Commercial in Confidence

Client Report No. **38658**

Nectria fuckeliana infection of nursery plants

Margaret Dick

THE JOINT FORCES OF CSIRO & SCION

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Client: **Forest Biosecurity Research Council**
Contract No:

CSIRO SCION

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EXECUTIVE SUMMARY

- No symptoms of disease developed in either *P. radiata* or *Pseudotsuga menziesii* nursery plants following inoculation with *Nectria fuckeliana.*

- When inoculated alone *Nectria fuckeliana* was re-isolated from 96% of treated *Pinus radiata* seedlings and from 68% of *P. radiata* cuttings.

- Inoculating *P. radiata* plants with *N. fuckeliana* spores mixed with those of a common saprophyte reduced the recovery rate of *N. fuckeliana* to 20% and 50% for seedlings and cuttings, respectively.

- *Nectria fuckeliana* was re-isolated from *P. menziesii* only following a very high inoculum dose.

- The fungus could not be re-isolated from branches and stems that were inoculated when already dead and the tissue colonised by other fungi.

- Plants were harvested for examination 6 weeks after inoculation and the survival of the fungus for longer periods of time, and after new leaders have formed, is unknown.

- Results in a field environment may differ from those obtained in a containment room as weather and competing microorganisms often have a marked effect on fungal survival and infective capability.

- A trial to examine infection in a natural situation was established in August 2005.

Objective

The objective of this work was to determine if nursery seedlings could become infected with N. fuckeliana, either symptomatically or asymptomatically, and carry the fungus to new locations.

Further Work

A small follow-up field trial to examine infection in a natural situation is recommended.

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INTRODUCTION

The first confirmed record of the wound pathogen, *Nectria fuckeliana* infecting *Pinus radiata* was from Otago in 1996 (Dick 2003) and it has since been found extensively in Otago and Southland. It was recorded in South Canterbury in 2004. In the period 1997- 2004 many seedlings and cutting of *P. radiata* were transported from South Island nurseries for planting out in the North Island. Although there are no records of the fungus from the North Island, and a special survey carried out in the North Island in 2004 did not confirm the presence of the fungus, there is concern that *N. fuckeliana* may have been, or may in the future be, transported to the North Island via the nursery material and become established in the plantations there.

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 then the wound could become infected. The possibility that dead portions of nursery plants could become saprophytically colonised with *N. fuckeliana* if inoculum were available has also been considered. A series of experiments to test the infection of nursery plants were therefore conducted.

In the absence of *N. fuckeliana* in the North Island experimental work was carried out in the containment facility at Ensis in Rotorua. Conditions in containment do not parallel those found in the nursery in that the environmental conditions are constant and, unless other fungi are applied to the plants, there is reduced competition from other micro-organisms. These differences from field conditions may compromise results. To attempt to address this some experiments were carried out with another fungus inoculated with *N. fuckeliana.*

MATERIALS AND METHODS

Plant material

Pinus radiata seedlings (sown on 16 October 2003)

Origin: GF113/104/A,
Plants were potted (two per liver pail) with a mix of fine and medium grade potting medium without fertiliser.

P. radiata cuttings (set 23 June 2003)

Origin: GF Plus 98/816.
Plants were potted (two per liver pail) with a mix of fine and medium grade potting medium without fertiliser.

Pseudotsuga menziesii (Douglas fir) seedlings (sown 19 May 2003)

Origin: 02/094

Plants were potted (three per liver pail) with a medium mix with 7kg/m^3 osomocote exact.

Inoculum

Nectria fuckeliana isolate NZFS 980 was grown on 2% MEA at 25°C for 7 days in the dark. Conidiospores were scraped from the surface for preparation of a spore suspension in sterile water. This was placed on a shaker for 7 days for conidiospore multiplication. The spore concentration (determined using a haemocytometer) was adjusted to 1 x 10^7 spores/ml. Part of this solution was serially diluted with sterile distilled water to give additional suspensions of 1 x 10^5 and 1 x 10^4 spores/ml.

An isolate of *Cladosporium cladosporioides*, a saprophytic fungus frequently found associated with young *P. radiata* plants in nurseries, was treated in the same way and a suspension containing 1 x 10^5 spores/ml prepared.

Inoculation methods

Apex and branches: The top of the plant was cut off to mimic routine topping carried out in nursery. Within 15 minutes the cut surface was inoculated with a 20 μl droplet of spore suspension. On some plants with side branches the tip of the branch was removed in the same manner.

Side branches: A lower side branch was sprayed with the contact herbicide paraquat (3% paraquat with 0.5% surfactant (pulse) added). After 30 days the dead branch was sprayed with approximately 0.5 – 1 ml of spore suspension containing 1 x 10^6 spores/ml.

Stems: Three adjacent fascicles were removed from the mid stem and a 5μl droplet of spore suspension placed in contact with each wound onto the primary leaf left behind.

Treatments

Treatments and the number of replicates for each spore concentration applied are shown in Table 1. The number of replicates varied between treatments as some plants had more than one suitable inoculation point whereas others did not. Uninoculated control plants were treated with sterile, distilled water.

Plants were held in a controlled environment room in containment at 21°C, 60% RH and a 12/12 hour light cycle. All plants and pots were enclosed in a plastic humidity chamber for 48 hours after inoculation to promote spore germination. Pots were then removed from the humidity chamber and watered as required to maintain soil moisture.

Table 1: Treatments and number of replicates

Treatment	Relicates		
	P. radiata **seedlings**	***P. radiata*** **cuttings**	***P. menziesii*** **seedlings**
Cut tops. 1x 10^7 spores/ml	11	10	15
Cut tops. 1x 10^5 spores/ml	4	19	12
Cut tops. 1x 10^4 spores/ml	4	12	4
Cut tops. *N. fuckeliana* + *Cladosporium*	5	26	18
Cut tops. Water control	8	13	14
Stem inoculation. 1x 10^5 spores/ml	8	12	4
Stem inoculation. Water control	4	3	3
Side branch killed. 1x 10^5 spores/ml	4	5	4
Side branch killed. Water control	2	4	2

ASSESSMENTS

Plants were visually assessed for signs of discolouration at weekly intervals.

Plants that had side branches sprayed with herbicide were harvested 4 weeks after inoculation. The above ground part of the plant, including the side branches were cut into 20 mm segments, surface sterilised in 30% hydrogen peroxide and plated onto 2% MEA. Plates were evaluated at 7 and 14 days for identification of fungal colonies.

The remainder of the plants were harvested 6 weeks after inoculation A 3 cm length was cut from the leader of each plant that had been topped and a 5 cm piece of stem, centred on the inoculation point, cut from each inoculated stem. The length of discolouration, either from the cut apex or around a stem inoculation point was measured to the nearest half millimetre. Each stem piece was surface sterilised in 30% hydrogen peroxide before being plated onto 2% MEA. Plates were evaluated at 7 days for identification of fungal colonies.

RESULTS

The herbicide spray applied to branches proved to be more damaging than anticipated and both inoculated and control treatments had began to die when the plants were harvested. *Nectria fuckeliana* was not reisolated from any of these plants, either from the inoculated branch or the main stem. The tissue was colonised by a number of different fungi, these were recognisable saprophytes.

Results from the topped and stem inoculated plants are given in Tables 2 – 4. Plants remained quite healthy in appearance. Discoloration associated with the cut tops was minimal and there was no difference between inoculated and uninoculated plants. No discolouration developed in response to the mid-stem inoculations.

Nectria fuckeliana was re-isolated from 26 of the 27 (*Pinus radiata* seedlings that were inoculated with *Nectria* alone, but from only one of the 5 plants inoculated with a mix of *Nectria* and *Cladosporium*. Recovery from *P. radiata* cuttings treated in the same way was generally lower with *Nectria* re-isolated from 36 of the 53 cuttings that were inoculated with *Nectria* alone, but from 13 of the 26 plants inoculated with a mix of *Nectria* and *Cladosporium*.

Nectria fuckeliana was recovered from Douglas fir seedlings only at the highest inoculum dose.

Table 2: Recovery of *N. fuckeliana* from *Pinus radiata* seedlings

Treatment	No. inoculations	% Nectria recovered	Average length of discolouration (mm)
Cut tops. 1x 10^7 spores/ml	11	91	2.0
Cut tops. 1x 10^5 spores/ml	4	100	1.5
Cut tops. 1x 10^4 spores/ml	4	100	2.0
Cut tops. *N. fuckeliana* + *Cladosporium*	5	20	1.5
Cut tops. Water control	8	0	2.0
Stem inoculation.1x 10^5 spores/ml	8	100	0
Stem inoculation. Water control	4	0	0

Table 3: Recovery of *N. fuckeliana* from *Pinus radiata* cuttings

Treatment	No. inoculations	% Nectria recovered	Average length of discolouration (mm)
Cut tops. 1x 10^7 spores/ml	10	100	2.0
Cut tops. 1x 10^5 spores/ml	19	79	1.5
Cut tops. 1x 10^4 spores/ml	12	75	2.0
Cut tops. *N. fuckeliaia* + *Cladosporium*	26	50	1.5
Cut tops. Water control	13	0	1.5
Stem inoculation.1x 10^5 spores/ml	12	16	0
Stem inoculation. Water control	3	0	0

Table 4: Recovery of *N. fuckeliana* from Douglas fir seedlings

Treatment	No. inoculations	% Nectria recovered	Average length of discolouration (mm)
Cut tops. 1x 10^7 spores/ml	15	60	1.5
Cut tops. 1x 10^5 spores/ml	12	0	1.5
Cut tops. 1x 10^4 spores/ml	4	0	1.5
Cut tops. *N. fuckeliana* + *Cladosporium*	18	0	1.5
Cut tops. Water control	14	0	1.0
Stem inoculation. 1x 10^4 spores/ml	4	0	0
Stem inoculation. Water control	3	0	0

DISCUSSION

The recovery of *Nectria* from Douglas fir only when plants were treated with a very high spore dose suggests that this species may not be a capable host to this fungus. Field inoculations of Douglas fir aged 2-4 years have been undertaken to test the susceptibility of older plants and for comparison with *P. radiata*.

Although there was no evidence of symptom development, *Nectria* was recovered from the majority of *P. radiata* plants from around the inoculation point. Conditions under which the plants were held were however highly artificial. In nature we can expect weather and competing microorganisms to have a marked effect on fungal survival and infective capability. This was partly illustrated by the reduced recovery of *Nectria* from plants where spores were mixed with those of another fungus, and the non-recovery from tissue that was dead prior to being inoculated. Plants were harvested for

examination 6 weeks after inoculation; and the survival of the fungus for longer periods of time, and after new leaders have formed, is unknown.

A small follow-up field trial to examine infection in a natural situation is recommended. This should involve either planting seedlings adjacent to an infected stand, or exploiting an operational planting that is next to an infected stand. Plants will be topped after planting to expose fresh wounds and part of the trial inoculated. Harvesting and examination of material will be staged over a period of at least 12 months. In August 2005, a field trial was established in a newly-planted stand in Otago to examine infection of seedlings in the field.

REFERENCE

Dick, M.A. 2003: *Nectria fuckeliana* infection of conifers: A review from a New Zealand perspective. Internal Forest Research Report for the Nectria Working Group.