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Susceptibility of *Pinus nigra* and *Cedrus libani* to Turkish *Gremmeniella abietina* isolates

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Abstract

Gremmeniella abietina causes shoot dieback and stem cankers on conifers throughout Northern hemisphere. The aim of this study was to investigate the virulence of Turkish *G. abietina* isolates in a field experiment.

The lower branches of 15-20-year-old *P. nigra* and *C. libani* in a plantation site at 1,050 m a.s.l. in Isparta were inoculated at 1-2-month intervals during September-January. Five isolates obtained from high altitude mountainous forests were used. Each isolate was inoculated into two branches per tree and repeated ten times on both tree species at each inoculation date. The branches were sampled at the end of February, and in August, and lesion lengths in the inner bark measured.

The mean lesion length on *P. nigra* and *C libani* were 10.6 ± 0.8 and 3.8 ± 0.2 mm in February and 17.6 ± 1.4 and 7.8 ± 0.8 mm in August, respectively.

Differences in the mean lesion length between the isolates were small. Nevertheless, there were significant differences between the isolates on *P. nigra* in November and January inoculations, and on *C. libani* at all four inoculation times.

The mean lesion lengths for all isolates at both sampling dates was the highest ($p < 0.01$) in December inoculations for both *P. nigra* (22.0 ± 1.9 February; 32.9 ± 2.9 August) and *C. libani* (5.6 ± 0.7; 11.3 ± 1.2). There was no difference between the September and January inoculations on *P. nigra,* despite the almost six-fold difference in incubation period. During the December inoculations, the trees were most likely in winter dormancy, i.e. unable to defend themselves, which would explain the large lesions.

Key words: temperature; inoculation date; mycelial growth; incubation period.

Resumen

Susceptibilidad de *Pinus nigra* y *Cedrus libani* a aislados turcos de *Gremmeniella abietina*

La *Gremmeniella abietina* causa la muerte regresiva de brotes y cancros sobre coníferas en todo el hemisferio norte. El objetivo de este estudio fue investigar la virulencia de aislados turcos de *G. abietina* en un experimento de campo. Se inocularon las ramas más bajas de *P. nigra* y *C. libani* de 15 a 20 años de edad en un sitio de plantación a 1.050 m snm en Isparta a intervalos de 1-2 meses entre septiembre y enero utilizando cinco aislamientos obtenidos de bosques de las zonas montañosas altas. Cada aislado se inoculó en dos ramas por árbol y se repitieron diez veces en las dos especies en cada fecha de inoculación. Se tomaron muestras de las ramas al final del mes de febrero, y en agosto, y se midieron la longitud de la lesión en la corteza interna. La longitud media de la lesión en *P. nigra* y *C. libani* fueron 10,6 ± 0,8 y 3,8 ± 0,2 mm en febrero y 17,6 ± 1,4 y 7,8 ± 0,8 mm en agosto, respectivamente. Las diferencias en la longitud de la lesión media entre los aislados eran pequeñas. Sin embargo, hubo diferencias significativas entre los aislamientos de *P. nigra* en inoculaciones de noviembre y enero, y en *C. libani* en los cuatro tiempos de inoculación. La longitud media de la lesión para todos los aislamientos en ambas fechas de muestreo fue la más alta ($p < 0{,}01$) en las inoculaciones de diciembre tanto para *P. nigra* (22,0 ± 1,9 de febrero, 32,9 ± 2,9 de agosto) como para *C. libani* (5,6 ± 0,7; 11,3 ± 1,2). No hubo diferencias entre las de septiembre y enero en *P. nigra*, a pesar de la diferencia de casi seis veces en el período de incubación. Durante las inoculaciones de diciembre, los árboles estaban probablemente en letargo invernal, es decir, incapaces de defenderse, lo que explicaría las lesiones de gran tamaño.

Palabras clave: temperatura; fecha de inoculación; Crecimiento micelial; periodo de incubación.

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Introduction

Gremmeniella abietina (Lagerb.) Morelet [anamorph: *Brunchorstia pinea* (Karst.) Hohn.] is an ascomycete which causes shoot dieback and main stem cankers on more than 40 coniferous species (Stephan and Schulze, 1987; Butin, 1995). In Turkey, the fungus was previously reported on native *Pinus halepensis* Mill. individuals and from an exotic *Pinus elderica* Medw. plantation (Spaulding, 1961; Soylu *et al*., 2001). Recently it has been reported from Lakes District of Turkey on western Taurus Mountains at about 1,700-2,100 m a.s.l. on *Pinus nigra* J. F. Arnold subsp. *pallasiana* (Lamb.) Holmboe (Lehtijärvi *et al*., 2010a,b). It was also observed on *P. sylvestris* L. at Black Sea Region above 1,800 m a.s.l. on lower branches of young trees. However, to our knowledge, the virulence of the Turkish *G. abietina* isolates has not been studied.

Susceptibility of conifers to *G. abietina* is to some degree under genetic control of the host (Roll-Hansen, 1972; Nevalainen and Uotila, 1984; Stephan *et al*. 1984; Dietrichson and Solheim, 1987; Aitken, 1993; Hansson 1998; Sonesson *et al*., 2007). However, the role of environmental factors in disease development is essential; severe epidemics occur exclusively under environmental conditions which both predispose the host and favour the spread and survival of the fungus (Donaubauer, 1972; Petäistö and Kurkela, 1993). Although such factors as topography, microclimate, stand structure and nutrient imbalances affect the development of disease locally, widespread *G. abietina* epidemics usually appear after cool and rainy growing seasons with low light intensities (Petäistö and Kurkela, 1993; Karlman, 2001; Petäistö and Heinonen, 2003; Thomsen, 2009).

Host susceptibility can be tested by inoculating the hosts either with spores or mycelium of the fungus. The first method is more natural as the fungus must be able to both cross the structural barriers and endure biochemical defence inside the living tissues (Patton *et al*., 1984). The second method tests only the degree of the biochemical defence but it works also in environmental conditions that do not predispose the trees for infection (Ranta *et al*., 2000). The susceptibility of conifers varies at different stages of shoot development and annual rhythm of the host; therefore the timing of inoculations is important (Roll-Hansen, 1964; Yokota *et al*., 1974; Barklund and Unestam, 1988; Aitken, 1993; Petäistö and Kurkela, 1993). While inoculation with spores is the most successful in the first half of growing season (Petäistö and Kurkela, 1993) the optimal time for inoculations with mycelium is the dormant period of the host (Patton *et al*., 1984).

The aim of this study was to investigate the susceptibility of *P. nigra* subsp. *pallasiana* and *C. libani* A. Rich. against *G. abietina* at different times during autumn and winter in an inoculation experiment in field conditions.

Material and Methods

Cultures of the fungus were obtained from pycnidia developed on dead branches of naturally infected Anatolian Black pine (*Pinus nigra* Arnold ssp. *pallasiana*) and Scots pine (*P. sylvestris*) collected from high altitude mountainous areas in the Black Sea region and the Lakes District of Turkey. Five *G. abietina* isolates obtained from five pycnidia were used in inoculation experiments (Table 1). The isolates were grown on oatmeal agar (OMA, Difco, Difco Laboratories) medium at 18 ± 1 °C in dark.

Field inoculations were repeated four times; at 15th of September, 8th of November, 27th of December 2010 and 27th of January 2011, in a *P. nigra* and *C. libani* plantation site near the campus area of Süleyman Demirel University, Isparta at 1,050 m a.s.l. The density of the stands was approximately 1,300 trees per hectare. The mean height and diameter at breast height of the pines were 5.0 m (range 2.5-7 m) and 13.5 cm (range 6.5-21.5 cm), respectively. The corresponding figures for the cedars were 3.7 m (range 2-5.5 m) and 5.7 cm (range 2-9 cm). Each of the five *G. abietina* isolates was in-

Table 1. Origin of *Gremmeniella abietina* isolates

Isolate name	Host species	Collection Location	Altitude of collection (m)
GaIPs	*Pinus sylvestris*	Mt. Zigana,Trabzon, Turkey	1,845
GaZPs	*Pinus sylvestris*	Mt. Ilgaz, Çankırı, Turkey	1,900
GaDgPn1	*Pinus nigra* subsp. *pallasiana*	Mt. Dedegöl, Isparta, Turkey	2,010
GaDgPn2	*Pinus nigra* subsp. *pallasiana*	Mt. Dedegöl, Isparta, Turkey	1,780
GaDgPn3	*Pinus nigra* subsp. *pallasiana*	Mt. Dedegöl, Isparta, Turkey	1,960

Table 2. Two-way ANOVA table for the lesion length for both host species and harvest times

Host	*Pinus nigra*				*Cedrus libani*			
Source	**d.f.**	**MS**	***f*-value**	***p*-value**	**d.f.**	**MS**	***f*-value**	***p*-value**
Harvest times - February								
Isolate (I)	5	555.947	9.904	0.000	5	150.384	33.176	0.000
Inoculation Date (Id)	3	2,703.183	48.156	0.000	3	74.849	16.512	0.000
I*Id	15	189.060	3.368	0.000	15	56.389	12.440	0.000
Harvest times - August								
Isolate (I)	5	349.177	2.794	0.018	5	78.077	1.925	0.091
Inoculation Date (Id)	3	5042.769	40.345	0.000	3	659.900	16.267	0.000
I*Id	15	359.748	2.878	0.000	15	42.337	1.044	0.412

d.f., degree freedom; MS, mean square.

oculated into two branches per tree and repeated ten times on both tree species at each inoculation date (totally: 5 isolates × 2 branches × 10 trees × 2 tree species × 4 inoculation dates = 800 branches in 80 trees). Branch diameter was measured at the inoculation point and the bark surface disinfected with 70% ethanol. Thereafter the outer bark and phloem were removed with a 4-mm diameter cork borer and inoculated with 4-mm diameter agar plugs cut from the edge of 4-6-week-old *G. abietina* colonies. Control shoots (2 × 10 × 2 × 4 = 160) were inoculated with sterile OMA. Wounds were tightly wrapped with parafilm.

Half of the inoculated branches (i.e. 400 + 80) were sampled at the end of February 2011 and the remaining ones six months later in August, which resulted in incubation periods of variable length (Table 3). The outer bark around the inoculation point was removed with a sterile scalpel and the lesion length measured. Re-isolation of *G. abietina* isolates was attempted from 10% of the inoculated shoots. Small pieces of tissues were cut from the edges of necrotic areas with a sterile scalpel. The pieces were plated onto 2% malt extract agar (MEA) and incubated in the dark at 15 °C for 3 weeks.

Table 3. Mean lesion lengths (mm) on branches inoculated with five *Gremmeniella abietina* isolates

Isolate	Pine				Cedar			
	September	**November**	**December**	**January**	**September**	**November**	**December**	**January**
February								
GaIPs	$4.70 \pm 0.68^{a\,B}$	$8.20 \pm 1.39^{ab\,B}$	$26.00 \pm 4.87^{a\,A}$	$4.70 \pm 1.16^{bc\,B}$	$3.40 \pm 0.34^{a\,A}$	$2.10 \pm 0.28^{b\,BC}$	$2.90 \pm 0.43^{bc\,AB}$	$1.00 \pm 0^{b\,C}$
GaZPs	$3.70 \pm 0.58^{a\,B}$	$4.60 \pm 0.67^{ab\,B}$	$21.40 \pm 2.88^{a\,A}$	$5.90 \pm 0.77^{b\,B}$	$3.80 \pm 0.44^{a\,BC}$	$6.50 \pm 1.52^{a\,B}$	$13.20 \pm 1.35^{a\,A}$	$1.10 \pm 0.23^{b\,C}$
GaDgPn1	$3.90 \pm 0.60^{a\,B}$	$13.80 \pm 3.77^{a\,AB}$	$24.60 \pm 4.84^{a\,A}$	$4.30 \pm 0.99^{bc\,B}$	$4.60 \pm 0.56^{a\,A}$	$2.80 \pm 0.13^{b\,AB}$	$4.60 \pm 0.88^{b\,A}$	$1.00 \pm 0^{b\,B}$
GaDgPn2	$4.40 \pm 0.67^{a\,B}$	$12.30 \pm 3.27^{a\,AB}$	$21.30 \pm 5.14^{a\,A}$	$11.50 \pm 2.08^{a\,AB}$	$3.30 \pm 0.42^{a\,B}$	$3.20 \pm 0.39^{b\,B}$	$5.10 \pm 1.48^{b\,AB}$	$7.00 \pm 0.92^{a\,A}$
GaDgPn3	$4.00 \pm 0.63^{a\,B}$	$12.60 \pm 2.37^{a\,A}$	$16.80 \pm 3.20^{ab\,A}$	$2.70 \pm 0.42^{bc\,B}$	$3.40 \pm 0.37^{a\,A}$	$3.70 \pm 0.79^{b\,A}$	$2.10 \pm 0.69^{bc\,A}$	$1.50 \pm 0.56^{b\,A}$
Control	$3.20 \pm 0.29^{a\,A}$	$2.20 \pm 0.33^{b\,AB}$	$2.10 \pm 0.23^{b\,B}$	$0.90 \pm 0.23^{c\,C}$	$1.20 \pm 0.20^{b\,A}$	$0.90 \pm 0.18^{b\,A}$	$0.10 \pm 0.10^{c\,B}$	$0 \pm 0^{b\,B}$
All isolates*	4.14 ± 0.28^{C}	10.30 ± 1.20^{AB}	22.02 ± 1.89^{A}	5.82 ± 0.68^{C}	3.70 ± 0.20^{B}	3.66 ± 0.40^{B}	5.58 ± 0.72^{A}	2.32 ± 0.40^{B}
August								
GaIPs	$11.00 \pm 2.67^{a\,B}$	$9.80 \pm 1.53^{ab\,B}$	$48.60 \pm 9.02^{a\,A}$	$16.20 \pm 1.95^{a\,B}$	$3.60 \pm 0.37^{a\,B}$	$4.90 \pm 0.55^{a\,B}$	$13.60 \pm 2.89^{a\,A}$	$8.80 \pm 1.99^{a\,AB}$
GaZPs	$4.60 \pm 0.56^{a\,B}$	$12.80 \pm 2.16^{ab\,B}$	$34.78 \pm 6.95^{ab\,A}$	$12.00 \pm 2.18^{a\,B}$	$4.80 \pm 0.36^{a\,A}$	$6.30 \pm 1.73^{a\,A}$	$10.10 \pm 1.79^{a\,A}$	$6.20 \pm 0.83^{a\,A}$
GaDgPn1	$7.90 \pm 1.53^{a\,B}$	$19.20 \pm 2.68^{a\,A}$	$20.78 \pm 2.36^{b\,A}$	$14.10 \pm 2.69^{a\,AB}$	$5.80 \pm 0.85^{a\,A}$	$6.30 \pm 0.60^{a\,A}$	$9.50 \pm 2.01^{a\,A}$	$8.80 \pm 2.32^{a\,A}$
GaDgPn2	$10.20 \pm 4.27^{a\,B}$	$13.80 \pm 2.72^{ab\,B}$	$28.80 \pm 4.97^{ab\,A}$	$12.60 \pm 2.12^{a\,B}$	$5.30 \pm 0.30^{a\,A}$	$10.20 \pm 2.59^{a\,A}$	$11.80 \pm 3.14^{a\,A}$	$11.90 \pm 2.77^{a\,A}$
GaDgPn3	$10.10 \pm 2.26^{a\,B}$	$14.50 \pm 3.12^{ab\,B}$	$30.50 \pm 4.66^{ab\,A}$	$17.80 \pm 2.28^{a\,B}$	$4.60 \pm 0.22^{a\,A}$	$5.00 \pm 0.42^{a\,A}$	$11.50 \pm 3.70^{a\,A}$	$6.40 \pm 1.66^{a\,A}$
Control	$8.30 \pm 2.27^{a\,A}$	$8.80 \pm 1.81^{b\,A}$	$17.44 \pm 4.80^{b\,A}$	$15.40 \pm 2.89^{a\,A}$	$5.60 \pm 0.87^{a\,A}$	$5.40 \pm 0.64^{a\,A}$	$18.70 \pm 4.06^{a\,A}$	$10.10 \pm 2.91^{a\,AB}$
All isolates*	8.76 ± 0.28^{B}	14.02 ± 1.16^{B}	32.90 ± 2.93^{A}	14.54 ± 1.02^{B}	4.82 ± 0.23^{C}	6.54 ± 0.68^{BC}	11.30 ± 1.22^{A}	8.42 ± 0.92^{AB}

Averages in the same column with the same lowercase letter are not significantly different ($p > 0.01$) according to Duncan's test.
Averages in the same row with the same uppercase letter are not significantly different ($p > 0.01$) according to Duncan's test.
* Average lesion length in branches inoculated with *G. abietina* only (excluding the control inoculations).

The mean temperature and relative humidity, as well as degree-days above 5 °C were calculated from data obtained from the Turkish State Meteorological Service for each incubation period starting from the beginning of inoculations till the date of harvesting.

Data were analyzed using the factorial design ANOVA, in which, each host species and each harvest time was analyzed separately. Six levels of isolate factor (GaIPs, GaZPs, GaDgPn1, GaDgPn2, GaDgPn3, and the control) and 4 levels of inoculation time factor (September, November, December and January inoculations) were used. Duncan's multiple range test was used in order to determine the differences between the group means. The analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL, USA).

Results

February harvest

The mean lesion lengths on both *P. nigra* and *C. libani* in February harvest were significantly different among inoculation dates and isolates as well as their interaction. On the other hand, in August harvest, the mean lesion lengths were significantly different among inoculation dates but not among isolates, and the interaction between isolates and inoculation dates was significant only for *P. nigra* ($p < 0.01$) (Table 2). The mean lesion lengths caused by the *G. abietina* isolates were larger than those measured for control inoculations on both *P. nigra* and *C libani* in February harvest (Table 3). There were also statistically significant differences in mean lesion length between the inoculation dates ($p < 0.01$). Both *P. nigra* and *C. libani* were found to be the most susceptible against *G. abietina* when inoculated in December; the mean lesion length for all isolates was significantly larger in December inoculations for both *P. nigra* (22.00 ± 1.9SE) and *C. libani* (5.58 ± 0.72) than at the other inoculation dates. While the shortest lesions on *P. nigra* were found in both September and January inoculations, the shortest ones on *C. libani* were found only in January inoculations ($p < 0.01$) (Table 3). The length of the incubation period did not explain the differences in lesion length; there was no statistically significant difference between the September and January inoculations on *P. nigra*. In contrast to the January inoculations the mean lesion length in September was not different from the control. On *C. libani* the situation seemed to be the opposite (Table 3).

There were some differences in lesion length between the isolates ($p < 0.01$), but virulence of the isolates varied in the different host–inoculation date combinations.

The daily mean temperature (°C) and relative humidity (%) between 15 September and 28 February ranged from –3.1 to 20.9 °C and 39.5 to 99.0%, respectively. The daily mean temperatures were above 10 °C until the first week of December. The daily mean temperatures were fluctuating mainly between 0 and 5 °C, or below 0 °C after December and January inoculations (Figure 1). Degree–days (d.d.) above 5 °C decreased rapidly from 741.1 for September inoculations to 9.9 for January inoculations (Table 4).

August harvest

In August harvest the lesion lengths were larger both in *G. abietina* inoculated branches and control than in February harvest. On *P. nigra* the lesions sizes in

Table 4. Effective temperature sums (degree-days), mean temperatures and relative humidity during incubations until first sampling in February

Incubation durations, days	Effective temperature sums (degree-days, threshold 5 °C)	Temperature (°C)	Relative humidity (%)
166 (09.15.2010-02.28.2011)	741.10	7.40	84.20
112 (11.8.2010-02.28.2011)	230.00	8.40	75.05
64 (12.27.2010-02.28.2011)	26.00	8.80	83.75
32 (01.27.2011-02.28.2011)	9.90	3.45	75.15

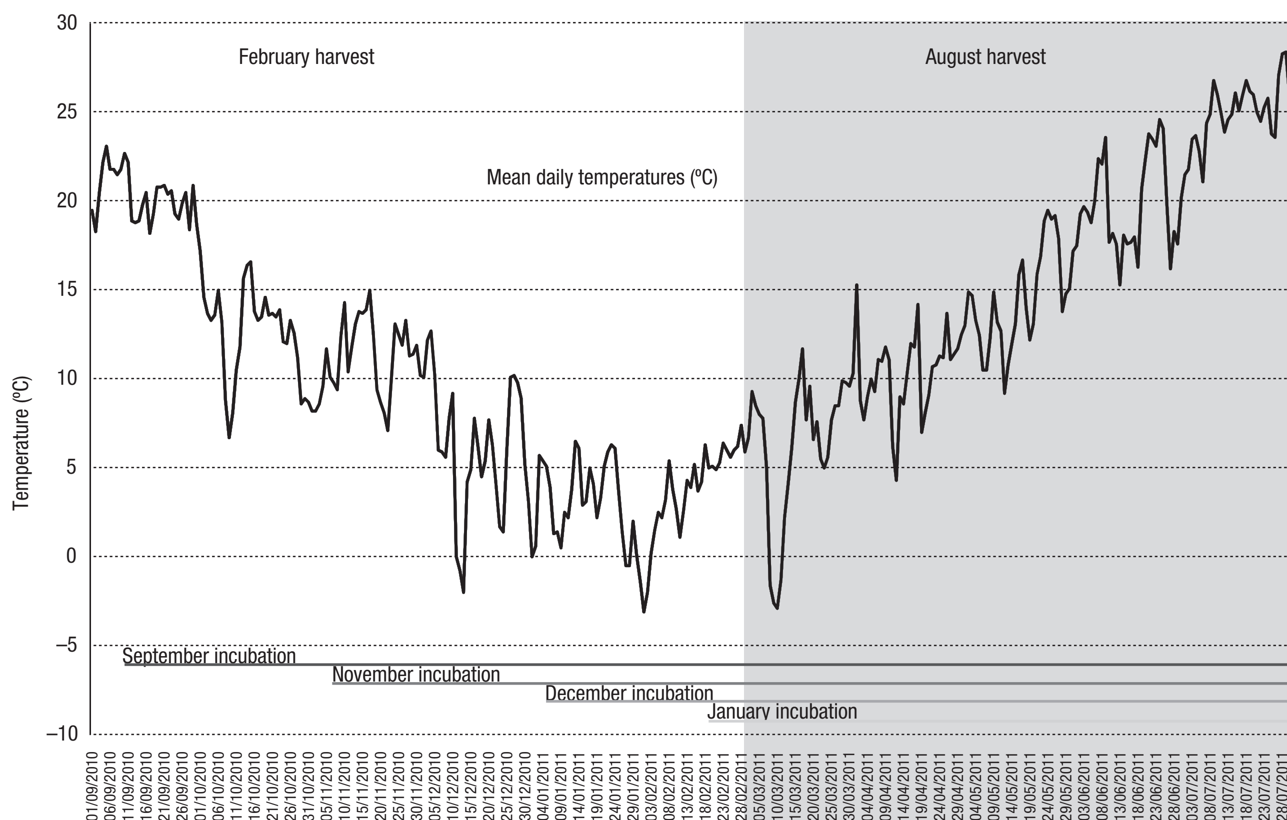

Figure 1. Mean daily temperatures (°C) during incubations.

G. abietina inoculated branches were 112, 36, 49 and 150% larger in August harvest in September, November, December and January inoculations, respectively. On *C. libani*, in turn, the lesion sizes were 30, 79, 103 and 263% larger, respectively. However, also the lesion lengths in controls were larger and the difference in relation to those in the *G. abietina* inoculated branches smaller than in February. As a result, the differences in lesion lengths between the *G. abietina* inoculated branches and controls were not significantly different with two exceptions. Results on *C. libani* were similar: the differences observed in February had disappeared.

On both hosts the lesion lengths were 2-3-fold larger in control inoculations done in December and January than those done in September and November. However, the differences were not statistically significant.

Discussion

Although both *P. nigra* and *C. libani* were found to be susceptible in the February sampling, the symptom severity on black pine was almost 3-fold higher than that on cedar. To our knowledge *G. abietina* has not been reported to occur on *C. libani*. Nevertheless, this species was reported to be susceptible against *Heterobasidion annosum* sensu lato (Fr.) Bref. (Lehtijärvi *et al*., 2011) and *Sphaeropsis sapinea* (Fr.) Dyko et Sutton (Doğmuş-Lehtijärvi *et al*., 2009) in mycelial inoculations – without being their natural host.

The length of the incubation period did not explain the differences in lesion length in the February sampling; there was no statistically significant difference between the September and January inoculations on *P. nigra,* despite the almost 6-fold difference in incubation period. Although, the difference was larger in the August sampling neither September nor January inoculations differed from the controls.

The temperatures during the first two inoculation dates were within the adequate intervals for plant growth, and therefore the defense mechanisms probably were still active. This could explain the increase in susceptibility of *P. nigra* and *C. libani* from September to December inoculation. During December inoculations and onwards, in contrast, the trees were most likely in dormancy, i.e. unable to defend themselves.

It is known that both virulence of *G. abietina* and host resistance are dependent on weather conditions (Roll-Hansen, 1964; Blenis *et al*., 1984; Barklund and Unestam, 1988; Marosy *et al*., 1989; Karlman *et al*., 1994, Terho and Uotila, 1999). Although spore dispersal and infection of the host occur during the growing season, *G. abietina* is able to grow into the living host tissues firstly during host dormancy (Patton *et al*., 1984). In an inoculation experiment using conidiospores, Marosy *et al*. (1989) found that a period of at least 44 conducive days, i.e. days with temperatures remaining between –6 and +5 °C, was necessary for symptom development. In the present study, the inoculation of the fungal mycelium into wounds reaching to the phloem bypassed any natural barriers present in the bark. However, once inside the living tissues, growth of the fungus in the inner bark can be expected to be controlled by the same defence mechanisms irrespective of how the fungus entered the host. Therefore, the conducive day concept should apply to both penetration of and growth within the living tissues. Several mycelial inoculation experiments support this view. Roll-Hansen (1964) found out that mycelial inoculations on the stems of Scots pine in late winter gave larger necrosis than those done in early spring when the soil had thawed indicating that the cold weather promoted mycelial colonization of the bark tissues. Terho and Uotila (1999) found an increasing trend from August to October in canker and necrosis lengths on 2-m-tall Scots pines inoculated with mycelium with two weeks intervals. Although the daily mean temperatures during the inoculation period in their study (August–October) were similar to those in November–January in the present study, the pattern in susceptibility of the trees was different. In their study, the longest cankers and lesions were formed on trees which were inoculated the latest, in October, while in the present study the lesion length peaked in December inoculations. The result was the same in both February and August sampling indicating that the reduction in lesion length from December to January inoculations was not because of the short incubation period from January to February in the first sampling. The short lesion lengths in January inoculations indicate a lower susceptibility of the host compared with December inoculations despite the continued low temperatures and probably dormancy as well. By the February sampling the number of conducive days for the December inoculations was 42 (estimated from mean daily temperatures below +5 °C) and for January inoculations 21. After February there was only eight more conducive days. As the lesion lengths for January inoculations in August were not significantly different from controls it seems likely that only the December inoculations were performed within the susceptibility period predicted by the conducive day concept (Marosy *et al*., 1989). Alternatively, the 3.8-fold difference in mean lesion lengths (22 vs. 5.8 mm) could be explained by a 2.6-fold difference in degree–days above 5 °C (26 vs. 9.9). Application of a threshold temperature of 5.9 °C would result in a ratio 3.86, which is almost identical with that of the lesion lengths. If the growth rate of the fungal mycelium within the host tissues during the host dormancy were temperature limited, the colder weather from January inoculations onwards would explain the shorter lesions. However, owing to the fact that *G. abietina* is able to grow at temperatures down to about –6 C (Marosy *et al*., 1989) and that the lesion lengths for January inoculations in August sampling did not differ from control, this alternative seems less likely.

The larger lesion lengths in the controls in the August sampling indicate that active defence reactions occurred also because of wounding alone after February sampling when the weather had become warmer. Wounding during the dormancy seemed to be more damaging as indicated by the longer lesions on both tree species in the December and January controls, although the difference was not statistically significant. Ceased fungal growth during the warm weather in summer could explain the smaller relative differences between the lesions on *G. abietina* inoculated branches and the controls (cf. Patton *et al*., 1984; Marosy *et al*., 1989).

The results of the current study clearly showed that the most sensitive period for both host species against *G. abietina* colonization was December. This was most likely a result of host dormancy and appropriate temperatures for fungal growth within the host tissues. More evenly distributed inoculation intervals and shorter incubation periods could have given more accurate results about when the susceptible period could have started and ended. Although both *P. nigra* and *C. libani* were found to be susceptible, the symptom severity on black pine was almost 3-fold higher than that on cedar. Moreover, the lesions were relatively short on *C. libani*, possibly because it may not be a natural host of *G. abietina*. The isolates used in the present study were obtained from *P. sylvestris* and *P. nigra*, and therefore could be expected to be adapted to infect pines, but not *C. libani*.

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