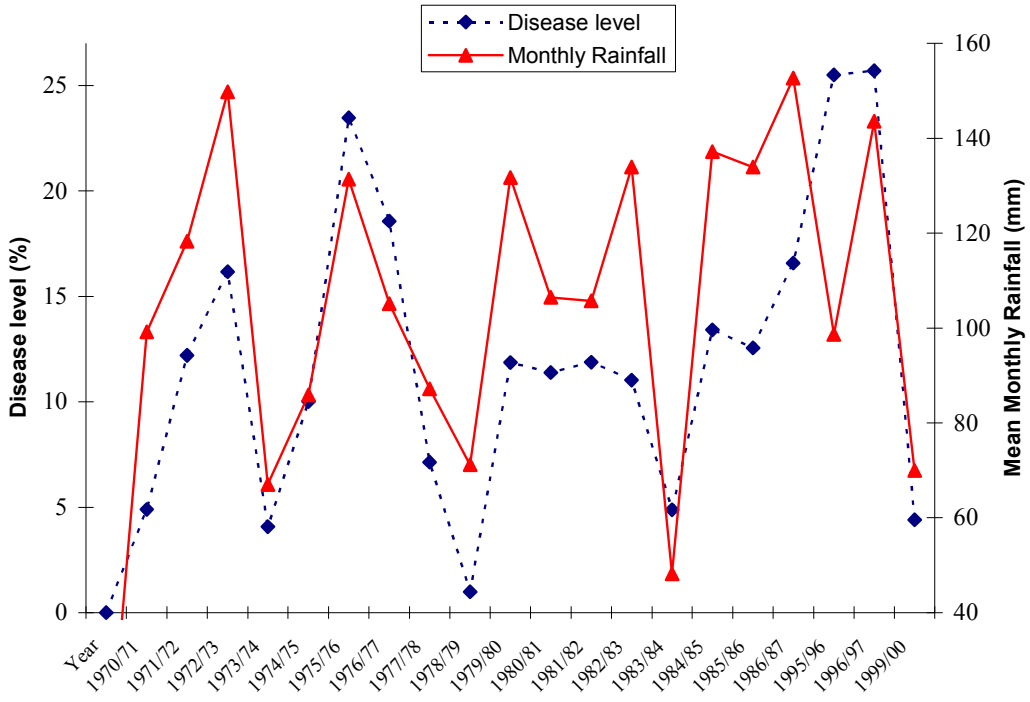

Fig. 7 - Relationship between summer rainfall (November-February) and Dothistroma needle blight.

Plantations with susceptible tree species that are subject to high rainfall or moisture conditions are liable to infection and although suited to temperate conditions, *Dothistroma* is present in areas that are covered in snow for a significant part of the year such as the Austrian and Barvarian Alps (Maschning & Pehl 1994; Kirisits, T. pers. comm. 2007). Kirisits & Cech (2007) reported an occurrence of Dothistroma needle blight on Swiss stone pine (*Pinus cembra*) trees at elevations between 850 and 1200 m asl. in the surroundings of the village Lutzmannsdorf in the upper Mur valley in Styria. This area has long snow cover from October to February/March.

In addition to suitable temperature and needle moisture, high inoculum densities are also important for infection (Bulman *et al*. 2004). The exact level of inoculum in the field is not known but Gadgil (unpubl. data) found that lesions developed with approximately 2000 conidia per needle after inoculating seedlings and keeping them in controlled conditions. There was a linear relationship between number of lesions formed per needle and number of conidia, with an R^2 of 0.70. In contrast, early laboratory work by Gibson *et al.* (1964) found that germination levels were lower under high densities. Light intensity is also known to increase the severity of the infection within the tree. Experiments have shown that the influence of light is related to the effect on the host plant rather than the fungus as light has no affect on either the germination of conidia or the early growth of the fungus on the needle surfaces (Gibson *et al.* 1967; Ivory 1970; Gadgil & Holden 1976).

Infection period

In controlled conditions the pre-reproduction period (time elapsed between inoculation and appearance of conidia-bearing stromata) can be as short as 2 weeks (Gadgil & Holden 1976). Two-year-old cuttings were kept under controlled conditions, viz. four temperature regimes (day/night temperatures 24°/16°, 20°/12°, 16°/8°, and 12°/4°C) and four leaf-wetness periods (8, 24, 48 hours, and continuous moisture) and inoculated with *Dothistroma pini*, applied as a conidial suspension. Germination percentage of conidia increased at higher temperatures but was not affected by wetness. Infection occurred under all treatments, was greater on needles >1 year old than on needles <1 year old, and was greatly increased under continuous moisture at 20°/12°. Stromata appeared sooner with higher temperatures and longer leaf-wetness periods with the optimum continuous moisture at 24°/16° resulting in stromata after 2 weeks. Curiously, although 24°/16° resulted in the shortest pre-reproduction period the 20°/12° regime gave significantly more infection than the other 3 temperature regimes tested at continuous moisture (Gadgil 1974).

In a more complex experiment, Gadgil (1977) studied the effect of varying wet and dry periods. In the first experiment inoculation with *D. pini* conidia was followed by:

1. continuous leaf wetness for 92 days
2. short leaf wetness periods of up to 8 h interspersed with 24 h dry periods
3. a 24 h dry period followed by continuous wetness for 91 days
4. no wetness period at all.

Infection, as shown by stromata bearing conidia of *D. pini*, was seen in all treatments but the percentage of infected needles was low except when continuous leaf wetness was provided for 91 or 92 days.

In the second experiment inoculated foliage was allowed to dry for 0-60 days before it was remoistened. The percentage of infected needles was highest in plants given 0-, 2- and 7-day dry periods, followed by those given the 14-day treatment, and was lowest with 30- and 60-day dry

periods. The pre-reproduction period of the fungus was 19-21 days on plants given the 0-, 2-, 7- and 14-day dry period treatments and 35 and 70 days, respectively, with 30- and 60-day dry periods. In the treatments involving 7- to 60-day dry periods, stromata were noticed within 10 days of rewetting of foliage. It appears that stromata formation, rather than germination and penetration, is inhibited by the absence of leaf surface moisture. Following on from that, it is apparent that once infection occurs the fungus can undergo a latent period when conditions are dry, but once rainfall resumes fruit bodies can form rapidly.

In nature, the period between infection, the first appearance of lesions, and later fruit bodies, varies depending on climate conditions and the time of year. Ivory (1972) found that symptoms can occur after 4½ weeks in summer and 10 weeks in colder months. In New Zealand, Gilmour & Crockett (1973) periodically exposed potted seedlings in a young *P. radiata* stand of infected with *D. pini* at Kaingaroa over a 4-year period, with subsequent incubation under dry greenhouse conditions. They showed that: appreciable infection did not occur unless the mean temperature was more than or equal to 10^{o}C over a wetness period of more than or equal to 15 hours and for infection at lower temperatures longer wetness periods were necessary. The minimum incubation period varied from a few weeks (after midsummer exposure) to several months (after midwinter) in the field, and was 4 - 9 months in the greenhouse (at 15 deg ±2.5°C). Gilmour (1981) showed that the length of the pre-reproduction period in the field was negatively correlated with mean air temperature (R^2=0.603). The period was longest at 15-16 weeks for seedlings exposed to infection in May, June, and July; and shortest for seedlings exposed in December at about 5 weeks.

In New Zealand, the main period of infection on current foliage in the central North Island therefore occurs from November to February, late spring to late summer, (Gilmour 1981) although the infection period can extend into April or May (Bulman unpubl. data). Visible infection is present all year round but is most predominant between September and October before the new flush of needles advances (Bulman *et al.* 1994).

Artificial inoculation

Dothistroma is a slow growing fungus that can be grown on most media types, although malt agars have been found to produce the best results (Gibson *et al.* 1964; Rack and Butin 1973; Gadgil, 1974; Gillman 1996). Conidia are generally produced abundantly in young cultures. Dothistromin is also produced in culture and appears as a dark reddish-brown pigment. Continued culturing of *Dothistroma* frequently results in a gradual loss in virulence and there is considerable variation in colony morphology, including sectoring, loss of dothistromin production and reduced sporulation (FRI 1970; Barnes *et al.* 2004). There is also considerable variation in growth rates, sporulation and dothistromin production between isolates of *Dothistroma* (Bradshaw *et al*. 2000, Barnes *et al*. 2004; Bradshaw & Zhang 2006). *Dothistroma* can grow in a wide range of temperatures and light does not affect growth (Ivory 1970). However, optimal temperatures for both conidia production and mycelial growth range from 16-20°C (Gibson *et al.* 1964; Ivory 1970; Rack & Butin 1973; Gillman 1996).

A variety of different artificial inoculation protocols for *Dothistroma* have been investigated (Gadgil 1967a, 1974, 1976; Parker 1972; West 2004; Barron 2006). For all protocols developed temperature, humidity and light have been shown to be important factors for obtaining successful inoculations. In general, temperature ranges between 18-20°C, high humidity with minimum leaf wetness periods and 12 hour light cycles have been found to produce the most reliable results (Gadgil 1967a, 1974, 1976; Parker 1972; West 2004; Barron 2006). Successful artificial inoculations have been performed

using fresh field inoculum and conidia obtained from cultures, both wild type and mutant strains. Although replication of these experiments within laboratories can be successful, reproduction between laboratories appears to be problematic, with the incidence and severity of infection varying both within and between experiments (Van-Aelst Bouma 2002; Devey *et al*. 2004; West 2004; Barron 2006). As a result of these issues with reliability, *Dothistroma* is considered be a difficult pathogen to perform artificial pathogenicity tests with and thus, field trials are more commonly used. Inoculations of several susceptible host species have been successfully performed in field trials (Gadgil 1967a; Peterson 1978; Gilmour 1981; Devey *et al*. 2004; Muir & Cobb 2005; Bulman *pers. comm.*).

Dothistromin

Dothistromin is a fungal toxin which is produced in large quantities by *Dothistroma* and is a non-specific toxin capable of killing or damaging cells from plant, algae, bacteria, and animals. In 1970, the solid, non-crystalline, red pigment produced by *Dothistroma* was purified and found to consist of two closely related compounds, $C_{18}H_{12}O_9$ and $C_{18}H_{12}O_8$. The name dothistromin was proposed for the former compound which was present in the greater amount (Bassett *et al*. 1970). This dothistromin extract was shown to be able to kill *Pinus attenuata* tissue *in vitro*, suggesting a role in pathogenesis (Bassett 1972). Although dothistromin is implicated in the development of disease symptoms, its exact mode of action in natural lesions has not been elucidated.

Role of dothistromin on infection process and lesion induction

Dothistroma enters pine needles through the stomata and then colonises the mesophyll both inter- and intra-cellularly (Gadgil 1967b). Initially, it was thought that the mesophyll tissue is killed by dothistromin in advance of growth by the fungus. Dothistromin affects tissue by causing a leakage of cytoplasm resulting in cell death (Forest Research Institute 1976). In bacteria dothistromin can also interfere with RNA and protein synthesis (Harvey *et al.* 1976). Aqueous solutions of dothistromin, prepared by mixing the toxin with calcium (Ca) or magnesium (Mg) ions at pH5, applied to pine callus cultures or detached needles killed cells caused *Dothistroma*-like lesions to develop. The lesions were limited and never killed the entire needle or callus, despite an excess of dothistromin applied. It was thought that the low levels of Ca and Mg in plant tissue solubilise dothistromin allowing it to diffuse through the plant tissue in advance of the fungus (Forest Research Institute 1976). Lesions similar to those produced by *Dothistroma* appeared within 5 days of dothistromin application into pine needles and lesion length varied with needle age (maximum in young needles) and possibly the season (Franich *et al.* 1986). Cells adjacent to those killed by dothistromin also died, expanding the lesion size, and accumulate benzoic acid which prevented colonisation of those cells by the fungus. A linear dose-response relationship was obtained for benzoic acid injected into *P. radiata* needles suggesting it is a mechanism of resistance of *P. radiata* to *Dothistroma* (Franich *et al.* 1986). It was suggested that the plant response, in the form of benzoic acid, was responsible for the most of the needle damage rather than the direct effect of dothistromin.

Dothistroma-like lesions can be artificially induced in *P. radiata* seedlings by using 10-100 ng of dothistromin into puncture wounds in needles. Light activation has been required for toxicity *in vitro* (Shain & Franich 1981; Stoessl *et al.* 1990; Debnam 1994). However, natural lesions caused by *Dothistroma* in New Zealand contain considerable more dothistromin (1-10 μg) than that required for artificial induction of wounds (Shain & Franich 1981). Dothistromin levels in needles infected with *Dothistroma* in New Zealand can reach levels about one hundred times the lethal level of toxin (Forest Research Institute 1976). Isolates of *Dothistroma* produce differing levels of dothistromin in

culture. A survey of a collection of worldwide isolates found that those from Germany produced extremely high levels of the toxin and, to a lesser extent, some of the isolates from the USA. Levels in culture were >500 times and >40 times as much as the New Zealand strain, respectively (Bradshaw *et al.* 2000). Dothistromin production of isolates from New Zealand was found to be highly variable within replicates but not significantly different either between isolates or collection locations (Gadgil 1996). Debnam & Narayan (1994) measured 3,633 lesions and found that the average amount of dothistromin per lesion was 107 ± 85 ng with a range of 0-437 ng, significantly lower than that found by Shain & Franich (1981).

Anti-bodies against dothistromin have been produced and used to develop a competitive enzyme-linked immunosorbent assay (ELISA) for the quantification of dothistromin (Jones *et al.*, 1993). Subsequently, an anti-idiotypic monoclonal antibody that mimics the toxin dothistromin has been developed (Jones *et al.* 1998). Dothistromin binding sites within small vesicles in P. radiata embryos using immunohistology for light and electron microscopy have been located and a 40-kDa peptide was identified that reacted specifically with a dothistromin-mouse albumin conjugate (Jones *et al.* 1998).

Dothistromin is a difuranoanthraquinone derivative that has the furobenzofuran moiety in common with structural similarity to the aflatoxin precursor versicolorin B. Aflatoxins are known mutagens, toxins and carcinogens. Intermediates from the aflatoxin and sterigmatocystin biosynthetic pathways have also been identified in *Dothistroma* (Danks and Hodges 1974) and several genes have been identified which show similarities to the genes involved in aflatoxin and sterigmatocystin production (Bradshaw *et al.* 2002; Bradshaw *et al.* 2006). Detectable products of dothistromin metabolism are CO_2 and oxalic acid and degradation is thought to involve peroxidase catalysed oxidation of dothistromin by H_2O_2 (Franich *et al.* 1986).

A variety of *Dothistroma* mutants have been made by targeted gene replacement and that are severely impaired in dothistromin production. These mutants have confirmed the involvement of several of the genes identified in the dothistromin biosynthesis pathway (Bradshaw *et al.* 2002; Bradshaw *et al.* 2006). The Bradshaw group at Massey University, New Zealand are using these transformants to investigate whether dothistromin is required for the development of needle blight symptoms (Bradshaw 2007a). Mutant strains of *Dothistroma* were made that do not produce dothistromin, and these were tested for their ability to infect pine needles. The dothistromin-deficient mutants were shown to cause needle blight, demonstrating that dothistromin is not needed to initiate infection (Bradshaw 2007b). This finding supports Bulman (2007) who stated that the role of dothistromin is uncertain and a relationship between dothistromin and pathogenicity or virulence in the field has never been established. Debnam *et al*. (1994) found no significant correlation between field resistance to Dothistroma needle blight and the length of lesions induced in needles injected with dothistromin.

Dothistromin genes are expressed at an early stage of growth in culture, suggesting a role in the first stages of plant invasion by the fungus or a role in competition with other microorganisms on the needle surface prior to hyphal invasion (Bradshaw and Zhang 2006b; Schwelm and Bradshaw 2006). This early production of dothistromin contrasts secondary metabolite production in other fungal species which usually occurs during the late exponential or stationary phase (Bradshaw 2007a). The Bradshaw group at Massey University, New Zealand are currently determining whether this early dothistromin production also occurs *in planta* and the interaction of dothistromin with phyllosphere fungi (Bradshaw 2007a). Genes responsible for the production of dothistromin lie in small groups alongside other types of genes in the genome (Bradshaw and Zhang 2006a). This is

also different from what occurs in most other fungi that produce toxins, where the genes are clustered together, allowing for co-regulation in some cases.

Effect on human health

Dothistroma produces a toxin, dothistromin, in culture and in nature. Dothistromin is structurally similar to the aflatoxin precursor versicolorin B and there are similarities between the biosynthetic pathways involved in the production of dothistromin and aflatoxin. Aflatoxins are potent mycotoxins that are also known to be mutagens and carcinogens (Bradshaw 2004). Similarly, dothistromin has been shown to be highly toxic, capable of killing or damaging cells from plant, algae, bacteria or animals (Harvey *et al.* 1976; Stoessl *et al.* 1990; Jones *et al.* 1995). In most studies, light activation has been required for toxicity (Stoessl *et al.* 1990). Dothistromin has also been shown to be a mutagen but there is no evidence to show that dothistromin is a carcinogen (Elliot *et al.* 1989; Skinnider *et al.* 1989; McLarin & Ferguson 1985; Ferguson *et al.* 1986). However, as aflatoxin is a known carcinogen and forestry workers are known to be high risk for the development of certain cancer types (Kawachi *et al.* 2007), there is a possibility that dothistromin is carcinogenic. In view of the potential, but unknown, risk of dothistromin, guidelines to minimise exposure of forestry workers to dothistromin were recommended in 1984 (Bulman *et al*. 2004). It was recommended that all stands where disease levels were 15% or more be sprayed, if workers were to be operating there within 12 months. The guidelines are now not followed.

Assessments of the amount of dothistromin in the field have found that workers are exposed to varying amounts dothistromin. The amount of dothistromin detected on either swabs taken from the skin or a patch attached to a forest worker was between 0 – 516 ng dothistromin which was calculated to give rates of dothistromin deposition from between >0.01 ng/minute – 3.11 ng/minute (Briggs 1984). In general the highest levels of dothistromin were recorded on clothes. The amount of dothistromin in the air (recorded from filters) was also very variable ranging from 0.0 – 10.0 ng/l (Briggs 1984; Gadgil & Franich 1984). Rates of dothistromin were found to vary depending on seasonal and climatic variation, and the level of *Dothistroma* infection in the stand. The highest level of dothistromin detected was during August (Briggs 1984). The amount of dothistromin in run-off from tree canopy after rainfall was high, ranging between >100 to 6500 ng/l and the amount of dothistromin recorded in water samples within or near to infected stands were less than 400 ng/l (Gadgil & Franich 1984). In a report prepared by Bates (1984) the level of dothistromin that could be considered to be unsafe was calculated to be 50ng/kg body weight/day, based on the lowest level of orally ingested aflatoxin shown to cause cancer in animals. This corresponds to 3500ng/day for a 70 kg worker.

Although the levels of dothistromin associated with forestry workers and in environmental samples is lower than concentrations known to cause mutagenesis or toxicity, the effect of long term exposure to low levels of this toxin is unknown. Reviews of cancer incidence amongst forestry workers suggested that the increase in cancer was more likely to be the irritant effect of dust rather than dothistromin. However, dothistromin could not be entirely ruled out as a contributing factor (Elliott 1989).

A survey of a collection of worldwide isolates of *Dothistroma* found that isolates from Germany produced extremely high levels of the toxin and, to a lesser extent, some of the isolates from the USA. Levels were >500 times and >40 times as much as the New Zealand strain, respectively (Bradshaw *et al.* 2000). If these strains were introduced to New Zealand, the high levels of

dothistromin produced might have serious human health implications as the levels of dothistromin exposure in the field may exceed what would be considered safe.

Effect on host

Dothistroma needle blight causes growth loss and mortality. Disease outbreaks are not a recent occurrence, with mortality and severe needle blight recorded in North America in the 1950s; and Africa, Chile, and New Zealand in the 1960s. Gibson (1974) suggested that its increasing importance in the northern hemisphere, where it is probably endemic, is not so easily explained. Woods (2003) described outbreaks in British Columbia; and Brown (2007) reported outbreaks and marked increase in disease distribution and incidence since 1999 in Great Britain. This increase coincided with an upsurge in distribution and severity of the disease in France in the late 1980s and early 1990s (Villebonne & Maugard, 1999).

Mortality

In natural *P. contorta* stands on southern Vancouver Island mortality of a number of 4- or 5-year-old trees was observed. Mortality of 7- and 8-year-old *P. radiata* averaged 60% in three years, while mortality of *P. muricata* averaged 13% (Parker & Collis 1966).

Dothistroma needle blight was epidemic in a *P. nigra* and *P. ponderosa* plantation in Nebraska in 1950, and again in 1963-65 (Peterson 1967a). Wagener (1967) reported that blight was first noted in California in 1964, and some *P. radiata* had apparently been killed by the disease in 1966. Thompson (1966) stated that 40% of *P. contorta* trees in a Christmas tree plantation in Oregon were dead or dying because of Dothistroma needle blight. In a cool and moist coastal region of northern California, mortality in two 7- to 9-year-old *P. radiata* plantations increased from 0.2% of the trees in March 1967 to 67.6% in April 1969 (Cobb *et al*. 1969). In the early 2000s, *Dothistroma* was reported to be causing extensive defoliation and mortality in native lodgepole pine (*Pinus contorta* var. *latifolia*) in northwestern British Columbia, Canada. It was the most prevalent pest in a survey of 100 randomly selected lodgepole pine plantations and has caused considerable crop tree mortality. The disease even caused mortality in 55-year-old lodgepole pine trees (Woods 2003). Disease severity was such that mortality was extensive in managed plantations and also mature lodgepole pine trees in the area were succumbing, which was an unprecedented occurrence (Woods *et al*. 2005).

At age 20, Dothistroma needle blight had caused >90% mortality in 12 of 21 *Pinus ponderosa* plantations established in various parts of Illinois in 1941 and 1942, and 5 years later 8 of the remaining 9 plantations had reached or were entering this stage of infection (Lorenz 1972).

In Kenya, Gibson *et al*. (1964) reported tree death after severe blight, with a lag of about 2 years between first occurrence of blight and first mortality. They suggested trees debilitated by defoliation were readily invaded by *Armillaria* sp., which probably accelerated the onset of mortality. Dubin & Statley (1966) in Chile reported 17% mortality in a plantation of 800 *P. radiata*.

In New Zealand, *Armillaria* spp. have been associated with increased mortality in heavily infected stands (Shaw & Toes 1977, Woollons & Hayward 1984, Sweet 1989). Etheridge (1967) showed that severely defoliated trees were susceptible to *Armillaria* and green pruning wound pathogens. Etheridge (1967) found that Dothistroma needle blight, alone or in combination with secondary

attack by Armillaria root rot or *Diplodia*, was responsible for tree mortality of less than 2%, and deaths only occurred in areas where defoliation exceeded 85%. This is in keeping with other observations in New Zealand that mortality attributable to Dothistroma needle blight alone is very uncommon (Gilmour 1967b, Bulman, L. S. unpubl. data from Scion Forest Health database).

Growth Loss

Gill (1963) showed that defoliation by Dothistroma needle blight in Tanzania affected height and diameter growth of a young *P. radiata* stand. Gibson *et al*. (1964) reported studies where diameter growth of plots in two plantations was significantly lower ($p<0.01$) in trees with a disease rating of 6, compared with trees that had a disease rating of 4. The experiment was monitored for 1 year. There was no significant difference in height increment. However, in another experiment, significant differences ($p<0.05$) in height increment were found when related to disease severity. Whyte (1976) found no response in height increment from spraying and concluded height is a poor indicator of loss from defoliation by Dothistroma needle blight.

Gibson *et al*. (1964) commented that results from field trials indicated a considerable lag between onset of severe defoliation and resulting growth loss. Van der Pas (1981) demonstrated a clear lag between disease progress and growth response and suggested that it may be explained by an additive effect of reduced photosynthetic capacity caused by successive defoliation events and a decrease of photosynthates translocated to the roots.

The literature is very consistent with regard to the disease level at which growth loss occurs. The following studies agree that 25% of foliage has to be affected before losses are measurable. Hocking & Etheridge (1967) reported studies on *P. radiata* in Tanzania. Disease severity was linearly related to stunting in very young trees, and sigmoidally related to losses of height and diameter increments in 3-year-old trees, the most rapid losses occurring with 25-75% blighted foliage. Whyte (1969) came to similar conclusions where studies on *P. radiata* stands in New Zealand infected with *D. pini* or artificially defoliated have shown that subsequent loss of increment per acre is hardly affected until defoliation becomes >25% of the rising 1-year-old foliage on 50% of the total number of trees in the stand. Christensen & Gibson (1964) presented work showing the effect of defoliation over 13 months on the height and diameter increment of young *Pinus radiata*. Diameter growth was reduced to a greater extent than height. Serious loss was expected in a crop where > 25 % of the foliage had been killed or was moribund. Growth almost ceased at 75 % defoliation. In New Zealand it is recommended to spray only when 25% of the crown is defoliated (Bassett 1972). This recommendation was based on the increment loss figures from Whyte (1969).

Van der Pas (1981) demonstrated that the relationship between volume loss and disease level was approximately proportional, e.g. an average disease level of 50% over a period of at least 3 years resulted in volume loss of 50%. There was also a negative linear relationship between diameter increment and disease level (Fig. 8, diameter increment 1988-1996 and average disease 1986-1996, Bulman L. unpubl. data). At higher disease levels a non-linear relationship may be expected. Similar results were obtained in Australia where for every 1% loss of foliage above 25% there was a 1% loss in volume growth up to 75% foliage loss (Old & Dudzinski 1999).

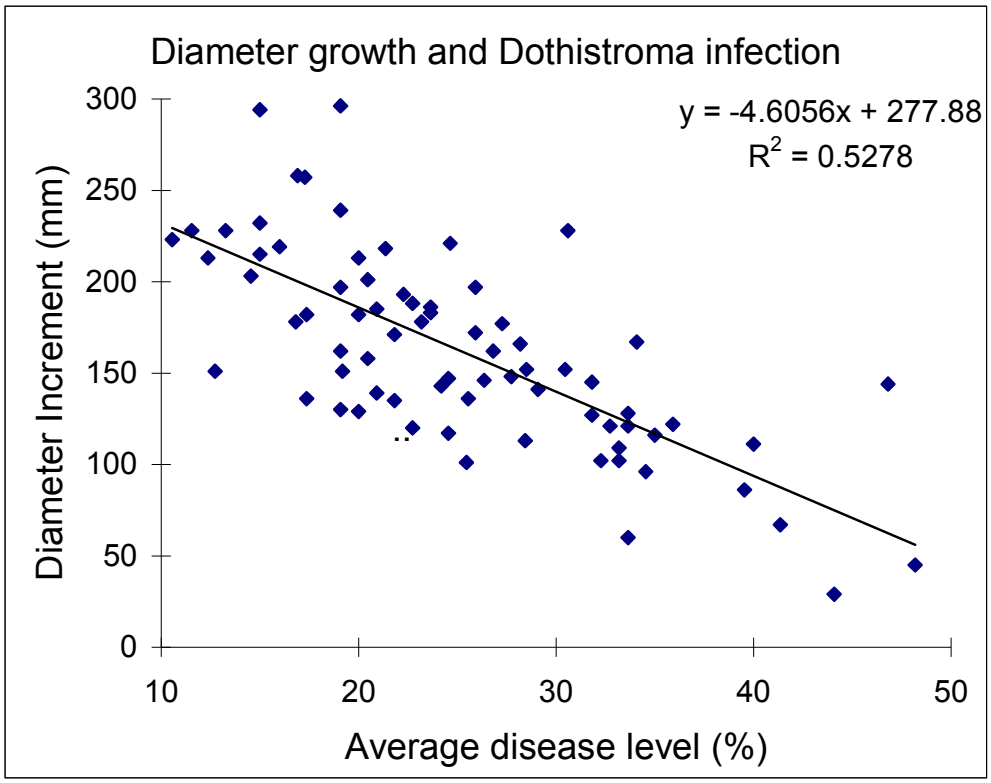

Fig. 8 – Relationship between diameter growth 1988-1996 and disease level 1986-1996

Economic Impact

Estimates of annual economic loss due to Dothistroma needle blight in New Zealand vary. Sutton (1971) estimated that the spray programme in New Zealand at three or four operations a rotation would only increase the wholesale price of timber by 1% and FOB price of newsprint by 0.4%. Sweet (1989) estimated that increment losses from Dothistroma needle blight were insignificant because of regular aerial spray applications of fungicide. Therefore, he considered that economic losses from Dothistroma needle blight amounted to the cost of spraying only, approximately $1.2 million per annum.

New (1989) estimated cost of Dothistroma needle blight control at $1.6 million, and conservatively using a stumpage of $20/$m^3$ and low overall disease levels of 10% severity resulted in losses of 225,000 m^3 per annum which at a stumpage of $20/$m^3$ gave a loss of $4.5 million. Bulman (2007) stated annual loss is in the order of $24 million.

Control

Chemical control

Spray trials
Overseas, chemical control of Dothistroma needle-blight was shown to be effective. The ability of a range of fungicides to eradicate the fungus or protect hosts was tested in a series of trials in Africa in the early 1960s. Fungicide investigations started as a joint programme between Tanzania and Kenya in 1963 and field screening of a wide range of fungicides was wound up by the end of 1964. Depending on the trial, treatments were applied using handsprayers, mist sprayers, or aircraft. In the aerial spray trials the two treatments were 1.8 kg of copper oxychloride (59% a.i). with a sticker at 45 litres/ha, and 2.27 kg of copper hydroxide (40% a.i.) at 22.5 litres/ha. Overall, the copper fungicides showed best results and it was concluded that application of copper fungicides may provide economic control of Dothistroma needle blight (Gibson *et al*. 1966).

Hocking (1967) reported that in laboratory and field tests of 38 fungicides several showed high contact activity. Triphenyl tin or copper-based compounds gave good field protection. Two widely spaced annual applications were recommended for protection in areas with two periods of heavy rainfall. Hocking (1965a, 1965b) demonstrated that copper fungicide provided a protectant and eradicant role by killing existing conidia and killing existing infections in order to prevent future production of conidia.

In the United States, several studies on chemical control were done. Thomas & Lindberg (1954) stated that 3-5 applications, depending on the rainfall, of fungicide between May and mid-July gave control of Dothistroma needle blight on *P. nigra,* with Bordeaux mixture gaving the best results. Peterson (1965) presented the results of tests made in 1963 to compare the effectiveness of four fungicides (Bordeaux mixture, zineb, captan, and Puratized agricultural spray) in controlling *D. pini* in a 30-year-old *Pinus nigra* plantation. Best results were obtained with the Bordeaux mixture but, since *D. pini* can infect previous seasons' needles as well as newly developing ones (which had not previously been reported), it was recommended that further studies were needed to determine the most effective number and timing of applications. Peterson (1967a) demonstrated that in small handspray trials, copper fungicide gave almost 100% protection for one growing season, when applied twice 3 to 6 weeks apart. In tests at Ames, Iowa, spraying with TC-90 (copper salts of fatty and rosin acids), benomyl, or Bordeaux mixture effectively controlled Dothistroma pini on shoots of 15-year-old *Pinus nigra*. Addition of polyvinyl chloride enhanced the effectiveness of benomyl (Epstein 1970).

In the U.S.S.R., Schischkina & Tzanava (1966) showed that 3 handspray applications of 1% Bordeaux mixture 20 days apart provided good control in *P. pityusa* in Georgia (33% infection compared with 83% infection in the unsprayed control).

In late 1965 Gibson visited New Zealand to advise on the Dothistroma situation and relate his experience with the disease in Kenya. He recommended that, based on the Kenya situation, the use of fungicide applied at an early stage of disease development when inoculum is sparse and re-infection rate is low would provide effective control (Gibson 1965).

Aerial spray trials were started in New Zealand in 1965 (Gilmour 1965b) and the first operational spraying was carried out in 1965-66 (Olsen 1971). Gilmour carried out two series of trials in 1965 and 1966 to test various copper formulations, dosage rates and type of diluent (Gilmour & Noorderhaven 1973). In 1967, Gilmour established a series of trials to examine the influence of time of application on disease development (Gilmour & Noorderhaven 1971). The first series of trials showed that adequate control for 2 years was obtained with 2 applications, 3 months apart, of cuprous oxide at 2.24 kg copper metallic equivalent in 56 litres water/ha. Over 1 year a single application was almost as effective as 2 applications, but the one in February was only half as effective as the December one. It was suggested that fungicide effectiveness was independent of the initial disease intensity. There was no difference between oil and water formulations.

For time of application, Gilmour & Noorderhaven (1971) showed that November was the most effective time for a single application of cuprous oxide, while application in October and December provided the most effective double spray treatment. The double spray treatment was slightly more effective than the single one.

Series of spray trials were carried out throughout New Zealand to confirm the optimum time of application at a regional level (Bartram 1976, Jelinek 1976, Knowles 1976, Stoodley 1976). In summary, the best time to spray is between October and November with warmer areas sprayed earlier than cooler areas (Bulman *et al.* 2004).

Gilmour *et al.* (1973) carried out an analysis of the spray programme from 1966 to 1973. They found that only 10.6% of 13,560 ha sprayed in one large plantation have required a second spray in 7 years and concluded that one or two applications per rotation may be necessary to control the disease. Bulman (unpubl. data) carried out an analysis from two periods (1981 and 1986) of the frequency of spraying in stands aged 9 to 16 years old growing in a central North Island forest, using spray data from 1972 to 1986. He found that 61% of stands were sprayed only once or twice since planting, and 17% of the stands had never been sprayed.

Research into techniques of aerial application has lead to increases in the efficiency of controlling Dothistroma needle blight in *Pinus radiata* plantations. Attempts to improve application are not new. In 1966, a trial was carried out to determine the effect of height on droplet pattern. The trial concluded that spray drift would be considerable under even light wind conditions at heights of over 30 m. It was suggested that additional spray runs be made along gullies Bowers and Bay (1966). Increased efficiency has been brought about by the reduction of spray volume/ha, the addition of oil to the spray mix and the use of rotary atomizers to produce fine spray droplets (Ray & Vanner 1987, 1988). Advances in spray technology allowed that rate of carrier to be reduced from 4.16 kg (50% copper) in 50 litres of water per ha to 20 litres of water per ha. The latter rate was applied from 1982 to 1984, after which the application rate was reduced to 1.14 kg (75% copper), or 1.66 kg (50% copper), in 2 litres of oil made up to 5 litres with water, per hectare (Bulman *et al* 2004).

Richardson & Ray (1996) and Richardson (2000) carried out work on developing optimal strategies for aerial application of fungicide to control Dothistroma needle blight. Specifically, the effect of a number of application variables on the optimum bout width, and within-block spray deposition, was tested. It was shown that the recommended 20 m bout width was appropriate for the worst-case scenario of low headwind speed and low release height, but when spraying in a crosswind bout width could be increased to 40-50 m (Richardson & Ray 1996). Richardson (2000) found that the current spraying strategy may not be delivering application rates to specification. That spraying is effective suggests that actual application rates could be reduced further if application efficiency

were increased. Model predictions with high winds over rough terrain require validation. Also, droplet size has a large influence on drift and actual droplet size produced during operational spraying needs to be determined.

Application costs contribute from 60% to 80% of the total cost of Dothistroma control. Therefore there is a cost incentive to improve the efficiency of the application by, for example, reducing total spray volumes. Similarly, there may be opportunities to reduce rates of both copper and mineral spray oil. In addition, substituting mineral oil by vegetable oil might have environmental benefits. Two copper products (cuprous oxide and copper oxychloride) and two spray oils (Caltex winter spray oil and BP Dothi oil) have been used in operational Dothistroma control. A recent study (Gous and Richardson 2006) evaluated the application characteristic of a range of alternative products and overall spray mixes. The study showed that sprays containing cuprous oxide and either BP Dothi oil or Syntol mineral oil had the most desirable spraying characteristics. Also, increasing the oil percentage in the spray mixture resulted in greater spread on the leaf surface. These improvements in spray formulation may result in reduced cost of spraying, but new formulations have to be field tested.

Chemical activity

Gibson & Howland (1970) noted that drainage water from treated foliage was more toxic than the fungicide on the foliar surface itself to conidia of *D. pini*, indicating a possible detoxifying effect of the foliage. The pathogen is highly sensitive to Cu; overall results showed an LD 50 value in the region of 0.9 ppm. Cu. Since surface water on treated plants transports copper in quantities toxic to germinating conidia, treatment may be expected to reduce the capacity of infected trees to produce inoculum as well as the viability of existing conidia.

Franich (1988) reported the effect of copper on *Dothistroma*. Copper fungicide (as 50% cuprous oxide w.p. formulation) applied to *Pinus radiata* needles is solubilized by complexation and oxidation processes to give potentially more than 30 mg/litre of cupric (Cu^{2+}) ions in aqueous solution. Bioassays using *Dothistroma pini* conidia showed that exposure to 20 mg/litre Cu^{2+} in the presence of *P. radiata* needle aqueous exudates (which stimulate germination and support fungal growth) for periods as short as 1.5h was sufficient to kill the spores. Lower concentrations (10 mg/litre of Cu^{2+}), while not greatly reducing conidial germination rate, substantially reduced germ tube length and affected the hyphal anatomy, while 5 mg/litre Cu^{2+} prevented production of conidia by mycelium grown in vitro. Low dose rates (0.1-5 mg/litre) affected mycelium metabolism, causing a 5-fold increase in secondary metabolite (mainly dothistromin) synthesis, but did not reduce germination. Cu^{2+} concentrations in water films on the *P. radiata* needle surface need, therefore, to be above 10 mg/litre to prevent infection from taking place, or above 5 mg/litre to prevent re-infections from secondary conidia.

Gadgil (1986) investigated the effect of copper sprays on production of spores by *Dothistroma pini*. In the laboratory, germination on seedlings dropped from over 90% to less than 1% one day after spraying. A field experiment was set up to determine the effect of operational spraying. At the beginning of the experiment in April about 250 spores pre needle were produced. Numbers peaked in June/July with 4,500 per needle. In the unsprayed block numbers dropped to 750 per needle in December and rose sharply to 2000 in February, with 90% germination. In the sprayed block, number of viable spores in December and January dropped to 50 per needle, and in February germination rose to 40% but number of spores was still low. The effectiveness of fungicide appears

to be due to a double action – protecting new foliage from germinating spores and reducing inoculum by killing the spore-producing fruiting bodies.

Fungicide Persistence

Gibson *et al*. (1966) reported that just over 10 months after fungicide application, foliage analysis showed copper content of 600 ppm. He considered this is remarkable. Gilmour & Noorderhaven (1973) found appreciable amounts of copper residues on needles after 2 months and 254 mm rain, but they were almost completely eroded after 3 months and 432 mm rain.

Experiments were made over a two-year period at Muguga, Kenya, to determine the retention of fungicides, applied by a knapsack sprayer, on the foliage of young *Pinus radiata* (Gibson & Howland 1970). Five preparations containing basic copper oxychloride or cuprous oxide showed little difference in rate of removal by weathering after application at the same initial concentration of Cu. In one experiment, Lovo (amine stearate formulant) significantly improved the retention of technical copper oxychloride. Several experiments with Perenox (cuprous oxide formulation) at concentrations of 0.08-1.5% copper showed that there was no simple relation between rainfall and rate of removal by weathering. Within experiments, rates of removal did not differ significantly with different initial concentrations, suggesting that a small number of applications at a high concentration might prove more economic than a larger number at a lower concentration.

Gous *et al*. (2007) showed that as oil concentration in the spray formulation increased, overall copper persistence increased. After 17 weeks (the maximum period tested) about 20% of the initial deposit was still present. However, with a larger droplet size (80 μm VMD versus 65 μm VMD) produced for the high oil concentration (86% by volume at 3 litres per ha equivalent), it is possible that the observed results reflect a droplet size effect rather than an oil type effect.

The implications of these findings are that there is potential for reducing total spray volumes for Dothistroma control because as oil concentration increases, overall copper persistence increased. This is an important result because it suggests that reducing total spray volumes from 5 litre/ha to 3 litre/ha is worth evaluating in field studies. Such a reduction in total spray volumes would produce significant cost savings.

Economics of Spraying

Gibson (1967) provided one of the earliest reviews of the economic of spraying and outlined the difficulties associated with estimating the benefits of spraying. Firstly, the level of disease control needs to be determined; then the difference in productivity between unsprayed and sprayed susceptible species and non-susceptible species taking into account site factors and end use needs to established. Furthermore, the cost of spraying has to be factored in. Gibson (1967) suggested that calculations based on those considerations indicate it will be possible to provide economic control of Dothistroma needle blight in many parts of Kenya, but the majority of plantations were at altitudes between 2000 and 3000 m asl where topography and altitude made low flying inaccessible and inefficient. It was concluded that aerial application of fungicide in Kenya was not a feasible control option.

van der Pas *et al.* (1984) estimated the compounded cost of spraying. For example, a stand with a rotation of 25 years that was sprayed at ages 4, 5, and 6, assuming a 10% discount rate and spraying

costs of $15/ha, will give a discounted value of disease control of approximately $300/ha. In other words, the additional revenue needed to justify control is $300/ha.

Gibson (1971) showed that a high degree of protection in young *P. radiata* was afforded by relatively light fungicide applications, though more information on the growth of *P. radiata* was needed for accurate assessment of the benefits of the treatment. Nevertheless, aerial spraying was recommended for East Africa. Results of trials showed that application of 4kg/ha applied in two operations annually would be economically justified (Gibson 1972). However, by 1974 it was concluded that while economic control by aerial application of copper fungicide had been achieved in New Zealand, this means of control was impractical in Africa.

In 1970 the cost of one application and fungicide was estimated to be $7.17/ha (Sutton 1970), but this cost was not related to the value of additional wood produced from disease control. The total cost of the programme to control Dothistroma needle blight within the Pinus radiata stands of Kinleith Forest, New Zealand from 1966 to 1988 was NZ$18.4 million (1988 dollars). Control costs in 1988 were substantially less than they were when spraying began because of improvements in application techniques and reduction in spray volume. The average cost of spraying in 1966 was estimated to be $44.72/ha in 1988 dollars, compared with $15.59/ha in 1988. Spray records for 40 stands (10 300 ha total) showed that the average spray frequency per hectare per rotation was 5.45 (range 2.10-10.30). Yield information was available for 10 of the 40 stands and there was no correlation between expected yield and spraying frequency for these areas, and the economics of control could not be demonstrated one way or other (Dick 1989).

Woollons and Hayward (1984) showed that in trials carried out over 5 years at Kinleith, annual spray resulted in 6.0m/ha more basal area than unsprayed controls. This difference arose from reduced diameter increment and increased mortality in the unsprayed areas, but the standard operational treatment of spaying at 25% infection did not provide adequate protection. The dominant 300 stems/ha in unsprayed areas showed no difference in basal area or height compared with the dominant 300 stems/ha from annually sprayed areas. Similar findings were shown in van der Pas *et al*. (1984) where in four aerial spray trials differences in basal area between sprayed and unsprayed stands could only be demonstrated after 1 or more years of disease reduction. However, thinning eliminated basal area differences between treatments. In a series of trials carried out in the mid 1980s to the early 1990s no growth differences between sprayed and unsprayed blocks could be shown after pruning and thinning in spray trials at four out of five sites (Bulman 1993).

In Chile, Alzamora *et al*. (2004) carried out a study that involved physical and economic evaluation of chemical control for Dothistroma in varying silviculture schemes. The trial examined several management and control methods. The objective was to evaluate the efficiency of these methods in limiting damage caused by Dothistroma in the context of overall practical and economic effectiveness. The results indicated that chemical control was effective, but the higher costs involved meant that they exceeded the value of the recovered volume of timber.

The evidence against spraying on economic grounds is reasonably compelling, but in heavily infected stands there is a case for spraying to reduce overall inoculum levels. There is a concern among foresters that if spraying was stopped disease would increase to a level where mortality becomes common. In the trials discussed in Bulman (1993) four blocks of approximately 50 ha were not sprayed from 1985 to 1993. Mortality due to Dothistroma needle blight was extremely rare. In unthinned stands, or if the entire crop needs to be protected, growth response to spraying is likely and spraying may be justified.

Effects of copper application on the environment

Dothistroma needle blight in New Zealand is controlled by the spraying of either cuprous oxide or copper oxychloride (Bulman *et al.* 2004). Up to 200 tonnes of fungicides have been used in any one spray season. Spraying generally takes place between October and November (Bulman *et al.* 2004). In 1946, a study suggested that pasture lands in New Zealand were considered to be copper deficient and a copper metal equivalent (1.4 kg/ha) was applied, which was claimed to have eliminated scouring and increased meat production (Ray 1984). However, there are environmental concerns over the impact from repeated application of copper on the pollution of streams, lakes, pastures, agricultural crops and forests. Although copper is an essential trace element, excessive amounts are poisonous. As a result of this concern, the impact of copper on freshwater resources, aquatic life, animals and vegetation has been investigated.

Fish (1968) made a study of a stream that drained areas of *Pinus radiata* forest, both before and after standard spraying with a formulation of 2.08 kg copper active ingredient per ha in 50 l water for control of Dothistroma needle blight. Samples were taken one month before a single spray in November 1966, one week after the November 1966 spray, and one week after a single November 1967 spray. The dissolved copper content of the water was very small in all samples, but the fine particulate debris filtered from the water carried detectable quantities of copper. Although the amount of this seston decreased sharply after the first spraying in 1966, its copper content increased by several times after spraying began. No such effect was found among animals (snails, caddis fly larvae, etc) investigated. These results suggest that, although some of the copper applied as a spray to the catchment makes its way into the drainage waters, the aquatic fauna either does not consume this material, or if it does, the additional copper is not accumulated in the animals' bodies. This work was subsequently repeated and extended in the early 1970s and again, aerial spraying of copper oxychloride (50% copper) was found to have no effect on stream fauna over a two year testing period (Forest Research Institute 1976). In that study, the amount of copper dissolved in water was measured in three sampling areas before spraying, after spraying, and at 3-monthly intervals for the next 2 years. The copper content in water varied from 0.01 ppm to 0.09 ppm, the highest level being measured in the unsprayed area.

In 1998, the Forest Owners' Association through the Dothistroma Control Committee commissioned a report to review copper toxicity on aquatic life. A review on whether copper sprays could have an adverse effect on aquatic life in streams draining sprayed pumice land catchments was carried out. No field measurements were undertaken. The review concluded that spraying is likely to result in levels of copper that markedly exceed acute criteria (United States Environmental Protection Agency criterion of 3.9µg/l for acute (1-12 hour) exposure and 1.4µg/l for chronic exposure) when spray enters streams directly. These criteria were developed after comparing sensitivity ranges to copper of a wide variety of aquatic organisms (Collier & Hickey 1998). However, it also stated that the presence of riparian vegetation can markedly reduce the levels of copper entering streams directly following spraying. This report also presented unpublished data from Rowe (Landcare) that described copper concentrations in streams following spraying at 4.16 kg 50% copper oxychloride in 50 litre water per hectare at the Maimai Catchment area south of Reefton in Westland. High concentrations of copper (300µg/l) were detected in streams where young trees were present and there was no riparian vegetation, one hour after spraying. The acute toxicity level of 3.9µg/l was exceeded for 12 hours following spraying. If mature trees and/or riparian vegetation was present little copper was detected in streams.

Copper has been shown to build up in horticultural systems after years of repeated spraying. Alva & Graham (1991) reported some old orchard sites contained up to 370 kg/ha of metallic copper in the soil. In France, rates of 200-500 mg per kg of soil have been found (Brun *et al*. 1998). The amount of copper sprayed in horticulture is considerably more than that applied to pine forests for the control of Dothistroma needle blight. For instance, avocado orchards in New South Wales have had copper fungicides applied at up to 15 times per year at rates of 3-6 kg/ha (Van-Zwieten *et al*. 2004).

Niklinska *et al*. (2006) tested the effect of heavy copper pollution on soil microorganisms in forest soil organic layers. The study was conducted in mixed *P. sylvestris* and *Quercus* sp. forests in southern Poland. The annual mean copper input, from two large copper smelters in the area, reaches about 32 kg/ha. Intensive industry started in the area in 1969. Several microbial indices such as microbial biomass, basal soil respiration, community level physiological profiles, and the pollution-induced community tolerance approach based on Biolog EcoPlate assay, were used to examine the effect of copper in soil. Although the treated site was heavily polluted with copper, neither basal soil respiration, nor microbial biomass or physiological profiles, differed from the unpolluted controls. This might be explained by either limited availability of copper or developed resistance to pollution. An effect was detected only by tolerance measurements using the Biolog assay, and then tolerance was only very weakly expressed (only one (D-cellobiose) out of 31 substrates was significantly different at the copper polluted sites compared with the unpolluted site).

In contrast to some spraying in horticultural systems, spraying for Dothistroma needle blight control is generally carried out 2 to 5 times during a 25 year rotation at a rate of 0.86 kg/ha. In 2007 the Dothistroma Control Committee agreed to fund a study to test the hypothesis that copper builds up in soil after repeated spray application in sufficient quantity to cause environmental damage.

Silvicultural control

Thinning and pruning
Thinning and pruning reduce disease levels. After thinning, the stand is opened which results in greater air circulation and the foliage dries at a faster rate than in an unthinned stand. This reduction in leaf wetness period slows the rate of infection. Thinning increases the distance between trees and this reduces the effectiveness of rain-splashed spores, most of which travel only short distances. Highly susceptible small trees are removed in preference to less susceptible trees (Bulman L. S. unpubl. data), thereby lowering the amount of inoculum available to continue the infection process. The removal of susceptible trees reduces the impact of the disease on overall stand volume.

Pruning removes infected foliage and lowers the inoculum available to initiate new infection. Foliage on the ground is rapidly colonised by other fungi (Gadgil 1970, Gibson *et al*. 1964) and so infected needles produce spores for only a short period after pruning, and the spores that are produced are unlikely to travel from the ground to infect foliage on the tree. In contrast, Gibson *et al*. 1964 stated that there was some evidence that pruning may accelerate the onset of mortality in affected stands. In one highly infected plantation unpruned plots had 2.8% mortality compared with 8.8% mortality in pruned plots one year after treatment. The pruned height and percent crown removed were not given, and it was stated that further experiments to check the relationship between pruning and mortality did not give conclusive results.

Marks & Smith (1987) examined the effect of low pruning and canopy closure on Dothistroma needle blight in 7- and 5-year-old radiata pine plantations in north eastern Victoria. Pruning reduced

the level of infection within rows close to the edge of the plantation of 7-year-old trees, possibly because of improved stand ventilation, but this effect rapidly disappeared with increasing distance from the edge. When the mean crown separation was <1.0 m Dothistroma needle blight increased rapidly in the 5-year-old stand. For a separation of >2.5 m the disease was held at a very low level. Tree height and crown diameter *per se* did not affect disease levels. The results overall suggested that silvicultural control by pruning, spacing and thinning is practicable even on sites favourable for the disease.

In 1984, Martin McKenzie established a silviculture trial to examine the effect of pruning and tree density on Dothistroma needle blight in an unsprayed stand at Kinleith Forest. Pruning reduced disease levels for at most 2 years, and there was no difference in disease levels between plots stocked at approximately 830 stems/ha compared with plots at approximately 430 stems/ha at age 8 (Bulman 1990). Hood & Ramsden (1996) established a trial in Queensland to examine pruning and thinning. They could not demonstrate treatment response after two years. Both trials used small treatment plots; of 0.119 ha and 0.069 ha (Hood & Ramsden), and it is likely that these were too small to reduce inoculum and consequently achieve reduction in disease levels.

In Australia, Neumann *et al*. (1993) demonstrated a relationship between stocking, Dothistroma severity and attack by Sirex woodwasp.

As early as 1965 it was suggested that Dothistroma needle blight was more severe in densely stocked stands. It was observed by a group of Australian Foresters who visited New Zealand in December 1965 that there was heavy infection on natural regeneration at 2000-4000 stems/ha at 15 years of age. An uninfected area nearby had much lower stocking. They also made the observations that all dominants above 15m height were relatively unaffected compared with their smaller neighbours and that shaded areas appeared to be less favourable for the disease. Pruning was assumed to slow disease progress (Marks 1966). In Nebraska, Van Haverbeke & Boldt (1968) described outcomes when rows of *Pinus ponderosa* and *Juniperus virginiana* were released after nearly 20 years of suppression by hardwoods. They stated that release caused a reduction of incidence of *Dothistroma pini*.

In a study carried out by Dick (1989), 40 stands in Kinleith Forest were sprayed on average 5.5 times (range 2.1-10.3) between 1970 and 1988. The stands selected by Dick were generally grown for pulpwood and maintained high stockings of between 660 and 1630 stems/ha at age 10. Rawley (1990) stated that current silvicultural intention for Kinleith Forest is to production thin to a final stocking of 375 stems/ha at age 11-13 from an initial stocking of about 1000 stems/ha. An analysis carried out in 1987 (Bulman, unpubl. data) of spraying carried out at Kaingaroa Forest found stands at Kaingaroa were not sprayed as often as those in Kinleith. The analysis covered two periods (1981 and 1986) and determined the frequency of spraying in stands aged 9 to 16 years old. Only one stand out of the 384 selected for the 1981 period was sprayed 6 times since planting, 61% of stands were sprayed only once or twice since planting, and 17% of the stands had never been sprayed. Summer rainfall (November to February) is similar at Kinleith and Kaingaroa (458 mm and 482 mm respectively). Stands at Kaingaroa Forest were usually managed on sawlog or framing regimes and were less heavily stocked than stands at Kinleith. Shirley & Coker (1985) stated the annual programme of waste thinning and pruning at Kaingaroa involves some 35,000 ha. Spraying is less frequent where silvicultural management has been carried out.

Bulman (1993) suggested that thinning-to-waste resulted in decreased disease levels, and van der Pas (1984) showed that response to spraying could not be demonstrated at 4 sites where pruning and

thinning were carried out to prescription. It was shown that thinning removed the most susceptible trees and pruning suppressed disease levels for at least one season.

Age resistance by use of vegetatively propagated aged material

It was proposed that vegetative propagation of mature material can be used as a technique to improve resistance in the field. A clonal stand in Rhodesia , propagated by air-layering from a single 7-year-old tree, showed exceptional vigour and complete resistance to *Dothistroma pini* at 21 months. Control seedlings planted in the same vicinity were very severely diseased after the same period (Barnes 1970). A similar study was conducted by Garcia & Kummerow (1970) where ten-month-old *P. radiata* seedlings were grafted with scions taken from 5-, 10-, 15- and 25-year-old trees and artificially inoculated with *D. pini*. Infection decreased with increasing age of the trees that provided the scions. Ades & Simpson (1990) in Australia monitored the relative resistance in the field to needle blight caused by Dothistroma of juvenile cuttings compared with seedlings. Fifty-two clones in twenty-two full-sib families were represented in the field trial at Yarralumla, ACT, Australia. All cuttings were taken from hedged ortets which were four years from seed, but had been cut back periodically to one metre high since age two. One-year-old seedlings, from a seed orchard, where most of the parents of the clones were represented, were used as controls. The trial was assessed for needle blight infection six years after planting. Mean infection of all cuttings was 21.1%, compared with 29.8% for seedlings. This difference was attributed to the greater maturation age of the cuttings. It was calculated that if the best 10% of clones were selected, infection could have been further reduced to 12%, a level where other disease control measures would be considered unnecessary.

Shade

Field observations and experiments in the Kenya Highlands showed that the severity of Dothistroma blight in young *P. radiata* was reduced where the host was grown in shade. The effect was attributed largely to a reduction of the sporulation of the pathogen on infected tissues under those conditions. However, the growth of young *P. radiata* was seriously checked by shade, and the development of those observations as a field control measure was rejected (Gibson 1966).

Control by application of fertiliser

The effects of sulphur and nitrogen applications were examined by Lambert (1986) in Australia. Growth, foliar nutrients and *Dothistroma* infection were studied for 7 yr following application of S (300 kg/ha as gypsum) and N (100 and 400 kg/ha as ammonium nitrate) to a 4-yr-old *P. radiata* plantation in Nundle State Forest, New South Wales. Gypsum increased foliar sulphate for at least 7 years, but no direct effects on growth were seen. The major effects of N were stimulation in volume growth by the low rate and a depression in volume growth by the high rate of application. The negative effect of high rates of N was attributed to induced S and P deficiences. Induced S deficiency led to accumulation of arginine in the foliage. *Dothistroma* infection was higher on plots receiving N and was significantly correlated with foliar arginine concentration. This study was probably prompted by Eldridge *et al.* (1981) who found that in a study of the effect of environment on disease incidence and severity, soil parent material appeared to be the dominant factor with highest average infection being on sulphur-deficient basalt soils. Other factors such as soil depth also showed some effect. It was suggested that particular soil parent materials produce stress factors leading to increased foliage content of certain amino acids that enhance the growth rate of the invading fungus.

In Chile Contreras (1988) found no response in Dothistroma needle blight to fertiliser application at planting. Disease was only successfully reduced by annual application of fungicide.

In New Zealand, a fertiliser trial was set up in June 1965, in which two heavy applications of NPK and Mg, and Mg alone, were applied. In February 1966 defoliation in the treated plots was about 15% lower than in controls, but by June 1966 mean defoliation was 40% in treated and untreated trees (Gilmour 1967c).

Resistance

Tree resistance

The first work on resistance to Dothistroma needle-blight was started in Africa in 1963 and reported in Paterson (1966), who suggested that selection for resistance to *Dothistroma pini* by *P. radiata* had been relatively successful. Peterson (1967a) described a study in 30- to 32-year-old plantations of *P. nigra* and *P. ponderosa* in Nebraska in 1963-65. While not looking at resistance specifically, he noted that in most trees, current needles, initially resistant, became susceptible in midsummer but in some trees they remained resistant. It was also noted that general resistance varied greatly between individual trees. Paterson (1968) described a successfully intensified programme in East Africa of plus-tree selection which included selection of *P. radiata* for resistance to *Dothistroma pini*. Searches of 24,699 acres of plantations in 1962-67 yielded 216 plus-trees (a mean selection pressure of 1/68,600). Ivory & Paterson (1969) also reported progress in breeding for resistance to Dothistroma needle blight in East Africa. They showed that it is possible to select *P. radiata* with inherent blight resistance on the basis of the appearance of the phenotype. Scion material from such select trees was also found to possess a greater degree of resistance than similar scion material from non-select trees. Their work indicated that resistance was transmissible to seedling progeny and that its heritability is very high. The relationship between vigour and resistance was shown to be linked, in that resistant trees were more vigorous than their random neighbours. However progeny from only one of six plus-trees selected for vigour were found to possess enhanced resistance.

In USA, attempts to identify resistant species and varieties started in the late 1960s. Selection trials with potted seedlings aged 8 months to 2 years placed in an infected *Pinus radiata* stand in Del Norte County, California, indicated that *P. muricata* (at least the northern race tested) was apparently resistant; and Guadalupe Island and Cedros Island pines (*P. radiat*a var. *binata* and *P. muricata* var. *cedrosensis*) were intermediate in resistance and may be of interest in resistance breeding of the highly susceptible *P. radiata*. Resin accumulation round the site of infection in *P. muricata* var. *cedrosensis* prevented the formation of stromata, suggesting a possible connection between resinosis and resistance, perhaps related to the number of resin ducts in needles-few in *P. radiata*, numerous in *P. muricata*, and intermediate in the two other taxa (Cobb & Libby 1968).

Libby *et al*. (1968) describe the location, environment, numbers and gross physical characteristics of the pines on Cedros and Guadalupe Islands and plantations of these pines elsewhere. Preliminary tests showed that island pines were much less susceptible to *Dothistroma pini* than mainland *P. radiata* populations and are therefore of high genetic interest in a breeding programme.

In New Zealand, selection for resistance started in 1966 when 66 phenotypically 'resistant' trees were selected from plantations heavily infected with *Dothistroma pini.* When clonally propagated by

cuttings 21 trees retained resistance. However, only 5 of these clones were acceptable in growth, branching and stem straightness. In a second study, highly significant clonal variation in resistance to natural infection was found in hedged clonal archives from 2- and 3-yr-old 2nd-generation ortets. Variation was also strong in hedges of clones aged 18-23 yr. A third study, involving a diallel cross among 25 seed orchard clones, showed marked genetic differences among 5-yr-old trees in resistance to natural infection. *Dothistroma* infection was shown to be a moderately heritable trait (Wilcox 1982). Burdon & Bannister (1973) found that among mainland populations, the Cambria provenance was more susceptible to Dothistroma blight.

Promising results were gained by Carson (1989) who assessed Dothistroma needle blight on *Pinus radiata* in New Zealand in 9 progeny trials ranging from 2 to 10 years old. Resistant families could be identified from all assessments, and rankings were consistent over sites and years. Heritabilities were moderately high, specific combining ability (SCA) was very small in comparison with general combining ability (GCA), and there was no indication of substantial genotype × location interaction. Genetic gain expected from seed orchard progeny from performance-tested first-generation parents was calculated from progeny test data to be about 11-12%. Carson suggested that the actual reduction in disease with disease-resistant stock may be greater because of the epidemiological effect which occurs when all trees in a stand are resistant. Dungey (pers. comm.) suggested gain may not be quite so high. She found damage due to *Dothistroma* infection was moderately heritable and well correlated across the three sites tested. Correlations between damage levels and DBH indicated that this disease was having a significant negative effect on growth, consistent with previous studies by Carson (1989). Maximum predicted gain from selection was around 9%, which dropped to 6-7% with inbreeding constraints, lower than the 11-12% predicted by Carson. However, Dungey suggested that selection and breeding within the *Dothistroma* resistant advanced-generation population she tested would continue to achieve significant gain in resistance.

Dick (1989) estimated that a 15% decrease in average stand infection from using the *Dothistroma* resistant breed would result in a 56% reduction in spray costs but was uncertain whether the resistant breed would provide gains or losses in yield. Carson *et al*. 1991 stated that estimated benefits from a *Dothistroma* resistant breed comprised of a 15% decrease in average stand infection, a 56% reduction in spray costs, and an unspecified reduction in growth loss - these assumptions are presumably based on Dick (1989). Resistance to Dothistroma needle blight is a trait that has been recognised in the GF Plus scheme developed by the Radiata Pine Breeding Company. Current ratings for Dothistroma resistance use data from 10 trial sites assessed 18 times at ages varying from 2-9 years (RPBC 2006).

To date, the operational performance of this breed has not been documented.

At the biochemical level, Franich *et al*. (1986) suggested host response may provide resistance. They found that the magnitude of response of needles to dothistromin was mildly correlated with observed field resistance to *D. pini* among 7-yr-old control-cross progeny comprising 50 full-sib *P. radiata* families. This correlation supported the hypothesis that a rapid response to dothistromin by accumulation of benzoic acid is a mechanism of resistance of *P. radiata* to *D. pini*. However, Debnam *et al*. (1994) found no significant correlation between field resistance and length of lesions induced in needles injected with dothistromin. Debnam & Narayan (1994) determined the dothistromin content of naturally occurring Dothistroma needle blight lesion in a clonal trial at Kaingaroa Forest. From 3,633 lesions the average amount of dothistromin per lesion was 107 ± 85 ng with a range of 0-437 ng. The results showed that dothistromin production was extremely variable within and between clones. There was no significant correlation between any of the

variables measured (lesion length, number of fruit bodies per lesion, amount of dothistromin per gram lesion tissue weight, amount of dothistromin per lesion) and measured resistance to Dothistroma needle blight in the field. It was concluded that it was not possible to determine the potential resistance of the tree by measuring response of individual needles to the fungus.

Age resistance

For *Pinus radiata*, increasing resistance to Dothistroma needle blight with age has been well documented. Ivory (1968) and Gibson (1967) stated that the highly susceptible *P. radiata* develops resistance with increasing age. The generally accepted age where resistance is shown is 15 years, but this can vary depending of inoculum density, environment, and weather. Early reports proposed that tolerance of *Dothistroma* by *P. radiata* is related to tree height, rather than to age, with the peak infection rate occurring at 3-6 ft (Hocking 1965, Hocking & Etheridge 1967, Ivory 1968).

In New Zealand, it was quickly noted that *P. radiata* older than 15 years appeared to be resistant (Poole 1965). These observations were supported by results from a trial carried out in Rotoehu Forest (Lawrence 1977). Twenty trees in each of five plots were established in three compartments. Two of the compartments were planted in 1960, the other in 1963. Trees were assessed annually in October from 1971 to 1976. Disease levels decreased over the duration of the trial, where in 1971 approximately 15% of trees were assessed at 1-5% infection compared with over 80% of trees in 1976.

Analysis by the Soil Bureau in Wellington showed that needles from 38-year-old *P. radaita* contained more manganese, silica, and iron than those from 3-year-old trees (Sievwright 1968). Tea blister rust was reduced by internal application of nickel (Venkata Ram 1964). Because of these findings Sievwright (1968) tested the effect of manganese, silica, nickel, copper, and iron applied to the soil of potted seedlings that had been inoculated with a Dothistroma spore suspension. The foliar levels of manganese, iron, and nickel were increased but copper and silica were not satisfactorily raised. The increase in micro-elements had no effect on infection of young *P. radiata* and all plants tested showed symptoms 8 weeks after inoculation.

In the late 1970s and early 1980s a considerable amount of work on age resistance was carried out at the Forest Research Institute. Franch & Wells (1977) examined the buffer capacity, measured at pH 6.2, of aqueous homogenates of 1-yr-old needles from *P. radiata* trees aged 5, 10, 15, 20 and 40 yrs. They found that the buffer capacity increased with tree maturity. They found that high buffer capacity of mature-tree needle homogenates did not appear to be a property directly related to mature-tree resistance to *D. pini*.

In the United States, Walla & Peterson (1976) found that needle wax was not an important factor in fungal development. Chloroform removed similar amounts of surface wax from needles of *P. nigra* and *P. sylvestris* whether or not they were susceptible to *D. pini*. Wax was similar in amount on young needles and on older needles. Germination of *D. pini* conidia was similar on naturally inoculated resistant and susceptible needles. More germ tubes grew towards stomata on susceptible needles than on resistant ones but this difference was not great enough to account for differences in susceptibility. However, Franich *et al*. (1977) showed that on *P. radiata* the surface topography and stomatal structure of 1-year-old needles differed from trees of different ages. The stomata of needles from mature trees were smaller (10-15 µm) than those from young trees (15-20 µm). While the yield of crude wax from needles of trees of all ages was similar (0.2%), there was more of the acidic component in the wax of mature tree needles. It was also found that in mature tree needles the

stomatal antechamber was frequently occluded with wax. They suggested that these wax occlusions in mature tree *P. radiata* needles may contribute to age resistance by way of a physical barrier. Franich (1977) also suggested that the wax coating may contribute to the increasing resistance to infection by *Dothistroma pini* with increasing tree age. Waxes of primary needles and cotyledons consist of fatty acids, esters and secondary alcohols. A large proportion of wax of secondary needles consists of resin acids, found in quantity in older trees. Franich suggested that they were probably involved in preventing hyphal penetration by blocking stomatal pores. Perhaps Walla & Peterson and Franich's results were compatible and may explain why age resistance is seen in *P. radiata* but not in *P. nigra* and *P. sylvestris*.

The effects of monoterpenes were then examined. Mixtures of volatile compounds were obtained from *P. radiata* foliage of populations of young *Dothistroma* susceptible and mature, resistant trees. Gas-liquid chromatographic analysis showed that the mixtures consisted mainly of 13 monoterpene hydrocarbons. Yields of volatile compounds from young trees were about twice that from mature trees. The monoterpene mixtures stimulated both the germination of *D. pini* spores, and mycelial growth when incorporated into liquid shake cultures at 10, 100 and 300 p.p.m. Inhibition of mycelial growth occurred only at 1000 p.p.m. It was concluded that the monoterpene composition does not bear any simple relationship to mature tree resistance (Franich *et al*. 1982).

Franich *et al*. (1983) then looked at the fungistatic effects of fatty acids on age resistance. Bioassays using *D. pini* as test organism showed that a long chain fatty acid (stearic), omega-fatty acids and oxidized resin acids were highly fungistatic. The compounds inhibited both spore germination and mycelial growth in vitro. The effect of the compounds on *D. pini* in vivo was studied by inoculation in growth cabinets of *P. radiata* rooted cuttings where foliage had been sprayed with acetone, and thereby depleted of epicuticular and stomatal pore fatty and resin acids. The mean infection level recorded for acetone-treated plants was about twice that of control plants, suggesting that these compounds could be pre-infection factors contributing to resistance of mature pines.

Franich's work was discontinued under directive from the New Zealand Forest Service and he never had the opportunity to confirm initial findings that epicuticular wax and resin acids were responsible for age resistance in *P. radiata*.

Interactions with other fungi

Garrido *et al.* (1982) noted that trees having ectomycorrhizae of the Russulaceae were not attacked by *D. pini*, *Diplodia pinea*, or *Armillaria*. Extracts from these fungi or from needles of mycorrhizal trees strongly inhibited spore germination of those pathogens. Needle extracts from non-mycorrhizal trees were not inhibitory.

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Physiological Needle Diseases of Pine

Margaret Dick

Introduction

The abiotic causes of premature needle death are many and varied. Some are readily identified and well-documented. These include damage caused by mineral salts, misapplied pesticides and herbicides, air pollutants, nutrient deficiencies and environmental damage. Other needle disorders are more cryptic and considerable research effort may be required to establish relationships between the effects observed and the inciting factors. The most common and well-understood causes are briefly outlined. There are many publications describing and discussing these disorders (see Sinclair *et al.* 2005) for a comprehensive range of literature). The records of cryptic damage, and the research undertaken to determine causal relationships are discussed in more detail.

Recognisable abiotic disorders

Salt - sodium chloride

Overseas where deicing salt is applied to roads damage to trees along highways is a frequent occurrence. Chronic minor salt damage to foliage of trees along coastlines occurs as spray, and aerosols from bursting bubbles at wave tops, are blown ashore. Damage from storms may extend for a considerable distance inland. Sodium, chloride, and other ions can enter a plant when it is wet and accumulate in the tissue. Needles turn brown, the necrosis beginning at the tips and progressing towards the base. Damage is caused mainly by the toxic chloride ions. Chloride is translocated towards tips of twigs and foliage where it accumulates. Sodium and other ions also enter the tissue but accumulate more slowly and their concentration in salt-damaged plants is less correlated with the extent of necrosis than is the chloride concentration. Symptoms of salt spray damage in *Pinus radiata* are of needle-tip necrosis and also of lesions along the needle length (Sinclair *et al.* 2005).

Other salts

These salts do not cause direct damage to needles, they are absorbed by the roots and the damage to needles is incidental. However, this type of damage may be easily confused with primary damage and is included here for that reason. Other inorganic chemicals that cause damage include various salts and ions of boron, copper, manganese, nickel, zinc aluminium, arsenic and lead. Chlorosis and necrosis of needle tips are typical symptoms of toxicity for several minerals and are not diagnostic for a particular ion. In New Zealand boron toxicity (death of immature needles) has been observed following applications to redress B deficiency (Will 1985).

Nutrient deficiencies

As above, nutrient deficiencies do not cause direct damage to needles. However, this type of damage may be easily confused with primary damage and is included here for that reason. The most

frequently encountered deficiencies in New Zealand are nitrogen, phosphorus, boron, and magnesium. Less common are manganese, copper, zinc, potassium, iron and calcium (Will 1985). Most deficiencies are manifest by needle chlorosis of some form. It may be uniform over the whole tree (N) or primarily in the needle tips (Mg, P, K). Shortened needles and/or needle distortion may also occur (P).

Induced deficiency can result from a variety of inputs. Root destruction (physical or disease) may lead to insufficient root mass remaining for uptake of nutrients. Silvicultural activities such as pruning may remove a source of nutrients (e.g. Mg) able to be translocated and required by developing foliage. Changing soil conditions such as pH, e.g. through rising water table after heavy rain when there is an underlying alkaline rock structure, may alter availability of some minerals. For instance, Mn becomes unavailable at pH above 6.5 (Peter Beets pers. comm.).

Herbicide

Herbicide damage is usually, but not always recognisable. Hormone herbicides, which act as plant hormones that disrupt plant processes cause aberrant growth that may be accompanied by chlorosis. Contact herbicides cause lesions of limited extent on needles and these can be mistaken for a pathogenic attack. Triazines cause needle chlorosis that may be either general or in patterns. Needle bleaching is the result of excessive Amitrole. Other patterns of needle death may also occur as the result of either uptake from the soil or from direct chemical contact (Sinclair *et al.* 2005).

Air pollutants

Severity of injury varies with concentration of the pollutant, duration of exposure, sensitivity of the plant, and environmental conditions before and during exposure. Injury can be acute or chronic. Acute injury is associated with short episodes of exposure to high concentrations of a pollutant which are rapidly absorbed and kill plant cells. Chronic injury results from continued exposure to a pollutant in amounts that do not initially kill tissues but accumulate to cause physiological disturbance. The main causes of pollutant damage are oxidants (e.g. ozone), sulphur dioxide and fluorides. Ammonia, chlorine and hydrogen chloride also cause occasional localized damage. Plants are injured by pollutants that accumulate in stagnant air during atmospheric inversions in valleys and basins, and in populated regions when there are stationary high-pressure weather systems. Several pine species in North America (*Pinus echinata, P. strobus, P. ponderosa, P. jeffreyi*) have exhibited reduced growth that correlated with increasing air pollution and could not be explained by any other means. Acute injury of conifer foliage is uncommon but has been recorded in some highly sensitive individuals of *P. strobus*. The immature needles have a zone of semi-mature tissue near the needle base that is ozone sensitive. Mesophyll cells in the zone collapse and a bleached appearance develops. A band of affected tissue forms and as the needle continues to grow this brown tissue separates the green needle tip from the green base. Necrosis then progresses towards the needle tip (Sinclair *et al.* 2005).

Lesions occurring on pine needles (*P. ponderosa* and *P. sylvestris*) within the fascicular sheath were described by Rice *et al.* (1986). Basal injury was most frequent on trees growing in areas with sources of aerial air pollution and had high levels of sulphur and fluoride within the fascicular sheath. The sulphur and fluoride concentration also increased with the age of the needles. The authors postulated that the pollutant residues dissolved in rain become concentrated at the base of the needle by capillary action and water evaporation, and are not transported within the needle by

physiological processes. Basal injury may occur as a result of acute exposure to acid deposition during the period of needle elongation, or chronic exposure over the entire period that the fascicle is retained. Injury of this nature has been induced by regular application of simulated acid precipitation (Rice *et al.* 1986; Huttunen & Laine 1983).

Air pollution is uncommon in New Zealand and has been recorded only occasionally in small numbers of trees adjacent to industrial sites.

Climatic

Severe water shortage in *Pinus* spp. during hot conditions may cause needles to lose turgor and droop at a point near the needle base where lignification is incomplete. Needles then either fade and turn brown or remain green and permanently bent. Similar symptoms can be caused by frost injury to this susceptible point of the needle, sometimes simply leaving a chlorotic band on the needle (Sinclair *et al.* 2005).

Drying of needles and twigs in winter and early spring is often linked to prior freezing injury. Symptoms include chlorosis and foliar browning. The symptoms commonly occur after warm dry windy weather (Sinclair *et al.* 2005).

Cryptic foliar disorders

A number of disorders of cryptic cause have been found, after attempts to locate and identify a pathogen or an invertebrate pest have failed, to be primarily the result of a sequence of climatic conditions. For some disorders research efforts have continued over many years yet disagreement over fundamental causal influences remains.

Red belt phenomenon

A climatically induced disorder of conifers known as “red belt” occurs episodically in parts of Europe and North America. Red belt phenomenon in its classical form consists of damage located in a narrow band at certain elevations on a mountain slope. It is caused by rapid changes in winter air temperature resulting in the freezing and thawing of tissues within a few minutes. Such injuries occur in the winter months and symptoms become apparent in late winter- early spring. There is always a preceding temperature inversion between the valley bottom and the slope above. An air stream lifts the cold air at the valley bottom to the warmer upper slopes so quickly that normal hardening cannot occur (Hansen & Lewis 1997).

Needle fleck

Needle flecks that cannot be attributed to pollutant damage, fungal infection or sap-sucking insects are extremely common, but do not appear to have any detrimental effect on the tree. Observations made in several locations have shown that flecks are more abundant on older needles and are always on exposed needle surfaces; they are common on trees of different species growing side-by-side, and are most frequent on trees growing at over 500m. The condition is therefore considered to be best characterized as winter weather injury (Miller & Evans 1974).

Needle droop

An abnormal drooping of the needles of current season growth of *P. resinosa*, resulting in premature loss of most of the affected needles was recorded in a number of locations in the USA during the 1930s (Davis *et al.* 1937). Current season needles were bent sharply downwards, the bend occurring within the needle sheath. Needles were still green when drooping began, later they died and turned brown, most of them remaining attached. There was no sign of external injury and no evidence that insects or microorganisms were responsible. The symptoms were observed in late summer to early autumn. Over a number of years the condition was correlated with atypical weather conditions. Rainfall considerably above average fell in early to mid summer followed by above average temperatures and below average rainfall for the remainder of the summer..

Patton & Riker (1954) carried out further research into this disorder. They observed that on the affected shoots damage was greatest on needles at the tip and decreased towards the base. If the entire shoot was not killed sometimes needles at the base were only lightly affected. Lesions, often resin-filled occurred on some of the needles at the droop point. The tissue at this point was often swollen and the needle epidermis split exposing a brown cork-like tissue. In a range of trials they exposed trees to drought, water-soaking, beating artificial rain or high temperatures (36-43°C). Droop symptoms only occurred under conditions favouring rapid drying of succulent tissue, with the most severe and rapid symptoms developing under high temperatures. Under conditions of water shortage plus air currents that would promote rapid transpiration needles began to respond within a few hours.

Patton and Riker (1954) suggested that soil type and water relations of affected trees are directly involved in the cause of typical symptoms. Affected plantations were on sands or sandy loams and such soils generally have a narrow range of available water between field capacity and the permanent wilting percentage. Capillary movement is slow in soils drier than field capacity – it is probable that during periods of rapid transpiration the available water on soil particles in contact with the roots is removed much faster than it can be replaced by capillary movement. Thus there may be a zone around each absorbing root from which all available water has been removed. The ratio of absorption to transpiration is therefore a basic factor in the etiology of needle droop. Another factor is the degree of succulence of the tissues which is correlated with the weather conditions and may differ from year to year. When a combination of factors favourable to transpiration occurs, such as the high air temperature and low RH of a hot dry wind on a sunny day, a high rate of transpiration may be induced. This leads to an absorption lag, resulting in a serious internal water deficit and loss of turgor of succulent tissue.

Bergdahl and French (1976) believed that needle droop resulted from the inability of poorly developed root systems to acquire sufficient water during periods of moisture stress. Three plantations (aged 3, 4 and 8 years) were examined over two consecutive seasons with similar results obtained from each age class. Neither biotic factors, nor the widely believed summer frost theory were found to be able to account for the condition. Affected trees had poorly developed or malformed root systems. Symptoms were also correlated with coarse sandy soils that did not hold moisture well, and with competition for water from herbaceous groundcover plants. The poor root symptoms were attributed to either improper nursery practices before planting or to poor planting technique.

Semi-mature needle blight (SNB)

Foliage injury of eastern white pine (*P. strobus*) has been reported throughout the natural range of the species (in the USA) and has been discussed under a variety of names. This physiological needle blight is characterised by an orange-red discolouration of the distal portion of current season's needles (Linzon 1960; Dreisbach & Merrill 1990). Not all needles in a fascicle are affected, and affected needles may differ in the proportion of the needle killed. Affected needles remain green at the bases and remain attached to the shoots until normal shedding of the needles (Wenner and Merrill 1998). It was originally thought that the tips of the needles were affected first and they then progressively died back. However Linzon (1960) established that the blight starts part way along the needle in semi-mature tissue and the injury then spreads distally to the needle tips. Neither mature nor immature tissue was initially affected. Effectively the needle is girdled at one point and as a result the tissue beyond the dead band is killed. Needles continue to develop from the basal meristem until they reach maturity. The extent of the damage on individual needles depends on the age of the needle and the position of semi-mature tissue along the length of the needle at the time that the damaging environmental condition occurs (Linzon 1967). Successive attacks may occur in one season with new bands forming on needles that already exhibit dead distal portions.

The structure of semi-mature tissue and the way it differs from immature and from mature tissue was described by Linzon in 1962. Semi-mature tissue is distinguished by the start of the suberisation of the transverse and radial walls of the endodermal cells. There is no semi-mature tissue in the first two weeks of growth and these young needles are not susceptible to injury. The semi-mature tissue then occurs at the needle base until the needle stops growing after which the needle tissue is mature and not susceptible to attack.

The destruction of the semi-mature tissue has been attributed by various authors to ozone, sulphur dioxide, heat and water stress (Sinclair *et al.* 2005). In different years outbreaks of the disorder occurred at different times through the growing season. Sequences of weather conditions have been found to be correlated with the appearance of symptoms (Linzon 1960; Spaulding & Hansborough 1943)).

Wenner and Merrill (1998) re-examined the phenomenon and stated that their longitudinal sections showed that fungal hyphae were consistently associated with the disorder, and that one fungal species, *Canavirgella banfieldii*, which has a one-year life-cycle, predominated. Pathogenicity tests were not conducted but Wenner and Merrill (1998) suggested that the timing of fruit body maturation and spore release was consistent with the apparent timing of infection and symptom development observed on symptomatic trees in the field. They implied that the disorder is, in fact, due to fungal infection. No other studies that might clarify this further have been sighted.

Spring needle-cast of Pinus radiata in Tasmania

Spring needle-cast of *Pinus radiata* is described as the rapid browning and collapse of mesophyll tissues of 1-year-old needles in spring followed by premature needle casting. It first appears about the time of canopy closure and occurs in stands that receive between 1200 and 2000 mm annual rainfall. Foliar sprays with chlorothalinol improved foliar retention (Podger and Wardlaw 1990a) indicating that one or more fungi are important components of the disorder. Podger and Wardlaw (1990a) postulated that undefined environmental conditions seasonally induce ephemeral stress which stimulates a ubiquitous endophyte, or a complex of endophytes, to secondary pathogenic activity. They rejected the results of studies in New Zealand that demonstrated that a similar

disorder was the result of infection by *Cyclaneusma minus* during wet autumn periods; the infection followed by a latent period over winter and symptom expression in the spring (Gadgil 1984).

Podger and Wardlaw (1990b) experimentally examined the role that deficiencies in various nutrients might play in the spring needle-cast disorder but could demonstrate no relationship. They also looked at the combined effects of fertiliser application and an early thinning regime that reduced stand density and increased air movement within the stand. Incidence of the spring needle-cast disorder was significantly lower in thinned treatments but this effect was not sustained long-term. It is unclear whether the short-lived effect was due to crown closure or some other factor.

Xylem wall collapse in water-stressed pine needles

Pines respond to soil drought by closing their stomata to reduce water loss. Stomata close in response to hydraulic signals with early cavitation events in the leaf veins triggering the closure (Sperry 2000; Salleo *et al.* 2001; Cochard *et al*. 2002).

Cochard *et al.* (2004) microscopically examined the response within needles of four *Pinus* spp. to drought conditions and observed .collapsed tracheids in the dehydrated needles of all four species. An overall shrinkage of the needle occurred as the xylem collapsed which was found to be the result of negative sap pressures, rather than compressive forces exerted by the surrounding tissues. (Tyree, 1976).

Xylem cell wall collapse in *P. nigra* needles was found to precede cavitation in the stem. This needle collapse can be reversed though the length of time that collapse of tracheid walls may be sustained without permanent needle degrade is not known. Cochard *et al.* (2004) suggested that the needle response was triggered by minor cavitation occurring in the stem and that the stomatal closure at that point had the effect of considerably increasing tracheid hydraulic resistance to water flow therefore minimizing the risk of xylem dysfunction in more downstream parts of the tree.

Diagnosis of physiological needle disease

The macroscopic responses of pine needles to various physiological stresses can appear very similar regardless of the causal factors. Drought, temperature stress, salt or herbicide toxicity and air pollutants cause much the same type of injury. Even when good historical background data is available causes are not always definitive. Accurate field diagnosis can thus be extremely difficult and is further complicated by differences relating to age of needles, the season in which injury occurs and differing susceptibility from genetic variation. Stewart *et al.* (1972) attempted to distinguish types of injury using histological techniques and examined needles of *Pinus ponderosa, P. strobus, P. sylvestris* and *Pseudotsuga menziesii*. Their macroscopic observations indicated that differences between the types of needle necrosis induced by different stress factors were inconsistent; often more variation occurred in the kind of kind of necrosis caused by a specific stress than between stresses. Generalisations could be made: for example salt toxicity produced tip and intercostal necrosis with necrosis sometimes developing both distally and basally and ozone produced a characteristic chlorotic or necrotic fleck.

Microscopically there were many similarities. All stresses caused a general hypertrophy of the epithelial tissue of the resin canals. Granulation of mesophyll cells was also a general response. Hypertrophy of phloem cells and transfusion parenchyma was caused by all stresses in some

instances but was most consistent for necrosis caused by natural senescence, drought or fluoride pollution. Collapse of the mesophyll cells best characterized necrosis caused by ozone, sulphur dioxide, salt or boron toxicity. Stewart *et al.* (1972) concluded that the best value of histological studies was the elimination of certain pathogens, thereby narrowing the possible causal agents. Examination could also ascertain when certain air pollutants were not responsible for a particular injury; for example, the absence of mesophyll collapse can eliminate sulphur dioxide as a cause of necrosis. Conversely, however, the presence of mesophyll collapse, because of its ubiquitous nature, could not establish aetiology. Stewart *et al.* (1972) concluded that despite a few specific variations the response of pine needles to most stresses is fundamentally similar and the application of pathological anatomy to field diagnosis appears to be limited.

Wenner and Merrill (1998) in studying the needle blight of *P. strobus* commented that although they made cross sections of conifer needles as part of their study these were of limited value. They felt that longitudinal sections, although properly oriented sections are extremely difficult to make, were much more informative and were necessary to detect fungal hyphae prior to tissue collapse. Using this method they compared longitudinal and cross sections from needles collected from the field and with those from trees experimentally subjected to high levels of ozone. They confirmed the conclusions of Stewart *et al.* (1972) that many of the changes within the needles were a general response to abiotic stress and were therefore not diagnostic. Their studies of field collected material led them to conclude that histological examination of pine needles can serve as one more diagnostic tool when diagnosis is sufficiently critical to warrant the time and cost. However examining such a small portion of a needle, or a tree, can only supplement and never substitute for a thorough field diagnosis based on the total syndrome.

Physiological needle blight (PNB) of Pinus radiata in New Zealand

Periodic severe seasonal needle cast of radiata pine of unknown origin has been recorded in a number of locations throughout New Zealand since the early 1980s.

Symptoms of the disorder are:

- Red-brown discoloration over a large part of the crown – visible from at least 500 m distance. From this distance the disorder may easily be confused with Dothistroma needle-blight.
- Drooping needles. Current needles are not affected, one-year-old needles often form khaki or red-brown bands in the early stages of decline, then turn entirely red-brown, droop from the branches but (unlike Cyclaneusma needle-cast) still remain firmly attached. Cyclaneusma-like yellowing is not present.
- Localised distribution. Nearly all trees in parts of affected stands may be diseased. Often trees growing in gullies and gully systems running down from ridges are most severely affected (Figs. 9 and 10). Disease is more localised than that caused by Cyclaneusma needle-cast where affected trees are usually scattered throughout the entire stand.
- Disease first appears in winter and peaks in September/October/

Fig. 9 – Distribution of PNB in an affected stand of *Pinus radiata* in Northland (29 Sept 2002)

Fig. 10 – Older trees affected on the East Cape, with typical red-brown affected foliage (18 October 2005).

Affected needles in the early stages of breakdown often have one or more khaki-coloured or red-brown bands, at any point along the length of the needle. In other cases the whole needle discolours in a more uniform fashion. Drooping dead needles remain attached to shoots and twigs for some time. Casting of many of the affected needles subsequently occurs and in some cases individual trees may lose all of their foliage. The symptoms usually appear from late winter to spring with new defoliation not usually occurring during summer. The newly flushing foliage is generally not affected. Severe outbreaks of this disorder are almost always restricted to trees older than 12 years. Symptoms can look dramatic and the cause of some concern for forest owners. Trees that suffer repeated episodes of late winter defoliation will remain thin-crowned. Where episodes are less frequent, recovery of the crowns occurs.

Table 2 – Locations where PNB outbreaks have been reported

Biological Region	Year	Location	Comments
Auckland	1995	Mahurangi and Onetai	Widespread needle-cast
	1998	Mahurangi	Heavy needle-cast
Bay of Plenty	1995	Mamaku complex, Matata, Manawahe, Rotoma, Paengaroa complex, Rerewhakaaitu	Localised in higher part of block. Unusual needle-cast.
	1998	Kinleith, Endean, Manawahe, Rerewhakaaitu	Needle-cast that looks like Dothistroma from a distance, orange colour. Heavy needle-cast.
	1999	Rotoehu	Foliage brick red. Similar in appearance to Strasseria.
Buller	1998	Nemona	Moderate to heavy
	2002	Paparoa	Moderate to heavy in localised areas
Coromandel	1998	Tairua	
	2002	Coroglen, Tairua, Whangapoua	As seen from an aerial survey
Gisborne	1981	Wharerata	Puhipuhi-type dieback
	1983	Wharerata	Unknown top and tree death, as seen in 1981
	1985	Wharerata	Severe needle-cast. Looks like Dothistroma – needles retained but brown and drooping.
	1995	Wharerata	Trees dying from bottom upward. Symptoms from air and ground are Dothistroma-like.
	2000	Wharerata	Typical Strasseria/Cyclaneusma needle-cast
Northland	1981	Glenbervie Puhipuhi	Needle browning and dieback
	1984	Glenbervie Puhipuhi	Needle browning and death
	1989	Glenbervie Puhipuhi	Needle browning and death
	1995	Glenbervie Puhipuhi	Very heavy needle-cast, needles are rotting
	1996	Waipu	Needle-cast, same as seen at Mahurangi in Nov-Dec 1995.
	1998	Karaka, Oromahoe, Pouto, Waimatenui, Waipu	Heavy needle-cast. Up to 90% damage.
	2000	Avoka, Forsythe Downs, Kairara, Okaharau, Opouteke, Waipu	Heavy needle-cast. Up to 65% severity.
	2001	Glenbervie, Mangatawa, Maropiu, Taheke, Te Hapua	Heavy needle-cast – a combination of Strasseria and Cyclaneusma.
	2002	Maropiu, Maungatapere, Monteiths, Opapaki, Utakura, Waima, Waipu, Whangarei	Varying intensity, with higher parts of the stand affected more. Strasseria very prevalent. Heavy incidence. Heavy incidence and severity.
Nelson	1998	Lee Valley, Richmond Hills	Unusual needle-cast.
Rangitikei	2002	Paparangi, Waimarino	Heavy needle-cast. Hard to tell if Dothistroma or Strasseria. Heavy infection at base of gully.

Marlborough Sounds	1995	McLaren Bay	Unusual Cyclaneusma symptoms as seen in BP and East Coast.
	2002	McLaren Bay	Many trees with dead needles up to 80% along the road. Almost looks like fire scorch.
Taranaki	1998	Coulson Rd, Egmont Village, Lake Mangamahoe0	Stand edge trees show a higher level of needle-cast, some trees almost 100%. Majority of trees showing severe needle death. Typical of symptoms found in this region this spring and early summer.
Taupo	1995	KK	Dead needles on lower crown, Not Dothistroma, needles retained on tree.

Biological Region	**Year**	**Location**	**Comments**
Taupo	1998	Kinleith, Whakamaru	Unusual Cyclaneusma symptoms, red expression. Looks like Dothistroma. Needle-cast symptoms look similar to Dothistroma from a distance.
Westland	1998	Butlers, Kaniere, Mahinapua, Waimea	Heavy defoliation. Some trees have 80% defoliation. Dieback is on-going, but not Dothistroma, although symptoms are very similar. Severe needle-cast, looks as though needles are rotting on the branches.
	2002	Waimea	Strasseria infection
Wanganui	1999	Lismore	Bad needle-cast in gullies and heads of gullies. Looks like Dothistroma from a distance. Up to 80% needle-cast. Localised on either side of the gully.
Wellington	1996	Gordon Kear	Unusual symptoms, like those seen in Crohane last year.
	1998	Puketiro, Spicer, Valley View	Needle-cast very pronounced, worst seen in years.
	2000	Valley View	Needle-cast.
Waikato	1998	Onewhero	Localised heavy infection. Needle-cast widespread in this block.

Several fungi form fruiting bodies on the needles as the tissue collapses. Those most commonly identified are *Strasseria geniculata*, *Ceuthospora* sp, *Lohodermium* spp., *Cyclaneusma* spp., *Phomopsis* spp, *Sclerophoma pityophila, Pestalotiopsis funerea* and *Aureobasidium* spp. Isolations made from the needles in the early stages of discolouration yielded those listed and a number of other non-sporulating fungi that have not been identified. Those identified are all widespread in New Zealand and have a role in the breakdown of senescent tissue. They may also act as pathogens (albeit weak) when environmental conditions are stressing the host plant and favouring fungal growth. When this condition was first examined in New Zealand *S. geniculata* was so frequently associated with the early stages of needle breakdown that it was considered to be a likely causal agent. Over time, as it became apparent that the *S. geniculata* association was episodic and that during some outbreaks other fungi predominated, this view was discarded.

A study of historical records carried out in 2002-03 (Bulman & Dick 2003) showed a correlation between outbreaks of the disorder and prolonged rainfall during June or July. In the absence of a clear pathogenic agent they suggested that the disorder may be physiological in origin. In experimental work to test the theory that prolonged needle wetness might be responsible (Dick & Bulman 2004) *P. radiata* cuttings with a physiological age of seven years, and from four clones, were placed under a spray system which provided regular, though not continuous, water to the foliage for 20 weeks. Needles were wet for an average of 120 hours of each week. Needle discolouration was not observed in the first eight weeks but after that irregular browning on an increasing proportion of needles began to occur. Microscopic examination of needles with discoloured, but still turgid, zones showed loss of plasmids from the mesophyll cells and the

collapse of some of these cells. There was no measurable difference between the four clones. Isolations were made from both green and discoloured needles and only known saprophytes (most frequently *Pestalotiopsis* sp. and *Lophodermium conigenum*) were obtained.

Fungicide applications reduced the level of needle death in trees subjected to prolonged periods of needle wetness. Some fungal endophytes that had been associated with the disorder in the field were not present in needles, as plant had been reared in the glasshouse away from air-borne spores. The observed effect of the fungicide treatment may be elimination or reduction of a fungus, or a suite of fungi, that are capable of accelerating the breakdown of tissues severely stressed by abiotic conditions. Once needle deterioration has begun, for whatever reason, non-pathogenic fungi can accelerate the breakdown.

The condition is currently referred to in New Zealand as 'physiological needle blight' or 'PNB'. Current research is directed towards determining if the disorder could be due to restricted water uptake because of root death in anoxic soils. The effect of weather conditions over winter on the root-shoot water potential gradient in radiata pine was examined in the field during 2006 and 2007. Root temperature, air temperature, and humidity have been suggested as factors which may affect the plants' ability to maintain a suitable water potential gradient between roots and shoots. To date this research has not provided useful results because weather thought to be conducive to PNB was not experienced in the forests where experiments were established and the disease did not appear. This work is ongoing.

The causes of non-biotic needle breakdown outlined in this review have been considered as potential causes of the physiological needle blight of radiata pine in New Zealand. Most have been quickly discounted as the distribution of the symptoms spatially and over time show no correlation with herbicide applications, air pollution or nutrient deficiency.

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Fungal Endophytes in Conifers

Rebecca Ganley

Introduction

This section does not discuss pathogenic fungi but focuses instead on endophytic fungi. As such the reader may wonder why this chapter was included in a review of needle cast diseases in New Zealand. Endophytes may play a future role in control of needle cast diseases and the chapter was included here to provide an overview of fungal endophytes in trees.

Specialised pathogens are not alone in infecting plants. Endophytic fungi also infect plants, although as non-pathogenic colonists. Traditionally these endophytic fungi have often been viewed as fungal pathogens that have colonised their host in a cryptic or latent manner. However, recent studies have shown that endophyte assemblages present in the host tree are closely related to, but distinct from fungal pathogens (Ganley *et al.* 2004). Although some pathogens can live for a short period of time without causing disease symptoms in their host, i.e. *Fusarium circinatum* (Storer *et al.* 1998) or may not cause disease on some hosts within susceptible species ever, i.e. *Cyclaneusma minus* (Gadgil 1984), colonisation of that type, often termed endophytism, is fundamentally different from the behaviour of a true endophyte. In essence, pathogenic fungi that undergo a latent phase in their life cycle could be considered "casual endophytes" versus "permanent endophytes" which live their entire life within the host and are not known to have a pathogenic state. For the purpose of this review, only those fungi that would be considered permanent, non-pathogenic endophytes will be considered.

Function of endophytes

Fungal endophytes have been ubiquitously identified in the living tissue of all woody trees studied to date (Carroll & Carroll, 1978; Petrini & Carroll, 1981; Arnold *et al.* 2000). A mutualistic relationship between the endophytes and the plant host has been suggested as the endophytes live within the tissue without causing any apparent disease symptoms (Carroll 1988, Petrini 1991). It has been hypothesised that the plant provides nutrients to the endophytes and, in return, the endophytes may confer resistance to the host from attack by pests or perform other beneficial functions (Miller 1986; Hata & Futal 1995; Arnold *et al.* 2003). However, the ecological context of endophytes may be considerably broader than their mutualistic role with the host plant. In some non-conifer plant species, endophytic fungi have been shown to perform novel ecological functions (e.g. thermotolerance (Redman *et al.* 2002)), influence community biodiversity (Clay & Holah 1999, Matthews & Clay 2001), and directly enhance plant growth (Ernst *et al.* 2003). It is likely that fungal endophytes in conifer species could provide similar functions.

Only a few studies have looked at the function of fungal endophytes in conifers. In *Pinus monticola*, induced resistance conferred by foliar fungal endophytes, has been demonstrated against the pathogen *Cronartium ribicola*, the casual agent of white pine blister rust. Seedlings colonised with native assemblages of fungal endophytes had greater survival rates than control seedlings and the levels of resistance observed were equivalent to those currently selected in polygenetic breeding programs for white pine species against this disease (unpublished data). The exact mechanism

behind this induced resistance is unclear. It has been proposed that this form of resistance occurs when the endophytes stimulate an induced systemic resistance (ISR) response, which is an enhanced defensive capacity (Delaney 1997). Whether the biochemical pathways activated by this resistance response are the same as those triggered by major gene or polygenic resistance is unknown. Nevertheless, induced resistance is often considered durable resistance, as once activated it provides resistance for a prolonged period and often against multiple pathogens (van Loon *et al.* 1998). It is possible that application of this form of resistance could help stabilise traditional breeding practices by applying multiple defences for pathogens to overcome.

A similar mechanism for defence against insects by fungal endophytes has also been observed. In Balsam fir (*Abies balsamea*) and red spruce (*Picea rubens*) needles, several endophytes have been isolated that produce secondary metabolites that are toxic to spruce budworm larvae (*Choristoneura fumiferana*) (Clark *et al.* 1989). The exact mechanism behind this resistance is still not understood, although a mutual antagonism between the host and the endophyte fungus has been suggested. Studies between plant host calli and the endophytes have shown that the endophytes excreted non-specific herbicidal metabolites which caused necroses, growth inhibition and death of the host calli (Peters 1998). Furthermore, the host calli were shown to produce non-specific antifungal metabolites.

In addition to resistance, fungal endophytes in conifers may be required for early tissue decomposition. The presence and position of these fungi in the needles makes them ideal contenders as early decomposers (Müller & Hallaksela, 1998). After initial infection of the host, the growth of the endophytes usually diminishes and is restricted to very small tissue areas (Suske & Acker 1986; Deckert *et al.* 2001). It is likely that the endophytes remain in this slow growing state until injury or natural senescence of the needle triggers growth and proliferation.

There are vast arrays of other functions that endophytes in conifers could potentially be involved in. However, eliciting these roles can be difficult due to complexity of the research as well as the long-lived nature of conifer species. Nevertheless, the importance of fungal endophytes in mediating many ecological processes has been demonstrated in some conifers as well as a variety of other plant species. Essentially these endophytes are providing an extended genotype for their hosts and form an integral part of the extended phenotype or symbiotic community of a plant (Whitham *et al.* 2003).

Diversity and abundance

Fungi can encompass the range of known symbiotic lifestyles with their hosts and, potentially, indirectly with their community. Thus, identifying what components of the fungal community are present within a host system is central for determining how they can influence overall growth and productivity. The majority of plant endophytes that have been identified are ascomyceteous, although, within this class there is a high degree of diversity of fungal species (Frohlich & Hyde 1999; Arnold *et al.* 2000). In general, endophytes are considered selective and are usually limited to only one or few taxonomically closely related hosts. However, certain genera of fungi have appeared regularly in the majority of host trees sampled. In particular, these have included; *Cryptocline, Cryptosporiopsis, Hormonema, Lophodermium, Phomopsis, Seiridium*, and *Phyllosticta* (Carroll & Carroll 1978, Petrini & Fisher 1988, Hata & Futal 1996, Stone *et al* 1996). The isolation of ascomyceteous fungi alone is likely to be a gross underestimation of the total diversity present as a total DNA approach in grass, versus culture-based analyses predominantly

performed in trees, yielded sequences that represented all fungal phyla (Chytridiomycota, Zygomycota, Basidiomycota and Ascomycota)(Vandenkoorhuyse *et al.* 2002).

Certain genera of fungi have appeared habitually in the majority of conifer hosts and have been the focus of most studies. Within the *Pinus* genus, members of the *Rhytismataceae* have been the most abundant endophytic fungi isolated from needles (Carroll & Carroll 1978, Hata & Futal 1996, Deckert and Peterson 2000, Guo *et al.* 2003, Ortiz-Garcia *et al.* 2003, Ganley & Newcombe 2006). However rare or undefined endophytes, which have also been isolated, are often overlooked or considered inconsequential. For instance, in *P. monticola*, western white pine, Carroll & Carroll (1978) identified only one endophyte, *Leptostroma*, and another study in an arboretum in Japan, only the non-rhytismataceous endophyte *Phialocephala* was isolated (Hata & Futal 1996). Yet, in a more recent, sequence-based study, at least 21 discrete taxa and 10 different orders of fungi were identified (Ganley & Newcombe 2006). Although the majority of these endophytes were Rhytismataceous, many of these endophytes were novel or highly divergent from other known taxa. It is likely that the rare endophytes could be highly host specific and it has been postulated that they could have important specialised roles within the host and potentially, the surrounding forest community.

Investigation of fungal endophytes in tropical plants has shown a high species richness (Arnold *et al.* 2000, Frohlich and Hyde 1999), which has led to the hypothesis that these endophytes may be hyperdiverse. It is possible that the same could be true for temperate forest systems. Although early studies only identified a few fungal species in temperate forest trees(Carroll & Carroll 1978, Hata & Futal 1996), more recent studies have identified a plethora of fungal endophytes (Ganley & Newcombe 2006, Kauhanen *et al.* 2006, Stefani & Bérubé 2006). Furthermore, most studies have only focused on needle endophytes, not taking into account the fungal assemblages present in other tissue types such as bark, stems or roots. When investigated, such other tissue has also found to contain a wide array of fungi (Petrini & Fisher 1988, Hoff *et al.* 2004).

Although fungal endophytes have been isolated from all plant species investigated, the identification of these endophytes is clouded in ambiguity. In the absence of traditional species delimitations appropriate for endophytes, estimates of their diversity have been based on the 'morphospecies' concept or morphological similarity to known species. As fungal endophytes frequently have highly variable morphology, lack sexual reproduction structures and frequently do not sporulate in culture, identification based on these methods can be problematic. Furthermore, identification is hampered by the low number of known fungal species, the majority of which are generally pathogens as these are the conventional focus of most mycological systematists. Such processes can also be extremely time-consuming, particularly when novel species are involved. Alternatively, identification based on the ribosomal DNA (rDNA) region of the genome (Tehler *et al.* 2003, White *et al.* 1990) provides an efficient and dependable method of identifying taxa, especially so when combined with traditional methods. Sequence-based analyses have been central in resolving the previous assumption that endophytes are known or delimited parasites that are undergoing a latent phase, for which environmental or host cues might trigger overt parasitism (Ganley *et al.* 2004). Instead, it was shown that fungal endophytes are genetically distinct from known pathogens of their host plant or its congeners, even though they may be morphologically similar. These methods have also shown that the high numbers of endophytes isolated from plant tissue are likely to be a series of genetically distinct individuals rather than propagation of one genetic clone (Ganley & Newcombe 2006). Such findings have huge implications for understanding the dynamics of these symbionts in their hosts and their influence on the surrounding community.

Endophyte transmission

The majority of fungal endophytes that have been studied in woody trees are believed to be transmitted horizontally via asexual spores based on the low frequency of fungi present in seed tissue (Ganley & Newcombe 2006, Bloomberg 1966, Pugh & Buckley 1971; Wilson & Carroll 1994) and the lack, but successive colonisation, of endophytes in first year leaves (Johnson & Whitney 1989, Wilson 1996, Kaneko & Kaneko 2004). Although a few endophytes have been isolated from seeds of woody plants (Bloomberg 1966, Pugh & Buckley 1971, Wilson & Carroll 1994, Ganley & Newcombe 2006) it is unknown whether these endophytes are systemic within the host. Nevertheless, the ecological importance of fungi in seed is unquestioned, as such seedborne endophytes are disseminated with their hosts, and thus have a unique opportunity to develop in young seedlings. For the fungal endophytes that are not seedborne, spores are thought to disseminate through air or water (Wilson & Carroll 1994), although, it has been speculated that some insects can disperse endophyte inoculum. Infection of the endophytes is limited to discrete regions of tissue where they remain in a slow growing state in the host accumulating very little fungal biomass areas (Suske & Acker 1986). It is then thought that senescence of the leaf triggers the endophytes to grow and sporulate. However, no studies so far have found a correlation between endophytes isolated from healthy needles and those found in needle litter. The transmission of endophytes in woody trees contrasts with grasses, where endophytes are predominantly seedborne and spread through the plant producing substantial fungal biomass (Clay & Schardl 2002).

Needle colonisation: *Within and between hosts*

In all plant hosts studied to date, multiple species of fungal endophytes have been identified from leaf tissue. In conifers, fungal endophytes have been shown to be restricted to discrete portions of tissue in the needles where they remain in a slow growing state, accumulating very little biomass (Suske & Acker 1986). Micrographs of fungal endophytes in needles have shown very limited mycelia of endophytes within the needles (Sherwood-Pike *et al.* 1986, Suske & Acker 1986). It is assumed that the endophytes remain in this state until injury or natural senescence of the needle triggers growth and proliferation. Restricted infections allow for many different species of fungal endophyte to occupy the same needle, especially if specific endophytes are localised in the apoplast and others in the symplast (Stone 1987, Stone 1988, Boyle *et al.* 2001, Ganley & Newcombe 2006). Thus, it would be expected that each needle could potentially host a diverse array of endophytes.

Fungal endophytes that have been isolated from the same needle have been found to be genetically distinct, which suggests that the high frequency of endophytes present is not a single genetic clone that has propagated through out the needle (Ganley & Newcombe 2006). Instead the numerous endophytes are most likely to have occurred from independent colonisation events. Furthermore, multiple infections of needles with different fungal strains could be species specific. Some studies have shown that endophyte frequency along the needle is dependent on the host species. In eastern white pine, the distal portion of the needle was found to produce the most hyphal outgrowth (Deckert & Peterson 2000), whereas, in black spruce the proximal region of needles contained the most endophytes (Johnson & Whitney 1989). Thus, it is likely that endophytes are highly specific at the host-species level and, as well, among the host population.

The frequency and diversity of dominant as well as other less frequent fungal species present in the needle tissues is a result of complex interactions of stand age, geographical location and seasonal influence. In general, older trees contain a more diverse and dense array of endophytes than younger trees (McCutcheon & Carroll 1993). Furthermore, the incidence of colonisation of fungal species

increases with needle age, with bud and current-year needles more likely to be endophyte-free than other needle age groups (Johnson & Whitney 1989). However, younger trees can acquire comparable diversity when exposed to inoculum from older trees (McCutcheon & Carroll 1993). This is attributed to an increase in the diversity and exchange of wind-blown spores between trees due to the increased canopy height of older stands. In support of this, analysis of seeds has shown that the frequency of endophytes is very low, yet the diversity and density of endophytic fungi in young seedlings from old growth forests is high (Ganley & Newcombe 2006). Thus, the majority of fungal endophytes must disseminate horizontally and widespread colonisation of the needles must occur within the first few years after germination.

The diversity of endophytes from trees within managed stands or from isolated trees is significantly lower than that observed in trees located near old growth stands (Ganley & Newcombe 2006, McCutcheon & Carroll 1993). Studies have also shown that there is an unnaturally low diversity of endophyte inoculum and fungal strains in large clear-cut regions that have been replanted with nursery trees (Petrini 1991). Likewise, the diversity and type of fungal species present in trees in urban plantings and exotic locations is quite different from that occurring naturally (Ganley & Newcombe 2006, Petrini *et al.* 1982, Stone *et al.* 1996). The endophyte assemblages found in seedlings from nurseries has also been shown to be very different from seedling in forest locations, although once outplanted in natural settings it is likely that the nursery seedlings would acquire similar arrays of endophytic fungi (Ganley & Newcombe 2006). The ability of trees to acquire high levels and a wide array of fungal endophytes in exotic locations was highlighted in a recent study of *Larix sibirica*, Siberian larch, where the highest diversity of fungal endophytes was found in an old, introduced stand rather than in a natural area of larch (Kauhanen *et al.* 2006). Other factors that have been shown to affect endophyte colonisation are seasonal influences and elevation. Carroll and Carroll (1978) showed the infection frequencies of endophytes in Douglas-fir collected over a wide geographical range were positively correlated with elevation. The distribution of rainfall is also closely correlated with infection levels as moist conditions are required for germination of endophyte spores on the leaf tissue (Collado *et al.* 1999, Wilson & Carroll 1994).

Implications for import/export

Fungal endophytes provide a multitude of issues surrounding the importation or exportation of plant material. In many instances because fungal endophytes are morphologically similar to known pathogens, confusion can arise in the identification of different species or *forma specialis*. This was recently highlighted in Citrus in which a ubiquitous endophyte of many woody plant species was confounded with the citrus black spot fungus, *Guignardia citricarpa* (Baayen *et al.* 2002). The non-parasitic endophyte does not cause citrus black spot, yet it was subject to needless quarantine restrictions. The implementation of molecular identification tools has helped with the monitoring of this pathogen. Likewise, the importation of material that contains fungal endophytes is also problematic if these organisms are unknown or restrictions could be applied if they contain new introductions of biological organisms. However, as many of these fungal endophytes may be beneficial to their hosts, this could have adverse effects. Also possible is the hybridisation of an endophyte with a closely related pathogen resulting in an increased host range. These issues highlight the importance of understanding the function and diversity of endophytes present in forest communities, both native and exotic, so informed decisions can be made in regards to their importation status.

Discussion

Fungal endophytes represent a substantial but widely unrecognised component of forest communities, essentially providing an extended phenotype for their hosts. Fungal endophytes have been shown to be involved in mediating resistance within their host and it is likely that further studies will demonstrate a role in a diverse array of mutualistic functions. The interaction of symbionts and their hosts is undoubtedly an intriguing and cryptic relationship. Furthermore, the existence and recognisation of this symbiotic community provides a new layer of interaction for understanding and discerning plant-pathogen systems but also highlight the importance of endophytic fungi in diversity-ecosystem function roles.

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Cyclaneusma Needle-Cast

(refer to Bulletin 222 for more detail)

Lindsay Bulman

Pathogenicity

In a pathogenicity trial, rooted cuttings of three clones taken from 7-year-old trees and seedlings of *Pinus radiata* were inoculated with ascospores and macerated mycelia of *Cyclaneusma minus* and kept for 3 months in growth rooms at different temperatures. All cuttings belonging to two clones exhibited typical symptoms of the disease and the fungus was re-isolated from needles taken from these cuttings. Both types of inocula were effective. Needles from cuttings of the third clone yielded very few isolates of *C. minus* and they did not show any symptoms of the disease. Seedlings were not infected by the fungus. It was concluded that *C. minus* is pathogenic to older plants but not to seedlings of *P. radiata.*

Infection period

Samples of needles cast in a 10-year-old stand of *Pinus radiata* were collected over fortnightly periods and the numbers of spore-bearing apothecia present in the litter layer were determined. There were two peak periods of needle cast - a major one in spring and a minor one in autumn. The greatest numbers of apothecia were found in autumn-winter (May to August). Records from a Hirst spore trap set up in the same stand showed that airborne ascospores of *Cyclaneusma minus* occurred most frequently in autumn-winter and that ascospore release was dependent on rainfall. Monthly isolations from needles showed that current season's needles were first colonised by *C. minus* in autumn-winter (May-June) when they were about 8 to 9 months old and by *Lophodermium* spp. about 2 months later. Most of the infected needles were shed when they were about 1 year old but some were retained until the following winter. The peak infection period is during autumn-winter, if rainfall is frequent and daily mean temperatures are above 10^{o}C during that period then severe needle-cast may be expected the following spring.

Cyclaneusma species variation in New Zealand

Collections of *Pinus radiata* needles showing symptoms of Cyclaneusma needle-cast were made over two periods (1977 to 1983 and 1996 to 1998) throughout New Zealand. Differences in apothecium length and characteristics of *Cyclaneusma* grown on agar cultures indicated that there are at least two morphological types of *C. minus* in New Zealand; these are termed *C. minus* 'verum' and *C. minus* 'simile'. *Cyclaneusma minus* 'verum' was the most common type identified and *C. minus* 'simile' was found more often in the North Island (particularly the central North Island) than in the South Island.

Distribution of Cyclaneusma needle-cast

Fifteen forests totalling 70,000 ha of *Pinus radiata* were surveyed for Cyclaneusma needle-cast in 1983, 1984, and 1985. Disease severity was highest in the 11- to 20- year-old stands and lowest in the 1- to 5-year-old and over 25-year-old stands. Disease severity generally increased from 1983 to 1984 to 1985 but there was significant variation between regions. The Northland, Gisborne, Bay of

Plenty, and Taupo biological regions had the highest disease intensity, and Canterbury and Nelson had the least disease. Records from the Forest Health Database generally confirmed the findings of the aerial surveys. No difference in disease incidence between years could be demonstrated.

Severity of needle-cast appears to be related to micro-site, as disease was more severe on sites prone to mist and low cloud. Therefore, even in regions where the overall severity of Cyclaneusma needle-cast is low, severe needle-cast can occur. Conversely, the disease may not be a problem on some sites within a high-risk region. Fig. 11 shows data collected from aerial surveys carried out in 2005 and 2006 where there is a mild relationship between elevation, autumn rainfall, and disease level at the East Cape.

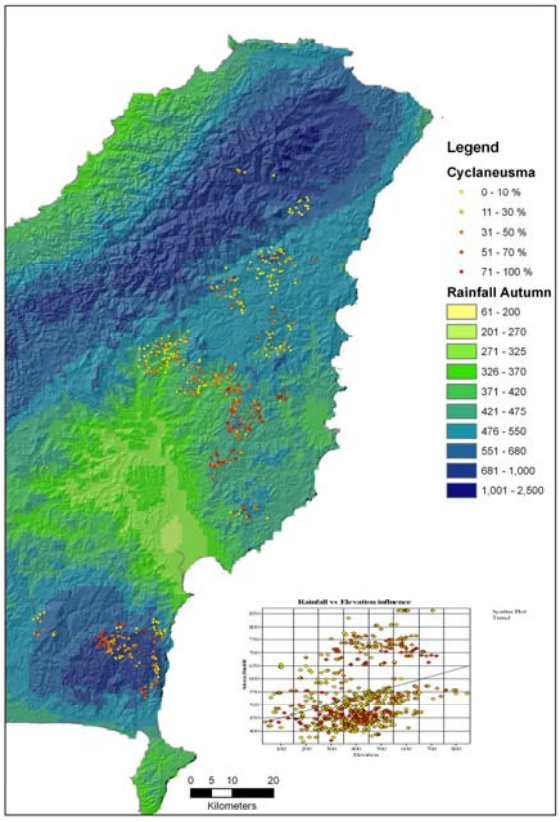

Fig. 11 – Cyclaneusma needle-cast and elevation and autumn rainfall at the East Cape

Effect on growth and economic loss

A trial was carried out to determine the effect of Cyclaneusma needle-cast on volume growth of *Pinus radiata*. Twenty pairs of 9-year-old final-crop trees were selected in a stand of *Pinus radiata* heavily infected with *Cyclaneusma minus* at Kaingaroa Forest in 1977. Each pair consisted of one heavily diseased tree and one healthy tree. Disease severity, in terms of percentage of crown infected, was recorded annually. Relationships between disease severity and growth were studied by complete stem analysis at tree age 15 years. Annual volume increments of the diseased trees were significantly reduced from age 7 onwards. Reduced diameter growth in the lower parts of the stem of the diseased trees caused small changes in the relative diameter distribution. The relationship

between disease severity and volume loss showed a reduction of the average volume increment of approximately 60% at an average disease severity of 80%.

Stand growth was projected to age 30 for various proportions of diseased trees. For each 10% increase in the proportion of diseased trees a reduction in total volume of 10-14 m^3/ha and a subsequent reduction in revenue at clearfelling of $600-$700/ha is predicted when disease severity of affected trees averages 59% over a 6-year period. When 50% of the crop is diseased, a reduction in revenue of between $3,200/ha and $3,600/ ha may be expected. Losses in wood volume, attributable to the disease, of 5% per annum in the forests sampled during the aerial surveys were predicted for stands aged between 6 and 20 years. Growth losses of 6.6% per annum for the *Pinus radiata* estate aged between 6 and 20 years were estimated and financial loss due to the disease is estimated to be of the order of $51 million per annum. Aerial surveys undertaken in 2005 and 2006 provided similar disease data to those gather in the mid 1980s, apart from Northland where disease levels were lower, and the central North Island where disease levels were significantly higher.

During October and November 1994, trials to test the effect of needle-cast caused by *C. minus* on growth of *P. radiata* were established at Otago, Nelson, Wellington, East Cape, and Auckland. An additional trial was established in Northland in 1995. In 1994 and 1995, disease was not apparent at Nelson and the East Cape, plot trees were selected at these sites in November 1996. At Auckland, disease levels of the susceptible trees averaged 67% from 1994 to 1999, compared with 12% for the 'healthy' trees. Respective increments were 95 mm and 157 mm, a difference of 62 mm over 5 years. Growth differences were obvious at Northland where the susceptible trees grew 81 mm compared with 107 mm for the 'healthy' trees over 4 years. At Wellington the trees chosen as susceptible had average disease levels of 58% (1994-99) compared with 6% for the 'healthy' trees and respective increments were 73 mm and 121 mm. Cyclaneusma needle-cast significantly reduced growth of individual trees at these sites.

When regressions of increment and disease levels from Auckland and Wellington were compared, the intercepts were significantly different but the slopes were similar, suggesting that the effect of disease on diameter increment was the same at both sites. An average disease level of 60% over 6 years resulted in a 50% diameter increment loss.

Control

Fungicides were screened for ability to control needlecast of *Pinus radiata* caused by *Cyclaneusma minus*. Undetached shoots were dipped in water-based suspensions at fortnightly intervals for 20 months from the time of flush. Significant improvements in levels of needle retention were achieved with dodine (86% retention), anilazine (76%), benomyl (74%), and dichlone (66%), in comparison with untreated controls (39%). Injections of acidified aqueous solutions of carbendazim into stems of 8- to 10-year-old trees resulted in reductions in foliage yellowing, needle loss, and numbers of *C. minus* colonies isolated from the foliage. Needle loss was reduced two- to three-fold on 0- to 1-year-old shoots after one season of injections; after 2Ê consecutive years of injections needle loss was reduced seven-fold on 1- to 2-year-old shoots. Yellowing and defoliation were also reduced by injections of the nonfungicidal compounds ortho-phenylenediamine and L-arginine monohydrochloride, implying that not all the effects of carbendazim are a consequence of its fungicidal properties.

Aerial applications of benomyl (0.25 kg/ha) in an emulsion of water (6 litres/ha) and BP crop oil (4Ê litres/ ha) in June and July failed to check the disease in a 10- year-old *P. radiata* plantation. In a

second trial, six monthly aerial applications of dodine from April to August reduced disease incidence and severity but the high cost of spraying could not be justified by the small reduction in disease levels.

Assessment of a trial with different final crop stockings and varying proportions of unpruned followers showed that stocking density or pruning had no practical effect on incidence or severity of Cyclaneusma needle-cast. Trials to test the effects of applying five different thinning ratios at five crop ages on the incidence of Cyclaneusma needle-cast were established on two sites at Kaingaroa Forest in 1985. Trees were first thinned at ages ranging from 4 to 9 years, and again at 10 years, when susceptible trees were selected for thinning. Delayed thinning had a significant effect at one site where disease was prevalent. At this site, plots thinned at ages 4 and 5 had mean disease levels over the period 1989 to 1999 of 21% (disease severity exceeded 30% in 28% of the trees) compared with 10% (severity over 30% in only 3% of the trees) for the plots thinned at ages 7 and 8. The early-thinned plots had lower dbh (433.9 mm) than the late-thinned plots (452.6 mm). Treatment differences might have been even greater if disease selection had not been carried out during a second thinning in 1991.

Delayed thinning at the other site, which had consistently low disease levels, initially resulted in reduced growth; however, the late-thinned plots outgrew the early-thinned plots during the period since final thinning, thereby eliminating any differences. Delayed thinning with selection for disease resistant final-crop trees should be beneficial on sites with high disease.

Tree stocking density or pruning had no practical effect on incidence or severity of Cyclaneusma needle-cast. This is because the spore production and dispersal process of *Cyclaneusma minus* results in sufficient inoculum to ensure infection and severity of infection is not dependent on the length of needle moisture periods.

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