Neonectria fuckeliana infection of *Pinus radiata* nursery stock

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Abstract Nectria flute canker is a disease of *Pinus radiata* stems caused by *Neonectria fuckeliana*. The fungus is not known in the North Island, leading to concern that it may be transported north via asymptotically colonised nursery stock. Wounded *P. radiata* seedlings and cuttings were exposed to natural inoculum from an adjacent infected forest, and half were artificially inoculated. No disease symptoms were recorded throughout the trial. *Neonectria fuckeliana* was re-isolated from 24% and 7% of inoculated seedlings and cuttings respectively 3 months after inoculation, and at 12 months from 3.5% of the remaining inoculated seedlings only. *Neonectria fuckeliana* was not isolated from uninoculated seedlings or cuttings exposed to natural inoculum. These results indicate that nursery plants have little capability of retaining *N. fuckeliana* within the tissue even when directly inoculated. Nursery stock is therefore unlikely to be a pathway for transport of the fungus.

Keywords *Neonectria fuckeliana*, *Pinus radiata*, nectria flute canker.

INTRODUCTION

Nectria flute canker, caused by the ascomycete *Neonectria fuckeliana* (C. Booth) Castl. & Rossman, is a stem disease of *Pinus radiata* D. Don occurring in the southern regions of New Zealand (Bulman 2007a, b; Dick & Crane 2009; Gadgil et al. 2003). It has also, more recently, been reported from *P. radiata* plantations in southern Chile (Morales 2009).

The long, narrow cankers that form on *P. radiata*, commonly referred to as flute cankers, develop around pruned branch stubs. The cankers vary in size but can extend for several metres above and for a shorter distance below a branch stub. Although tree crowns generally remain healthy, affected trees are susceptible to

decay and wind breakage at infected whorls, and wood quality can be affected (Crane et al. 2009).

Neonectria fuckeliana is native to Northern Europe and North America where it has been recorded principally as a wound invader of species of *Picea* and *Abies*. It is considered to be an endophytic coloniser of sapwood or sometimes a weak pathogen of these hosts (Roll-Hansen & Roll-Hansen 1979; Schultz & Parmeter 1990; Vasiliauskas & Stenlid 1998). Likewise in New Zealand, canker formation is invariably associated with stem wounds.

The fungus was first reported to be established in New Zealand by Gadgil et al. (2003). Herbarium specimens and isolates held in

Scion collections (NZFRI-M and NZFS) from Dunedin and Southland Crosby regions (Crosby et al. 1976) were examined and these dated back to 1997 (Gadgil et al. 2003). *Neonectria fuckeliana* has since been found extensively in Otago and Southland, and in a few locations in South Canterbury and Mid Canterbury (Crane et al. 2009). There are no records from the North Island. During the period 1997–2004 thousands of *P. radiata* seedlings and cuttings from South Island nurseries were planted out in locations in the North Island. Members of the forest industry became concerned 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.

There has been no evidence of nectria disease of nursery plants or in plantations prior to pruning, and as *N. fuckeliana* is considered to be a wound invader intact seedlings or rooted cuttings are very unlikely to have the opportunity to become infected. However, it is common practice in New Zealand forest nurseries to standardise the height of the plants before lifting by removing the top. If topping is carried out there is the theoretical possibility that, if there were an inoculum source nearby, the wound could become infected. Typically topping is carried out some weeks before lifting and the wounds have calloused over before plants leave the nursery for establishment in plantations.

The objective of this study was to determine if *P. radiata* nursery-age plants could become infected with *N. fuckeliana* and, if so, whether the colonisation was durable and if infected plants would express disease symptoms.

MATERIALS AND METHODS

A newly planted site adjacent to a *N. fuckeliana*-infected stand of *P. radiata* was selected in Berwick Forest, Otago and four 12 × 12 m blocks were marked out within an area of approximately 1 ha. Two hundred and twenty un-topped 18-month-old *P. radiata* seedlings and 220 cuttings from a local nursery were planted in alternating rows of cuttings and seedlings with 1 m spacing between rows and also between individual plants in a row. Each of the 4 blocks contained 11 rows of

10 plants. Two blocks had 5 rows of seedlings and 6 rows of cuttings; the others had 6 rows of seedlings and 5 rows of cuttings.

Neonectria fuckeliana isolate NZFS 980 was grown on 2% malt extract agar (MEA) Petri plates at 25°C for 7 days in the dark. Conidia of the synanamorph *Acremonium* sp. were scraped from the surface of the plates to seed flasks of a 0.5% malt extract solution for preparation of a spore suspension. Flasks were placed on a shaker for 7 days for multiplication of the conidia. The spore concentration, determined using a haemocytometer, was adjusted to 5×10^3 spores/ml.

One month after planting in August 2005 all plants were topped, i.e. approximately 20 mm of the apical shoot was removed with secateurs. The same day half of the plants were inoculated by applying a 25 µl droplet of spore suspension (containing approximately 125 spores) to the cut top using a micropipette. In blocks 1 and 3 inoculum was applied to all plants in rows 1, 2, 5, 6, 9 and 10. In blocks 2 and 4 inoculum was applied to all plants in rows 3, 4, 7, 8, and 11. Equal numbers of seedlings and cuttings thereby received inoculum or remained uninoculated. The uninoculated plants served as a control for comparison of symptom development and plant growth and could also potentially become infected naturally by *N. fuckeliana* spores from the adjacent stand.

At the end of November 2005, 3 months after establishment of the trial, plants were assessed in the field for symptoms of dieback or canker. Half of the 110 plants from each treatment were then harvested for examination and isolations in the laboratory. The needles were stripped from each stem and the extent of discolouration from the cut top measured. The top 80 mm lengths of stem were surface sterilised in 10% hydrogen peroxide before being cut into 5 mm lengths and placed onto 2% MEA 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 establishment of the trial for all of the remaining plants, although a 20–40 mm length of stem below the wound point was sampled for isolations rather

than the 80 mm length plated after the 3 month assessment. At 12 months a trace of discoloured tissue could be found in some plants when the stems were dissected; in others the inoculation point had been incorporated into the stem and was identified only by a slight distortion and the association with the whorl. Measurements of the extent of discoloured tissue were therefore not made at the 12 month assessment.

A generalised linear mixed model with a logit link function was used to analyse the probability of a positive isolation of *N. fuckeliana*. As *N. fuckeliana* was only reisolated from one uninoculated plant (a seedling at 3 months) only the effect of plant type was considered in the model. The length of discolouration in the stem was analysed using a mixed model. Separate variance estimates for each plant type and inoculation treatment combination were estimated in order to account for heterogeneity of variance according to Littell et al. (2006). In analysing both the probability of a positive isolation and the length of the discoloured stem, the block consisting of five seedlings at each time period was considered to be the experimental unit. The block was treated as a random effect, while the material type (cutting or seedling) and the inoculation (with or without) were considered fixed effects. All analyses were conducted with SAS software, Version 9.2 of the SAS System for Windows, SAS Institute Inc., Cary, NC, USA.

RESULTS

No symptoms of dieback, stem lesions or infection of any kind were observed at either of the two assessments. At 3 months the cut top of the plants was readily identifiable, although at 12 months the wound was often obscured by the rapid growth of the uppermost whorl of branches. The wound had largely occluded and in some cases could barely be detected.

At 3 months *N. fuckeliana* was isolated from 24% and 7% respectively of the 55 inoculated seedlings and 55 cuttings sampled (Table 1) and from only one of the 110 uninoculated plants (55 seedlings and 55 cuttings) tested at this time. There were no differences between the two plant

types ($P=0.213$). At 12 months after inoculation *N. fuckeliana* was recovered from just 2 of the 55 inoculated seedlings. These data were not analysed.

Table 1 Percentage of *Pinus radiata* plants infected with *N. fuckeliana* after 3 and 12 months ($n = 55$ for each treatment combination at each time)

Treatment	3 months	12 months
Seedlings – inoculated	24	3.5
Seedlings – not inoculated	2	0
Cuttings – inoculated	7	0
Cuttings – not inoculated	0	0

Discolouration in the stem associated with the cut was mostly consistent with normal response to an injury in both inoculated and non-inoculated plants. Average length of discolouration at 3 months (Figure 1) was highest in the uninoculated cuttings, but *N. fuckeliana* was not recovered from any of the plants in this treatment. A significant plant type by inoculation treatment interaction was observed ($P=0.020$), the uninoculated cutting having a significantly greater stain than any of the other treatment combinations.

Figure 1 Predicted mean length (mm) of discolouration for each treatment (with standard errors). CI= cuttings that had been inoculated, CU= cuttings that had not been inoculated, SI= seedlings that had been inoculated, SU= seedlings that had not been inoculated.

At 3 months the top 20–40 mm of all of the stems, including those from which *N. fuckeliana* was recovered, were colonised by other fungi. Most of these could be identified, at least to genus, and are known saprophytes or endophytes commonly associated with *P. radiata*. At least two fungi were obtained from each stem section with up to five species from some sections. The most frequently obtained saprophytic fungi were *Aureobasidium* sp., *Pestalotiopsis funerea* (Desm.) Steyaert, *Epicoccum purpurescens* Ehrenb., *Alternaria* sp., *Trichoderma* sp., *Botrytis* sp., *Cladosporium* sp., *Gliocladium* sp. and *Sclerophoma* sp.

At 12 months isolation of any fungi from the stem sections was rare.

DISCUSSION

There was no sign of disease on any of the seedlings or cuttings at any time, when assessed at 3 months or 12 months after inoculation. However, colonisation of the stem of some plants by *N. fuckeliana* had occurred although the fungus was obtained only from the top 20 mm of the stem adjacent to where the spore suspension had been placed. Highest numbers were in the inoculated seedlings that were harvested at 3 months. The percentage of plants from which *N. fuckeliana* was recovered dropped markedly at 12 months.

Plants had barely grown during the 3 month period from inoculation to first harvest whereas it was apparent at the second harvest that there had been vigorous growth in the intervening 9 months. No measurements were made so this growth cannot be quantified. It is possible that some biochemical aspect of vigorous growth was inhibitory to the fungus and that it was generally unable to survive in the tissue although this is speculation. There was also little recovery of the other colonising fungi that were detected during the 3 month isolations.

Discolouration in the stem tissue associated with the cut top at 3 months was largely consistent with normal response to an injury. Three of the uninoculated cuttings had extensive discolouration but even after removing these

from the analysis the uninoculated cuttings still had significantly longer mean discolouration than uninoculated cuttings or the seedlings. The reason for this is not known. It is possible that, compared to seedlings, cuttings have a different transpiration rate related to the different root system and when cut the apical portion dried out more quickly. This could lead to a physiological response or to enhanced colonisation by one of the saprophytic fungi recorded that resulted in the discolouration.

Neonectria fuckeliana was isolated from only one of the 220 uninoculated plants. This positive result was possibly due to cross-contamination from the inoculated plants rather than from natural inoculum from the surrounding stand.

In plantation trials that examined the influence of season on the formation of nectria flute cankers, winter pruning and inoculation of pruned stubs with conidia consistently resulted in more infection than summer pruning and inoculation (Bulman 2007a). Spore trap data (Crane et al. 2009) have shown a trend towards low numbers of ascospores over spring and summer, increasing during the autumn and winter months. For these reasons the trial reported here was established in late winter. It is possible that more of the inoculated plants may have been colonised under different environmental conditions. However, there was little invasion of the stem of those plants that did return a positive result and it is likely that the tissue is not suitable for *N. fuckeliana* colonisation at this age.

Only one isolate of *N. fuckeliana* was used in the trial. NZFS 980 was obtained from a severely cankered *P. radiata* in 2002 and has been used for inoculation experiments of trees of pruning age (5–8 years) with resultant canker formation (M.A. Dick, unpublished data). Variability in the aggressiveness of individual isolates has not been examined although variability in tree response to inoculation (measured by canker extent) has been recorded (M.A. Dick, unpublished data). Isolate variability will be assessed in future experimental work.

In literature on *N. fuckeliana* from overseas there is no record of the fungus colonising

nursery stock or very young plants. Research into the ecology of *N. fuckeliana* (Crane et al. 2009) 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 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 New Zealand Forest Health Inspectors (SPS Biosecurity) who were 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 and Southland had been planted over the preceding 8 years (BJ Rogan, SPS Biosecurity, pers. comm.). Samples collected by the inspectors were examined in the Scion Forest Health Reference Laboratory. No evidence of *N. fuckeliana* was found at that time nor has it been identified in these locations during subsequent annual inspections (Scion Forest Health Database, unpublished data).

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. These results, along with the fact that forest nurseries are usually some distance from an inoculum source demonstrates that nursery stock is extremely unlikely to become infected and to be 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.

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