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Article

Two-Year Survival of *Gremmeniella abietina* Conidia Collected on Branches Left on the Ground after Pine Harvesting

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Abstract: In 1972, it was reported that viable ascospores and conidia of *Gremmeniella abietina*, North American race, were present on dead branches up to 10 months after they were killed. In Sweden, the survival period of conidia of *G. abietina*, European race, was reported to be over 18 months. We investigated the viability of conidia produced by *G. abietina*, European race, over a 2-year period in eastern Canada. Infected branches with pycnidia were left on the ground in June 2006. Branches were sampled every month during three growing seasons. Conidia germination was tested monthly and showed a very high rate of germination (76%–98%) from July 2006 to August 2007. Very few pycnidia were detected in the fall of 2007 and in May 2008. In June and July 2008, no pycnidia could be observed, the shoots being in an advanced stage of decomposition. In light of these observations, it is recommended to delay pine plantation until after two growing seasons following harvesting of diseased pine trees.

Keywords: *Pinus resinosa*; *Gremmeniella abietina*; disease prevention

1. Introduction

The fungal pathogen *Gremmeniella abietina* (Lagerb.) Morelet causes shoot blights and cankers, symptoms of a disease known as scleroderris canker, mainly found on two-needle pines. In North America, the European race of *G. abietina* (GaEU) was first reported in New York State in 1975 on

red pine (*Pinus resinosa* Ait.) and Scots pine (*Pinus sylvestris* L.) [1]. The names "European race" or "European strain" were given to distinguish this exotic pathogen from the native North American one [2]. It is equivalent to the A type [3] or the large tree type [4] of *G. abietina* reported in Europe. None of these designations (race, strain, type) have any taxonomical value [5]. In Canada, this so-called European race was first reported in Quebec in 1979 along the New York-Quebec border [6]. In 1983 and 1984, the disease was detected in 121 plantations north of the Ottawa River [7]. After harvesting red pine in some infected plantations located in that area, the question was raised about the survival period of the pathogens on slash left on the ground to assess when red pine could be replanted. In 1972, it was reported that viable ascospores and conidia of *G. abietina*, North American race, were present on dead branches up to 10 months after they were pruned [8]. In Sweden, the survival period of conidia of *G. abietina*, European race, was reported to be over 18 months [9]. The objective of this study is to measure the survival period of GaEU on infected red pine branches left on the ground after harvesting.

2. Materials and Methods

This experiment was conducted in a 35-year-old red pine plantation infected by GaEU and located at Chute-Saint-Philippe, north of Montreal, Province of Quebec, Canada (46°38′57″ N; 75°16′23.1″ W). Pines were planted in rows every 1.5 m with 2 m between rows. The site is a sandy soil and the topography is usually flat with few topographical depressions. A first thinning was done in 2004: one row of pines out of three was removed in the whole plantation. In June 2006, 125 branches bearing shoots with pycnidia of *G. abietina* were pruned from residual pines and left on the ground to mimic the harvesting situation. The diameter of the branches at the base was approximately from 1.5 to 3 cm and they were left on the ground in a thinned row with no grass partially lighted. Shoots attached to these branches were not directly in contact with the ground. Six branches bearing several shoots were sampled every month during the growing seasons. This sampling was done as long as pycnidia were visible on shoots. In the laboratory, infected shoots were left for 3 h in a moist chamber composed of a Petri dish with a filter paper moistened with sterile water. Conidia were collected with a sterile needle from the top of one pycnidium and spread with a laboratory loop on a Petri dish containing water agar. This was repeated four times, conidia being collected from a different pycnidium for each Petri dish. After 60 h of incubation, which is the optimal period to examine germination and before conidia are covered with mycelium, conidia germination was evaluated under the microscope. One hundred conidia were examined at random for each plate. The germination mean was calculated for each sampling date using the observations of conidia on the four plates.

3. Results

The observation period extended from July 2006 until pycnidia were no longer present on shoots in July 2008 (Table 1). Branches were collected 13 times over three growing seasons. Conidia germinated during the first two growing seasons (2006 and 2007). The germination rate remained high during the first growing season (2006). In 2007, the last conidia were observed in August. In May 2008, very few old pycnidia were present and the conidia sampled came from only one pycnidium.

From the latter, of the 200 conidia plated, only 10 germinated. In June and July 2008, no pycnidia could be found because of the advanced degradation of shoots.

Table 1. Sampling dates of branches with mean germination percentage of 400 conidia observed for each sample date from 2006 to 2008.

Sampling Date	Conidia Germination (SD *)
12 July 2006	76 (14.1)
22 August 2006	89 (12.4)
27 September 2006	93 (3.2)
1 November 2006	89 (3.5)
15 May 2007	98 (1.7)
7 June 2007	96 (1.6)
6 July 2007	96 (2.2)
21 August 2007	95 (0.8)
26 September 2007	NE **
1 November 2007	NE
15 May 2008	5 (5) ***
17 June 2008	NE
7 July 2008	NE

* SD, Standard deviation; ** NE, None evaluated (absence of conidia); *** Based on 200 conidia.

4. Discussion

As expected, conidia showed a very high germination rate in 2006 during the first growing season, this high rate continuing even up until November that year. In 2007, the germination rate was also very high from May to August but no conidia could be found from September to November 2007 as pycnidia appeared dry and empty. This indicates that the pathogen was able to produce new pycnidia during the second growing season in 2007. The fact that *G. abietina* stopped producing pycnidia in September 2007, in contrast to the previous year where it continued until late in the fall (November), suggests that the dry climatic conditions observed at that time could have affected sporulation, even though it is most likely the result of the degeneration of the pathogen into the decaying shoots. In support of this, in May 2008, when climatic conditions were favourable to sporulation, it was almost impossible to find any pycnidia on shoots containing conidia. This means that new pycnidia were not produced during the third growing season after pruning. This was confirmed by our subsequent observations in June and July 2008 where no pycnidia could be observed on shoots that were all in an advanced stage of decomposition. Our results strongly suggest that the degeneration of the pathogen began in the fall of 2007. This two-year life cycle of the anamorph stage is in accordance with studies of *G. abietina* in Sweden where the pathogen appeared to have a biennial life cycle [10]. In a study similar to ours conducted in Sweden, it was shown that 18-month-old slash produced as much conidia as fresh slash [9]. Based on these observations, it is recommended to postpone planting *P. sylvestris* seedlings to the third vegetation period after sanitary clear-cut. In North America, the only observation concerning the survival of conidia and ascospores were related to the North American race of *G. abietina* over a period of less than one year [8]. In light of our observations, and in accordance with the study carried out in Sweden [9], it is recommended, in eastern North America, to delay pine planting

until after two growing seasons following harvesting of pine trees infected with GaEU. Another possibility would be to chip all residual material from infected pine after the final harvesting.

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Author Contributions

The first author has designed and supervised the collection of samples in the field and the process of material in laboratory. The second author has contributed to the writing of the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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