A Conducive Day Concept to Explain the Effect of Low Temperature on the Development of Scleroderris Shoot Blight

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ABSTRACT

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The in vitro growth of *Gremmeniella abietina* at −6 C both in the presence or absence of ice crystals was confirmed. Red pine seedlings artificially inoculated with the North American serotype, exposed to natural field conditions and artificially manipulated field conditions, developed symptoms of Scleroderris shoot blight when exposed to 44 or more days in which the temperature remained between −6 and +5 C or snow completely covered the seedlings or tree parts—a conducive day. Thus, an extended period of relatively mild canopy temperature during the winter appears to favor disease development. The conducive period—a period in which 44 or more conducive days occurred—could either occur in the winter after inoculation, or over the two winters after inoculation. The apparent latent period in the disease cycle may result from the need for winter conditions before symptom development can occur. The occurrence of symptoms primarily on lower branches, and the restriction of the disease to latitudes that receive sustained snow cover in the Lake States are consistent with this observation. Comparison of literature descriptions of outbreaks of the disease to weather records revealed a strong association between conducive periods, usually single conducive winters, and the occurrence of symptoms. The natural range of the disease may be restricted by the need for recurrence of conducive periods within 3 yr to avoid breaking the disease cycle.

Additional keywords: Ascocalyx abietina, Brunchorstia pinea, psychrophilic fungi, *Pinus resinosa, Scleroderris lagerbergii.*

Scleroderris shoot blight, caused by the Ascomycete *Gremmeniella abietina* (Lager.) Morelet (= *Ascocalyx abietina* (Naumov) Schlaepfer-Bernhard, anamorph = *Brunchorstia pinea* (Karst.) Hohn.) is a pathogen on members of the Pinaceae in the northern Lake States, northeastern regions of the United States, Canada, Europe, and Japan. Three serotypes of the fungus—the North American (NA), the European (EU), and the Asian—have been identified (13). The NA and EU serotypes occur in North America.

The NA serotype affects small pine trees and lower branches of larger trees, to about 2 m above ground level (8). Trees smaller than about 2 m can be killed by the disease; taller trees will usually survive the infection (7,14), as only one internode per year is typically killed. Disease associated with the NA serotype continues to cause losses throughout red pine (*Pinus resinosa* Ait.) and jack pine (*P. banksiana* Lamb.) plantations north of about 45° N latitude. Although the disease caused by this serotype

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has occasionally been found south of 45° N latitude, these infections have usually been transient (14). Detailed accounts of losses in North American nurseries and plantations due to this serotype have been published (10 and references therein).

It is widely accepted that environmental conditions are important in the development of epidemics of Scleroderris shoot blight in the Lake States. However, progress toward determining which environmental conditions are most critical to disease development has been hindered by a disease cycle that is nearly a year long. Most attempts to relate the occurrence of this disease in North America to environment have focused on spring/summer spore production and dispersal, on the assumption that the amount of disease will be positively related to inoculum levels (11,12,37,38). The role of environment in restricting the disease to northern latitudes and to lower branches of host trees, and in affecting the length of the latent period, has not been adequately explained. Full symptom development, including twig death and basal reddening, has not been observed under artificial conditions or under field conditions outside the known range of the disease (4,42), despite successful fungal penetration (5,22). This suggests that the environmental factors limiting success of such inoculation attempts may be the same factors that limit the range of the disease in nature, and that these factors probably affect some process other than spore dispersal and initial penetration. For example, environmental conditions may be critical for the rapid fungal colonization of tissue that occurs during midwinter. Despite such indications, only limited attention has been given to the effect of autumn, winter, and early spring environmental conditions on the development of Scleroderris shoot blight.

The EU serotype of *G. abietina* was first isolated in North America in the early 1970s (14), when the disease caused widespread mortality of mature trees in New York State (27,34,44). This serotype, which commonly causes upper crown damage and mortality of mature trees in Europe (2,3,9,31,36), affects many members of the Pinaceae and is associated with disease as far south as 43° N latitude. The absence of the NA serotype from diseased upper crowns and the predominance of the EU serotype in areas where there is disease in upper crowns led to the suggestion that the EU, but not the NA, serotype is capable of causing disease in upper crowns. Since its discovery in New York, the EU serotype has been detected as far west as eastern Ontario. The question arises as to whether the apparent westward "spread" of the EU serotype in North America presents a serious threat to the coniferous resources of the northern Lake States and much of western North America. Differences in symptom expression associated with the two serotypes may be due to differences in pathogenicity and host range. Alternatively, it is possible that they are due to environmental differences where the two serotypes predominate, or to some combination of genetics and environment.

An association between snow cover and disease on young trees involving both the NA and EU serotypes has been observed in North America, Europe, and Japan (17,24,28,32,48–51). Such an association might be explained by the nearly constant temperatures and higher humidities beneath the snow as contrasted with the much colder and drier ambient conditions above the snow. *G. abietina* is able to grow at temperatures slightly below freezing (13,16,52). Ettlinger (16) reported that *G. abietina* would grow at −5.8 C. Thus, the pathogen might be capable of colonizing tissues under snow cover. The longer young trees are completely buried in snow, the longer the period during which the fungus might grow in a dark, humid environment at or slightly below 0 C (11,49,50).

We propose that a sufficient duration of temperatures near freezing must prevail before development of Scleroderris shoot blight symptoms will occur in infested trees. In the case of disease in lower branches only, we propose that snow cover might be critical in moderating temperature. In areas where upper crowns of mature trees are affected by the EU serotype of the pathogen, temperatures near freezing might occur in the absence of snow.

The objectives of our study were to investigate the in vitro growth response of isolate ASL 4-b-3 of *G. abietina* to temperatures near or slightly below freezing, and to determine the importance of extended periods of near-freezing temperatures to the epidemiology of Scleroderris shoot blight.

MATERIALS AND METHODS

Spore suspensions (approximately 1.0×10^6 conidia/ml) of isolate ASL 4-b-3 prepared from V-8 broth flask cultures (23) were used for all conidial inoculations. Conidia were not more than 2 wk old when used, and their viability was 90% or greater. Inoculum was applied to the current-year growth of red pine seedlings after candles had begun to flush but before needle elongation. By brushing in an upward motion with a No. 1 artist's paint brush (4), between 1×10^5 and 2×10^5 spores per seedling were applied to the stems and bract surfaces. Immediately after inoculation seedlings were misted with water. Inoculations were conducted in late afternoon when feasible. When seedlings were inoculated earlier on a warm sunny day, or if heavy rainfall was occurring or predicted, the seedlings were shielded from the elements with aluminum foil for 24 hr.

"Branch" inoculations were accomplished by suspending infected branches on a vinyl-coated wire screen approximately 0.6 m above the seedlings (40). The infected branches were collected from a heavily diseased red pine plantation in Bayfield County, WI, and remained in place throughout the summer and fall, and, in the case of the local climate experiment, the winter.

Symptom assessment. Symptoms were assessed at monthly intervals throughout the spring and summer of the year after inoculation by the "pull test" technique (29), based on the unique symptom of loose fascicles associated with infection by this disease. Ten loose fascicles per tree (if available) were plated on V-8 agar + 100 mg of streptomycin per liter or one drop of 80% lactic acid per plate. Basal sections of needles from each fascicle were placed on two plates, one of which was incubated at 4 C and the other at 18 C. Probable colonies of *G. abietina* were transferred to V-8 agar slants and incubated at 18 C under a 16-hr photoperiod at $100\ \mu E\ m^{-2}\ sec^{-1}$ light intensity to favor sporulation (23). On the basis of results from preliminary isolation trials, a symptomatic seedling was considered infected if a sporulating isolate was obtained in slant culture, or if at least five nonsporulating isolates, tentatively identified as *G. abietina* by mycelial and growth characteristics, were obtained from the 10 isolation attempts. Unless the symptoms were atypical or otherwise subject to question, trees with more than 10 loose fascicles were considered infected without attempts at isolation. The term infection is used in this sense throughout this paper. Penetration is used to refer only to initial bract penetration by the fungus. Colonization refers to mycelial proliferation within stem and needle tissue, and symptom expression or symptomatic is used when referring to probable disease incidence, as evidenced by loose fascicles, needle-base reddening, or shoot or seedling mortality.

Temperature, precipitation, and snow cover data were obtained from the U.S. National Climatic Data Center (46) unless otherwise noted. Climatic data for Canada were obtained from Environment Canada (15), and for Europe from Meteorologische Abhandlungen (25).

Mycelial growth at low temperatures. Isolate ASL 4-b-3 was tested for growth and viability at temperatures near or below freezing, in the presence or absence of ice. Insulated baths (45 × 54 cm) with Neslab (Neslab Instruments, Inc., Portsmouth, NH) Endocal EN-850 coolers and Exacal EX-300 controllers or with EN-350 coolers and EX-400 temperature controllers were used to maintain temperatures within ± 0.1 C. Ten V-8 agar plates inoculated with a 3-mm-diameter mycelial plug cut from the outer edge of an actively growing colony were placed in each of four ethylene glycol baths at +4, −1, −6, and −10 C. Each plate was sealed with Parafilm, then enclosed individually in three separate plastic bags, each secured with a rubber band. The plates were placed in the baths such that each plate was submerged while the sealed end of the plastic bags was not, to avoid seepage of ethylene glycol into the plates. An additional 10 inoculated plates were placed in a −6 C incubator for 24 hr to induce ice nucleation before placement in the −1 C bath. Plates at +4 and −1 C were checked for growth after 14 days, plates at −6 C after 31 days, and those at −10 C after 42 days. The plates in which ice had been induced to form before incubation at −1 C were thawed, incubated at −1 C for an additional 14 days, and again examined for growth.

To clarify the role of ice as opposed to supercooled fluid water in growth of the fungus at −1 C, five plates each were either 1) inoculated without freezing of the plates, 2) inoculated after freezing of the plates, 3) inoculated with previously frozen inoculum, or 4) frozen and inoculated with previously frozen inoculum. Plates were checked for growth after 14 days at −1 C.

Local climate experiment. To investigate the disease response of inoculated seedlings both north and south of 45° N latitude, nine sites throughout Wisconsin and upper Michigan, ranging from 100 to about 450 cm of mean annual snowfall (19,45), were selected. Locations and latitudes of the sites are shown in Table 1. Weather data for the Trout Lake plot were collected by personnel at the University of Wisconsin Trout Lake Center for Limnology. Data for each of the other plots were taken from a nearby National Climatic Data Center volunteer data collection station. Ninety-eight seedlings were planted on a 2.0 × 0.6 m area of ground at each of the nine locations. Seedlings used were containerized red pine obtained from Potlatch Corporation (Cloquet, MN). Trees were approximately 8 mo old at the time of planting. Half of the seedlings at each site were inoculated with a spore suspension and half were placed under branch inoculum in June 1984. All seedlings inoculated with a spore suspension were reinoculated in late July, and seedlings in four of the plots (Sturgeon Bay, Antigo, Alberta, and Newberry) again in early October. The experiment was repeated in 1985 with new seedlings, and with the addition of one site. Half of the trees were inoculated with a spore suspension and half placed under branch inoculum in June 1985. Because of high seedling mortality during the summer of 1985, replacement seedlings were planted and inoculated in September 1985. In addition to the usual symptom assessment, further symptom development on the original seedlings, 2 yr after inoculation, was also assessed in 1986.

Conducive days. The number of conducive days was estimated as the number of days during which the ambient temperature remained entirely between +5 C and −6 C, or the trees or tree parts were completely covered with snow subsequent to inoculation.

Snow cage experiment. To investigate the effect of the presence or absence of snow cover on the development of Scleroderris shoot blight, snow cover conditions at a northern and southern Wisconsin location (Copper Falls State Park and Blackhawk Ski Club, respectively) were modified. An attempt was made to prevent snow accumulation at Copper Falls where heavy snow cover is common and the disease occurs naturally, and to use artificial snow to cover the seedlings at Blackhawk, where natural snow cover is typically light and the disease does not occur.

TABLE 1. Infection and mortality of red pine seedlings in the 1985 local climate experiment as related to number of conducive days[a]

			Conidial inoculation		Branch inoculation	
Location	Latitude	Number of conducive days	Percent infection	Percent mortality	Percent infection	Percent mortality
Spooner	45°49′	17	0	0	0	0
Antigo	45°8′	27	29	29	0	0
Trout Lake	46°2′	41	0	0	0	0
Hancock	44°7′	43	0	0	0	0
Janesville	42°40′	60	8	3	0	0
Newberry	46°20′	63	100	100	100	100
Sturgeon Bay	44°52′	65	39	14	16	11
Mellen	46°26′	65	20	9	59	33
Alberta	46°39′	79	93	75	89	89

[a] Each plot consisted of 98 containerized seedlings, half of which were inoculated with a spore suspension and half of which were exposed to branch inoculum, as described in the text.

Two plots of 25 4-yr-old seedlings obtained as 2-0 bare root stock, potted immediately after lifting, and held at the University of Wisconsin campus until planting, were established at each of the two locations. Half of the seedlings in each plot at the two locations were inoculated with a spore suspension in June 1984; the remaining seedlings were exposed to branch inoculum between June and October 1984. At Blackhawk, the natural snow that fell on one plot (the no-snow plot) was manually removed so that the crowns of the seedlings were always exposed to ambient conditions. Artificial snow was not needed on the snow plot, since snow cover was unusually deep in southern Wisconsin during 1984–1985. At Copper Falls, a wire-mesh cage (18 × 14 mesh) was placed over seedlings in the no-snow plot to prevent snow accumulation.

Average hourly and daily maximum and minimum temperatures were sensed with thermistors placed within the canopy 40 cm above ground in the center of each plot, recorded with a CR-21 (Campbell Scientific, Logan, UT) data logger, and stored in a solid state storage module (Campbell Scientific model SM64). An automatic photographic system developed by A. Alberga was adapted to record snow cover at each plot (1). The experiment was repeated in 1985 with 49 containerized seedlings per plot, with thermistors placed 20 cm above ground. Artificial snow was again unnecessary at the Blackhawk site. Seedlings from the 1984–1985 experiment in both plots at the two locations were not reinoculated in 1985, but were exposed to natural snow conditions during the winter of 1985–1986.

Roped-tree experiments. To determine if disease symptoms would develop in the upper crowns of young plantation trees if they were covered with snow throughout the winter, three 6-yr-old plantation trees in Bayfield County, approximately 2 m in height in 1984, were inoculated with a spore suspension in June 1984. The tops of two of these trees were pulled to the ground and secured in such a way that they would be covered by winter snowfall. The third tree was left standing throughout the winter. The trees were assessed for symptoms in the spring and summer of 1985. The same trees were reinoculated in July 1985 and assessed for symptoms during the spring and summer of 1986.

In the summer of 1986, a similar experiment was done with three 8- or 12-yr-old trees at each of two locations in Bayfield County. One tree at each site was inoculated and pulled to the ground, one was inoculated and left standing, and the third was pulled to the ground but not inoculated. This 1986–1987 experiment was repeated at two locations in 1987–1988.

Vertical microclimate experiment. To determine the potential for symptom expression in the upper crown of a large cone-bearing tree in the presence of adequate inoculum, a 15-yr-old plantation tree in Bayfield County was inoculated in June 1984 at 16 locations throughout the crown, from approximately 0.5 to 3.0 m above ground. The current-year growth of eight branches was inoculated with a spore suspension; the remaining eight branches were inoculated by tying a 20-cm-long infected red pine twig to the surface of the branch. In November 1986 an additional 10 branches close to the ground were inoculated with a spore suspension. The experiment was repeated during 1986–1987 and 1987–1988 at a nearby location, by using a spore suspension to inoculate 26 leaders on two branches at the base of the tree and five leaders on one branch at approximately 1.7 m above ground.

Conducive weather conditions as an indicator of the range and symptoms of Scleroderris shoot blight. We examined five reports of artificial inoculation of seedlings including nine location-year combinations (4,22,39,42,43), four field situations in which disease symptoms occurred in lower but not upper crowns (20; Laflamme, *personal communication*) or were absent from lower crowns when expected (39), and six field situations in which disease symptoms were reported in the upper crowns of large trees (2,3,9,31,36,44). When the year of symptom expression was not specified (2,31), weather data for a number of seasons were examined. A total of 30 location-year combinations were examined.

We examined climatic data associated with the New York State epidemic of the mid-1970s. We used temperature data from Boonville, NY, one of two main centers of upper crown infection, for the winters of 1964–1965 through 1984–1985. For comparison, we examined similar data for Ashland, in northern Wisconsin, where the disease does not occur in upper crowns.

The potential for disease in western North America. We determined the number of conducive days, as calculated with a 60-cm minimum snow cover, for single winters or consecutive periods during the winters of 1979–1980, 1980–1981, and 1981–1982 for three high elevation locations in California and Oregon: Yosemite Park, CA (1,561 m), Twin Lakes, CA (2,386 m), and Crater Lake, OR (1,974 m). We also examined ambient air temperatures during the winters of 1982–1983, 1983–1984, and 1984–1985 for four locations along the northwestern coast of the United States, three locations on the western coast of British Columbia, and two locations along the southwestern coast of Alaska, and calculated the number of conducive days occurring at each location for a single winter, or for each conducive consecutive 2-yr period.

RESULTS

Mycelial growth at low temperatures. After 14 days, growth was observed in all plates held at +4 C, and in all plates in which the water remained supercooled at −1 C. All plates in which ice formation was induced before placement at −1 C were still frozen after 14 days at this temperature, and no growth was observed. After these plates were thawed and incubated for an additional 14 days at −1 C, none of the plates was frozen and growth was observed in all 10 plates. Seven of 10 plates at −6 C were supercooled and supported a slight amount of visible fungal growth. Of the three plates containing ice, two supported fungal growth that could only be observed microscopically; one had no detectable growth. All plates at −10 C contained ice and no growth could be detected; however, normal growth occurred once the plates were transferred to a warmer temperature.

When growth on supercooled medium was compared to that on frozen medium, the medium remained supercooled in all plates not deliberately nucleated (treatments [1] and [3]), and supported measurable growth after 14 days at −1 C, regardless of whether or not the inoculum plug had been prefrozen. Ice was present in the media in all plates that had been frozen initially (treatments [2] and [4]). Measurable growth was visible on the inoculum plugs that had not been prefrozen, but did not extend on to the surface of the frozen agar (treatment 2). Growth on the prefrozen inoculum plugs on frozen agar (treatment 4) was not apparent to the naked eye but could be detected microscopically. Our failure to observe growth in the frozen plates in the preceding −1 C experiment may have been due to the lack of microscopic examination.

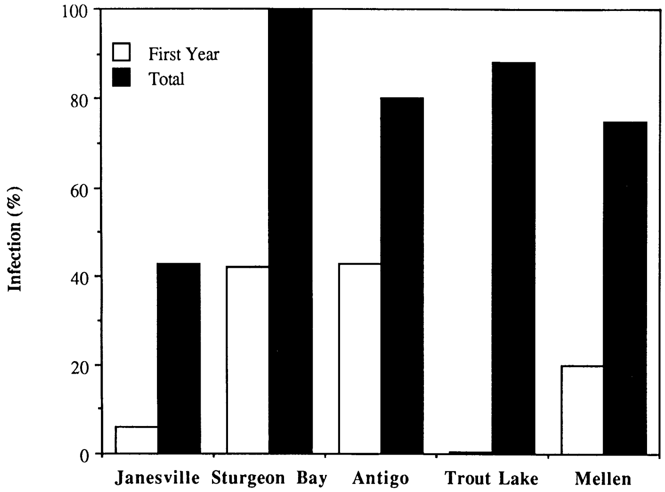

Fig. 1. Infection in 1985, the first spring (open bars), and 1986, the second spring (solid bars), after conidial inoculations in 1984 at selected sites in the local climate experiment. All trees were inoculated during June, and again in late July 1984. Trees at Sturgeon Bay and Antigo were inoculated again in early October 1984.

Local climate experiment. Disease incidence and mortality observed on seedlings in both the conidial inoculation plots and the branch inoculation plots ranged from 0 to 100% in both 1985 and 1986 (Tables 1 and 2). The presence or absence of infection could not be clearly related to the latitude of the plot. Of the three sites located south of 45° N latitude, infection occurred in 1985 on seedlings at two locations—Sturgeon Bay, just south of 45° N latitude, and Janesville, the southernmost location in the experiment. Of the six sites north of 45° N latitude, infection was absent in 1985 from seedlings at Spooner and at Trout Lake (Table 1). In the 1985–1986 experiment, infection occurred on all seedlings inoculated in 1985 except those at Janesville and Hancock (Table 2). Five of the original nine plots were followed through the second season and reassessed for further symptom development in 1986. The incidence of infection increased in all cases (Fig. 1). This increase was most dramatic at the Trout Lake site. Infection had been completely lacking at this location in 1985, but 88% of the seedlings showed infection in 1986.

In the presence of adequate inoculum, infection by the NA serotype of *G. abietina* occurred in areas both north and south of 45° N latitude. Environmental factors directly related to latitude, such as photoperiod, did not appear to be critical to disease development. An examination of temperature data for the summer of 1984–1985 did not reveal any differences in maximum temperature during the summer incubation period following inoculation that might explain the observed differences in disease expression at the experimental sites. The winters of 1984–1985 and 1985–1986 in Wisconsin were, however, somewhat atypical. Snowfall during 1984–1985 was lighter than normal in the northern part of the state, but much heavier than normal in the extreme southern part of the state. Snowfall during 1985–1986 was quite heavy and remained on the ground for a near record-breaking period in all but the extreme southern portions of the state.

Conducive days. On the basis of preliminary analysis of results of the local climate experiments we concluded that disease appeared most frequently after winters in which there were long periods during which canopy temperatures remained near 0 C, most frequently under snow cover. To quantify the possible role of temperature, we designated each day to which trees or tree parts were exposed to ambient conditions as conducive or nonconducive. A conducive day was defined as one in which the ambient air temperature remains between −6 and +5 C for the entire day, or the snow cover is enough to completely cover the trees or branches under consideration. The lower temperature

TABLE 2. Infection and mortality of red pine seedlings in the 1986 local climate experiment as related to number of conducive days[a]

			Conidial inoculation		Branch inoculation	
Location	Latitude	Number of conducive days	Percent infection	Percent mortality	Percent infection	Percent mortality
Janesville	42°40′	39	0	0	0	0
Trout Lake	46°2′	60	68	16	93	50
Hancock	44°7′	99	0	0	0	0
Sturgeon Bay	44°52′	100	25	25	50	50
Mellen	46°26′	101	61	52	33	28
Alberta	46°39′	101	100	100	100	100
Spooner	45°49′	102	88	75	17	11
Antigo	45°8′	104	...[b]	...[b]	33	33
Newberry	46°20′	107	54	50	0	0
Calumet	47°10′	116	86	58	58	18

[a] Each plot consisted of 98 containerized seedlings, half of which were inoculated with a spore suspension and half of which were exposed to branch inoculum, as described in the text.

[b] No seedlings remained to be evaluated.

limit is the lowest temperature at which the fungus has been observed to grow (above, and 13,16,52). The upper limit was chosen somewhat arbitrarily as that below which physiological activity of a tree might be severely reduced (47). This definition is used throughout this manuscript.

The number of conducive days to which the seedlings in the local climate experiment were exposed reflected the unusual snow conditions during the 1984–1985 and 1985–1986 winters. The number of conducive days between 1 October—when conducive days normally begin to accrue at the sites—and 28 February—when incipient symptoms should have developed (Patton, *unpublished*; Skilling, *personal communication*)—ranged from 17 to 79 during 1984–1985 and from 39 to 116 in 1985–1986 at the various plots (Tables 1 and 2). A critical number of conducive days appeared necessary for disease development. All plots except Antigo 1984–1985 exposed to 43 or fewer conducive days were completely free of disease, while all plots except those at Hancock 1985–1986 exposed to 60 or more conducive days exhibited typical disease symptoms. The branch inoculation technique led to less consistent infection over the 2 yr than did the conidial inoculation technique. Thus, the lack of disease on seedlings exposed to branch inoculum at Janesville in 1985 and Newberry in 1986 may have been due to poor inoculation. In any event, no infection with branch inoculation occurred with fewer than 44 conducive days.

Snow cage experiment. The snow cage at Copper Falls prevented snow accumulation on the seedlings during both seasons. The seedlings at both locations were exposed during the two winters to temperatures as low as −36 C. Snow completely covered the seedlings in the two snow plots during both seasons, where temperatures within the seedling canopies remained between −4 and 0 C throughout the winter of 1984–1985 and between −6 and 0 C throughout the winter of 1985–1986. In 1984–1985 the no-snow plots at Blackhawk and Copper Falls were exposed to 21 conducive days, and seedlings at both locations were symptom free (Table 3). Both snow plots were completely covered with natural snow between 8 January and 2 March. The trees were exposed to 67 (Blackhawk) or 70 (Copper Falls) conducive days, and disease symptoms developed at both sites. Because the trees were 6 years old in the summer of 1986 and the disease typically kills only one internode per year, little mortality occurred in these trees in the first season.

A heavy wet snowfall in the Madison area on 8 and 9 November 1985 left the seedlings planted and inoculated in 1985 encased in ice on the ground surface for the entire 1985–1986 winter, and seedlings in both the snow and no-snow plots developed symptoms (Table 3). The snow plots at Blackhawk and Copper Falls were each exposed to 101 conducive days, and high levels of disease incidence were observed in both. Because smaller seedlings were used during 1985–1986, much higher mortality occurred than in 1984–1985. At Copper Falls the no-snow trees did remain free of ice and snow throughout the winter, but some developed disease symptoms despite the low number of conducive days (24) to which they were exposed.

TABLE 3. Infection and mortality of red pine seedlings in the 1985 and 1986 snow cage experiments as related to number of conducive days[a]

Location	Number of conducive days	Conidial inoculation Percent infection	Conidial inoculation Percent mortality	Branch inoculation Percent infection	Branch inoculation Percent mortality
1985					
BH No-Snow	21	0	0	0	0
BH Snow	67	76	12	6	4
CF No-Snow	21	0	0	0	0
CF Snow	70	48	0	8	0
1986					
BH No-Snow	43[b]	36	36	50	46
BH Snow	101	83	83	86	7
CF No-Snow	25	9	0	0	0
CF Snow	101	62	56	33	29

[a] 25 4-yr-old (1985) or containerized (1986) seedlings were inoculated with a spore suspension, and 25 exposed to branch inoculum, at each location, as described in the text. BH = Blackhawk, CF = Copper Falls.

[b] Seedlings in the BH no-snow plot were embedded in a case of ice throughout the winter; therefore, the measure of conducive days does not adequately describe the conditions to which they were exposed.

Disease incidence on seedlings inoculated in 1984 increased dramatically after the second winter. These trees were not reinoculated in 1985; however, the plots at Blackhawk and Copper Falls were exposed to 46 and 90, respectively, additional conducive days during the winter of 1985–1986. Infection level in both snow plots increased to over 95%, while that in the no-snow plots, completely lacking in 1985, was over 75% in 1986 (Fig. 2). In 1986, symptoms on these trees appeared first on 2-yr-old needles, rather than on the 1-yr-old needles typically affected. By the end of the summer, both the 2-yr-old needles and the 1-yr-old needles had been killed.

Logistic regression of disease incidence on conducive days for all data obtained from the local climate and snow cage experiments estimated a positive correlation ($R^2 = 0.79$, $n = 26$, $P = 0.005$). In an effort to improve on our original definition of a conducive day, all temperature ranges between −12 and +8 C, in conjunction with a 20-cm snow cover, were considered for all plots in the local climate and snow cage experiments. Snow cover alone, between 0 and 40 cm, regardless of temperature, was also considered. Finally, each combination was considered both through the end of February and through the end of March. No significant improvement over the original logistic regression was obtained ($R^2 = 0.83$ for a maximum temperature of +1 C and a minimum temperature of −7 to −11 C vs. $R^2 = 0.79$ for the original definition; $n = 26$, $P = 0.005$). The original definition of a conducive day was thus retained as an indicator of the potential for expression of symptoms due to the NA serotype of *G. abietina*, although some alteration of either the lower or upper limit of the definition would not alter our result at all. The insensitivity of the regression to small changes in the temperatures used to define a conducive day, and the rather sharp demarcation between the numbers of conducive days in winters that precede disease (conducive winters) and those that do not, suggest that the likelihood of disease is less sensitive to small changes in "near 0 C temperatures" than to the duration of these conditions.

Roped-tree experiment. No symptoms were observed on any of the trees in the roped-tree experiment in the summers of 1985 or 1987 after winters in which no more than 25 conducive days had accumulated. In the summers of 1986 and 1988, inoculated trees pulled to the ground during the winter became infected; those remaining upright did not. In addition, the uninoculated tree pulled to the ground during the winter of 1987–1988 was also infected. The winters of 1985–1986 and 1987–1988 were

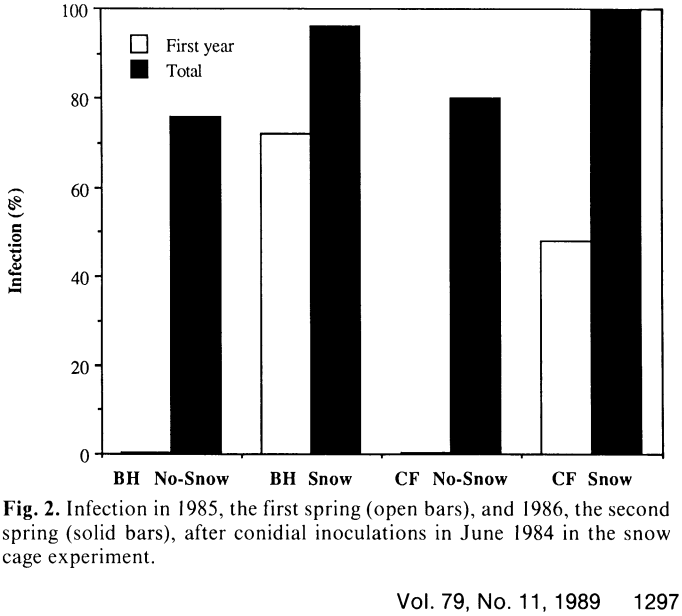

Fig. 2. Infection in 1985, the first spring (open bars), and 1986, the second spring (solid bars), after conidial inoculations in June 1984 in the snow cage experiment.

conducive when a snow cover of 30 cm or more was included in the calculation. None of the winters was conducive based on air temperature alone (Table 4).

Vertical microclimate experiment. The results of the vertical microclimate experiment parallel those of the roped-tree experiment; the numbers of conducive days for the two experiments were the same (Table 4). No symptoms were observed in 1985 after a winter in which 22 conducive days occurred. In 1986, typical symptoms were present on the lowest inoculated branch (conidial inoculum) after 44 conducive days, but not above the snow line (22 conducive days). No additional symptoms were observed on either tree in 1987 (25 conducive days). In 1988, three leaders near the ground on the second tree were infected after 71 conducive days (snow cover); all inoculated leaders higher in the crown were free of symptoms (27 conducive days without snow cover). Thus, the NA strain is capable of causing typical symptoms on large, cone-bearing trees if winter conditions are conducive.

TABLE 4. Presence (+) or absence (−) of disease, and number of conducive days to which trees were exposed, for trees in the roped-tree experiment

	Disease presence			Number of conducive days	
Season	Upright crowns inoculated	Roped crowns inoculated	Roped crowns not inoculated	With snow	Without snow
1984–1985	−	−	NA[a]	22	16
1985–1986	−	+	NA	44	22
1986–1987	−	−	−	25	25
1987–1988	−	+	+	71	27

[a] NA = not included in the experiment.

TABLE 5. Comparison of disease occurrence to number of conducive days: inoculation studies

			Number of conducive days		
			Temperature		
Disease location	Weather data location	Disease?	Only	Plus snow	Serotype[a]
Madison, WI (4)	Madison				
1977–1978		no	13[b]	13	NA
1978–1979		yes	5	52	NA
1979–1980		no	13	13	NA
Syracuse, NY (22)	Syracuse	no[b]	29[c]	29[c]	EU
Boonville, NY (39)	Boonville	yes	2[d]	2[d]	EU
Lake Clear, NY (39)	Gabriels	no	1[d]	1[d]	EU
St.-Ettiene, Qué.	Québec				
1964–1965 field (42)		yes	19	...[e]	EU
1965–1966 field (43)		yes	31	...	EU
1964–1965 greenhouse (42)		no	0	0	EU

[a] NA = North American serotype, EU = European serotype.

[b] Luley and Manion (22) demonstrated penetration by *Gremmeniella abietina*, but occurrence of full symptom development (loose fascicles, basal reddening, and twig death) was not reported.

[c] Data for 1 November 1981 through 30 January 1982 were used to calculate conducive days.

[d] Conducive days were calculated for 5 May 1976 through 31 July 1976.

[e] Specific snow data not available, but estimated to have provided over 43 conducive days.

The conducive day concept as an indicator of the range and symptoms of Scleroderris shoot blight. We compared disease symptoms reported in the literature to numbers of conducive days estimated from published weather records to assess the applicability of the conducive day model outside the Lake States and to the EU serotype of *G. abietina*.

In eight of nine cases of artificial inoculation, and each case of disease in the field for which the year was specified, typical symptoms developed only when the trees or tree parts had been exposed to more than 43 conducive days subsequent to inoculation (artificial inoculations) (Table 5) or during the winter preceding disease expression (field situations) (Table 6). In cases where the year in which symptoms were observed was not specified for a given location, at least half of the winters for which weather data were examined were conducive (Table 6).

In no single season during the 20-yr period examined did more than 43 conducive days occur at Boonville. Thus, the New York epidemic happened despite a complete lack of conducive winters. However, in both the snow cage and the local climate experiments, symptoms were observed in cases in which the first winter was not conducive but the second winter after inoculation was. When the possibility that disease in New York developed during a 2-yr conducive period was examined, a plausible explanation emerged. In Boonville all but two consecutive 2-yr periods between 1964–1965 and 1984–1985 had greater than 43 conducive days (Table 7). On the other hand, in Ashland, WI, seven 2-yr periods between 1964–1965 and 1984–1985 were conducive, but only two consecutive 2-yr conducive periods occurred during the 20 yr in question (Table 7).

The potential for disease in western North America. We examined temperature data for several locations in western North America, where the fungus does not yet occur, to examine the potential for disease should the pathogen move to that region. Winters at Yosemite Park, Twin Lakes, and Crater Lake were conducive during one, two, or all three of the years examined, respectively. Climatic conditions along the coasts of Oregon and Washington during the winters for which temperature data were examined were too warm to support disease development.

TABLE 6. Comparison of disease occurrence to number of conducive days: field studies

		Disease in:		Conducive days		
				Temperature		
Disease location	Weather data location	Upper crowns	Small trees or lower crowns	Only	Plus snow	Serotype[a]
Boonville, NY (39)	Boonville	no	yes	19	71	EU
Lake Clear, NY (39)	Gabriels[b]	no	no	10	20	EU
Ste.-Foy, Qué. (20)	Ste. -Foy	no	yes	42	92	NA
Kazabazua, Qué.[c]	Shawville	no	yes	37	48	EU
St. John's, Nfl. (44)	St. John's	yes	NR[d]	62	62	EU
Western Germany (36)	Munich	yes	NR	45	45	EU
Southern Austria (9)	Salzburg	yes	NR	49	...[e]	EU
Switzerland (3)	Zurich	yes	NR	64	...	EU
Great Britain (31)	Carlisle	yes	NR	15-61[f]	...	EU
Southern Scandinavia (2)	Oslo	yes	NR	30-79[g]	...	EU

[a] NA = North American serotype, EU = European serotype.

[b] Snow data were from Tupper Lake, New York.

[c] Laflamme, *personal communication.*

[d] Not reported.

[e] Snow data not available.

[f] Of six seasons examined, 1973–1978 and 1985–1986, three were conducive.

[g] Of seven seasons examined, 1973–1979 and 1985–1986, all were conducive except the winter 1978–1979.

Conducive winters or consecutive 2-yr periods during the same time did occur at Nanaimo, Bella Coola, and Prince Rupert, British Columbia, and Annette and Juneau, AK (Table 8). These days were conducive because of temperature alone.

DISCUSSION

The data presented above illustrate the importance of extended periods of temperatures near 0 C for development of Scleroderris shoot blight on red pine. In the Lake States, microclimate modification due to snow cover was the dominant factor in determining conditions suitable for disease. By manipulating the duration or location of snow cover to which red pine seedlings or branches were exposed during the winter, we were able to obtain symptom expression at latitudes south of the normal range of the disease (local climate and snow cage experiments) and at heights in the crown at which disease did not otherwise occur (roped-tree experiment), or to prevent disease symptom development (snow cage experiment). In none of our experiments could ambient air temperature above the snow alone explain symptom expression.

In all cases except one in our inoculation experiments, disease developed if 44 conducive days (roped-tree and vertical microclimate experiments) or more occurred in a winter. The trees in the exception (Hancock during 1985–1986) were apparently exposed to the fungicides Kocide and Duter, applied to nearby plots between 17 July and 28 August 1985, which may explain the lack of disease on these trees in 1986. Conversely, in all cases except three, disease failed to develop if 43 days (Hancock 1984–1985) or fewer were conducive. At the Blackhawk no-snow plot during 1985–1986, the number of conducive days calculated was based on temperatures sensed at 20 cm, well above the seedlings that were frozen into a layer of ice, and therefore does not accurately describe the conditions to which the seedlings were exposed. The seedlings at the Antigo site during 1984–1985 had an extremely high level of mortality within the first months after planting. Those seedlings surviving through the winter appeared to be very stressed. Whether or not the condition of the seedlings is related to disease development after only 29 conducive days is not known. The symptoms after only 25 conducive days at the Copper Falls no-snow plot in 1986 may be indicative of a low probability of symptom development after fewer than 44 conducive days. On the basis of all of these results, taken together, we define a winter conducive to Scleroderris shoot blight as one in which 44 or more conducive days occur between 1 October and 28 February. This definition would have predicted the outcome of 30 of the 32 year-location combinations in our inoculation experiments for which we feel that the data are reliable, within and without the natural range of the disease, under natural or modified conditions.

Our findings may explain many of the characteristics unique to the disease caused by the NA serotype of *G. abietina*. Because branches closest to the ground are covered with snow for longer periods in a given season, and are therefore exposed to more conducive days, one would expect to find symptoms here more often than on higher branches. Although areas both north and south of 45° N latitude often experience extremely cold winter temperatures, the northern areas usually have significantly more snow and therefore have a much higher likelihood of exposure to more than 43 conducive days in any given season. It is here, then, that the disease would be able to maintain itself over time. Since conducive days as defined here do not begin accumulating until early fall, both in the Lake States and other parts of North America, if more than 43 such days are needed for tissue colonization and symptom expression, the existence of a long latent period between initial penetration and symptom expression the following spring is quite understandable.

The occurrence of symptoms in 1986 on 2-yr-old but not 1-yr-old seedlings in the snow cage experiment, 2 yr subsequent to inoculation, suggests that the usual 9-mo latent period associated with the disease cycle of *G. abietina* may occasionally be longer. It appears that the lack of symptom development on these seedlings in 1985 was not due to poor inoculation or penetration, but rather due to an absence of conditions favoring further host tissue colonization during the winter of 1984–1985. The fungus was apparently able to remain quiescent in the host tissue for almost 2 yr, causing symptom expression only after being exposed to favorable conditions during the second year after inoculation. Our conducive winter concept, then, might be extended to include a consecutive 2-yr period in which 44 or more conducive days accumulate over two consecutive winters. (We do not have direct evidence on which to choose a number of conducive days necessary for a conducive 2-yr period, and have assumed that about 44 is sufficient.) Such an interpretation might also explain the occurrence of symptoms on 2-yr-old but not 1-yr-old needles observed by Laflamme in 1984 (20).

Conducive 2-yr periods based on ambient air temperature (assuming no snow cover) might explain the occurrence of disease symptoms in the upper crowns of mature trees in New York State in the early 1970s. Of the 20 2-yr periods for which temperature data were examined, only two were not conducive (Table 7). These two periods (1977–1978 + 1978–1979 and 1978–1979 + 1979–1980) coincide with the decline in high levels of disease and the end of widespread mortality in New York noted by B. Schneider (*personal communication*) and others (17,26).

From Tables 1, 2, 3, and 7, it is clear that conducive winters do occur outside the natural range of the disease. If the disease is restricted by the necessity of conducive winters for symptom development, why is it normally absent from Madison and Janesville (Tables 1 and 2), or from upper crowns of trees near Ashland (Table 7)? Once symptoms appear, inoculum, as conidia, may be formed from pycnidia during the summer or fall following

TABLE 7. Number of conducive days at Boonville, NY, and Ashland, WI, for one or two winters ending in year shown, as determined by temperature alone

	One winter		Two winters	
Year	Boonville	Ashland	Boonville	Ashland
1964–1965	15	6		
1965–1966	41	30	56	36
1966–1967	22	16	63	46
1967–1968	26	22	48	38
1968–1969	33	23	59	45
1969–1970	17	15	50	38
1970–1971	27	14	44	29
1971–1972	27	24	54	38
1972–1973	31	30	58	54
1973–1974	29	25	60	55
1974–1975	39	22	68	47
1975–1976	19	17	58	39
1976–1977	29	17	48	34
1977–1978	18	17	47	34
1978–1979	19	9	37	26
1979–1980	21	24	40	33
1980–1981	25	17	46	41
1981–1982	22	20	47	37
1982–1983	28	39	50	59
1983–1984	40	28	68	67
1984–1985	29	14	69	42

TABLE 8. Number of conducive days, as determined by temperature alone, at select locations along the western coast of North America

Location	1982–1983	1983–1984	1984–1985
Brookings, OR	0	1	0
Newport, OR	0	1	4
Hoquiam, WA	4	9	16
Bellingham, WA	11	15	26
Nanaimo, B.C.	12	17	47
Bella Coola, B.C.	73	47	64
Prince Rupert, B.C.	21	27	43
Annette, AL	37	42	43
Juneau, AL	84	67	76

symptom expression, or in the spring, 1 yr after symptom expression. Ascospores may follow conidia by 1 yr. Thus, a single infected twig may produce inoculum during three consecutive summers. If a conducive period does not follow any of the three critical summers, the disease cycle will be broken, and any new infection must result from inoculum from some other source. Thus, the southern boundary of the disease in small trees will probably be strongly related to the frequency of occurrence of three consecutive nonconducive periods intervening between conducive winters and the rate of southerly spore movement. The likelihood of disease in the crowns of large trees will be very low if inoculum is prevented from building up in these crowns by frequent 3-yr interruptions between conducive 2-yr periods. After interruptions of the disease cycle, only the relatively small amount of inoculum carried aloft from infections of small trees or lower branches of larger trees will be available to cause infections in upper crowns. Luley and Manion (22) have demonstrated that deposition of conidia of *G. abietina* diminishes quite strongly with distance. A low level of disease occurring infrequently could easily be overlooked or confused with symptoms caused by *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton in Sutton (= *Diplodia pinea* (Desm.) Kickx) or *Sirococcus conigenus* (D.C.) Cannon and Minter (= *Sirococcus strobilinus* Preuss). We did in fact isolate *G. abietina* from branches with typical symptoms approximately 10 m above ground in northern Wisconsin in July 1983, a finding consistent with our model's predictions. The 2-yr period 1981–1983 was conducive in northern Wisconsin (Table 7).

G. abietina is capable of growth at subfreezing temperatures (13,16,53), and grew more rapidly on media supercooled to −6 C than on media at this temperature containing ice. A similar growth response to low temperatures was shown by Gill and Lowry (18), who demonstrated growth on nonfrozen media at −5 C of two species of *Cladosporium* and one of *Penicillium* causing black spot spoilage of meat. Growth was also reported on frozen media, but only at extremely low rates.

The ability of *G. abietina* to grow in vitro at temperatures slightly below 0 C is consistent with its growth within host tissue in midwinter. Siepman (35) observed hyphae in living, chlorophyll-containing parenchyma cells of bud scales, and in cells of the epidermis, hypodermis, and cortex parenchyma. Patton et al (29, *unpublished*) were unable to confirm intracellular colonization of epidermal or hypodermal cells. Because the fungus can grow more rapidly in supercooled fluid (intracellular, or xylem ray tissues [6,48]) than in the presence of ice (extracellularly), there may be a preference for intercellular growth during winter, although either mode of proliferation at low temperatures appears possible.

Our review of other reported occurrences of disease caused by both the NA and the EU serotypes suggests that our conducive day model may be a useful indicator of the potential for disease development involving other serotypes, hosts, and locations. For 28 of 30 location-year combinations that we examined, disease occurred only after the accumulation of more than 43 conducive days in a single winter. In one instance (Boonville 1976, see Table 5) our model failed to explain symptom expression, and in one instance (southern Scandinavia in 1978) symptoms were reported to be absent after what we calculated to have been a conducive winter. The failure of the model in the latter instance may be a reflection of the difficulty we encountered in determining the exact geographical location of the disease reported, and in obtaining weather data recorded near enough to the infection site to be an accurate reflection of the local microclimate. In each case for which disease occurred in upper crowns, the accumulation of conducive days was due solely to temperature. This is consistent with our conclusion that the effect of snow cover on disease development in seedlings and on lower branches is one of temperature modification.

If the presence of Scleroderris shoot blight in upper crowns represents a response of the fungus to environmental conditions, regardless of the serotype, then the threat of not only the EU, but also the NA, serotype to coniferous resources throughout northern North America may be real. Development of disease in areas where it does not currently occur would require the simultaneous presence of compatible pathogen and hosts and the occurrence of conducive conditions on a regular basis.

Damage caused by both serotypes of *G. abietina* has been severe on *Pinus* spp. In host range studies in New York, presumably with the EU serotype, ponderosa pine (*P. ponderosa* Laws.), lodgepole pine (*P. contorta* Dougl.), Jeffrey pine (*P. jeffreyi* Grev and Balf.), sugar pine (*P. lambertiana* Dougl.), and western white pine (*P. monticola* Dougl.) were shown to have potential for high susceptibility to the pathogen (41). In a separate study in Wisconsin, where only the NA serotype has been found, no disease resistance was found in more than 300 seed sources of ponderosa pine (Skilling, *personal communication*). Ponderosa and lodgepole pine frequently occur at higher elevations in the western United States and Canada where extended snow cover during the winter will make winters conducive, as was observed at Yosemite, Twin Lakes, and Crater Lake. If *G. abietina* were to be introduced into such areas (the NA serotype has been detected as far west as eastern British Columbia), the environment would favor Scleroderris shoot blight and may lead to the development of serious disease epidemics in young pine stands.

Douglas-fir (*Pseudotsuga menzesii* (Mirb.) Franco), sitka spruce (*Picea sitchensis* (Bong.) Carr.), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) are important forest components along the western coasts of Oregon, Washington, and British Columbia, and the Alaskan panhandle. Sitka spruce and *Pseudotsuga taxifolia* (Poir.) Britt. are reported hosts of *G. abietina* in nature (10,33), and all three western species have shown susceptibility, presumably to the EU serotype, in the New York host range studies (41). The frequency of susceptibility within these species and the extent to which they may be damaged if they are susceptible is not clear at this time. The relationship of the pathogen taxonomy to host range is currently being investigated (30). Nonetheless, our limited examination of temperatures at nine locations along the northwest coast of North America (Table 8) indicates that air temperature alone is sufficient to provide conducive winters at some locations. If the locations and years considered are representative of conditions in this area, and if *G. abietina* is capable of damaging these species, mature stands of Douglas-fir, Sitka spruce, and western hemlock may be in danger of succumbing to a disease outbreak such as that which occurred in New York, should *G. abietina* become established. Thus, measures to prevent introduction of either serotype of *G. abietina* to any area where it is not currently present may be appropriate.

The issue of whether the NA and EU serotypes differ with regard to pathogenicity and host range, or whether the differences in the symptoms observed in the field are the result of the environment where the two serotypes occur is not fully addressed by our studies. We have found nothing in the literature to suggest that the response to the environment of the EU serotype is greatly different from that which we found for the NA serotype. However, resolution of differences in pathogenicity, host range, and response to environment will require testing the two serotypes simultaneously at a number of sites.

LITERATURE CITED

1. Alberga, A. H., Marosy, M., Tanner, C. B., and Upper, C. D. 1987. A disk camera system for automatic recording of visual data: Snow depth in field plots. Phytopathology 77:927-929.
2. Barklund, P., and Rowe, J. 1981. *Gremmeniella abietina* (*Scleroderris lagerbergii*), a primary parasite in Norway spruce die-back. Eur. J. For. Pathol. 11:97-108.
3. Bazzigher, G. 1978. *Ascocalyx*-Triebsterben an Koniferen in der Schweiz. Pages 5-7 in: Proc. of IUFRO Working Party S 2.06.02: Canker diseases (Scleroderris), Munich, August 1978.
4. Blenis, P. V. 1982. Influence of environmental factors on the disease cycle of Scleroderris canker. Ph.D. thesis. University of Wisconsin, Madison, Wisconsin. 102 pp.
5. Blenis, P. V., Patton, R. F., and Spear, R. N. 1984. Effect of temperature on the ability of *Gremmeniella abietina* to survive and

to colonize host tissue. Eur. J. For. Pathol. 14:153-164.

6. Burke, M. J., Gusta, L. V., Quamme, H. A., Weiser, C. J., and Li, P. H. 1976. Freezing and injury in plants. Annu. Rev. Plant Physiol. 27:507-528.
7. Canadian Forestry Service. 1978. Dangerous dieback disease threatens Canadian conifer plantations. Pages 1-3 in: Forest Insect and Disease Conditions in Ontario, Spring 1978. For. Serv. For. Res. Newsl. GLFRC Spring 1978.
8. Canadian Forestry Service. Forest Insect and Disease Survey. 1981. Scleroderris canker. Pages 13-14 in: Forest Insect and Disease Conditions in Canada, 1980.
9. Donaubauer, E. 1974. Über das Kieferntriebsterben in Österreich (*Scleroderris lagerbergii* Gremmen und *Cenangium ferrunginosum* Fr.). (On the dieback disease of pines in Austria.) Pages 67-98 in: 100 Jahre Forstliche Bundesversuchsanstalt Wien.
10. Dorworth, C. E. 1971. Diseases of conifers incited by *Scleroderris lagerbergii* Gremmen: A review and analysis. Publ. Dep. Fish. For., Can. For. Serv. No. 1289. 42 pp.
11. Dorworth, C. E. 1972. Epidemiology of *Scleroderris lagerbergii* in central Ontario. Can. J. Bot. 50:751-765.
12. Dorworth, C. E. 1973. Epiphytology of *Scleroderris lagerbergii* in a kettle frost pocket. Eur. J. For. Pathol. 3:232-242.
13. Dorworth, C. E., and Krywienczyk, J. 1975. Comparisons among isolates of *Gremmeniella abietina* by means of growth rate, conidia measurement, and immunogenic reaction. Can. J. Bot. 53:2506-2525.
14. Dorworth, C. E., Krywienczyk, J., and Skilling, D. D. 1977. New York isolates of *Gremmeniella abietina* (*Scleroderris lagerbergii*) identical in immunogenic reaction to European isolates. Plant Dis. Rep. 61:887-890.
15. Environment Canada. Atmospheric Environment Service. 1964–1985. Monthly Record–Meteorological Observations in Canada. Vols. 49-70.
16. Ettlinger, L. 1945. Über die Gattung *Crumenula* sensu Rehm mit besonderer Berüscksichtigung des Crumenula-Triebsterbens der *Pinus*-Arten. Beitr. Kryptogamenflora Schweiz 10:1-73.
17. French, W. J., and Silverborg, S. B. 1967. Scleroderris canker of red pine in New York State plantations. Plant Dis. Rep. 51:109.
18. Gill, C. O., and Lowry, P. D. 1982. Growth at subzero temperatures of black spot fungi from meat. J. Appl. Bacteriol. 52:245-250.
19. Heiser, M. R., ed. 1978. Snow and Frost in Wisconsin, 1961-1977. Wisconsin Agriculture Reporting Service, USDA–Economics, Statistics, and Cooperatives Service, Madison, WI. 38 pp.
20. Laflamme, G. 1986. Symptôme inusité du chancre scléroderrien sur le pin rouge au Québec. (An unusual symptom of Scleroderris canker on red pine in Québec.) Can. J. Plant Pathol. 8:1-5.
21. Lang, K. J., and Schütt, P. 1974. Anatomische Untersuchungen zur Infektions-biologie von *Scleroderris lagerbergii* Gr. [*Brunchorstia pinea* (Karst.) von Hohn]. Eur. J. For. Pathol. 4:166-174.
22. Luley, C. J., and Manion, P. D. 1984. Inoculum potential of *Gremmeniella abietina* in New York. Pages 82-95 in: Scleroderris Canker of Conifers. Proc. Int. Symp. P. D. Manion, ed. Martinus Nijhoff/Dr. W. Junk Publishers, Boston.
23. Marosy, M., Patton, R. F., and Upper, C. D. 1988. Spore production and artificial inoculation techniques for *Gremmeniella abietina*. Phytopathology 79:1290-1293.
24. Martin, J. C. 1964. The red pine mortality problem in the Kirkwood Forest Management Unit, Sault Ste. Marie District, Ontario. Can. Dept. For., For. Entomol. Pathol. Branch. Inf. Rpt. 23 pp.
25. Meteorologische Abhandlungen. Berliner Wetterkarte. Neue Folge. Serie B Grundlagenmaterial. 1973-1986. Verlag von Dietrich Reimer, Berlin. Vols. 35-48.
26. O'Brien, J. T. 1970. Evaluation of Scleroderris canker on National Forest land in the Lake States. 1969 Interim Report. USDA–For. Serv. Rep. No. S-70-1. 7 pp.
27. O'Brien, J. T. 1984. Historical and current Scleroderris situations in the United States. Pages 26-31 in: Scleroderris Canker of Conifers. Proc. Inter. Symp. P. D. Manion, ed. Martinus Nijhoff/Dr. W. Junk Publishers, Boston.
28. Ohman, J. H. 1966. *Scleroderris lagerbergii* Gremmen. The cause of dieback and mortality of red and jack pines in upper Michigan plantations. Plant Dis. Rep. 50:402-405.
29. Patton, R. F., Spear, R. N., and Blenis, P. V. 1984. The mode of infection and early stages of colonization of pines by *Gremmeniella abietina*. Eur. J. For. Pathol. 14:193-202.
30. Petrini, O., Petrini, L., Laflamme, G., and Ouellette, G. B. 1989. Taxonomic position of *Gremmeniella abietina* and related species: A reappraisal. Can. J. Bot. (In press)
31. Read, D. J. 1966. Dieback disease of pines with special reference to Corsican pine *Pinus nigra* var. *calabrica* Schn. I. The nature of the disease symptoms and their development in relation to the crown and to aspect. Forestry 39:151-161.
32. Roll-Hansen, F. 1964. *Scleroderris lagerbergii* Gremmen (*Crumenula abietina* Lagerb.) and girdling of *Pinus sylvestris* L. Medd. Norske Skogforsoksves. 19:153-175.
33. Roll-Hansen, F., and Roll-Hansen, H. 1973. *Scleroderris lagerbergii* in Norway. Hosts, distribution, perfect and imperfect state, and mode of attack. Medd. Norske Skogforsoksves. 30:441-459.
34. Setliff, E. C., Sullivan, J. A., and Thompson, J. H. 1975. *Scleroderris lagerbergii* in large red and Scots pine trees in New York. Plant Dis. Rep. 59:380-381.
35. Siepmann, R. 1976. Ein Beitrag zur Infektionsbiologie des durch *Scleroderris lagerbergii* verursachten Schwarzkieferntriebsterbens. Eur. J. For. Pathol. 6:103-109.
36. Siepman, R., Lang, K. J., and Schönhar, S. 1975. Verbreitung des durch *Scleroderris lagerbergii* Gr. verursachten Schwarzkieferntriebsterbens in der Bundesrepublik Deutschland. Eur. J. For. Pathol. 5:185-189.
37. Skilling, D. D. 1969. Spore dispersal by *Scleroderris lagerbergii* under nursery and plantation conditions. Plant Dis. Rep. 53:291-295.
38. Skilling, D. D. 1972. Epidemiology of *Scleroderris lagerbergii*. Eur. J. For. Pathol. 2:16-21.
39. Skilling, D. D. 1977. The development of a more virulent strain of *Scleroderris lagerbergii* in New York State. Eur. J. For. Pathol. 7:297-302.
40. Skilling, D. D., and Waddell, C. D. 1974. Fungicides for control of Scleroderris canker. Plant Dis. Rep. 58:1097-1100.
41. Skilling, D. D., Schneider, B., and Fasking, D. 1986. Biology and control of Scleroderris canker in North America. USDA–For. Serv. Res. Pap. NC-275. 19 pp.
42. Smerlis, E. 1967. Occurrence and pathogenicity of *Scleroderris lagerbergii* in Québec. Plant Dis. Rep. 51:584-585.
43. Smerlis, E. 1968. Additional information on the pathogenicity of *Scleroderris lagerbergii*. Plant Dis. Rep. 52:738-739.
44. Sterner, T. E. 1984. Scleroderris canker, *Gremmeniella abietina* (Lagerb.) Morelet, in Canada–an overview. Pages 8-10 in: Scleroderris Canker of Conifers. Proc. Int. Symp. P. D. Manion, ed. Martinus Nijhoff/Dr. W. Junk Publishers, Boston.
45. Strommen, N. D. 1969. Michigan snow depths. Michigan Dept. of Agriculture, Michigan Weather Service. 17 pp.
46. U.S. National Climatic Data Center. 1964-1987. Climatological Data. Vols. 89-92.
47. Vegis, A. 1973. Dependence of growth processes on temperature. Pages 145-170 in: Temperature and Life. H. Precht, J. Christophersen, H. Hensel, and W. Larcher, eds. Springer-Verlag, New York.
48. Yokota, S. 1973. Report. Pages 16-18 in: Proc. IUFRO Working Party S 2.06.02–Canker Diseases (Scleroderris). Minneapolis, Minnesota, September 1973.
49. Yokota, S. 1975. Scleroderris canker of Todo-fir in Hokkaido, Northern Japan. III. Dormant infection of the causal fungus. Eur. J. For. Pathol. 5:7-12.
50. Yokota, S. 1975. Scleroderris canker of Todo-fir in Hokkaido, Northern Japan. IV. An analysis of climatic data associated with the outbreak. Eur. J. For. Pathol. 5:13-21.
51. Yokota, S., Uozumi, T., and Matsuzaki, S. 1974. Scleroderris canker of Todo-fir in Hokkaido, Northern Japan. I. Present status of damage, and features of infected plantations. Eur. J. For. Pathol. 4:65-74.
52. Yokota, S., Uozumi, T., and Matsuzaki, S. 1974. Scleroderris canker of Todo-fir in Hokkaido, Northern Japan. II. Physiological and pathological characteristics of the causal fungus. Eur. J. For. Pathol. 4:155-166.