CROSS INFECTIVITY OF SCLERODERRIS CANKER ON NATIVE AND EXOTIC CONIFERS IN NEWFOUNDLAND

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Summary

Scleroderris canker caused by the European race of *Gremmeniella abietina* variety *abietina* was first recorded in Newfoundland in 1979. In 1981, a red pine plantation near Torbay 10 km north of St. John's was destroyed by the disease. A quarantine was established on the Avalon Peninsula to reduce the possibility of an accidental introduction of Scleroderris canker to the remainder of the Island. The spread of the European race of *Gremmeniella* would be catastrophic for native red pine (*Pinus resinosa*) stands in central and western Newfoundland as they already constitute a rare and endangered species.

Scleroderris canker caused by *G. abietina* var. *balsamea* was also recorded on the Northern Peninsula in a Sitka spruce (*P. sitchensis*) plantation. The variety *balsamea* does not seem to infect pines. The objective of this study was to determine cross infectivity and virulence of the different *Gremmeniella* varieties among the native and exotic conifer species planted in Newfoundland.

We used two conidial concentrations to test cross infectivity and determine virulence of both the European race and the variety *balsamea*. Seedlings were inoculated by spraying a conidial suspension on the recently developed leaders of 5 different conifer species: red pine, jack pine, white spruce, black spruce and Japanese larch. Inoculated seedlings were placed in a dew chamber at 100% relative humidity and 18°C for 3 days, then transferred to a growth chamber at 4°C for 45-60 days. Seedlings were subsequently transferred to a greenhouse and were inspected for symptom expression. Infection was confirmed by placing needles from each seedling on 2% malt agar.

The European race of *G. abietina* was the more virulent with infection rates up to 40%. Red and jack pine were equally susceptible, followed by black spruce. Non-surface sterilized needles of all conifer species showed presence of *Gremmeniella* viable spores three months after inoculations. *G. abietina* var. *balsamea* was not found on any seedlings.

Presence of viable spores of the European race three months after inoculation suggests a great potential for every conifer species seedling to be a carrier of viable spores for months after *Gremmeniella* sporulating season. One seedling of black spruce was infected with *Gremmeniella,* however we believe this is representative of a potentially very low infection rate on non-pine hosts.

Key words: Scleroderris canker, *Gremmeniella*, inoculation.

Introduction

Scleroderris canker was first recorded in Newfoundland in 1979 (Singh et al. 1980), on Austrian pine (*Pinus nigra* Arnold) near St. John's and the disease has since been recorded in several localities on the Northeast Avalon Peninsula

(Hudak and Singh, 1984) (Figure 1). Cultures isolated from infected trees were identified as *Gremmeniella abietina* variety *abietina* (Laberg.) Morelet (European race). This variety is thought to be an accidental introduction recently in North America and displays high levels of damage.

In 1981, a red pine (P. resinosa Ait.) plantation near Torbay, 10 km north of St. John's, was destroyed by Scleroderris canker. In 1982 infected ornamental Scots pine (P. sylvestris L.), jack pine (P. banksiana Lamb.) and red pine were found along Salmonier Line 30 km west of St. John's (Sterner and Davidson, 1983). In both localities all diseased trees were cut and burned. In 1987 severe foliage damage was detected on Scots pine in an old nursery on Salmonier Line and Collier's Ridge west of St. John's (Moody, 1989). Most severe damage occurred on red pine while Austrian pine was moderately affected with some mortality. New infection sites occurred almost yearly in and around the St. John's area affecting only red, Scots and Austrian pines. In 1993, most Scots pine in the old nursery on Salmonier Line were dead or dying and were cut. A quarantine was established on the Avalon Peninsula to reduce the risk of an accidental introduction of Scleroderris canker to the remainder of the Island where planted and naturally occurring red pine stands exist. Transplanting of ornamental hard pines from nurseries and distributors in the St. John's area to outlying communities is a great concern and may spread this disease to other areas of Newfoundland.

On the Northern Peninsula of Newfoundland, we have identified a Sitka spruce (Picea sitchensis (Bong.) Carr.) plantation infected with *G. abietina* var. *balsamea* Petrini et al. The variety *balsamea* does not seem to infect pines (Moody, 1989). The source of this infection was thought to be an imported nursery stock from the Maritime provinces of Canada, but this is certain. The variety *balsamea* has never been found in the Maritime provinces and the disease was observed in only one of 26 plantations with the same nursery stock. Thus it is possible that the variety *balsamea* is endemic on native black spruce (P. mariana (Mill.) B.S.P.) on the Northern Peninsula of Newfoundland. The plantation was sanitized by removing severely infected trees and branches. However, symptoms reoccurred in 1990, 1991 and 1992, but he disease does not seem to be spreading.

In continental Canada there is another variety of *G. abietina* var. *abietina* (American race) thought to be native to North America which causes damage on the lower 2 metres of host trees. This variety has not been reported in Newfoundland.

Many diseases and insects accidentally introduced in Newfoundland have been transported on plant material such as seedlings, ornamentals and wood products. In the case of Scleroderris canker, the disease was probably introduced by imported infected seedlings or ornamentals. A breech in the current quarantine will probably originate from such material being transported across the isthmus from the Avalon Peninsula to the remainder of the island. Introduction of the European race *G. abietina* would be catastrophic for the viability of native red pine as they already constitute a rare and endangered species. Extinction of red pine is a distinct possibility if the quarantine is breeched. The objective of this study was to determine cross infectivity and pathogenicity of the European race of *G. abietina* and the *G. abietina* var. *balsamea* among five conifer species planted in Newfoundland and to determine the possibility of non-host conifer species as carriers of the disease.

Materials and Methods

The conifer hosts considered for this study were primarily seedling species produced in Newfoundland: red pine, jack pine, white spruce (*P. glauca* (Moench) Voss), black spruce and Japanese larch (*Larix leptolepis* (Sieb. & Zucc.) Gord.). One to two year old dormant container stock were transplanted into 5 inch pots with a vermiculite, peat and soil mixture. The seedlings were then placed in an incubator or greenhouse at 20°C (16 hrs light) and 15°C night for 3-4 weeks to break dormancy and obtain new leader growth required for the inoculation test.

The two isolates used were collected in Newfoundland (Table 1). Isolate #105 is the European race of *G. abietina* while isolate #618 is the variety *balsamea*. The *Gremmeniella* inoculum was obtained as described by Ylimartimo and Haansuu (1993), and consisted of 6.25 g barley, 18 ml water, 1 teaspoon of crushed red pine needles mixed in a 125 ml Erlenmeyer flask and left overnight to dissolve. The media was autoclaved the next day, left still for 3 days and autoclaved again. The media was inoculated with *Gremmeniella* and then incubated at 18°C under fluorescent light (16 hrs light\ 8 hrs dark). Conidia were usually numerous after 4-6 weeks.

A conidial suspension was prepared and then further diluted to obtain two concentrations: 250 spores per µl and 2500 spores per µl. These two conidial concentrations of the two isolates were used as inoculum. An atomizer was used to spray a 2 ml conidial suspension on recently developed leaders of 5 different conifer species. Ten seedlings were used for each combination and ten seedlings were sprayed with water as a control. Inoculated seedlings were placed in a Perceval 135-D dew chamber at 100% relative humidity (RH) and 18°C for 3 days for conidial germination and infection. Seedlings were then transferred to a growth chamber set at 70% RH, 4°C with 8 hrs light\ 16 hrs dark for 45-60 days. This latent period is the number of consecutive days necessary for a successful infection (Marosy et al. 1989).

Symptom expression was favoured by transferring seedlings into the greenhouse at 16 hrs light\ 8 hrs dark at 20°C for about 2-4 weeks. Seedlings were inspected daily for symptom expression. Infection was confirmed by placing 4 surface sterilized needles and 4 non-sterilized needles from each seedling on 2% malt agar. Petri plates were incubated for 3 weeks in the dark at 18°C. Candidate colonies were subcultured for confirming the presence of *Gremmeniella*.

Results

Incidence of infection varied greatly between the two *Gremmeniella* strains and among the different hosts (Table 2). Isolate 105 identified as European race *G. abietina*, was the more virulent with infection rates (on surface sterilized needles) up to 40 % on jack pine and 38% on red pine. Spore concentrations seemed to have little impact on the infection rate. Red pine and jack pine were equally susceptible, followed by black spruce. The black spruce seedling infected showed infection on one needle, and when later tested again it showed no signs of infection. Japanese larch and white spruce showed no infection. Non-surface sterilized needles of all host species showed presence of *Gremmeniella* even three months after inoculations with the higher spore concentration. Viable spores were evident only on pine species using lower spore concentration.

Isolate 618 from Sitka spruce and identified as *G. abietina* var. *balsamea*, was not found on either surface sterilized or non-surface sterilized needles. One needle on a jack pine seedling has been identified as infected by *G. abietina* var. *balsamea*, but this is probably due to experimental error.

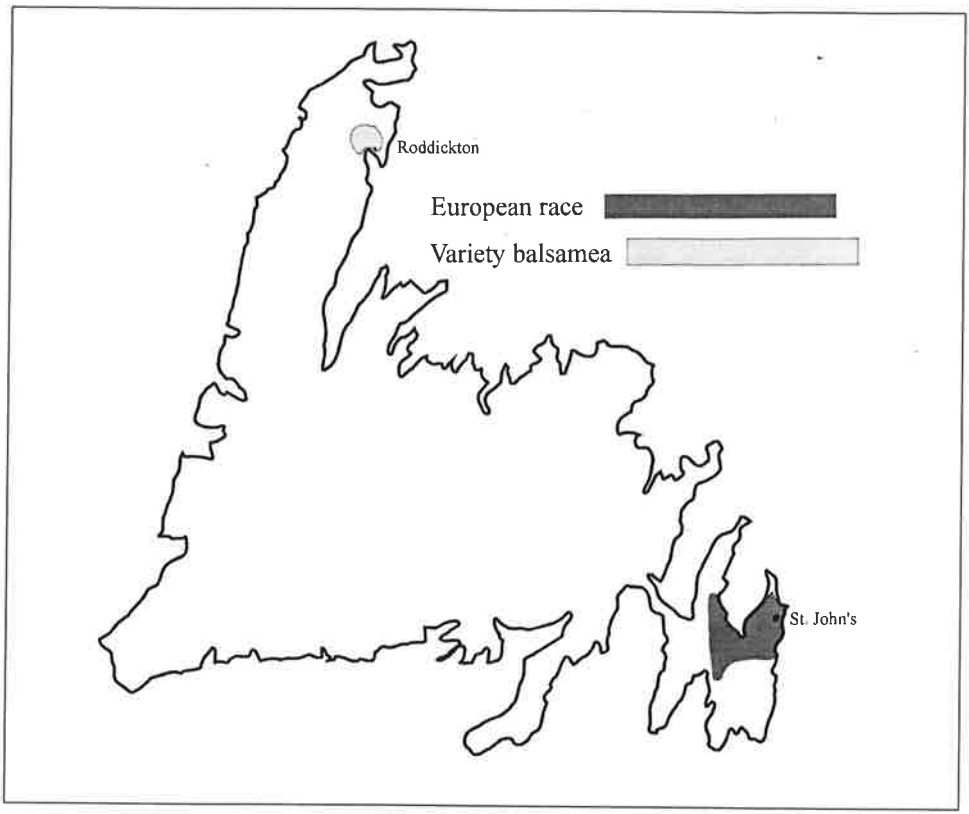

Fig. 1. Distribution of *Gremmeniella* varieties in Newfoundland.

Discussion

Spores of European race *G. abietina* were viable on every conifer host tested three months after inoculation. This suggests a great potential for every conifer species seedling to be a carrier of viable spores for months after sporulating season (June). To reduce the risks to nursery stocks, we have to minimize the inoculum rate in the air by eradication of host pines in and around the nursery. Since only conidia are produced with the European race and the conidia are disseminated over a short distance, it reduces the perimeter to be treated. Even though the number of conidia trapped from a site decreases rapidly as the distance from the inoculum source increases, Skilling et al. (1986) found it was still possible to collect a few conidia at a distance of 610 metres. Consequently the minimum distance to eradicate host trees to prevent the disease would be 0.6 kilometre around a nursery. To avoid infection of seedlings in transit we recommend restricting seedling transport to periods before or long after sporula-

tion season and treat seedlings with appropriate fungicide to minimize the risk of carrying viable spores.

Red and jack pine seedlings are highly susceptible to the disease and are also carriers. Thus no pine seedlings or pine material should be shipped out of the quarantine area. Jack pine seems to be an excellent carrier of the disease because it does not die immediately upon infection as red pine does because it is more resistant to the European race (Laflamme, 1993).

One seedling of black spruce was infected with *Gremmeniella*. We believe this is representative of a potentially very low infection rate on non-host conifer species to *G. abietina* var *abietina*. Considering that 60% of black spruce seedlings were also carriers of viable spores, this suggests caution regarding shipment of black spruce seedlings outside the quarantine area.

Viable spores of *G. abietina* var. *balsamea* were not present on any hosts after three months. Since these spores had a high germination rate immediately after inoculation (above 90%), eliminating the possibility of unfertile inoculum, we conclude that the spores of the variety *balsamea* do not survive on non-host species contrary to the variety *abietina*. The variety *balsamea* has only been found on spruce and fir (Petrini et al. 1989) and seems to need a wound to be able to infect its host. Also, the infection occurs on the shoot causing a canker (Laflamme, 1988) and not on needles as the variety *abietina*.

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Table 1. Origin and varieties of *Gremmeniella abietina* used for inoculations

Isolate No.	Variety (Serovar)	Origin,	Host	Date of collection
105	*G. abietina* var. *abietina* (European race)	St. John's, NF,	Scots pine	1981
618	*G. abietina* var. *balsamea*	Roddickton, NF,	Sitka spruce	1992

Table 2. Percentage of infection caused by two isolates of *Gremmeniella* on five different conifer species.

Host	Isolate 105		Isolate 618		Control
	Non-sterilized	Sterilized	Non-sterilized	Sterilized	
	250 spores per µl				
Japanese larch	0	0	0	0	0
Jack pine	70	30	0	10*	0
White spruce	0	0	0	0	0
Black spruce	0	0	0	0	0
Red pine	38	38	0	0	0
	2500 spores per µl				
Japanese larch	60	0	0	0	0
Jack pine	90	40	0	0	0
White spruce	30	0	0	0	0
Black spruce	60	10*	0	0	0
Red pine	12	12	0	0	0

*Only one needle on one seedling infected