Etiology and Real-Time Polymerase Chain Reaction-Based Detection of *Gremmeniella-* and *Phomopsis-Associated* Disease in Norway Spruce Seedlings

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Accepted for publication 7 July 2006.

ABSTRACT

Borja, I., Solheim, H.. Hietala, A. M., and Fossdal. C. G. 2006. Etiology and real-time polymerase chain reaction-based detection of *Grewmeniella-* and Phomopsis-associated disease in Norway spruce seedlings. (Phytopathology 96:1305-1314. 02)

In spring 2002, an unusual disease outburst was recorded on Norway spruce seedlings in southeast Norway. Extensive damage was recorded on 1- and 2-year-old Norway spruce seedlings that either had wintered in nursery cold storage or had been planted out in autumn 2001. The damage was characterized by leader shoot dieback and stem necroses on the upper or lower part of the shoot from 2001. *Grennneniella abietina* and a *Phomopsis* sp. frequently were isolated from the diseased seedlings. Internal transcribed spacer (ITS) ribosomal (r)DNA sequence analysis and random amplified microsatellites profiling indicated that the *G. abietina* strains associated with diseased nursery seedlings belonged to the large-tree type (LTT) ecotype of the European race of G. *abietina* var. *abietina,* and inoculation tests confirmed their pathogenicity on Norway spruce. Based on ITS rDNA sequence analysis, the *Phontopsis* strains associated with diseased seedlings did not represent any characterized *Phomopsis* spp. associated with conifers. The *Phontopsis* sp. was not pathogenic in inoculation tests, indicating that it may he a secondary colonizer. 1TS-based real-time polyrnerase chain reaction assays were developed in order to detect and quantify *G. abietina* and Phomopsis in the nursery stock. We describe here the *G.* abietina-associated shoot dieback symptoms on Norway spruce seedlings and conclude that the unusual disease outburst likely was related to the *G. abietina* var. *abietina* epidemic caused by the LIT on large Scots pines in 2001.

Additional keywords: Picea abies.

Gremmeniella abietina (Lagerb.) M. Morelet is one of the most serious pathogens on pines in the Northern hemisphere. It causes a range of symptoms known as the Brunchorstia dieback or Scleroderris canker. G. abietina comprises two described varieties: var. *abietina* associated with a number of conifers and with a large geographic distribution area, and var. *balsamea* known only from Quebec, Canada, where it is associated with Ahies balsamea, Picea glauca, and P mariana (25). As reviewed by Hamelin and Rail (12), G. abietina var. abietina, based on morphological, serological, and genetic studies, has been divided into North American, Asian, and European races. The European race of *G*. abietina var. abietina consists of two ecotypes named the "small-tree type" (STT) and the "large-tree type" (LIT) (16), also previously described as biotypes A and B. respectively (30). Based on pairing tests, STT and LTT are regarded as genetically isolated (32). The LTT is most common in 15- to 40-year-old Scots pine (Pinus sylrestris L.) trees in southern Scandinavia and Finland (15.31). where it causes dieback of current-year shoots in the entire crown. The STT occurs on young Scots pine trees in northern Scandinavia and at higher elevations in the south, where it causes perennial cankers on the parts of the tree covered by a lasting snow layer during the winter (19).

Besides pines, G. *abietina* also may attack Norway spruce (Picea abies (L.) Karst.) where the infection occurs mainly on the previous year's leader shoot and results in stem girdling and top dying (20.28). During the outbreaks in the southernmost part of Norway in the early 1980s, *G. abietina* damaged Norway spruce trees and saplings grown as the understorey below Scots pine (2,28).

The symptoms of Gremmeniella spp. damage on trees (here defined as having a diameter >10 cm at breast height) differ from those on saplings (>1 m tall) and seedlings (<1 m tall). On Scots pine seedlings, damage caused by G. abietina can be identified readily due to the characteristic umbrella-like folding of needles on the infected leader shoot (4,22). Regarding Norway spruce seedlings, there are no reports describing *Gremmeniella* infection on seedlings while still in nursery production (≤2 years old). Barklund and Hellgren (I) explained this by a long latent period after the infection and reported that shoot dieback symptoms on seedlings usually develop after they have been planted out. The symptoms at this stage are described as being similar to shoot dieback caused by *Sirococcus* conigenus (DC) P.F. Cannon & Minter (5).

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DOI: 10.1094/PHYTO-96-1305