Nectria infection of nursery stock: Berwick Forest Trial

by

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THE JOINT FORCES OF CSIRO & SCION

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Nectria Nectria infection of nursery stock: Berwick Forest Trial

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Contract No:

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EXECUTIVE SUMMARY

Objective

The objective of this study was to determine if *Pinus radiata* nursery plants could become infected with *N. fuckeliana*, either symptomatically or asymptomatically, and thereby be capable of carrying the fungus to new locations.

Key Result

Results from field trials show that nursery plants have little capability of retaining *N. fuckeliana* within the tissue even when directly inoculated with spores. Nursery stock is therefore extremely unlikely to represent a pathway for transport of the fungus.

Summary

There are no records of *N. fuckeliana* from the North Island but there is some concern that the fungus may have been, or may in the future be, transported to the North Island via nursery stock and consequently become established in plantations there.

Nectria fuckeliana does not infect through intact tissue; it requires an entry point. It is common practice to top plants in the nursery beds and this would theoretically provide an infection court for *N. fuckeliana* for a short period of time before the wound heals.

In a field trial established in Berwick Forest, adjacent to a stand with *N. fuckeliana* infected trees, nursery plants were topped and half were inoculated with a conidial spore suspension.

Three months after inoculation *N. fuckeliana* was re-isolated from 24% and 7% of inoculated seedlings and cuttings respectively. The fungus was recovered from 5-15 mm of the stem directly below the cut top where the spore suspension was applied. *Nectria fuckeliana* was also isolated from one of 55 uninoculated seedlings sampled at this time but not from any of the uninoculated cuttings. Twelve months after inoculation *N. fuckeliana* was re-isolated from 3.5% of inoculated seedlings only. No symptoms of disease developed in either seedlings or cuttings at any time.

It is likely that the positive result from one uninoculated seedlings at the 3-month evaluation was due to cross-contamination from the inoculated plants rather than from natural inoculum from the surrounding stand.

As *N. fuckeliana* is spread primarily by water-splash it would be necessary for infected trees to be located in close proximity to a nursery in order to provide the necessary inoculum. As forest nurseries are usually some distance from an inoculum source, and trial results show that nursery plants have little capability of retaining *N. fuckeliana* within the tissue even when directly inoculated, nursery stock is extremely unlikely to represent a pathway for transport of the fungus.

Application of Results

Ensure the removal of infected trees growing within 0.5 km of a nursery that is supplying plants to an area free of *N. fuckeliana.*

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Information for Ensis abstracting:

Contract number	
Products investigated	*Nectria fuckeliana*
Wood species worked on	*Pinus radiata*
Other materials used	
Location	Berwick Forest, Otago

INTRODUCTION

The first confirmed record of the wound pathogen, *Nectria fuckeliana,* was from Otago in 1996. *Nectria fuckeliana* has since been found extensively in Otago and Southland and in a few locations in South Canterbury and Mid Canterbury. During the period 1997- 2004 thousands of *P. radiata* seedlings and cuttings were sourced from South Island nurseries for planting out in locations in the North Island. There are no records of *N. fuckeliana* from the North Island but there is some concern that the fungus may have been, or may in the future be, transported to the North Island via nursery stock and consequently become established in plantations there. A further concern has been that plants could become infected with *N. fuckeliana* while young, and continue to carry the fungus within the stem without showing any signs of disease.

As *N. fuckeliana* is a wound pathogen (Vasilauskas & Stenlid 1998) intact seedlings or cuttings are very unlikely to have the opportunity to become infected. If topping of nursery material is undertaken there is the theoretical possibility that, if there is an inoculum source nearby, the wound could become infected. The possibility that dead portions of nursery plants could become colonised with *N. fuckeliana* if inoculum were available has also been raised.

In tests carried out in the Ensis Containment Facility in Rotorua *Nectria* was readily reisolated from nursery stock 6 weeks after inoculation to a freshly created wound. No disease symptoms were apparent and the fungus was only recovered from the area around the wound. *Nectria* could not be re-isolated from branches and stems that were inoculated when already dead and the tissue colonised by other fungi. Results of these tests were discussed in Ensis Report No. 38658 (May 2005).

It is probable that results from inoculation tests undertaken in a field environment may differ from those obtained indoors as both weather and competing microorganisms often have a marked effect on fungal survival and infective capability. Hence a trial to examine infection in a natural situation was established in August 2005.

MATERIALS AND METHODS

Site and plant material

A newly planted site adjacent to a *Nectria*-infected stand of *P. radiata* was selected in Berwick Forest. Stands on the four boundaries of the site were:

North Cpt 50/02 1991 *P. radiata*
East Cpt 44/13 2004 *P. radiata*
South Cpt 51/03 1998 *P. radiata*
West Cpt 50/03 1992 *P. radiata*

Seedlings were 18 months old from an all-rounder CP seedlot. Cuttings were 99/387 or 99/385 seedlots.

Four plots were established by Wenita Forest Products Ltd in July 2005.

Trial layout

A total of 220 *Pinus radiata* seedlings and 220 cuttings were distributed in a randomized block layout with alternating rows of cuttings and seedlings. Each of the four plots contained 11 rows of ten plants.

Preparation of inoculum

Nectria fuckeliana isolate NZFS 980 was grown on 2% MEA at 25°C for 7 days in the dark. Conidia (*Acremonium* stage) were scraped from the surface for preparation of a spore suspension in sterile water. This was placed on a shaker for 7 days for conidial multiplication. The spore concentration (determined using a haemocytometer) was adjusted to 5×10^3 spores/ml.

Treatments

1. Seedlings - inoculated
2. Seedlings - uninoculated
3. Cuttings - inoculated
4. Cuttings - uninoculated

All plants were topped after planting (Fig 1) and half (treatments 1 and 3) were inoculated immediately after topping. A 25 μl droplet of conidial spore suspension was applied to the cut top. At a spore concentration of 5×10^3 spores/ml this provided a mean 125 spores to each inoculated plant.

The uninoculated plants served as a control for comparison of plant growth and symptom development, and could also potentially become infected by *Nectria* spores from the adjacent stand.

Fig 1: *P. radiata* plants at Berwick Forest. All plants were topped

Assessments

Three months after trial establishment, at the end of November 2005, half of the plants from each treatment were collected for examination and isolations in the laboratory. The needles were stripped from each stem and the extent of discolouration from the cut top measured. Eighty mm lengths of stem were then surface sterilised in 30% hydrogen peroxide before being cut into 5 mm lengths and placed onto 2% malt extract agar in Petri plates which were incubated at 22°C. Plates were evaluated at 7 days for identification of fungal colonies. This process was repeated 12 months after inoculation (August 2006) for all of the remaining plants. A 20-40 mm length of stem below the wound was sampled for isolations rather than the 80 mm length plated previously.

RESULTS

Appendix 1 contains the interim report, prepared in January 2006, of results from the 3 month sampling.

At 3 months the cut top of the plants was readily identifiable whereas at 12 months, in August 2006, the wound was often obscured by the rapid growth of the uppermost whorl of branches. The wound had generally occluded and in some cases could barely be detected. In some plants a trace of discoloured tissue could be found when the stems were dissected; in others the inoculation point was identified only by a slight distortion when the stem was dissected. Measurements of the extent of discoloured tissue were therefore not made at the 12 month assessment.

Recovery of *N. fuckeliana* from the plants on both sampling occasions is given in Table 1. At three months *N. fuckeliana* was recovered from 24% and 7% respectively of seedlings and cuttings and from only one of the 111 uninoculated plants (55 seedlings and 55 cuttings) tested at this time. After a further 9 months *N. fuckeliana* was recovered from just 2 of the 55 inoculated seedlings (~3.5%).

All of the stems were colonised by several fungi, including those from which *N. fuckeliana* was recovered. Most of the fungi could be identified, at least to genus, and are known saprophytes commonly associated with *P. radiata*. At least two fungi were obtained from each stem section with the number of species per section ranging up to 5. The most frequently obtained saprophytic fungi were *Aureobasidium* sp., *Pestalotiopsis funerea*, *Epicoccum nigrum*, *Alternaria* sp., *Trichoderma* sp., *Botrytis cinerea, Cladosporium* sp., *Gliocladium* sp. and *Sclerophoma* sp.

Table 1: Recovery of *N. fuckeliana* from seedlings and cuttings

Treatment	% *Nectria* recovery at 3 months	% *Nectria* recovery at 12 months
Seedlings - inoculated	24	3.5
Seedlings - uninoculated	2	0
Cuttings - inoculated	7	0
Cuttings - uninoculated	0	0

DISCUSSION

Nectria fuckeliana does not infect through intact tissue; it requires an entry point. It is common practice to top plants in the nursery beds and this would theoretically provide an infection court for *N. fuckeliana* for a short period of time before the wound heals.

There was no sign of disease on any of the seedlings or cuttings at any time during the twelve months following inoculation. Discolouration in the tissues was consistent with normal response to an injury. Average length of discolouration at three months was 3.9 mm (all treatments) and was highest in the uninoculated cuttings. The average for the uninoculated cuttings was skewed by 3 plants with extensive discolouration (Appendix 1). This discolouration was not associated with *Nectria* infection. For those plants from which *Nectria* was recovered the fungus was obtained only from the tissue adjacent to where the spore suspension had been placed.

Nectria fuckeliana was obtained from only one of the 220 uninoculated plants. It is possible that this positive result was due to cross-contamination from the inoculated plants rather than from natural inoculum from the surrounding stand.

The number of inoculated plants (110 in total) carrying the fungus 12 months after inoculation (2 of 55 seedlings plus 55 cuttings) was extremely low and is in marked contrast to results obtained when potted plants held in the Ensis Containment Facility were inoculated (Dick 2005). Although there was no evidence of symptom development, *Nectria* was recovered from around the inoculation point of the majority of *P. radiata* plants. Conditions under which the plants were held were however highly artificial as there were no competing microorganisms and no effect of weathering. In nature other microorganisms influence both fungal survival and infective capability. Likewise weather conditions occurring in the natural environment can affect spore germination, plant penetration and fungal survival. This was partly illustrated in the experiment with potted plants in containment. Recovery of *Nectria* from plants where the inoculum of *Nectria* spores was mixed with spores of another (saprophytic) fungus was markedly reduced, and there was no recovery from tissue that was dead prior to being inoculated (Dick 2005).

In literature on *N. fuckeliana* from overseas there is no record of the fungus colonising nursery stock or very young plants (Dick 2003).

Research into the ecology of *N. fuckeliana* has shown that the spores produced on the stems of infected trees are exuded when conditions are wet and are dispersed by water-splash. This mode of dispersal markedly reduces the chances of spores travelling long distances and finding wounds through which infection can take place. Nurseries that are not sited close to an inoculum source are unlikely to be exposed to spores.

In 2004 Forest Health Inspectors trained to identify the signs and symptoms of *N. fuckeliana* infection undertook a special survey of North Island and northern South Island forests in which nursery stock from Otago/Southland had been planted. No evidence of *N. fuckeliana* was found (Bulman 2005).

CONCLUSIONS

Young plants of *P. radiata* do not readily retain *N. fuckeliana* within the stem even when conditions for fungal colonisation are optimised (by application of a spore suspension directly to an infection court). The results of this trial, along with the fact that forest nurseries are usually some distance from an inoculum source demonstrates that nursery stock is extremely unlikely to represent a pathway for transport of the fungus. The absence of *N. fuckeliana* from northern stands where southern nursery stock has been planted further supports this premise.

Acknowledgments

Aaron Gunn of Wenita Forest Products Ltd arranged the site at Berwick Forest and for the seedlings and cuttings to be established in the plots. Mark Kimberley of Ensis advised on trial design. Rita Tetenberg, Judy Gardner and Chrystal Kelly carried out much of the laboratory work. The help of all these people is very much appreciated. The Forest Biosecurity Research Council and FRST funded this project.

References

Bulman, L.S. 2005: National Nectria Survey. Ensis report No. 37701.

Dick, M.A. 2003: *Nectria fuckeliana* infection of conifers. A review from a New Zealand perspective. Ensis report No. 10154

Dick, M.A. 2005: *Nectria fuckeliana* infection of nursery plants. Ensis report No. 38658

Vasiliauskas, R.; Stenlid, J. 1998: Fungi inhabiting stems of *Picea abies* in a managed stand in Lithuania. Forest Ecology and Management 109: 119-126

APPENDICES

Appendix 1 – Infection of nursery stock: Berwick Forest Trial. Interim report January 2006

Background

The first confirmed record of the wound pathogen, *Nectria fuckeliana* was from Otago in 1996 and it has since been found extensively in Otago and Southland and in a couple of locations in South Canterbury. During the period 1997- 2004 thousands of *P. radiata* seedlings and cuttings were sourced from South Island nurseries for planting out in a range of locations in the North Island. Although there are no records of *N. fuckeliana* from the North Island there is concern that it may have been, or may in the future be, transported to the North Island via the nursery stock and become established in the plantations there. An additional concern has been that plants could become infected with *N. fuckeliana* while young, and continue to carry the fungus within the stem without showing any signs of disease.

As *N. fuckeliana* is a wound pathogen intact seedlings or cuttings are very unlikely to have the opportunity to become infected. If topping of nursery material is undertaken there is theoretically the possibility that, if there is an inoculum source nearby, the wound could become infected. The possibility that dead portions of nursery plants could become colonised with *N. fuckeliana* if inoculum were available has also been raised.

In tests carried out in the Ensis Containment Facility in Rotorua *N. fuckeliana* was readily reisolated from nursery stock 6 weeks after spores were applied to a freshly created wound. No disease symptoms were apparent and the fungus was only recovered from the area (within 10 mm) around the wound. *Nectria* could not be re-isolated from branches and stems that were inoculated when already dead and the tissue colonised by other fungi. Results of these tests were discussed in Ensis Report No. 38658 (Dick 2005).

It is probable that results from inoculation tests undertaken in a field environment may differ from those obtained indoors as both weather and competing microorganisms often have a marked effect on fungal survival and infective capability. Hence a trial to examine infection in a natural situation was established in August 2005.

Objective

To determine if nursery plants could become infected with *N. fuckeliana*, either symptomatically or asymptomatically, and thereby capable of carrying the fungus to new locations.

Methods

A newly planted site adjacent to a known infected stand of *P. radiata* was selected in Berwick Forest for a trial in a randomised block layout. Four plots containing in total 220 *Pinus radiata* seedlings and 220 cuttings were established. All plants were topped after planting and half were inoculated immediately after topping. A 25 μl droplet of spore suspension was applied to the cut top. The concentration of the spore suspension was 5 x 10^3 spores/ml giving a 125 spore application to each inoculated plant.

Treatments

1. Seedlings - inoculated
2. Seedlings - uninoculated
3. Cuttings - inoculated
4. Cuttings – uninoculated

Assessment

Half of the plants from each treatment (55 of the 110 plants/treatment) were collected for isolations in the laboratory 3 months after trial establishment. The needles were stripped from each stem and the extent of discolouration from the cut top measured. Stems were then surface sterilised and placed onto artificial media. The recovery of *Nectria* and the extent of stem discolouration are given in Table 1.

Results

All of the stems were colonised by several fungi, including those from which *N. fuckeliana* was recovered. Most of the fungi could be identified, at least to genus, and are known saprophytes commonly associated with *P. radiata*. In most cases there were at least two fungi obtained from each stem section and sometimes up to five.

Table 1: Recovery of *N. fuckeliana* from seedlings and cuttings

Treatment	% *Nectria* recovery	mm discolouration (average)
Seedlings inoculated	24	2.91
Seedlings uninoculated	2	2.93
Cuttings inoculated	7	3.02
Cuttings uninoculated	0	6.87

Comments

Apart from a couple of trees that had not survived establishment there was no sign of disease on any of the seedlings or cuttings. Discolouration in the tissues was consistent with normal response to an injury. Average length of discolouration was highest in the uninoculated cuttings but was still minor. The average for the uninoculated cuttings was skewed by 3 plants with extensive discolouration.

Three months after inoculation, *N. fuckeliana* was recovered from 24% and 7% respectively of seedlings and cuttings. *Nectria* was recovered from only 1 of the 111 uninoculated plants tested.

Nectria was only obtained from the tissue in the top of the plants. It had not grown beyond the top couple of cm.

It is more likely that the positive *Nectria* result from an uninoculated plant was the result of cross-contamination from the inoculated plants than from natural inoculum from the surrounding stand.