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G. Laflamme and R. Blais

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Article abstract

In the early 1980s, more than 90% of mortality caused by *Gremmeniella abietina*, European race, was recorded in red pine (*Pinus resinosa*) plantations 200 km northwest of Montreal, Quebec, Canada. Surrounding jack pines (*Pinus banksiana*) did not appear to be affected. Consequently, foresters began to plant the affected areas with jack pine seedlings. In 1988, plots of 100 jack pines were established in three of the four selected plantations. As reference, red pine seedlings were planted in 1989 under similar conditions in the fourth plantation. Observations were carried out annually from 1989 to 1992. Mortality of red pine seedlings reached 70% in 1992 while all jack pines on the three experimental sites were free of the disease except for a tip blight, a distinctive feature allowing race identification in the field. The North American race symptoms were present at a very low incidence, but began to increase on site I in 1992. More than 10 years after planting, the jack pine trees still show resistance to the European race of *G. abietina* while all the red pines died.

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## Resistance of *Pinus banksiana* to the European race of *Gremmeniella abietina*

Gaston Laflamme and Robert Blais<sup>1</sup>

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In the early 1980s, more than 90% of mortality caused by *Gremmeniella abietina*, European race, was recorded in red pine (*Pinus resinosa*) plantations 200 km northwest of Montreal, Quebec, Canada. Surrounding jack pines (*Pinus banksiana*) did not appear to be affected. Consequently, foresters began to plant the affected areas with jack pine seedlings. In 1988, plots of 100 jack pines were established in three of the four selected plantations. As reference, red pine seedlings were planted in 1989 under similar conditions in the fourth plantation. Observations were carried out annually from 1989 to 1992. Mortality of red pine seedlings reached 70% in 1992 while all jack pines on the three experimental sites were free of the disease except for a tip blight, a distinctive feature allowing race identification in the field. The North American race symptoms were present at a very low incidence, but began to increase on site I in 1992. More than 10 years after planting, the jack pine trees still show resistance to the European race of *G. abietina* while all the red pines died.

### [Résistance du *Pinus banksiana* à la race européenne de *Gremmeniella abietina*]

Au début des années 80, plus de 90 % de mortalité causée par *Gremmeniella abietina*, race européenne, a été relevée dans des plantations de pin rouge (*Pinus resinosa*) localisées à environ 200 km au nord-ouest de Montréal, Québec, Canada. Les pins gris (*Pinus banksiana*) avoisinants ne semblaient pas attaqués. Les forestiers ont donc reboisé ces aires avec des semis de pin gris. Des parcelles d'études de 100 pins gris ont été établies dans trois plantations sur les quatre sélectionnées en 1988. Comme références, des semis de pin rouge ont été plantés dans des conditions semblables dans la quatrième plantation en 1989. Les observations annuelles se sont déroulées de 1989 à 1992. La mortalité des semis de pin rouge atteignait 70 % en 1992 alors que les pins gris des trois sites expérimentaux n'étaient pas attaqués, à l'exception d'une brûlure à l'extrémité des pousses, caractéristique qui permet d'identifier la race au champ. Les symptômes de la race nord-américaine étaient présents à un taux très bas; leur incidence a augmenté sur le site I en 1992. Plus de 10 ans après la plantation, les pins gris montrent une résistance à la race européenne de *G. abietina* alors que tous les pins rouges sont morts.

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1. Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., P.O. Box 3800, Sainte-Foy, Quebec, Canada G1V 4C7; e-mail: glaflamme@cfl.forestry.ca

## INTRODUCTION

*Gremmeniella abietina* (Lagerb.) Morelet, the causal agent of Scleroderris canker of conifers, is a damaging fungal pathogen in the pine plantations and tree nurseries in North America, Europe and Asia (Gibbs 1984; Laflamme 1993; Laflamme and Lachance 1987; Setliff *et al.* 1975; Skilling *et al.* 1986; Yokota *et al.* 1974). Dorworth and Krywienczyk (1975) recognized three races of *G. abietina* based on serological tests. These three races (North American (NA), European (EU) and Asian) are confined to the *abietina* variety of this species (Petri *et al.* 1989). Later, these three races were confirmed as being divergent based on their ecological, etiological and molecular characteristics, but are considered morphologically indistinguishable (Bernier *et al.* 1994; Hamelin *et al.* 1993; Petri *et al.* 1989, 1990). The NA and EU races are present in North America while the Asian race has only been reported in Japan. The NA race is believed to be indigenous to this continent based on its wide distribution, its ecological adaptations and the high levels of genetic variability (Hamelin *et al.* 1993; Laflamme 1993). The NA race only infects pine shoots that are under snowcover (Marosy *et al.* 1989), thus only seedlings or lower tree branches of a tree are affected. The EU race also begins by infecting shoots on the lower part of the tree but it can subsequently develop in the upper crown of large trees, thereby causing more damage than the NA race. The fact that the EU race killed large red pine (*Pinus resinosa* Ait.) trees in New York State, U.S.A. (Setliff *et al.* 1975) and many younger plantations in Quebec, Canada (Laflamme and Lachance 1987) has caused great concern that the disease could affect all pine species. Host range studies conducted on most conifers present in the United States showed that almost all of them, with the exception of *Thuja* species, were susceptible (Skilling *et al.* 1986). The possibility that both *Pinus banksiana* Lamb. (jack pine) and *Pinus contorta* Dougl. var. *latifolia* (lodgepole pine) species would become invaded by the EU race has been apprehended (Dor-

worth and Muir 1993), as both species cover a very large geographical distribution.

In 1984, a study was undertaken on the epidemiology of Scleroderris canker in red pine plantations northwest of Montreal and Ottawa, where the EU race had just been identified (Laflamme and Lachance 1987). Although red pines were severely damaged by the pathogen, the surrounding or mixed jack pine seedlings or trees, planted or from natural regeneration, were apparently not affected. Following these observations, foresters decided to plant jack pine under or near red pines infected with the EU race in a few of these plantations. These appear to be very good sites on which to study the behaviour of the EU race of *G. abietina* on this tree species. The objective of this study was to measure, over many yr, the impact of the EU race on jack pine.

## MATERIALS AND METHODS

The four different experimental sites were located in a region extending from 120 to 200 km northwest of Montreal (Canada). On each site, the inoculum source consisted of red pines planted in the late sixties with a height of over 3 m and with *G. abietina*, EU race, infecting more than 80% of these trees (Table 1). The prevalence of this race was verified in three different yr between 1984 and 1988 and the presence of shoot blight over 2 m in height in the crown of red pines (Table 1) is characteristic of the EU race (Laflamme *et al.* 1998). One permanent plot of 100 jack pine seedlings was delineated on sites I, II and III in 1988. Site IV was planted with red pine seedlings in 1989 and set up as reference; sites I, II, III could not be planted with red pines as these would have been dominated by the jack pines present. Field observations began in 1989.

On site I, the 100 jack pine seedlings were growing under residual living infected red pines (Fig. 1). On site II, the 100 jack pine seedlings were planted in a clearcut area near a few residual infected red pines. On site III, the 100 jack

**Table 1. Site characteristics of the four red pine plantations used as inoculum source of *G. abietina*, EU race, for the infection of pine seedlings. The height of red pines and the infection height in the crown of these red pines were measured in 1988**

Site	Long. - Lat.	Year of seedling plantation	Height	
			Red pines (m)	Infection (m)
I	(46°53'N, 75°16'W)	1986	3 - 4	3
II	(46°53'N, 75°16'W)	1985	3 - 4	3
III	(46°39'N, 75°16'W)	1985	7 - 8	5
IV	(46°23'N, 74°47'W)	1989	5 - 6	4

pine seedlings were located in a clearcut area less than 2 m from the nearest diseased red pines. On site IV, 436 red pine seedlings were planted in an opening of about 3,000 m<sup>2</sup> in the middle of a plantation of diseased red pines, the lower branches of which had been pruned in 1984 in order to eliminate the presence of the North American race.

The disease was identified in the field according to symptoms and signs such as the occurrence of shoot blight showing greenish colouration under the bark, and the presence of cryptopycnidia (Cauchon and Lachance 1980). Isolates were grown from these cryptopycnidia for race identification, which was conducted by using 12% and gradient poly-

acrylamide gel electrophoresis (PAGE) as described by Petrini *et al.* (1989).

## RESULTS

From 1989 to 1992, no mortality of jack pine seedlings was recorded on sites I, II and III whereas mortality of red pine seedlings caused by *G. abietina* on site IV was very high, reaching 70% 4 yr after plantation (Table 2).

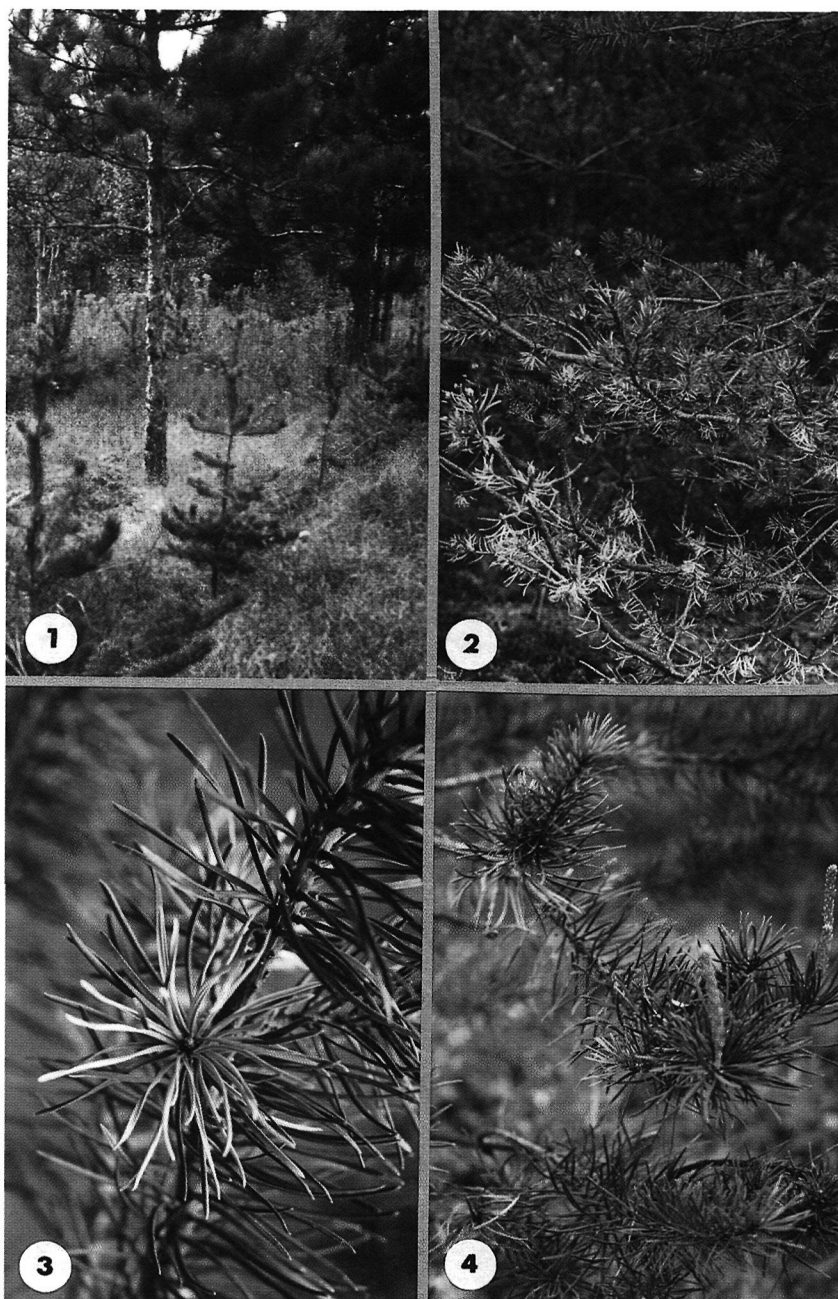
On jack pine, a symptom different from the shoot blight caused by the NA race (Fig. 2) and now attributable to the EU race was observed. The presence of pycnidia of *G. abietina* at the base of

**Table 2. Percentage of mortality and disease incidence caused by *G. abietina*, North American and European races, on red and jack pines, near or under red pine trees severely infected with the European race of *G. abietina*. Jack pines were planted on sites I, II and III and red pines on site IV**

Incidence	Site	Year			
		1989	1990	1991	1992
Mortality	I	0	0	0	0
	II	0	0	0	1 <sup>a</sup>
	III	0	0	0	0
	IV	-	39	53	70
European race	I	75	98	92	96
	II	10	38	39	52
	III	20	35	43	70
	IV	-	44	55	76
North American race	I	0	5	63 <sup>b</sup>	70 <sup>b</sup>
	II	0	0	5 <sup>b</sup>	6 <sup>b</sup>
	III	2	3	6 <sup>b</sup>	10 <sup>b</sup>
	IV	-	-	-	-

<sup>a</sup> Jack pine killed by the NA race of *G. abietina*

<sup>b</sup> Natural pruning of branches occurred, making it difficult to separate them from shoots killed by *G. abietina*.



**Figure 1.** Jack pine seedlings planted under red pine infected by the European race of *G. abietina* : Site I in 1989. (Photo : R. Blais)

**Figure 2.** Shoot blights on jack pine caused by the North American race of *G. abietina*. (Photo : G. Laflamme)

**Figure 3.** Tip blight on jack pine caused by the European race of *G. abietina*. (Photo : R. Blais)

**Figure 4.** Numerous tip blights on one sampled jack pine caused by the European race of *G. abietina*. (Photo : R. Blais)

the needles or on the buds, and of cryptopycnidia in the shoots were the signs of the disease; they were used to culture the fungus and then to identify the race. This symptom, a tip blight, was always associated with the EU race of *G. abietina* (Fig. 3). This tip blight never developed into a shoot blight. The fungus seemed to abruptly stop developing and the length of the dead infected part of the shoots measured usually less than 2 cm (Fig. 3).

With this typical symptom of the EU race on jack pine, it was then possible to collect information directly in the field on the incidence of both races of the disease. The incidence of EU race symptoms on jack pine seedlings was very high on all three experimental sites; the highest incidence was on site I where seedlings were growing directly under the infected red pine trees (Table 2). The frequency of symptoms per tree was relatively low (Table 3) except on site 1 (Fig. 4), where it reached, for instance, 50 tip blights on one sample tree in 1992.

## DISCUSSION

On site I, jack pine seedlings planted directly under the infected red pines and thus fully exposed to infection were literally showered with conidia. It is known that the EU race of *G. abietina* produces conidia only; these are dispersed by rain-splash and the dispersal range is limited (Skilling *et al.* 1986). In spite of this high infection pressure, all trees survived and in 1999, they had overgrown the dying red pines.

On sites II and III, jack pines were located some distance from infected red pines and may have been exposed to fewer conidia than on site I. This could explain why a slightly lower disease incidence occurred in these sites compared with site I. A trial has been undertaken to determine the range of dispersion of conidia. On site II, one jack pine was dead in 1990 from infection by the NA race (Table 2). The contamination was likely coming from natural jack pine stands in which the native NA race of *G. abietina* occurs at the

endemic level. The NA race produces ascospores that are responsible for long-distance fungus spread (Skilling *et al.* 1986). It could not come from the residual infected red pines because all those trees had been pruned at a height of 2 m prior to the jack pine plantation, and it is known that the NA race can only infect its hosts in the snow, usually less than 2 m above the ground (Marosy *et al.* 1989). Jack pine is not killed by the EU race even when trees are showing a high frequency of tip blight (Table 3). This explains why these jack pine trees, growing near or with red pines severely infected with the EU race, were not damaged (Laflamme and Lachance 1987).

This is the first official report of this typical symptom associated with the EU race on jack pine. After 10 yr of field observations, we considered this symptom as being a reliable means of identifying the occurrence of that race on this tree species without race identification through laboratory procedures; but one must still check the conidia under the microscope to verify whether *G. abietina* is present. Our observations also demonstrate quite clearly that jack pine shows a strong resistance to the EU race of *G. abietina*, while red pine is very sensitive to it. The contradiction with previous results (Skilling *et al.* 1986) where jack pine was rated highly susceptible comes from the rating system used by these authors in which seedlings that outgrew the infection were still counted as being infected. This strong resistance is quite surprising because, prior to its introduction into North America approximately 30 yr ago, jack pine had never coexisted with the EU race of *G. abietina*, to our knowledge, and thus could not have developed effective defence reactions in such a short period of time. For example, white pine blister rust (*Cronartium ribicola* J.C. Fisher) was introduced about 100 yr ago into North America (Maloy 1997) and almost no resistance has developed on white pine (*Pinus strobus* L.) against this exotic pathogen (Hoff *et al.* 1980). This strong resistance reaction from jack pine to the EU race of Scleroderris canker is a phenomenon that needs to be further studied.

**Table 3. Mean frequency of infected shoots per tree and per site (I, II and III) of *G. abietina*, EU race symptom, observed on 100 jack pines (maximum frequency on a given tree)**

Site	1989	1990	1991	1992
I	2 (5)	4 (8)	5 (6)	10 (50)
II	1 (2)	1 (2)	2 (10)	2 (10)
III	1 (2)	2 (3)	2 (6)	2 (5)

Our observations indicate that the EU race could progress towards western Canada through natural jack pine stands, as stated by Dorworth and Muir (1993) because jack pine can carry the disease through tip blights. But this is unlikely to occur for two reasons: first, the EU race only produced conidia which are disseminated over short distances, such as within an individual tree (Skilling *et al.* 1986), or the spread of the disease, for example, can be stopped by a 5 m wide road (Laflamme 1999); secondly, the spread of the EU race in North America is related to the distribution of infected seedlings from tree nurseries and today, the control of the disease in nurseries with phytosanitary inspection before shipping the seedlings should stop the spread of the disease to new locations (Laflamme *et al.* 1998). Nevertheless, to ensure that native lodgepole pine stands will not become infected by the EU race by another route, quarantine should be invoked to prevent any transportation of living pines originating from areas where the EU race is present. In the meantime on lodgepole pine, inoculation trials with the EU race have been undertaken to verify if such resistance could exist on this species, which is genetically related to jack pine. Lodgepole pines are seriously affected by Scleroderris canker in Sweden (Karlman *et al.* 1994). However, another amplotype of the pathogen, as defined by Hamelin *et al.* (1996), is most likely involved and thus care should be taken not to introduce this amplotype into Canada where jack and lodgepole pines could be affected.

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