Growth Phase of Bare-root Scots Pine Seedlings and Their Susceptibility to *Gremmeniella abietina*

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Petäistö, R.-L. 1999. Growth phase of bare-root Scots pine seedlings and their susceptibility to *Gremmeniella abietina*. Silva Fennica 33(3): 179–185.

Bare-root row-sown seedlings of Scots pine (*Pinus sylvestris* L.) in a forest nursery were inoculated with *Gremmeniella abietina* conidia at different times during their first and second growing seasons. The following spring, the proportion of diseased seedlings was different in various inoculation time treatments according to the age of the seedlings. The first year seedlings were susceptible to infection until late summer, whereas the second year seedlings were not. It is thought that this difference is due to the different growth rhythms of the first and second year seedlings. The difference in the susceptibility of bare-root seedlings to the disease in various growth corresponded to that reported earlier for container seedlings.

Keywords age, nursery, *Pinus*, *Scleroderris*
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Received 10 March 1999 **Accepted** 7 September 1999

1 Introduction

Seedling production of forest tree species in Finland has changed over the last decades, bare-root seedlings having been largely replaced by container seedlings. However, a considerable amount of Scots pine (*Pinus sylvestris* L.) seedlings are still produced as bare-root seedlings. In the bare-root seedlings production, row-sowing is used to avoid the laborious transplanting in tree nurseries.

Gremmeniella abietina (Lagerb.) Morelet is one of the most damaging pathogens found in forest nurseries in Finland (Kurkela 1967, Punter 1967, Teich 1972). The main dissemination time of *G. abietina* conidia in Finland is in the first half of the growing season (Nevalainen 1986, Petäistö unpublished data) from end of May to the beginning August. The ascospores disseminate later than conidia in the summer (Nevalainen 1986). The North American race produce abundantly ascospores, Skilling (1969) found their as well as conidia dissemination from May to September. The chemical control of this fungus has improved recently, but damage caused by this pathogen is still found in tree nurseries.

Table 1. The sowing time and inoculation times of the experiment seedlings in Experiments I–IV and times of length measurements of 40 seedlings in Experiments II–IV and the corresponding temperature sums, d.d.

Experiment and experiment years	Sowing time of the seedlings	Inoculation times	Times of length measurement and temperature sum
I 1995–96	8 June 1995	24 July 1995 * 22 Aug. 1995**	Measurement lacking (715 d.d.*, 1034 d.d.**)
II 1996–97	8 June 1995	19 June 1996, 17 July 1996, 22 Aug. 1996	20 June (282 d.d.), 17 July (517 d.d.), 22 Aug. 17 (932 d.d.), 17 Sept. (1100 d.d), 1 Oct. (1120 d.d.)
III 1996–97	5 June 1996	17 July1996, 19 Aug.1996, 17 Sept. 1996	22 Aug. (932 d.d.) 1996, 17 Sept. (1100 d.d.) 1996, 1 Oct. (1122 d.d.) 1996
IV 1997–98	5 June 1996	17 June 1997, 16 July 1997, 18 Aug. 1997, 17 Sept. 1997	17 June (272 d.d.) 1997, 16 July (617 d.d.) 1997, 19 Aug. (1051 d.d.) 1997, 1 Oct. (1333 d.d.) 1997

The susceptibility of container seedlings to this disease has been found to depend among other things on the growing phase (shoot and needles) of the seedlings (Petäistö and Kurkela 1993, Capretti 1990). Second year and older seedlings are very susceptible in the growth phase during the first half of the growing season. The first year container pine seedlings are grown until middle of July under plastic shelter and their shoots are also susceptible to this disease until late summer (Petäistö and Laine 1999). There is a study about first year container seedlings from Sweden, Hamnede (1980); she reported that the effect of late inoculations (August–September–October–November) decreased from August to November in Umeå, Sweden. However with first year bare-root seedlings there are no studies published previously.

The spore dissemination time of *G. abietina* and the susceptibility of the seedlings in the growth phase of shoot and needles are important factors in planning control. In addition, cold stress predispose the seedlings to the disease (Petäistö and Repo 1988, Petäistö and Kurkela 1993, Yokota 1975) and this also need to be taken in consideration in control.

The aim of this study was to determine the susceptibility of bare-root seedlings in the first and second year of growth to *G. abietina*.

2 Materials and Methods

In these experiments, Scots pine seedlings were of local, middle Finland, provenance. They were row-sown, at a density of about 43 seedlings/row meter, in five rows in a nursery bed, and covered with gauze (Lutrasil thermoselect, Freudenberg, Spinnvliesstoffe KG, D-67661 Kaiserslautern) until the end of June in the sowing summer. Five experiment plots (about $0.5\,m \times 0.5\,m$) / treatment were marked in the nursery bed. The plots in the seedling bed were randomly assigned to the inoculation treatments: inoculation times and no-inoculation (Table 1). There was also one plot for the seedlings whose length was measured during the experiment (see later). The seedlings were raised according to the nursery's practice but without fungicide and herbicide.

The seedlings were inoculated with conidia of *G. abietina*, B-type (Uotila 1983, Petäistö et al. 1996, isolates Pat2.1 and Kai1.2, from Dr. A. Uotila). The conidia were produced in vitro as described by Petäistö and Kurkela (1993). The

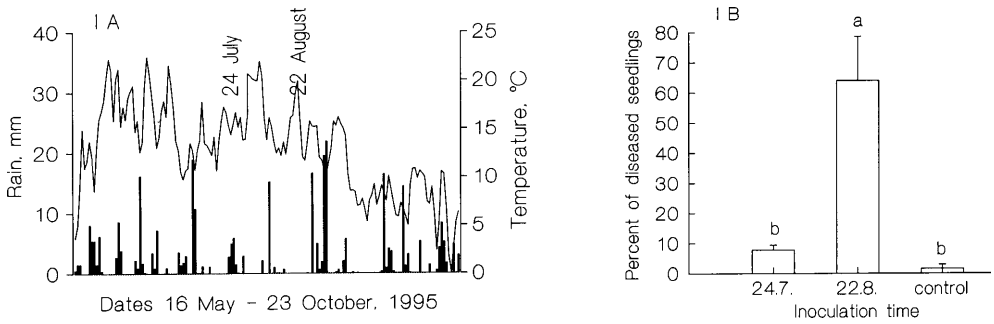


Fig. 1. Experiment I. A) The amount of rain (mm) and the temperature (°C) during the summer 1995. First year pine seedlings were inoculated on 24 July and 22 August 1995. B) The proportion of diseased seedlings in spring 1996 in different treatments: inoculated on 24 July 1995 and on 22 August 1995 and in noninoculated control (3). Bars indicate standard deviation.

conidia suspension was applied to the seedlings with a sprayer (Dosi-spot, Burchmeier and Cie. AG, CH-5444 Künten) using 3.2–3.6 × 10^6 conidia/100 cm^2. After inoculation, the surface on the seedlings was kept moist for two days by nursery irrigation sprayers. The inoculation times are shown in Table 1.

The seedlings were classified according to their condition into seven classes following spring: (1) healthy (bud healthy, growth started, colour of needles normal); (2) weakened (color of needles pale, the growth begins slowly); (3) diseased (base of needles brown, bud dead and resinous); (4) dead and diseased (shoot dead and symptoms of *G. abietina* visible); (5) dead (dead, no symptoms of disease); (6) top of the shoot dead (no clear symptoms of *G. abietina*); and (7) slightly diseased (symptoms only in the lowest needles). In the data analysis, condition classes 3, 4, 5 and 7 were merged into one diseased class.

In experiments II–IV the length of 40 seedlings both to growth initiation point and to needle point (needles pointed upwards) was measured repeatedly from June to October in experiments II and IV and from August to October in experiment III (Table 1). In each experiment the 40 seedlings stay in one external plot after the inoculation experiment plots in the seedlings bed.

The SAS® System was used for the data analysis. The data from the classification of condition were analysed using the GLM procedures (SAS/STAT Users Guide 1992) with the Tukey test, and data from the measurement of growth with repeated measures analysis of variance.

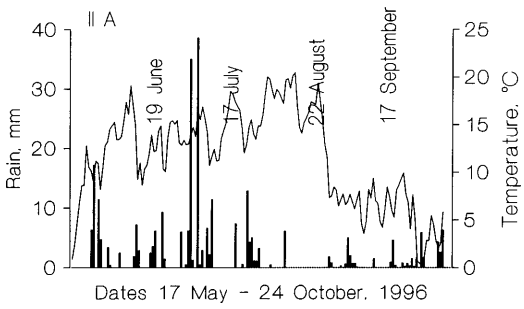
3 Results

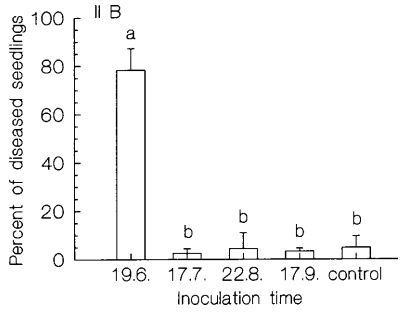
In Experiment I (1995–1996) in summer 1995 the temperature sum reached 1304 d.d., the average temperature sum in this area in the period 1972–1992 was 1205 d.d. There was no rainfall during the period 26 July–2 August, i.e. after the first inoculation on 24 July. After the second inoculation on 22 August rain fell during the week after inoculation and the temperature went down (Fig. 1A).

Experiment II (1996–1997) and III (1996–1997), in summer 1996 the temperature sum reached 1120 d.d. Rain fell during the week after the first inoculation of Experiment II (on 19 June), but not during the week after the second inoculation (on 19 July) nor the one after the third inoculation (on 22 August) (Fig. 2A, 3A). The temperature fell noticeably at the beginning of September. It rained more frequently at the beginning of July than later in the month and in August.

In Experiment IV (1997–1998), the temperature sum in summer 1997 reached 1333 d.d. Rain fell after the first inoculation and also after the second and fourth inoculation, but there was less rain after the third inoculation (Fig. 4A).

In Experiment I (1995–1996) first year seed-





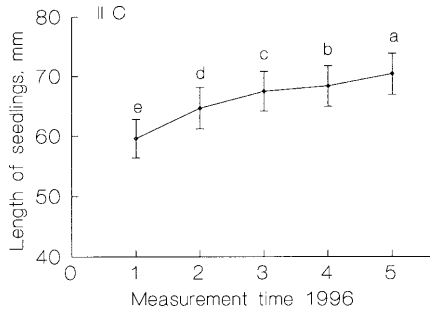
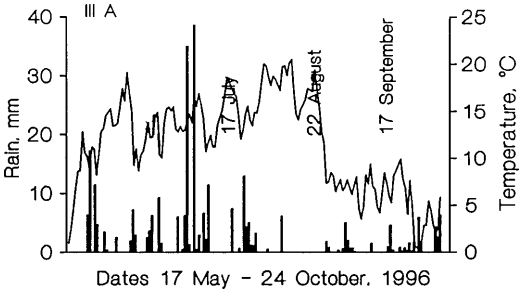
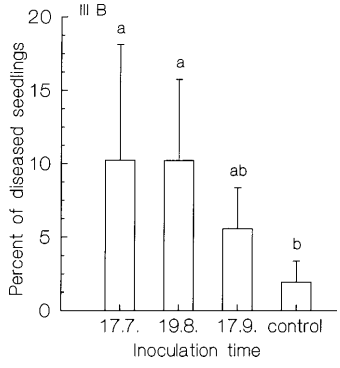


Fig. 2. Experiment II. A) The amount of rain (mm) and the temperature (°C) during summer 1996. Second year pine seedlings were inoculated between 19 June and 17 September 1996, Experiment II. B) The proportion of diseased seedlings in spring 1997 in five different treatments: inoculated on 19 June 1996, 17 July 1996, 22 August 1996 and 17 September 1996 and in noninoculated control. C) The lengths of the 40 second year seedlings measured on June–October 1996 at the same time as the inoculations (1–4) and 5) on 1 October. Bars indicate standard deviation.





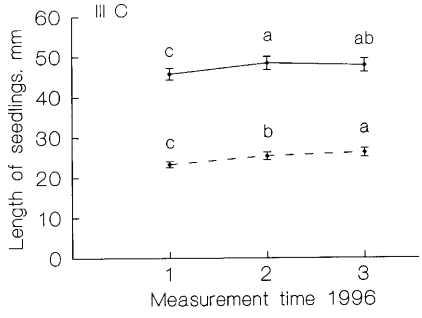
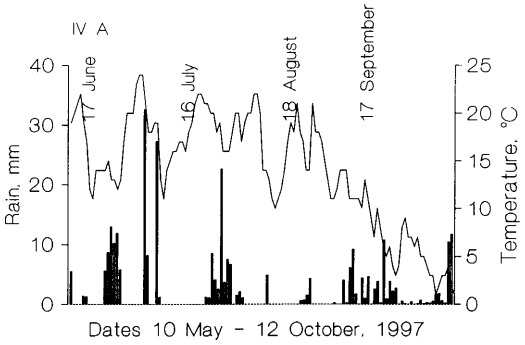
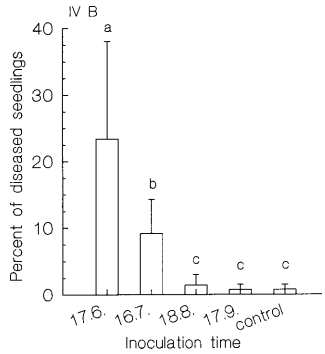


Fig. 3. Experiment III. A) The amount of rain (mm) and the temperature (°C) during summer 1996. B) The proportion of diseased seedlings in spring 1997, in different treatments: inoculated on 17 July 1996, 19 August 1996 and 17 September 1996 and noninoculated seedlings. C) The lengths of the 40 first year seedlings measured 1) on 22 August, 2) 17 September and 3) 1 October 1996. The measurements were made to top bud tip (– –) and to the tip of needles pointed upwards (——). Bars indicate standard deviation.





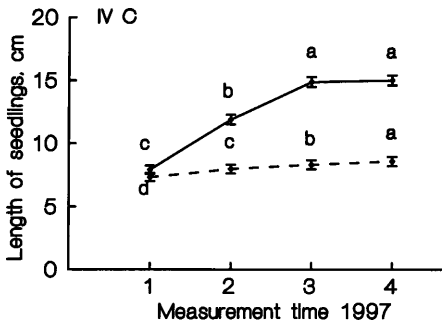


Fig. 4. Experiment IV. A) The amount of rain (mm) and the temperature (°C) during summer 1997. B) The proportion of diseased seedlings in spring 1998 in four different treatments: inoculation on 17 June, 16 July 1996, on 18 August 1996, on 17 September 1997 and noninoculed control seedlings; Bars indicate standard deviation. C) The lengths of 40 second year seedlings measured in 1997. The measurements were made to top bud tip (– –) and to the tip of needles pointed upwards (——) on 17 June, 16 July, 19 August and on 1 October. Bars indicate standard deviation.

lings inoculated on 24 July, resulted in a proportion of diseased seedlings about eight times smaller than did inoculation time on 22 August (Fig. 1B). The proportion of diseased seedlings among the seedlings inoculated on 22 August differed significantly ($p < 0.01$ level) from the proportion among the seedlings inoculated on 24 July and in controls, but the results of first inoculation time did not differ significantly from those of controls.

In Experiment II (1996–1997), second year seedlings were used. The June inoculation treatments differed from other treatments statistically significantly ($p < 0.01$). Disease occurred in about 80 % of the seedlings inoculated on 19 June. In the other treatments (inoculation times 17 July, 19 August and 17 September and control), disease occurred in only 2–4 % of the seedlings (Fig. 2B). The lengths of the seedlings are shown in Fig. 2C. There were statistically differences in the lengths between measurement times in pairwise comparison, $p < 0.0001$: only for the difference between the lengths measured in August and September the p value was < 0.0252.

In Experiment III (1996–1997), the first growing season seedlings were inoculated on 17 July, 19 August and 17 September 1996. The proportion of diseased seedlings in the July and August inoculation time treatments differed statistically significantly from control but not from each others and September inoculation treatment (Fig. 3B). However, the proportion of diseased seedlings among seedlings inoculated in July and August was bigger than in the other treatments ($p < 0.06$). The length of the seedlings to needle tips pointed upwards differed between August and September measurements but not between September and October measurements, $p < 0.0001$ and $p < 0.221$, and the length to the shoot tip differed between August and September to extent $p < 0.0014$ and between September and October to extent $p < 0.044$ (Fig. 3C).

Experiment IV was carried out in 1997–1998 using second year seedlings sown in spring 1996. The first inoculation (on 17 June) resulted in 23 % diseased seedlings in spring 1998, and the second inoculation (on 16 July) in 9 % diseased seedlings. The difference between the treatments

is statistically significant ($p < 0.01$) (Fig. 4B). The proportion of diseased seedlings in the second inoculation differed significantly from those of the third inoculation (18 August), the fourth (17 September) and controls, ($p < 0.05$), while there were no significant differences between the results of the third and fourth and controls. The shoot length measured in June, July, August and October to bud tip differed pairwise statistically significantly from each others ($p < 0.0001 – p < 0.0003$). The lengths measured to needle tip pointed upwards were different at each measuring time ($p < 0.001$), except the lengths in August and in October ($p < 0.189$) (Fig. 4C).

4 Discussion

The results of the experiments show that the period when pine bare-root seedlings are susceptible to *G. abietina* depends on their age. Pine seedlings in their first growing season continue to grow until late summer (Kanninen 1990, Cannell et al. 1976). In our experiments according the measurements the first year bare-root seedlings continued to increase little in length September–October. These first year seedlings inoculated in late August became diseased but not those inoculated in the middle of September. The inoculation at the beginning of September also caused disease in the first year container seedlings grown under plastic shelter to the middle of July (Petäistö and Laine 1999). In the present study, the appearance of the disease in seedlings inoculated in August but not in seedlings inoculated in July in Experiment I may be partly due to climatic factors: there was less rain after the July inoculation than after the August inoculation. However it is possible that the susceptibility of first year seedlings increased with increasing age as shown with container seedlings by Hamnede (1980) until seedlings begin to stop growing. Moreover the conidia may stick better on the shoots of bigger seedlings.

In the second year bare-root seedlings, inoculation in June caused disease while inoculation in August and September did not. Container pine seedlings show a similarity in susceptibility: the second year seedlings are not susceptible in late summer (Petäistö and Kurkela 1993, Petäistö and Laine 1999). The explanation for the different period of susceptibility of these seedlings compared to that of the first year seedlings could be the different growth rhythm and bud formation time of second year seedlings. In the some year old seedlings, growth stops at temperature sum about 500 d.d (Koski and Sievänen 1985, Petäistö and Kurkela 1993). These studies report that the length of the second year seedlings according the measurements to bud tip may change little after the measurement in August. The main growth phase is important to the susceptibility, however, it has been found that cold stress makes second year seedlings also susceptible to late summer infection (Petäistö and Kurkela 1993, Petäistö and Repo 1988).

In Finland the dissemination time of conidia is over by about at the beginning of August and the main dissemination time is June–July (Nevalainen 1986, Petäistö unpublished data). First year bare-root seedlings may get the disease during almost the whole summer, although the risk is bigger in second year seedlings, which are very susceptible to disease in the main dissemination time of the *G. abietina* conidia. The spores need moisture to release (Nevalainen 1986, Laflamme and Archambault 1990). In Europe, the later disseminating ascospores does not seem to occur as often as the ascospores of North American race in North America (Skilling et al. 1986).

Generally, the frequency of the disease was not so high in bare-root seedlings as in container seedlings. This may be caused by the higher humidity among the tightly-growing container seedlings after inoculation than in bare-root seedlings growing outdoors. Furthermore, it may be that the conidia drip off from the small bare-root seedlings more easily, and the few needles do not provide such a large surface area for the suspension to stay on the plant. The bare-root seedlings grown outdoors are shorter in their first growing season than are first year container seedlings grown first under plastic shelter (see Petäistö and Laine 1999). However, growth continues similarly until late summer in both types of seedlings in their first growing season.

As a general conclusion of this we know the susceptible period of first and second year bare-root seedlings and container seedlings to *G. abietina*. More knowledge is needed about the spore

dissemination times and effective control times to prevent this disease.

Acknowledgements

I am grateful to the staff of Suonenjoki Research Station, especially to Eija Lappalainen, Mervi Ahonpää and Urpo Paananen for technical assistance. I also wish to thank Prof. Timo Kurkela and Dr Risto Rikala for critically reading the manuscript and Juha Hyvönen for help in statistics.

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