NECTRIA FUCKELIANA INFECTION OF PINUS RADIATA IN NEW ZEALAND: RESEARCH APPROACH AND INTERIM RESULTS

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[*]**Abstract** —Stem malformation, typically developing after pruning, has become a problem in some *Pinus radiata* plantations in part of the South Island of New Zealand over the last 10 years. Infection through the pruned branch stub may result in extensive stain and decay within the stem, although tree crowns remain green and healthy. *Nectria fuckeliana*, a Northern Hemisphere fungus commonly recorded there as a saprophyte or weak pathogen of species of *Picea* and *Abies*, is the most commonly isolated fungus from affected trees. Nectria fuckeliana had not been recorded in New Zealand prior to 1996. Interim results from trials established to determine the effect of silviculture, stub treatment and environment on disease development indicate that pruning in winter results in more infection than summer pruning and that incidence is related to stub size. Aspects of the basic biology of the fungus such as spore production, dispersal and germination are being studied, with results so far indicating that *N. fuckeliana* is able to function over a broad range of conditions.

Introduction

Pinus radiata D. Don (Monterey pine) comprises 90% of the 1.8 million hectares of plantation forests (which cover 7% of the land area) in New Zealand. Selected as the primary plantation species for its fast growth, *P. radiata* typically has a rotation length of 25-30 years. Annual rings may reach over 25 mm in trees grown on high index sites and per tree recoverable volume averages approximately 2.4 m^3. Pruning within the first 8 years is widely undertaken, and although an added cost to forestry operations, the practice enables the production of a clear-wood butt log within the short growing cycle. Any infection or malformation of the butt log can result in sufficient degrade to relegate the log from a high return end-use to a lower value product, and it may become uneconomic to harvest the log at all.

In New Zealand, *P. radiata* has a history of stem infection through branch stubs by *Diplodia pinea* (Desm.) J. Kickx f. (syn. *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton), causing the disease 'Diplodia whorl canker'. Effects range from minor sapstain through extensive degrade to tree death following invasion of the stem at the whorl; the hyphae in the tissue effectively block water transport up the stem. The epidemiology of this disease was thoroughly studied during the 1980s (Chou 1987; Chou and MacKenzie 1988), resulting in effective recommendations for management based on the scheduling of pruning operations during cool, dry weather. In the mid 1990s an increasing incidence of stem malformation after pruning was reported in some plantations in the lower South Island in spite of following the Diplodia whorl canker management recommendations. External symptoms were identical to those caused by *D. pinea*, although tree death was rare and only associated with stem breakage at infected whorls. Wilt of the crown did not occur. A species of *Acremonium* was isolated from discolored sapwood and subsequently, with the formation of perithecia on the surface of branch stubs and on stem cankers, *Nectria fuckeliana* Booth was identified as the primary colonizer of cankered sapwood. This was the first record of *N. fuckeliana* in New Zealand.

Nectria fuckeliana is recorded in the Northern Hemisphere as a wound invader of conifers. In Europe and Scandinavia it is one of the most common inhabitants of wounded Norway spruce (*Picea abies* (L.) H.Karst.) stems, occurring in both stained and unstained wood (eg Huse 1981; Roll-Hansen 1962; Roll-Hansen & Roll-Hansen 1979, 1980, Vasiliauskas & Stenlid 1998). Other species of *Picea* and also of *Abies* are common hosts. In a 27-year-old stand of mixed *Picea* species in Scotland, substantial stem cankering associated with wood colonization by *N. fuckeliana* was recorded 3 years after a pruning and brashing operation (Laing 1947).

In North America, *N. fuckeliana* is usually found as a saprophyte on logging slash or fallen trees of both *Picea* and *Abies* species. It has also been occasionally isolated from dead or dying leaders of young *Picea glauca* (Moench) Voss, *P. mariana* (Mill.) Britton and *Abies*

balsamea (L.) Mill. (Smerlis 1969). Its ability to act as a pathogen to a number of conifer species, including five species of *Pinus*, was demonstrated in inoculation tests (Smerlis 1969; Ouellette 1972). *Nectria fuckeliana* was associated with cankers of white fir (*Abies concolor* (Gordon) Lindl. ex Hildebr.) in southern Oregon and northern California (Schultz and Parmeter 1990), and pathogenicity of the fungus was established with inoculation tests. In overstocked stands some suppressed trees were killed within a year of inoculation, whereas vigorous trees often contained the infection, and callus tissue developed over the injured area. There are no published records of natural infection of *Pinus* spp. from the Northern Hemisphere.

To increase understanding of this little-known disease in New Zealand, a number of trials have been established and a number of surveys undertaken. The immediate imperative has been to find an operational management strategy that will minimize the establishment of infection. The ultimate goal of this research is to understand the disease epidemiology, to enable informed decisions about internal quarantine and movement of forest products, and to prevent spread of the disease to other parts of New Zealand.

Distribution and host range of *Nectria fuckeliana* in New Zealand

Perithecia of *N. fuckeliana* have been observed and collected only in the southern part of the South Island. However, fruit bodies of *N. fuckeliana* do not form on all, trees and cankers, or 'fluting' typical of those associated with *N. fuckeliana* occur throughout the country. Therefore a broad national survey was undertaken to determine whether *N. fuckeliana* is present in forests outside the known infected regions. Where stem fluting was present, 80-100 mm long increment cores were taken 10-20 mm above the affected branch stub. Cores were surface sterilized in 10% hydrogen peroxide for 3-5 min, cut into sections, and placed on Petri plates containing 2% malt extract agar for incubation at 20°C. This technique has proved a satisfactory method of evaluating sapwood infection without the necessity of felling trees. A total of 202 stands were inspected and fluting was recorded in 27 (13.4%). *Nectria fuckeliana* was not isolated from any of the samples taken.

An intensive survey within the known infected area was conducted where individual trees in over 600 plots were assessed for fluting. Of the 12,300 trees assessed, 25% had some form of fluting. Follow-up surveys and detailed analysis relating fluting intensity with environmental factors are in progress. A delimiting survey over the

extension of the known infected area and beyond, to determine the distribution and extent of the fungus, is also underway.

Nectria fuckeliana has only been recorded from P. radiata in New Zealand. Although a number of species of *Picea* and *Abies*, the primary hosts overseas, have been tested in New Zealand, none have found favor for plantation forestry. Of the conifers other than *P. radiata* that are grown commercially, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) comprises just over 6% of the plantation estate, with a mixture of species from several genera making up the remaining 1.8% of plantation softwoods. Six species of conifers other than *P. radiata* have been inoculated with *N. fuckeliana* in a pilot trial to determine susceptibility of sapwood to colonization and any tendency to canker formation. These are *Pinus contorta* Loudon, *Pinus ponderosa* Douglas ex C.Lawson, *Pseudotsuga menziesii*, Cupressus macrocarpa Hartw. ex Gordon, *Sequoia sempervirens* (D.Don) Endl. and *Larix decidua* Mill. Results of the inoculations should be available within two years.

Ecology and epidemiology of Nectria fuckeliana

Early stages of infection

Although *N. fuckeliana* is the most commonly isolated fungus from affected trees, the relationship of the fungus to the disease is not completely understood. The nature of tree susceptibility to invasion requires further investigation. The *Acremonium* anamorph forms on the surface of cut infected wood and is produced in culture, but the primary inoculum in nature is thought to be the ascospores (Vasiliauskas and Stenlid 1997). An inoculation experiment to determine whether there is a difference in aggressiveness of these two spore states in causing disease is in progress. The early stages of infection will also be observed in inoculated trees after felling at regular intervals. Examination of infected wood by confocal and scanning electron microscopy will show the location of the fungus within the tissues of the tree and the type of cellular damage it causes.

Spore production and dispersal and relationship of disease to weather conditions

Knowledge of the basic biology of *N. fuckeliana* will allow forest managers to prune trees when infection risk is low. In New Zealand, viable ascospores are present in the perithecia in all seasons of the year. They also remain viable within the perithecia for many months of storage at

room temperature or 4°C. Therefore it is important to determine the conditions of their release into the environment. Preliminary laboratory and field observations show that the ascospores ooze out of perithecia under wet conditions. They germinate readily over a broad range of temperatures, with the optimum between 18 and 25°C. The ascospores can be successfully trapped using microscope slides coated with Vaseline (methods based on Ostry and Nicholls 1982). Weekly spore trapping is underway in two locations. Meteorological data will be correlated with the trapping results. It is hoped that these experiments will help to pinpoint the conditions under which infection is most likely to occur.

Survival of *N. fuckeliana* in debris

As it is common practice to thin the forests to waste rather than to extract thinned stems, large volumes of woody debris cover the forest floor in the years following thinning and pruning operations. With the invasion of decay fungi and the breakdown of wood by cerambycid larvae (primarily the native *Prionoplus reticularis* White) the material breaks down in less than 10 years. However the potential contribution that infected debris may make to inoculum buildup is unknown. It is also possible that material, such as firewood or poles taken from forests may carry living Nectria within the wood or as fruiting bodies on the surface. While the fungus remains confined to one geographical region we will attempt to prevent spread into new localities through restrictions on the movement of wood. An understanding of the behavior of *N.. fuckeliana* in woody debris may indicate ways to influence infection levels through reducing inoculum buildup. The objectives of this project are to a) determine the potential for thinning and pruning debris to become infected and subsequently provide an inoculum source; b) determine the length of time *N. fuckeliana* survives saprophytically in logs from trees that were infected while standing; and c) the longevity of fruit bodies and their ability to produce ascospores once the trees have been felled.

Managing the disease:

Pruned stub trial

Concurrently with the ecological and epidemiological studies of *Nectria* behavior, a trial to identify silvicultural options that will limit infection has been established. As for the other trials discussed, this work is still in progress and therefore only research concepts and interim results can be reported here.

Cankers can invariably be traced to a wound site and there is no evidence that *N. fuckeliana* can invade intact stems.

Pruning wounds are the primary entry point. Altering the timing of pruning operations to a period when fungal inoculum is not available, or to when tree susceptibility to fungal invasion is low, could substantially limit the incidence of pruned stub infection. Colonization of the sapwood, with subsequent decay by other fungi, may be stimulated by a separate suite of contributing factors. In addition to the season of wounding or pruning, variables affecting colonization could include the surface area of injury, its depth and height above ground level, and the length of time since injury occurred.

A trial designed to examine some of these variables was established in 2003. A standard pruning operation was carried out in summer and again in winter. Wound inoculation was carried out for comparison with natural infection. The trial will test when, and for how long, stubs are susceptible to infection, the effect of treating stubs with a physical barrier/fungicide combination, and the effect of wound size on infection and canker formation.

Plot layout: Twenty-six plots were established within 2 treatment blocks, one plot of each of the 13 treatments within each block. Treatments (Table 1) were randomly allocated within blocks. This layout was replicated on 2 sites (ridge top and valley). Plots were 400 m^2 and contained enough trees to select 12 trees with similar height, DBH and branching characteristics from the approximately 40 trees in each plot. This gave a total of 48 trees per treatment, with 24 trees per site, 12 trees in each of the 2 plots.

Table 1 – Pruned stub trial treatments

Summer treatment	No. of plots	No of trees
S1 No summer fungicide	4	48
S2 Summer fungicide	4	48
S3 Summer fungicide delayed 3 months	4	48
S4 Summer inoculation immediate	4	48
S5 Summer inoculation delayed 3 months	4	48
S6 Summer inoculation delayed 6 months	4	48
U Unpruned	4	48
Winter treatment		
W1 No winter fungicide	4	48
W2 Winter fungicide	4	48
W3 Winter fungicide delayed 3 months	4	48
W4 Winter inoculation immediate	4	48
W5 Winter inoculation delayed 3 months	4	48
W6 Winter inoculation delayed 6 months	4	48
Total	52	624

Inoculation: Spore suspensions were prepared by placing flasks of 0.5% malt extract solution seeded with *Acremonium* spores onto a shaker for 5-7 days (ambient temperature). Solutions were then diluted to 1 x 10^6 spores/ml. Each stub in the inoculated treatments was sprayed with 1/2-2 ml spore suspension applied with a trigger sprayer, the volume depending on stub diameter.
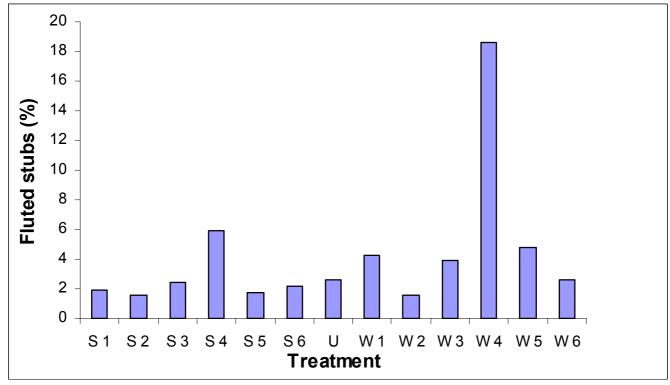
Figure 1: Percentage of pruned stubs with associated flutes

S = summer prune, W = winter prune, U = unpruned
1 = no fungicide, 2 = fungicide applied immediately, 3 = fungicide applied at 3 months, 4 = no fungicide, inoculated 0 months, 5 = no fungicide, inoculated 3 months, 6 = no fungicide, inoculated 6 months

After pruning, all branch stubs on 3 whorls per tree were measured and marked. The design allows some multiple replication among treatments; for instance, delayed inoculation after pruning acts as another control treatment until the inoculum is applied. The treatments were the same for summer and winter pruning; there are 288 trees in each treatment to compare pruning in summer and winter. A second-lift prune with the treatments repeated on two whorls was carried out on the same trees 2 years after the initiation of the trial.

Evaluations, undertaken on a stub-by-stub basis, where the assessor records stem fluting and fruit body formation are carried out every 3 months. Assessments of all study whorls will continue until December 2006, in order to confirm results and enable evaluation of treatments applied after the second-lift pruning. The low background level of stem flutes that are physiological in basis cannot be reliably separated from those associated with infection and all flutes are included in the assessment.

Results

Results 27 months after the establishment of the first stage of the trial are given here. For those treatments (S1,

S3, W1, W3) in which natural infection could be assessed, fluting was recorded, in at least one of the three whorls pruned during the first-lift, in 21 – 38% of trees. These figures are in the range of those that were recorded in the regional survey described above, in which fluting levels ranged from 0 - 80% in different stands, with an overall mean of 25%. The incidence of fluting was higher in trees that were pruned in winter. Winter pruning, without inoculation or fungicide, resulted in 38% infection compared with 21% for the summer pruned treatment. Percentage stubs infected is lower that percentage of trees

A total of 13,545 individual stubs are assessed every three months. Analysis of fluting on individual stubs is more sensitive than examining fluting incidence on individual trees and results are shown in Figure 1. Stubs inoculated immediately after winter pruning (treatment W4) had 18.6% infection, compared with 5.9% of those inoculated immediately after summer pruning (treatment S4). The rate of infection decreased significantly when inoculation was delayed by 3 or 6 months. Summer fungicide applied immediately after pruning resulted in 1.6% infection, identical to the corresponding winter fungicide treatment. Delayed fungicide application was slightly less effective, with 2.4% of stubs infected when fungicide was applied 3
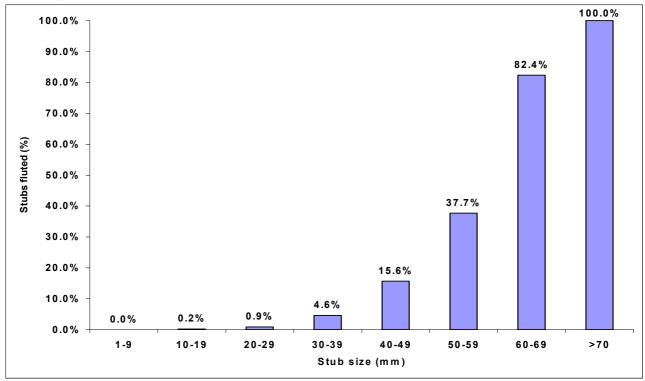
Figure 2: Percentage of stubs with associated flutes (excluding immediate summer and winter inoculation treatments).

months after summer pruning and 3.9% infection for application 3 months after winter pruning. Infection in the winter inoculation treatment was significantly different (P<0.0001) than the other treatments. Winter pruning and summer inoculation had the same level of infection, followed by the summer and winter fungicide treatments and summer pruning, although differences between the two groups were not statistically significant.

Fluting was rarely associated with stubs smaller than 30 mm diameter (Figure 2). Only 0.4% of the 8,826 stubs in this category (excluding treatments where inoculum was applied immediately after pruning) were fluted. It seems likely that natural inoculum alone will not usually initiate infection of stubs this size and artificial inoculation under optimal conditions is required. In the winter pruning treatment, 32% of the larger stubs (≥40 mm) had flutes compared with 16% for the summer pruning treatment, confirming the overall trend of increased fluting after pruning in winter. Fluting incidence was higher in the immediate winter inoculation treatment, when compared with the immediate summer inoculation, over all stub sizes.

Perithecial development is slow and rare. Perithecia were first seen 12 months after pruning and inoculation in summer, and 9 months after inoculation in winter. After 27 months, perithecia have been recorded on only 0.7% of trees.

The trial has now delivered some clear trends.

- Incidence of fluting is related to stub size.

- Winter pruning results in more infection than summer pruning.

- Inoculation immediately after pruning results in increased infection, in summer and in winter.

- Pruned stubs may be susceptible to infection for at least 3 months after pruning, as delayed fungicide application is ineffective.

- Fungicide application immediately after pruning reduces infection in both seasons.

- Fruiting bodies take at least 9 months to develop after treatment.

The key message is that pruning operations should not be undertaken in winter, and that fungicidal treatment of small stubs will not reduce overall disease incidence.

Conclusions and future research

This paper outlines research in progress on many aspects of the pine fluting disease associated with *N. fuckeliana* in New Zealand. In addition to the projects outlined, several other aspects are being considered. For example, it is not known whether appearance of the fungus in New Zealand resulted from a single or from multiple introductions. DNA analysis of the many isolates so far obtained will help to answer this question. Three genetics trials that were established some years ago to assess growth and wood characteristics of a number of breeding lines have been assessed for breeding values associated with resistance to *N. fuckeliana*.

Other aspects of the disease process also warrant further investigation. One characteristic of the disease is the formation of zones of dry, non-living cells in the sapwood in association with many of the pruned branch stubs. These zones initially form radially from the surface wound into the pith, and then longitudinally and primarily up the stem. They are apparent in stems felled up to 2 years after the pruning operation. This feature is postulated to be due to cavitation in tracheids at the wound surface under the influence of desiccation, with air moving through to adjacent tracheids resulting in loss of xylem function. The importance of dead wood zones in the infection process by *N. fuckeliana*, and by other fungal colonizers, has not been established but will be a future research focus.

Identification of a new canker disease in New Zealand *P. radiata* plantations initially caused alarm in the New Zealand forest industry. It is hoped that the results of these studies, however, on many aspects of the basic biology of the fungus and its relationship to its host in this new environment will enable plantation owners to manage the disease effectively. These studies may also have implications for other areas of the world where *P. radiata* is an important forest crop.

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