Client Report No. 38080

Nectria fluting disease of *Pinus* radiata: ecology and epidemiology

Patricia E. Crane

THE JOINT FORCES OF CSIRO & SCION

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Date: **June 2005**
Client: **Forest Biosecurity Research Council, FRST**
Contract No: **Milestone 1.4.1.4.4**

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EXECUTIVE SUMMARY

Pine fluting disease is thought to be caused by the introduced fungus *Nectria fuckeliana*. The disease is presently confined to the southern half of the South Island. A comprehensive work plan is presented to study the ecology and epidemiology of *N. fuckeliana*. The goals are to study (1) the organisms found in diseased wood and their interactions, the infection mechanism, and the early disease development in the host using artificial inoculation, (2) the cause of cambial death and anatomical changes in the infected wood, and (3) the influence of weather conditions on development of fruit bodies and spore production, dispersal, and germination of *N. fuckeliana*. Preliminary results have been obtained for several of these objectives. The results will be important to explain the present distribution, to prevent further spread, and for effective management of the disease.

Objective

To present a work plan to study the ecology and epidemiology of the *Nectria* fluting disease on *Pinus radiata*, and to present preliminary results.

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Information for Ensis abstracting:

Contract number	Milestone 1.4.1.4.4
Client Report No.	38080
Products investigated	
Wood species worked on	Pinus radiata
Other materials used	Nectria fuckeliana, other fungi and bacteria
Location	Otago, Rotorua

INTRODUCTION

Stem malformation that develops after pruning has become a serious problem in some *Pinus radiata* stands in the south part of the South Island in New Zealand. Research has begun on many aspects of the disease, including surveys of the distribution, and how the season of pruning affects susceptibility. However, many aspects of the basic biology, ecology, and epidemiology of the purported causal agent, *Nectria fuckeliana*, are still unknown. A 2-year research program to examine these issues was started in January 2005. Research goals were established, taking into account previous work done by research staff of Ensis, Rotorua, and other members of the *Nectria* Focus Group, and a survey of relevant literature. The ultimate goal of this research will be to reduce uncertainty about the behaviour of the disease; to aid in decisions about quarantine and movement of forest products; to contribute to the development of management strategies for affected forests; and to prevent spread of the disease to other parts of New Zealand. The purpose of this interim report is to outline the goals of the research program and to describe progress during the first 6 months.

RESEARCH GOALS AND PROGRESS

I. The infection mechanism and disease development in the host

Purpose: To understand the cause of the disease and how it spreads.

(1) Organisms associated with the disease

Previous isolations of microorganisms from infected wood of *Pinus radiata* have yielded several different fungi and bacteria. *Nectria fuckeliana* is the most common fungus associated with infected wood. The role of different microorganisms in disease etiology is not known. Isolations of organisms associated with the disease will be continued and compared with those done previously. This will give a more complete picture of the microbial ecology of flutes of different ages. It might be possible to relate the succession of organisms to time since pruning and to determine the spatial distribution of *N. fuckeliana* and other organisms within the diseased wood.

Progress: In January 2005, isolations were made from both healthy and diseased wood of three infected trees from Tokoiti Forest, south of Dunedin. *Nectria fuckeliana* was the most common fungus isolated from infected (fluted) wood, whereas this fungus was not found in healthy-appearing wood of the same disks. In late April, two infected trees were felled from the Tokoiti Forest for more detailed study than has previously been done. Isolates were taken from successive wood disks through entire flutes. Identification of all isolates is in progress.

Cultures will be preserved in the culture collection, Forest Biosecurity and Protection, Ensis, Rotorua (NZFS). This collection will be invaluable for complementary research on the variability of *Nectria fuckeliana* strains

present on the South Island, using morphological and molecular criteria, as well as experiments outlined below.

(2) The role of *Nectria fuckeliana* in the disease

The most common fungus isolated from flutes and branch stubs of infected radiata pine is *Nectria fuckeliana*. However, this fungus is known from Europe and North America as a weak wound parasite, mainly of spruce (*Picea* spp.) (e.g. Roll-Hansen and Roll-Hansen 1980, Vasiliauskas *et al*. 1996, Ouellette 1972). In Otago, the fungus has been found growing on pruned branch stubs in the absence of fluting. In other cases, there has been a failure to isolate the fungus from flutes (Margaret Dick, personal communication). The causal agent of the pine fluting disease needs to be confirmed by satisfying Koch's postulates: consistent isolation of the fungus from diseased trees, reproducing the disease symptoms artificially by inoculation, and reisolating the fungus. Experiments will be conducted to determine the role of *N. fuckeliana* in this disease following these criteria.

(a) Preliminary inoculation trial

Nectria fuckeliana produces three kinds of spores. Small, single-celled conidia (*Acremonium*-stage) are produced asexually in culture and on the surface of cut, infected wood. Occasionally, multicellular conidia are found associated with perithecia in nature, but this stage is not common. Sexually produced ascospores are produced in red fruiting bodies (perithecia) on the surface of colonized wood. This stage is likely the most important in spread of the fungus in nature. The purpose of the preliminary inoculation trial is to determine whether there is a difference in infectivity of the conidia and ascospores.

Progress: An inoculation trial was initiated in late April in the Flagstaff Forest, near Dunedin, to compare infectivity of ascospores and of the asexual spores from the *Acremonium* stage. Forty-five 6-year-old unpruned *Pinus radiata* were inoculated using either conidia, ascospores, or water (control). Three inoculation methods were used (15 trees of each): surface stem wound, deeper wound into sapwood, hole drilled into the end of a branch stub. Liquid inoculum was delivered into the wounds using a pipetter. Inoculated trees were examined in early June, but symptoms had not developed. Final assessment of the infections will be done in spring 2005.

(b) Inoculation trial to determine disease progress

In another trial, planned for spring 2005, young unpruned trees will be inoculated immediately after wounding using the most effective method and spore state determined from 2a, above. At monthly or bimonthly intervals, two trees will be harvested. Isolations will be done from the wood to recover the pathogen, and wood anatomical studies will be carried out to determine location of fungus and early damage symptoms in wood (see II, below).

(3) Comparison of *Nectria* and *Diplodia* disease development

Pine fluting disease and a stem disease caused by *Diplodia pinea* (*Sphaeropsis sapinea*) sometimes cause similar symptoms (Bulman and Dick

2004); initial infection stages of both will be compared. This will also complement work on molecular markers for these fungi (T. Ramsfield, personal communication).

At the same time as the inoculation trial above (2 b), several trees will be co-inoculated, on opposite sides of the trees, with *D. pinea* at the same time as inoculations with *N. fuckeliana*. They will be harvested at regular intervals, fungi reisolated, and disease development compared, using anatomical studies.

(4) Interactions of key organisms associated with pine fluting disease

In addition to *N. fuckeliana*, many other fungi and bacteria have been isolated from pine stems infected with the pine fluting disease, including common saprophytes, decay fungi, and bacteria. Observing their interactions may elucidate their roles in disease development. Understanding the competitive ability of *N. fuckeliana* may help to explain its common association with pruned branch stubs.

Paired cultures will be made of *N. fuckeliana* and other organisms and their interactions observed (e.g. competition, parasitism, growth inhibition or enhancement). Based on the cultural results, organisms will be chosen to pair on wood blocks. Sterile pine blocks will be placed on culture medium and inoculated at the same time at either end with two different organisms. In other experiments, one organism will be allowed to colonize a block before adding the second. The competitive ability of the organisms will be assessed by reisolating the fungi, and if necessary, by anatomical observations.

Progress: In paired cultures of *N. fuckeliana* and *D. pinea* at room temperature and 15C, dark pigment was induced in *D. pinea* when the cultures came into close contact; further growth of *D. pinea* was inhibited. In some cases, *N. fuckeliana* grew over the top of *D. pinea*, suggesting that *N. fuckeliana* is an aggressive competitor against *D. pinea* in culture. Some variability in the interactions was observed on different culture media.

II. Effect of key organisms on the host

Purpose: To determine the cellular location of the pathogen in the wood, and the cause of formation of “pathological whitewood” and death of the cambium (causing the fluting symptom).

Stem fluting is associated with death or damage to the cambium above pruned branch whorls. The role of *N. fuckeliana* in cell damage and death of the cambium needs to be elucidated. Anatomical studies of the early stages of infection may shed light on the processes that initiate fluting.

In cross sections of fluted stems, there is often a wedge-shaped area of whitish wood extending radially from a pruned branch stub to the pith. This “pathological whitewood” extends upwards into the flute, and *N. fuckeliana* is often confined to these areas. The *Acremonium* stage of *N. fuckeliana* often

fruits on the surface of the whitewood areas on disks within days of tree felling. It is not known whether the whitewood is a physiological response to the wounding after branch pruning or whether it is caused by cell death from fungal infection.

Samples from flutes and from comparable areas of healthy-appearing wood of the same disks will be compared using confocal microscopy and scanning electron microscopy. Anatomical studies will be done of older infections as well as the early stages at regular intervals after artificial inoculation.

Progress: Samples of cambium and sapwood from infected wood have been fixed in FAA (formalin – acetic acid – ethanol) in preparation for anatomical studies. Sectioning and staining methods for confocal microscopy have been tested by the Cellwall Biotechnology section, Scion, Rotorua.

III. Relationship of disease incidence to environmental conditions

Purpose: To understand the risk of disease development in new areas, the reasons for the current disease distribution, and how it is influenced by the biology of *N. fuckeliana* and by plantation management.

Little is known about the environmental conditions required by *Nectria fuckeliana* for development of fruiting bodies, spore formation, spore dispersal, and spore germination. Such information is crucial to the management of the disease. Experiments in both the laboratory and under field conditions will be conducted to elucidate the basic biology of the fungus and to relate biological events to weather conditions.

(1) Artificial production of perithecia and ascospores

Some isolates of *N. fuckeliana* produce fruit bodies (perithecia) readily in culture, but they are usually sterile, not producing ascospores. Earlier studies have shown that fruit bodies containing viable ascospores can be induced by culturing two compatible isolates together on pine twigs (M. Dick, personal communication; Roll-Hansen 1962). However, the conditions required for this are not completely understood. Production of ascospores in culture would provide a ready source of spores for other laboratory experiments; it would also increase our knowledge of conditions that induce fruit body production in nature.

Progress: In April 2005, five different isolates of *N. fuckeliana* were paired in all combinations on various media at room temperature in an attempt to induce fruit body formation. This experiment is still in progress and will be expanded when more isolates of the fungus become available. After successful production of perithecia and ascospores, new cultures will be made and placed in a variety of temperature and light conditions to determine more precisely the conditions required for these processes.

(2) Seasonal occurrence of ascospores in nature and their long-term viability

Perithecia will be collected regularly from infected trees, and the presence of viable spores assessed by germination on glass slides. Field-collected perithecia will be stored at room temperature, in a refrigerator, and in a freezer to test long-term viability. Ascospore viability will be assessed at bimonthly intervals.

Progress: Viable spores were present in perithecia collected in January, April, and June, suggesting that they may be present throughout the year, ready for release when conditions permit. Perithecia collected in summer (January) still contained viable spores after 3 months of storage at room temperature and in a refrigerator.

(3) The effect of temperature and light on spore germination

Study of spore germination in the laboratory will be used to determine field conditions and season of the year when infection of pine is most likely to occur.

Progress: Ascospores collected in nature were found to germinate easily on glass slides in droplets of water. Spore germination rate varied among ascospores from different perithecia in the same group. The color of perithecia indicates their level of maturity. Perithecia in a group on an infected tree vary in maturity, but a group likely produces viable spores over many months.

(4) The effect of pine resin on spore germination and growth

N. fuckeliana is primarily a wound pathogen that is able to grow and reproduce in highly resinous areas of pine wood, including branch stubs. The role of resin in growth of the fungus will be studied. Spore germination and growth of the fungus will be studied on media amended with pine resin and in enclosed chambers where the vapour from resin is present.

(5) Spore dispersal mechanism and correlation of the disease incidence with environmental factors

Understanding the mode of dispersal of ascospores is important to understanding the rate of spread and the current distribution of *N. fuckeliana*. It is assumed, based on European studies (Vasiliauskas and Stenlid 1997) and infrequent observation of conidia in nature, that ascospores are more important for spread of the disease. Little study of the spore dispersal mechanism has been made. A related pathogen, *Nectria galligena*, which causes a canker on apple, is vectored by rain and rain splash within infected trees (McCracken *et al*. 2003).

The seasons or weather conditions under which ascospores of *N. fuckeliana* are released and dispersed is not known. Spore trapping methods appropriate for this fungus will be investigated, and spore dispersal then studied in the field. Spores will be trapped on a regular basis for 1 year in two locations with high disease incidence. Spore trap results will be correlated with weather data

(e.g. rainfall, temperature, humidity) obtained by portable weather stations or from the NIWA database.

Progress: Laboratory and field observations show that ascospores are not forcibly discharged from the perithecia, but ooze out under moist conditions. As the air becomes drier, they remain attached in clumps on the perithecium and do not appear to disperse. However, they disperse readily when wetted, suggesting that water plays an important role in their dispersal.

Spores were successfully trapped on glass slides smeared with Vaseline, but were infrequent at distances greater than 1 m from the infected tree. They are often found in groups, suggesting dispersal together, as in water droplets.

Results and Conclusions

Preliminary studies of the basic biology and ecology of *Nectria fuckeliana* have provided insight into the techniques that will be appropriate for the rest of the research programme, and have yielded useful results about spore germination and dispersal of the fungus. Achieving the goals listed above is expected to contribute information relevant to the effective management of the pine fluting disease.

Acknowledgements

I thank Margaret Dick for extensive support in the laboratory and field and Lindsay Bulman and Tod Ramsfield for helpful discussions. Funding for this project is provided by FBRC (Forest Biosecurity Research Council) and FRST (Foundation for Research, Science, and Technology).

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