Sporulation of *Lirula macrospora* and Symptom Development on Sitka Spruce in Southeast Alaska

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ABSTRACT

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The timing of ascospore release of *Lirula macrospora* and development of symptoms in needles of different ages on Sitka spruce (*Picea sitchensis*) were studied for 2 yr at three sites in southeast Alaska. Ascospore release began with spruce budbreak and diminished when shoots were fully elongated. Sporulation peaked during the same week in 19 of 27 spore traps from 4 to 11 June 1987 and in eight of nine traps from 13 to 20 June 1988. The timing of peak sporulation was independent of site. Total spore deposit between April and August averaged 23.6 and 7.1 spores per square millimeter in 1987 and 1988, respectively. The pattern of symptoms and development of hysterothecia on infected needles of different ages was consistent throughout coastal Alaska. First-year needles presumably became infected during the period of sporulation but remained green through their first growing season. Infected second-year needles became reddish brown and developed immature hysterothecia, third-year needles became tan and harbored mature hysterothecia that sporulated in spring, and fourth-year needles were tan, had empty hysterothecia, and began to cast.

Spruce needle blight, caused by *Lirula macrospora* (Hartig) Darker, is the most damaging needle disease of spruce in western North America (2). In Alaska, Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is attacked by *L. macrospora* on the mainland and islands of the southeast, through coastal areas of the Gulf of Alaska, to the western edge of its natural range on Afognak and Kodiak islands (5,12). A high proportion of spruce in young forest stands in cutover areas is infected (11). The lower crowns of old-growth trees are also affected, and urban trees are sometimes severely diseased (4,13). *L. macrospora* also occurs on white spruce (*P. glauca* (Moench) Voss) in interior Alaska but does not appear to be causing much damage.

In Europe, Hartig (3) reported three different patterns of symptom development on spruce and hysterothecia of *L. macrospora*: The fungus sporulated from 3- and 4-yr-old needles attached to the tree and from 2-yr-old needles that had been cast. Also, he suggested that the timing of symptom and fungus development was dependent on particular local climatic factors. Walla (14) described the life cycle and timing of sporulation of *L. macrospora* on windbreak and landscape white spruce and Colorado blue spruce (*P. pungens* Engelm.) in North Dakota, where the fungus sporulated from attached fourth-year needles.

The disease had not been investigated in Alaska. The objectives of this study were to determine the patterns of symptom development on Sitka spruce and the timing of sporulation for *L. macrospora* in coastal Alaska.

MATERIALS AND METHODS

Study sites. Studies were conducted in 1987 and 1988 at the following three field sites near Juneau, Alaska: 1) False Outer Point (north Douglas Island), 2) Sandy Beach (south Douglas Island), and 3) 35 km north of Juneau on the mainland. Young Sitka spruce dominated the sites, but western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) was present in small numbers at each site. Three spruce trees (10–20 yr old) with similar levels of infection were selected for study at each site.

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Spruce phenology and weather. The phenological stages of spruce buds and shoots were recorded to determine if shoot or needle development coincides with sporulation. Bud width, bud length, shoot length, and needle length were measured to the nearest millimeter each week of sampling periods on the primary branch that emerged from each spore trap. All needle lengths were measured on needles that occur in the middle of the current year's shoot.

The relationship between date and bud length (before bud burst) and shoot length (after bud burst) as a percentage of the total shoot length at the season's end was graphed. Precipitation information was acquired from the National Weather Service's records (7,8) for the Juneau airport station.

Symptoms and signs. Symptoms of the disease on newly emerging, second-year, and older needles, as well as the appearance of hysterothecia, were noted each week on these plot trees during sampling periods and once a month during fall and winter. Hysterothecia were examined for ascospore development, using either a dissecting microscope or a compound microscope after hand sectioning. Terms used to describe needle age follow the suggestion by Peterson (9), i.e., needles produced in spring are "first-year needles," becoming "second-year needles" the following spring and "third-year needles" the spring after that, and so on.

Ascospore release. Ascospores were trapped at the three sites between late April and early August of both years to determine timing of sporulation. Spore traps were necessary to protect microscope slides and spore deposits from the heavy precipitation common to southeast Alaska. The traps were placed around branches bearing symptomatic needles with hysterothecia. All spore traps were placed at a height of about 1.4 m above the ground. In 1987, three traps were placed around each tree at 120-degree intervals, but only one trap per tree was used in 1988.

Coffee cans approximately 18 cm long and 16 cm in diameter were used as spore traps. After the tops and bottoms were removed, cans were placed horizontally over infected branches to cover infected needles. To collect disseminated ascospores, two microscope slides were placed in the bottom of each can. The slides did not need to be treated with a sticky substance because ascospores of *L. macrospora* have a gelatinous sheath that allows them to adhere to surfaces (14).

Slides were replaced with new slides each week and taken to the laboratory to determine spore numbers. A compound microscope with magnification of either ×100 or ×400 was used for counting spores. The ×100 magnification was used when the concentration of spores on slides was sparse ($<1/mm^2$). All spores resembling Funk's (2) description of *L. macrospora* that appeared in the field of view during four passes across the width of the slide were tallied. The area observed on microscope slides at each magnification was determined so that ascospore counts could be converted to number of spores per square millimeter.

The relationship of date to the number of ascospores counted was graphed for 1987 and 1988. Results of the numbers of ascospores, length of buds and shoots, and amount of precipitation were reported on the last day of the weekly interval.

RESULTS

Spruce phenology and weather. Overwintering spruce buds were about 4–5 mm long and began to swell in length and width before breaking. Active bud-

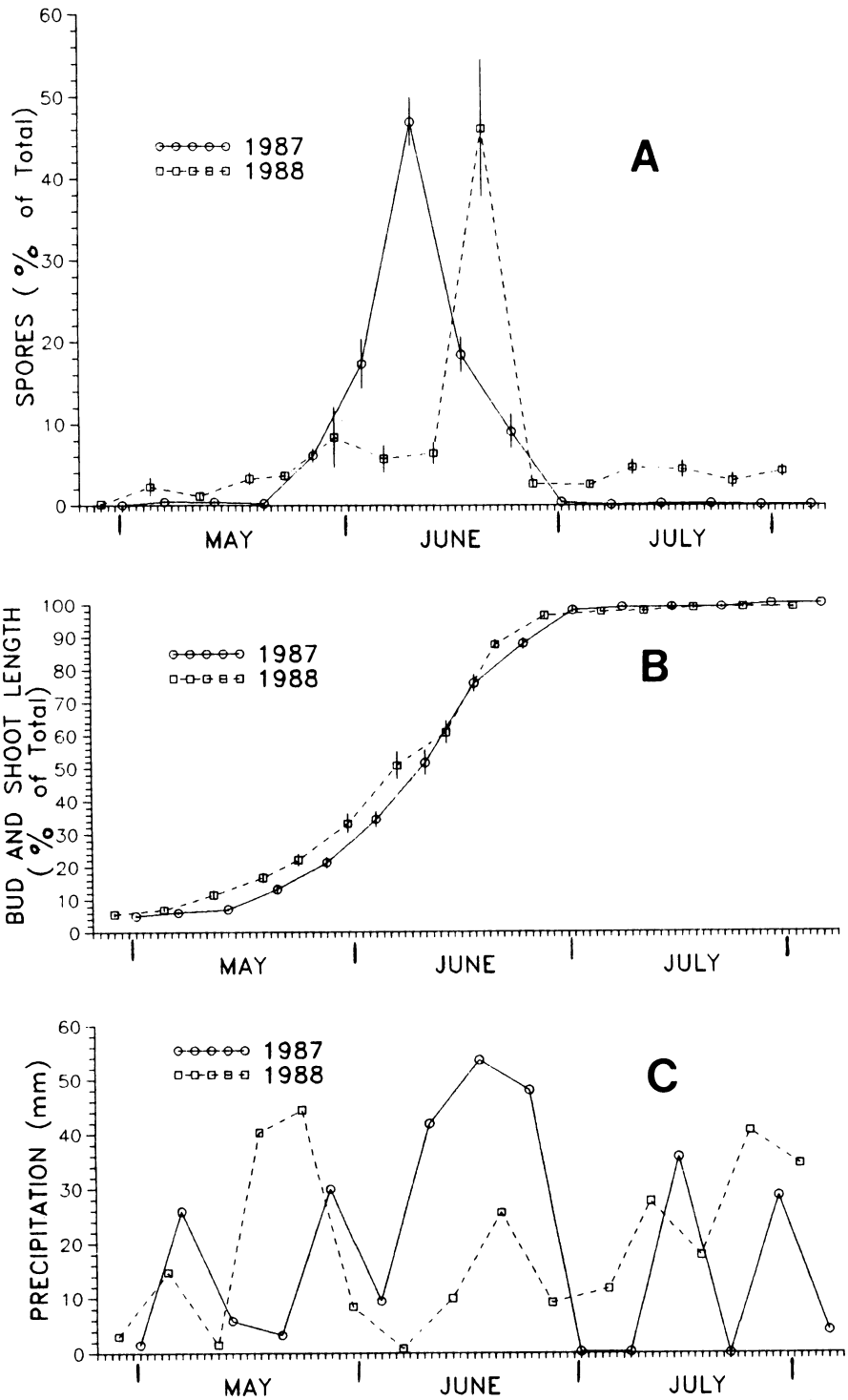

Fig. 1. (A) Mean percentage of ascospores (from seasonal total) of *Lirula macrospora* from 27 traps in 1987 and nine traps in 1988 near Juneau, Alaska, **(B)** mean percentage bud and shoot length (from the final, maximum shoot length) for Sitka spruce in the same traps for 1987 and 1988, and **(C)** total precipitation of the week (7,8). Error bars are mean ± one standard error.

break (splitting of bud scales) at all sites began during the week ending on 21 May in 1987 and on 18 May in 1988, and all buds had broken the following week for both years. Bud scales generally remained on the end of new shoots for 1 wk after budbreak. Thus, new first-year needles were not fully exposed to ascospores until late May of both years. Needle length was not correlated with ascospore release because needles reached full length ($\bar{x} = 17.8 \pm 3.1$ mm) within 2 wk after budbreak.

Spruce needles were bright green and flexible after emerging from buds. They became darker and more rigid during shoot elongation and were approaching the characteristic dark green of mature needles by the time shoot elongation ceased. Final length of spruce shoots (47–215 mm) was achieved by the last week in June for both sampling years (Fig. 1B). Little variation occurred in the timing of budbreak and shoot elongation of spruce among branches on the same tree, different trees, or different sites.

Precipitation occurred during most sampling weeks of both years (Fig. 1C). Notable periods of little or no precipitation were in late April and early and late July 1987 and late April, middle May, and early July 1988.

Symptoms and signs. First-year needles did not show any symptoms of disease until the following spring (Fig. 2). These second-year needles became reddish brown and most had hysterothecia initiating as colorless, raised structures along the needles' undersides in May and June. The immature hysterothecia became black during July. Some symptomatic needles did not develop hysterothecia. By winter, second-year needles appeared tan and hysterothecia contained immature asci. Ascospores appeared to be mature in March, but hysterothecia of *L. macrospora* were closed with hymenia unexposed until about the time of budbreak in May of each year. Some hysterothecia were apparently open before budbreak because a few ascospores were deposited on some traps in the initial sampling in late April. Hysterothecia contained very few asci with ascospores after sporulation had diminished by early August. Some third-year needles began to cast in fall, although many needles were retained for several additional years. Dead fourth- and fifth-year needles bearing spent hysterothecia were observed on many trees.

Ascospore release. In spore deposits observed microscopically, ascospores were tightly coiled or uncoiled in a sigmoidal shape and sometimes had accompanying germination structures as illustrated by Darker (1). Filiform spores of unidentified fungi were present, but the gelatinous sheath, rounded apices, and dimensions ($\bar{x} = 67 \times 5$ μm with sheath and 62×1.5 μm without sheath) of *L. macrospora* appeared distinctive. *Lophodermium piceae* (Fckl.) Hoehn., which has ascospores similar to those of *L. macrospora* and is common in mature spruce in coastal Alaska, was not observed in the study sites. The conidial stage of *L. macrospora*, not known to be infectious for this group of fungi (10), was not observed in spore traps.

The pattern of sporulation of *L. macrospora* was generally similar in 1987 and 1988. Ascospore release was sparse until late May, peaked in June, and dropped off sharply by early July (Fig. 1A). Peak sporulation was later in 1988 than in 1987; 70% of traps in 1987 and 89% of traps in 1988 had peak spore counts during 1 wk, 4–11 June in 1987 and 13–20 June in 1988. During those single weeks of peak sporulation, means of 47 and 46% of all the ascospores were deposited for the seasons of 1987 and 1988, respectively (Fig. 1A).

The limited variation in timing of peak sporulation was not associated by tree or site. The total number of ascospores from traps was greater during the season of 1987 ($\bar{x} = 23.6 \pm 19.6$ ascospores per square millimeter, range = 5.1–78.3) than during the season of 1988 ($\bar{x} = 7.1 \pm 4.3$ ascospores per square millimeter, range = 2.7–17.4). Maximum ascospore density from a single trap during a 1-wk interval was 34 and 15 spores per square millimeter in 1987 and 1988, respectively.

Ascospore release was initiated about the time that spruce buds broke (Fig. 1A,B). Peak sporulation occurred when spruce shoots were about 50% (in 1987) and 85% (in 1988) fully elongated. Once shoots reached their maximum length and needles began to appear dark green and feel stiff, sporulation had nearly ceased (Fig. 1A,B).

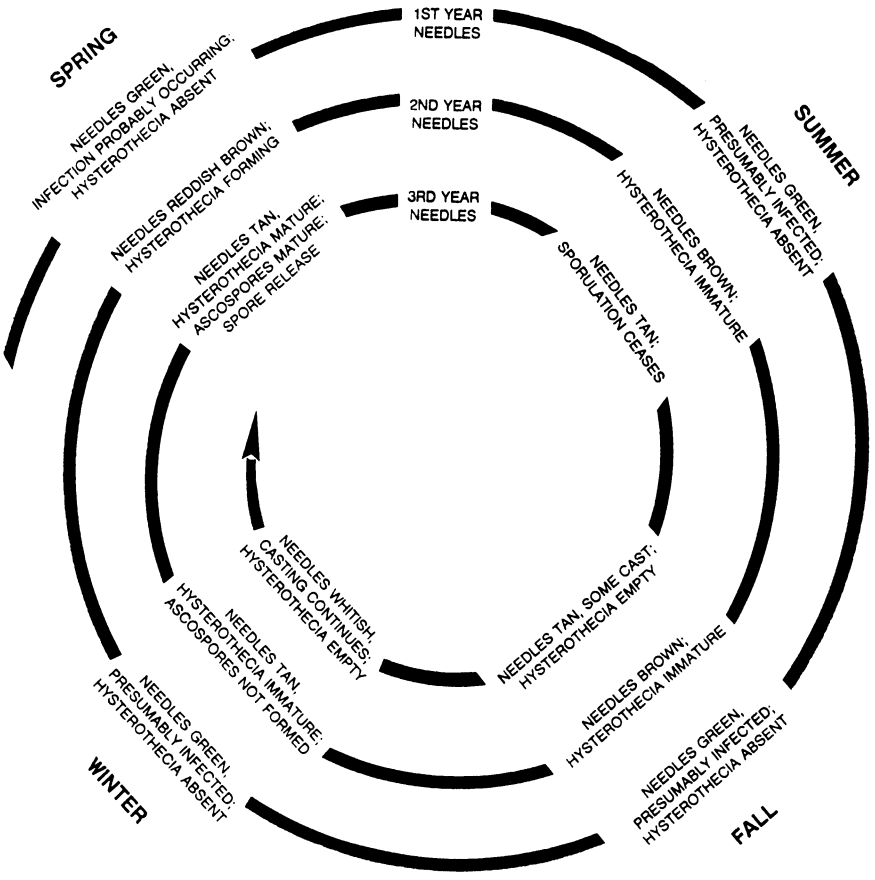

Fig. 2. Development of symptoms of spruce needle blight on Sitka spruce and maturation of hysterothecia of *Lirula macrospora* on needles of different ages in southeast Alaska.

DISCUSSION

The association of ascospore release of *L. macrospora* and spruce shoot elongation in spring demonstrated in this study is similar to Walla's (14) results for the same fungus in North Dakota. In North Dakota, however, continued sporulation by the fungus was sporadic and also was common after shoots were fully expanded. Scharpf (10) and Walla (14) report that precipitation is needed to initiate sporulation for this group of fungi.

In Alaska, the later peak in sporulation of *L. macrospora* in 1988 compared with 1987 may be attributed to weather factors. Sporulation was initiated about the same time both years. After initial spore release in 1988, however, the concentration of trapped ascospores dropped off for 2 wk and then developed maximum concentration the following week (Fig 1A). The period of diminished sporulation coincided with the least precipi-

tation of any sampling interval for that year. Sporulation resumed with the return to the typical cool, rainy weather. Because cool, wet weather prevails in coastal Alaska during spring, the fungus may experience only intermittent periods of interrupted sporulation.

The same pattern of symptoms on needles of different age classes (Fig. 2) was apparent whenever and wherever I have encountered the disease in coastal Alaska. The same branch often had all stages of symptom and hysterothecium development, but they were restricted to the different age classes of needles. The incidence of the disease on needles of different ages varied considerably, however. In 1988, for example, symptomatic reddish brown second-year needles, presumably infected by ascospore release in 1987, were extremely prevalent throughout southeast Alaska (13), but tan third-year needles were relatively uncommon.

Observations on the development of *L. macrospora* fruiting bodies and symptoms on spruce in coastal Alaska differ from those in the North Dakota study. Walla's (14) description of the disease on white and blue spruce in North Dakota was similar to the type 2 development outlined by Hartig (3). First-year needles were probably infected in spring but did not show symptoms until late summer, when they were second-year needles. Third-year needles developed immature fruiting bodies, but mature ascospores were produced and released in June to August on fourth-year needles. In coastal Alaska, *L. macrospora* has the shorter, Hartig type 1 disease cycle, where mature hysterothecia develop on attached third-year needles. Thus, the fungus requires one more year to complete its cycle in North Dakota. Whether the faster rate of symptom and fungal development in Alaska can be attributed to different host species, to fungal genetics, or to environmental factors is unknown.

L. macrospora appears to be well adapted to sporulating at the same time that succulent needles are emerging and elongating from buds. If these new needles are the only or the primary susceptible spruce tissues and if symptoms are latent for 1 yr, then ascospore release that coincides with the emergence of these needles in spring is an important aspect of successful infection by the fungus. Hartig (3) assumed second-year needles were the first to become infected because they were the first to develop symptoms. Walla (14), for *L. macrospora*, and McCain and Scharpf (6), for *L. abietis-concolor* (Mayor ex Dearn.) Darker, however, have shown that infection probably occurs on first-year needles. Second-year needles protected during sporulation developed symptoms of the diseases, suggesting these needles were infected as first-year needles.

Why many needles show symptoms characteristic of spruce needle blight but never develop hysterothecia, as do adjacent needles, is unknown. Scharpf (10) speculated that the lack or poor development of hysterothecia of *L. abietis-concolor* on some needles may be attributed to the presence of other fungi, especially a species of *Phoma*.

Efforts to control *L. macrospora* (e.g., with fungicides) in coastal Alaska should consider the timing of sporulation and the presence of susceptible tissues. Future studies should confirm evidence that first-year needles are the tissues infected by the fungus. Also, studies from other areas in coastal Alaska where spruce budbreak is earlier or later in spring could determine if a close association of sporulation with spruce bud and shoot development is consistent throughout the regional range of this fungus.

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