Timing of fungicide control of *Gremmeniella abietina* on Scots pine seedlings

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In the study in 2002 in central Finland Scots pine seedlings were protected against natural infection of *Gremmeniella abietina* using chemical application every two-weeks during the whole growing season, or during shorter periods in the growing season. Of the chemical application treatments, the disease occurrence in the following spring was smallest when the countered number of conidia in the coexistently collected rain samples was the highest. The dispersal values according the conidia dispersal model correlated positively with the immunologically (ELISA) analysed number of conidia in the rainwater samples. In northern Finland in 2000, the modelled values and analysed conidia number showed weak, non-significant positive correlation with each other. Weaker correlation might be due to the that the spore collectors were not located as close to the conidia sources as in the experiment in central Finland. Both conidia analysis and the conidia dispersal model appeared to be useful for timing control, in addition to the earlier results about the susceptibility phases of the seedlings.

Keywords: spore dispersal, model, *Pinus,* growth phase, temperature sum, monoclonal antibody

Introduction

Gremmeniella abietina (Lagerb.) Morelet is a serious pathogen especially for pines. The fungus disperses by means of conidia and ascospores. The two types occurring in Fennoscandinavia are the A- and B-type. Both types produce spores, but conidia are more common especially in the A type. According to Petäistö and Heinonen (2003), rain and the temperature sum are the main factors predicting the dispersal of conidia. Monoclonal antibodies against conidia of *G. abietina* can also be used as a tool for following conidia dispersal (Koistinen et al. 2000).

The susceptibility of container pine seedlings in their first and second growing season has been studied using artificial inoculation with fixed amounts of *G. abietina* conidia in Central Finland. The second-year seedlings were most susceptible prior to a temperature sum of about 800 d.d., and the first-year seedlings after about 800 d.d (Petäistö and Kurkela 1993, Petäistö 1999, Petäistö and Laine 1999). A fungicide, chlorotalonil (Bravo), has been shown to decrease disease occurrence when applied during the first 10 days after inoculation (Petäistö and Juntunen 2000).

The aim of this study was to study the possibilities of timing the control of *G. abietina* in the nursery by using a conidia dispersal model and immunological conidia detection (ELISA analysis on rainwater samples), and by taking the seedling susceptible phase into account.

Material and methods

Fungicide experiment

Seedlings and fungicide treatments

The experiment 2002 was performed at the Suonenjoki Research Station in central Finland. Scots pine, *Pinus sylvestris* L., container seedlings (Plantek-81F) were sown (local origin) on 29–30 April 2002, and moved outdoors on 24 June near 20-year-old Siberian cembra pines (*Pinus cembra* L.) that had diseased shoots bearing pycnidia of Scleroderris canker.

The seedlings were fertilized weekly with 0.1 % (1 July – 29 July) and 0.05 % (5 August – 26 August) Taimi Superex (NPK 19-4-20), 0.8 l per a tray. The height of the seedlings (the same sowing lot as the experimental seedlings) were measured in four trays, 9 seedlings/tray, on 24 June, 23 July, 7 August, 20 August and 6 September (Fig. 1).

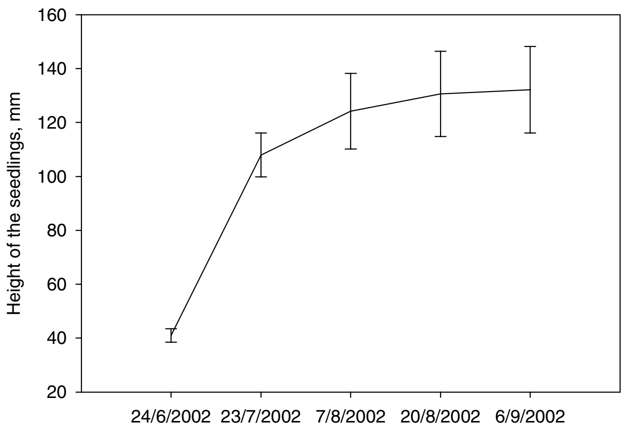

Figure 1. Height of the pine seedlings in summer 2002, Suonenjoki.

The fungicide chlorothalonil (Bravo®) was used (3 l/ha in 600 l/ha) to control Scleroderris canker infection during the summer. The five treatment (four trays per treatment) groups were:

1. fungicide treatments were performed on 26 June, 10 July, 24 July, 7 August, 21 August and 4 September and, at the corresponding times, and an additional four trays were treated with water,
2. fungicide treatments on 26 June and 10 July,
3. fungicide treatments on 24 July and 7 August,
4. fungicide treatments on 21 August and 4 September,
5. no fungicide treatment.

The effective control period for each fungicide treatment was calculated to be from one week before and to one week after the treatment (see Petäistö and Juntunen 2000).

The seedlings were examined on 7–9 May in spring 2003. They were classified as healthy, diseased (by *G. abietina*, brown needle base, dead bud, but also healthy needles), dead (by *G. abietina,* symptoms detectable), diseased by *Phacidium infestans* P. Karst. (mycelia found on seedlings in the spring, diseased seedlings in groups) and dead (cause not known). The number in each group was expressed as percent of seedlings.

Immunological conidia analysis on rainwater samples (ELISA)

G. abietina conidia are of the splash-dispersal type. The analysis using monoclonal antibodies was made on rainwater samples. Rainwater was collected using six plastic bottles, each with a funnel on top, placed around a diseased, about 20-year-old *P. cembra*. The funnels were covered with wire netting (mesh radius about 1.3 cm) to prevent larger litter from passing into the bottles. Rainwater was collected from the bottles and analysed weekly during 20 May to 4 November. The funnel and the bottle were rinsed with water (50–100 ml), which was added to the rainwater sample. The samples from the 6 bottles were combined to form one sample. The sample was passed through a tea gauze and stored in a plastic bag in a refrigerator until analysis by capture-ELISA (Koistinen et al. 2000).

Conidia dispersal model

Temperature (for calculating the temperature sum) and rainfall data were required for the conidia dispersal model (Petäistö and Heinonen 2003). Sensors connected to a minilogger at the experimental site measured air temperature and relative humidity during summer 2002. The rainfall data were obtained from the weather station of the Suonenjoki Research Station; temperature and relative humidity data were also available from this weather station.

Comparison of the conidia dispersal model and the ELISA results

As the conidia dispersal model was developed using data from central Finland, it was necessary to test whether it was applicable in more northerly areas. In 2000, the model was calculated using temperature and rainfall data from a more northerly area, Rovaniemi (weather station of the Finnish Meteorological Institute).

Rainfall samples were also collected in the Rovaniemi nursery in 2000 for ELISA conidia analysis. Rainfall samples were collected from 15 May to 28 August at five collection sites (two rainfall sample collection bottles at each point) at different points around the nursery. Weekly samples were packed in plastic bags and frozen, and the samples transported as frozen for the ELISA analysis. The samples from each of the five points were analysed separately. The results were combined for each collection time.

Data analysis

At the experimental site the trays and treatments were totally randomised. One-way ANOVA was used to test the disease occurrence between fungicide treatments. Correlation analysis was used to compare the disease occurrence as percent of seedlings (mean of tray means), values from the model and conidia numbers from the rainfall samples (ELISA).

Results

Disease occurrence in spring 2003

About half of the untreated seedlings were diseased in spring 2003; occurrence of the disease was lowest in chemical application treatment 1. In general, the disease was identified very easily because pycnidia of *G. abietina* had developed on the seedlings.

The treatments had a significant effect on the occurrence of the disease (Table 1). Treatment 5 (no fungicide) differed significantly ($p<0.001$) from treatments 1 and 2, but not from treatments 3 and 4 (Fig. 1). The lowest disease occurrence was in treatment 1 and 2.

Table 1. One-way ANOVA table of the effect of the fungicidal applications on the occurrence of disease expressed as a percent of diseased seedlings (mean of tray means).

	Sum of squares	df	Mean square	F	Sig.
Between groups	7810.058	4	1952.515	19.671	.000
Within groups	1488.903	15	99.260		
Total	9298.962	19			

Probability of conidia dispersal for summer 2002

The temperature and rainfall values during summer 2002 in Suonenjoki were needed for modelling the dispersal probability and the number of conidia (Petäistö and Heinonen 2003). In 2002 the main trend was that the dispersal probability values began to decrease after a temperature sum of 600 d.d., which occurred in the beginning of July (Fig. 2). The predicted daily conidia number and the dispersal probability values correlated 0.897, $p<0.001$, $n=187$). The sums of daily values were calculated for the effective periods of chemical application treatments 1–4 (Table 2).

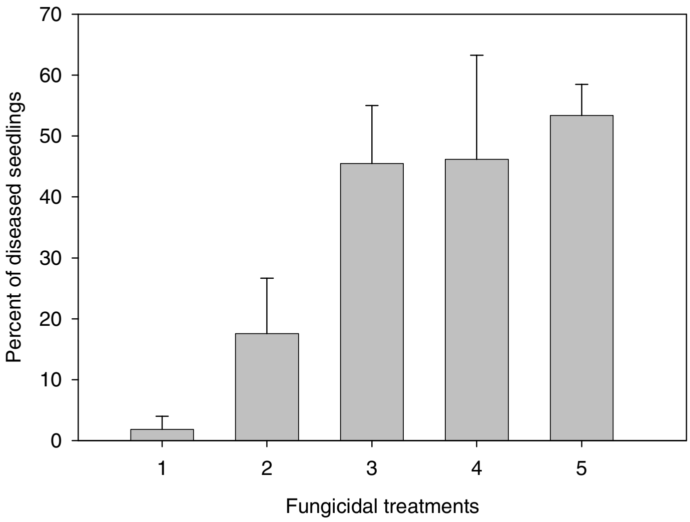

Figure 2. Percent of diseased seedlings in spring 2003 with fungicide treatments 1–5. The values are means of tray means. Vertical lines indicate standard deviation. Treatments with different letters differed statistically from each other.

Table 2. Summary of the results for Suonenjoki 2002. The effective period of the fungicidal applications, sum of the analysed conidia number (ELISA) and sum of the daily model dispersal probabilities (pm) and of the modelled number of spores (numbm) in the effective periods of fungicidal application and the disease occurrence in next spring (disease %).

Treatm	Effective period	dd-period	ELISA	pm	numbm	disease %
1	24.6.–11.9.	504–1434	17407406	32.14614	333.8778	1.86
2	24.6.–17.7.	504–771	7935100	15.14175	159.7183	17.55
3	17.7.–14.8.	771–1129	4203457	11.53306	109.0096	45.5
4	14.8.–11.9.	1129–1434	5632134	6.436966	73.79788	46.15
5	no		17528620	40.4996	443.1463	53.37

Number of conidia in rainfall samples, ELISA

The number of conidia was calculated as the total number in the collected rainfall sample. During the period 20 May to 3 November the total number of conidia was 22 826 470. Half of this number was in the samples collected up to 30 June (543 d.d.), while 99 % of the conidia was collected so far 30 August (Fig. 2).

As the seedlings were moved outdoors to the experiment site on 24 June, the numbers of conidia measured from this day onwards were used in the experiment. The fungicide treatments were performed on Wednesdays. Rainfall samples were collected weekly, on Monday morning. When calculating the number of conidia for each chemical application treatment the mean conidia values for one day (from the weekly value) were used for those days that were not coexistent with the effective period days.

About 45 % of the conidia recorded during the experiment period (from 24 June onwards) occurred in the samples of the effective period of treatment 2, and 24 % and 32 %, respectively, from the samples of the effective periods of treatments 3 and 4 (Fig. 2, Table 2).

Correlations between disease occurrence, dispersal probability and the ELISA results

During the period 20.5.–3.11., the weekly conidia number values (ELISA) correlated positively (0.4, $p<0.05$, $n=24$) with the weekly sum dispersal probability and the number of conidia (modelled).

In fungicide treatments 1–4, the disease occurrence (%) correlated negatively with the number of conidia (ELISA) (-0.9, $p<0.06$, $n=4$) of the corresponding effective periods and with the sums of the daily dispersal probabilities and the modelled number of spores during effective periods of the fungicide treatment (-0.9, $p<0.10$) (Table 3a and b).

Table 3a. Correlation between the weekly sums of the number of spores (ELISA), modelled dispersal probability (pm) and modelled spore number (numbm).

Suonenjoki, 2002		ELISA numb	summp	summnumb
ELISA numb	Pearson Correlation	1	.399	.407*
	Sig. (2-tailed)	.	.053	.049
	N	24	24	24
summp	Pearson Correlation	.399	1	.978**
	Sig. (2-tailed)	.053	.	.000
	N	24	24	24
summnumb	Pearson Correlation	.407*	.978**	1
	Sig. (2-tailed)	.049	.000	.
	N	24	24	24

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 3b. Fungicide applications 1–4, effective periods of these fungicide treatments, correlation between the sum of the number of spores (prevented ELISA) and sums of the daily modelled dispersal probability values (prevented pm) and sum of the modelled number of spores (prevented numbm) and disease occurrence in spring 2003.

Suonenjoki, 2002/2003		prevented ELISA	prevented mp	prevented mnumb	disease %
prevented ELISA	Pearson Correlation	1	.959*	.974*	-.910
	Sig. (2-tailed)	.	.041	.026	.090
	N	4	4	4	4
prevented mp	Pearson Correlation	.959*	1	.998**	-.911
	Sig. (2-tailed)	.041	.	.002	.089
	N	4	4	4	4
prevented mnumb	Pearson Correlation	.974*	.998**	1	-.926
	Sig. (2-tailed)	.026	.002	.	.074
	N	4	4	4	4
disease %	Pearson Correlation	-.910	-.911	-.926	1
	Sig. (2-tailed)	.090	.089	.074	.
	N	4	4	4	4

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Dispersal probability and ELISA results in northern Finland

The applicability of the model and the ELISA spore analysis was tested in Rovaniemi, North Finland, in 2000. According to the ELISA results, some of the spores were released already in week 22–28 May when the temperature sum was 54–83 d.d. The highest values occurred in the samples collected during 12–25 June and 17–23 July. The model gave a daily probability of over 0.1 for the first time in the last week of May.

The last conidia were found in the rainfall samples collected in 31 July – 7 August (651–715 d.d.) and, after this week, the model gave daily probabilities values of 0.3–0.8 (Fig. 3a and b). As the last rainfall samples were taken in the week 21–28 August, the possible number of conidia after this date could not be documented. The temperature sum reached its maximum value of 1100 d.d.

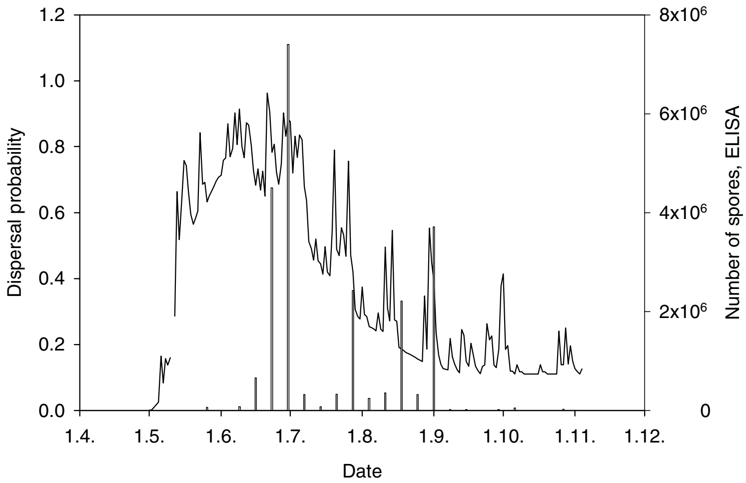

Figure 3a. The probability of conidia dispersal according to the model (Petäistö and Heinonen 2003) and the conidia number in the collected weekly rainfall samples (ELISA). Suonenjoki, summer 2002.

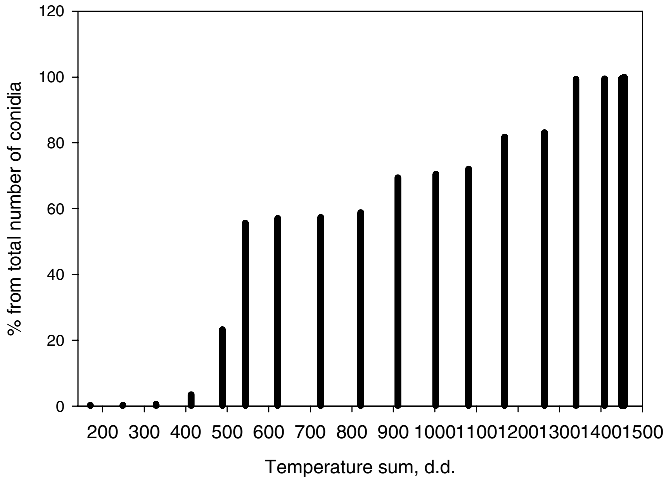

Figure 3b. Number of conidia as percent of the total number of conidia (ELISA) at different temperature sums. Suonenjoki, summer 2002.

in the middle of October, which was exceptional (about 20 days longer than the mean value) for the Rovaniemi area. The proportion of conidia out of the total number collected was 99.8% so far at a temperature sum of 590 d.d. (Fig. 4a and b).

The weekly sum conidia number (ELISA) correlated positively with the weekly sum of the dispersal probabilities 0.41, $p < 0.13$ (n=5) and with the weekly sum of modelled number of spores 0.35, $p < 0.20$ (n=15) (Table 4).

Table 4. Correlation between weekly spore number (ELISA), model dispersal probability (wprobabl) and model spore number (wnumber). Rovaniemi, 2000.

		ELISA	wnumber	wprobabl
ELISA	Pearson Correlation	1	.351	.407
	Sig. (2-tailed)	.	.199	.132
	N	15	15	15
wnumber	Pearson Correlation	.351	1	.814**
	Sig. (2-tailed)	.199	.	.000
	N	15	15	15
wprobabl	Pearson Correlation	.407	.814**	1
	Sig. (2-tailed)	.132	.000	.
	N	15	15	15

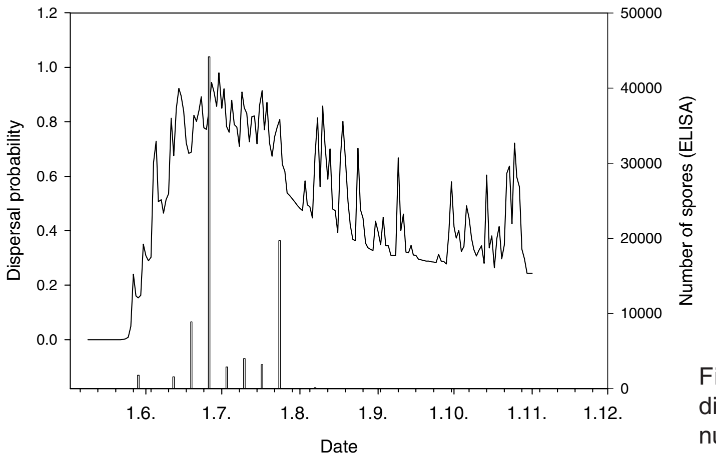

Figure 4a. The probability of conidia dispersal in Rovaniemi and the conidia number (ELISA). Summer 2000.

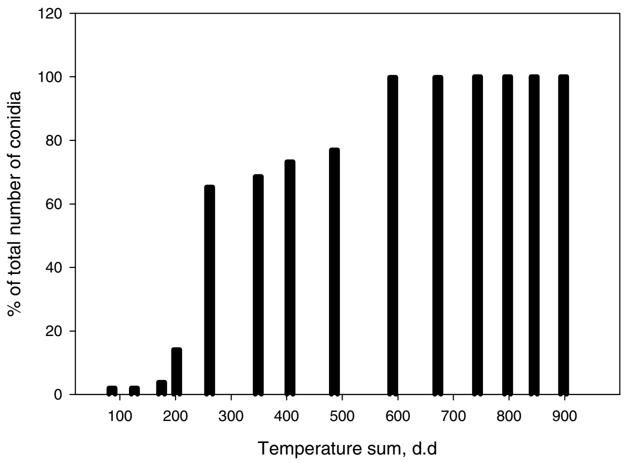

Figure 4b. Number of conidia as a percent of the total number of conidia in rainfall samples (ELISA) at different temperature sums. Summer 2000.

Discussion

G. abietina infects plants via spores. Weather factors affect the dispersal of the spores, as well as fungal penetration and the incidence of damage in the host plant. The correct timing of application control in forest nurseries is important in order to achieve optimum prevention of the disease (Petäistö and Juntunen 2000). Being able to use fungicide only when it is necessary would decrease the amount of fungicide applied and thus reduce the negative effects of fungicide on the environment (Landis et al. 1991). Information about the spore dispersal time and the susceptibility of the plant helps in optimising the timing of chemical application.

The Bravo fungicide treatments, applied every two weeks during the outdoor period of the seedlings, gave the best preventive effect. These fungicide treatments covered the period when 99 % of the total number of recorded conidia fell on the seedlings in the experiment site. In this two-week-interval treatment, the diseased percent on trays was about 3 % from the amount of diseased seedlings in non-controlled trays.

According to Petäistö and Juntunen (2000), this kind of fungicide treatment (about two weeks prior and after inoculation) considerably decreased the incidence of fungal damage. In addition, Sletten (1971) reported, using another fungicide on three-year-old bare-root transplanted pine seedlings, better effectiveness with a two-week fungicide interval than with only monthly fungicide treatment.

For the treatments with two applications, the smallest proportion of diseased seedlings occurred when the two fungicide applications were performed on 26 June and 10 July. Both the conidia number analysis and the model showed that conidia dispersal was more frequent during the effective time of this application than in the effective periods of the other two applications. In addition, the temperature sum had reached almost 800 d.d. during this prevention period and the first-year seedlings had obviously started to become susceptible to the fungus (Petäistö 1999, Petäistö and Laine 1999, Petäistö and Juntunen 2000).

Both of the later two-application treatments (24.7. and 7.8.; 21.8. and 4.9) obviously prevented about 14–15 % of the total disease occurrence (non treated). The corresponding ELISA conidia number proportions out of the total number were 24% and 32%, the model dispersal probability sum out of the total sum 28% and 16%, and the modelled number of conidia out of the total number 25% and 17%. Thus the modelled values and the ELISA conidia number values in the latest period did not seem to be exactly compatible. The seedlings might be less susceptible in the latest period (1129–1434 d.d.) than in the earlier. To the correlation analysis on the relationship between the conidia number and modelled values and disease occurrence did not include factor of susceptibility phases, which is a very important factor. However, general information about the growth phases and stress factors (e.g. cold, shade) is important for interpreting the correlations.

The modelled values and the ELISA conidia numbers recorded in Rovaniemi 2000 did not correlate as well as those in Suonenjoki 2002. One possible explanation for this is that the rainwater collectors in Suonenjoki were located in the immediately vicinity of the diseased trees, but not in Rovaniemi. The collection of rainfall in Rovaniemi should have been continued for a longer period because the growing season in 2000 was extremely long.

In conclusion, use of the model and/or conidia number analysis seemed to be worthwhile during the general main conidia dispersal time and when the seedlings are susceptible.

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References

Koistinen, K., Petäistö R.-L., Vartiainen S., Ehrbar K. & Kajander E. O. 2000. Monoclonal antibodies to specific antigens on *Gremmeniella abietina* spores. Mycologia 92(3): 421–429.

Landis, T.D., Campbell, S. & Zenzen, F. 1991. Agricultural pollution of surface water and groundwater in forest nurseries. In: Proc., Intermountain Forest Nursery Association. August 12–16, 1991, Park City, Utah, Rocky Mountain Forest and Range Experiment Station. General Technical Report RM-211. p. 1–15.

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

Petäistö, R.-L. 2005. Infection of Scots pine seedlings by *Gremmeniella abietina* during summer under different inoculum potential. Forest Pathology 35(2): 85–93.

Petäistö, R.-L. & Kurkela, T. 1993. The susceptibility of Scots pine seedlings to *Gremmeniella abietina*: effect of growth phase, cold and drought stress. European Journal of Forest Pathology 23: 385–399.

Petäistö, R.-L. & Laine, A. 1999. Effects of winter storage temperature and age of Scots pine seedlings on the occurrence of disease induced by *Gremmeniella abietina*. Scandinavian Journal of Forest Research 14: 227–233.

Petäistö, R.-L. & Juntunen, M-L. 2000. Timing fungicide applications for control of Scleroderris canker: Efficacy of fungicides applied to spores, hyphae and diseased seedlings. In: Lilja A. ja Sutherland J.R. (eds.). Proc. of the 4th Meeting of IUFRO Working Party 7.03.04 - Diseases and Insects in Forest Nurseries. Finnish Forest Research Institute, Research Papers 781: 189–200.

Petäistö, R.-L. & Heinonen, J. 2003. Conidial dispersal of *Gremmeniella abietina*: climatic and microclimatic factors. Forest Pathology 33(6): 363–373.

Sletten, A. 1971. Infection biology and chemical control of *Scleroderris lagerbergii* Gremmen on *Pinus sylvestris* L. Medd. Norske skogforsksvesen 29: 117–134.