Epidemiology of *Lirula abietis-concoloris* on White Fir in California

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ABSTRACT

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The disease cycle of *Lirula abietis-concoloris*, a needle pathogen of white fir (*Abies concolor*) in the central Sierra Nevada requires about 2 years for completion. New foliage was infected in the spring of 1984, but symptoms and signs did not appear until the summer of 1985. Hysterothecia began development in needles in late fall of 1985 and appeared mature by early spring. Opening of hysterothecia and release of ascospores did not occur in the field until May and June, and only then during periods of rain or overhead irrigation. In the laboratory, hysterothecia collected before May failed to open and cast spores under all temperature and moisture regimes tested, whereas those collected in May and June opened and dispersed spores at temperatures ranging from 5 to 28 C and for 11 days at 5–15 C. New foliage on trees receiving irrigation in June 1985 showed symptoms of heavy infection in 1986, whereas new foliage not receiving either rain or irrigation in May and June was nearly disease-free.

The pathology and control of many needle diseases of conifers have not been well studied, primarily because they often are uncommon in occurrence and cause relatively little damage to forest trees. Brown spot needle blight of southern pines caused by the fungus *Scirrhia acicola* (Dearn.) Siggers, and red band needle blight of pines caused by *S. pini* Funk & Parker are two notable exceptions (8,9). These diseases are responsible for mortality and growth loss of pines in North America and have received considerable attention from both scientists and managers (8,9).

In general, most needle diseases occur as sporadic outbreaks, seldom build up to epidemic proportions over large areas, and cause little damage or mortality to forest trees. However, for conifers in forest nurseries, and those grown as ornamentals or for Christmas trees, even low levels of needle disease can cause significant economic losses. Outbreaks of these diseases can seriously impair the aesthetic and economic value of not only individual trees, but also entire plantings (1,7,9,10,13). In California, a needle disease caused by *Lirula abietis-concoloris* (Mayr ex Dearn.) Dark. occasionally causes heavy economic losses in white fir (*Abies concolor* (Gord. and Glend.) Lindl.) grown for Christmas trees, although little tree damage or growth loss occurs in native forest stands (3,6,10). *Lirula abietis-concoloris* is not limited in distribution to California, but is a native fungus that occurs at endemic levels on several species of fir throughout much of western North America (11). When conditions are favorable, the disease often increases to epidemic levels. At present, the climatic (or microclimatic conditions) that favor disease outbreaks are unknown.

Conflicting reports occur in the literature on the life cycles of several species of *Lirula*. For *L. macrospora* (Hartig) Darker (= *Hysterium* (*Hypoderma*) *macrosporium*) on spruce (*Picea*) the life cycle in Europe is reported to range from 1 to 3 years (5). A 4-yr life cycle was reported for *L. macrospora* on *P. glauca* Moench and *P. pungens* Engelm. in North America (12). For *L. abietis* on *Abies* in North America, the life cycle has been reported to be 1 to several years (4), 2 to several years (2), 2–3 yr (R. S. Hunt, *personal communication*), and 2 yr (11).

The details of symptom development, maturation of fruiting bodies, spore release, or infection of *L. abietis-concoloris* are unknown. Therefore, this study was conducted.

MATERIALS AND METHODS

The study area selected was a plantation of white fir grown for Christmas trees in the foothills of the Central Sierra Nevada in California (10). Ten trees were selected at random along a strip through a heavily infected portion of the plantation. Symptoms and hysterothecial development were recorded on these trees at about 1-mo intervals from the summer of 1985 to the summer of 1986. At 3–5 day intervals in early spring of 1986, samples of 1984 foliage were removed at random from each test tree, taken to the laboratory, and examined microscopically for maturation of the hysterothecia, asci, and ascospores. Development of the asexual (pycnidial) stage of the fungus was not studied and is not known to function in the infection

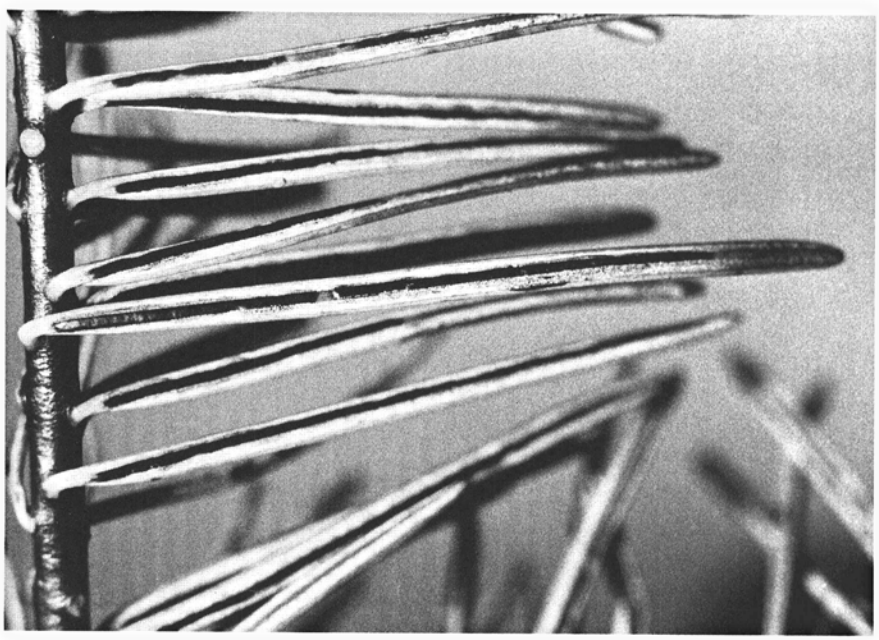

Fig. 1. The elongate, black hysterothecia of *Lirula abietis-concoloris* often extend nearly the full length of the infected needle.

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process.

To determine spore release in the field, two spore traps were placed on branches in each of 10 test trees. The trap consisted of clean glass microscope slides covered with a thin layer of vaseline and held on branches horizontally with a clothespin, vaseline side up. At the same 3–5 day interval mentioned above, the slides were removed, taken into the laboratory, and examined microscopically for ascospores.

A drop of acid fucsin and a 22 × 40 mm coverslip were placed on each slide and the slides were then examined at 100× with a binocular microscope for spores. A sample of the number of spores on the traps was made by counting the spores within the 100× field of view along the margins of the 22 × 40 mm coverslip on 5 of the 10 slides selected at random from each spore trapping interval. Therefore, at each examination period a total of 11.6 cm^2 of spore trap surface area was examined.

From 2–6 June, 1986 a portion of the study area was overhead-irrigated for about 12 hr a day to determine if viable spores were still present and capable of being released by the fungus a month after the last period of measurable rainfall in early May. The June sprinkling also was timed to correspond with the development of new, susceptible foliage to provide moisture necessary for infection. Temperature and relative humidity measurements were recorded on a chart hygrothermograph during spore trapping, and daily rainfall records were obtained from a local meteorological station.

Infection of 1986 foliage was determined in August 1987 by counting the number of infected and uninfected needles on each of 4 branches taken at random from 5 trees on irrigated and nonirrigated portions of the plantation.

To determine when hysterothecia are mature and ready to release spores, single needles bearing hysterothecia were collected at about 3–5 day intervals from the field and cemented to the inside of the lid of petri dishes containing 2% agar. One dish each was placed in the dark at 5, 10, 15, 18, 28 C and room temperature (23–25 C), and observed at least once a day for 5 days for hysterothecia maturation and spore release.

Other hysterothecia in petri dishes were observed for longer periods to determine the duration and number of spores released. In this test, five dishes each were placed at 5 and 15 C, and spore release was recorded at varying intervals for 11 days. To do this, a fresh agar dish was placed under each needle at each interval, and spores were counted with a microscope at 100× along five sample strips taken perpendicular to the needle for a total of 4.5 cm^2 of agar surface sampled. Germination also was determined for the spores released on agar at the various temperatures.

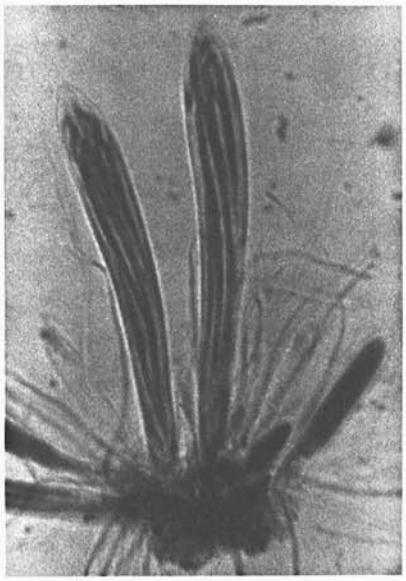

Fig. 2. The two asci in the center taken from within a hysterothecium contain what appear to be mature ascospores. Immature asci are seen to the immediate right of center. (430×)

RESULTS

Symptoms of disease on foliage infected in the spring of 1984 began to appear in June 1985. Infected needles turned brown and appeared slightly swollen along the lower needle midrib where hysterothecia develop. Some needles contained scattered black patches or bands and in a few cases an elongate, immature, black hysterotheum appeared to be developing on the lower surface along the needle midrib.

Symptoms appeared much the same in July and August. From late September to November, conspicuous, elongate, hysterothecia had developed on the lower surface of many infected needles (Fig. 1), but no asci were present. In early January 1986, asci were observed in some hysterothecia, but no spores had developed. By early February, some hysterothecia bore asci that contained what appeared to be mature spores, others contained asci with no spores. Most hysterothecia were well developed and contained some immature asci with spores by the end of February (Fig. 2).

Spore trapping began in mid-March,

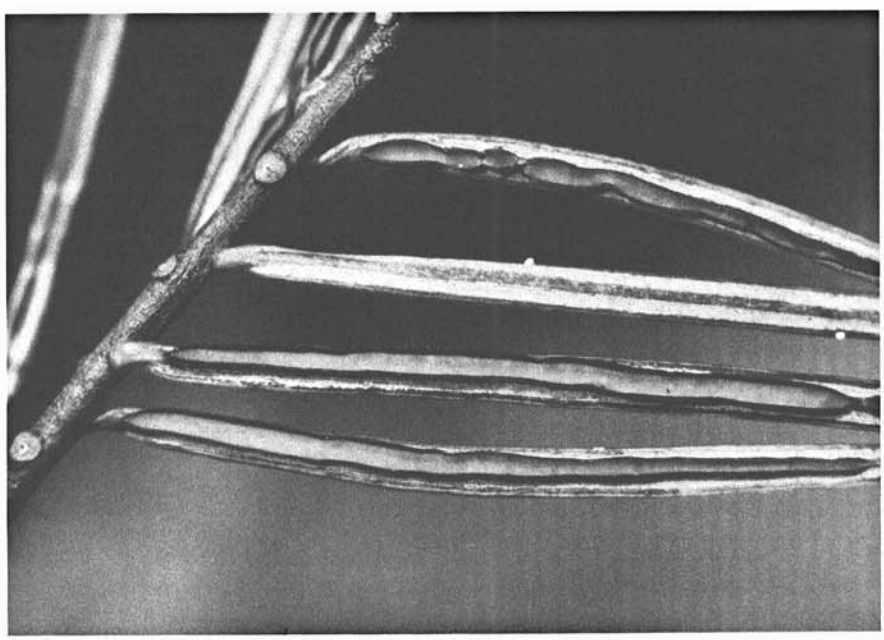

Fig. 3. Mature hysterothecia release spores along a longitudinal slit during periods of rain. Accospores are released from asci born on the cream-colored hymenial layer of the hysterothecium.

Table 1. Mean number of ascospores of *Lirula abietis-concoloris* dispersed per hour at various intervals over time in the laboratory at 5 and 15 C

Hour	Interval (hr)	5 C[a]	15 C[a]
0 (May 27, 9:00 a.m.)		...	...
1	1	5.0[b]	0
7	6	4.4	0.7
25	18	4.4	2.5
49	24	3.3	2.8
79	30	14.2	16.9
103	24	8.9	31.3
175	72	7.1	13.7
199	24	10.7	11.9
223	24	7.9	7.1
263[c]	40	6.8	2.0

[a] Spores per 4.5 cm^2 per hour.
[b] Based on only one of five dishes showing spores.
[c] The test was concluded after 263 hours because molds were developing on the needles and hysterothecia.

when most hysterothecia were well developed, but contained few mature asci with ascospores. By mid-April, most hysterothecia contained asci with what appeared to be mature spores. No spores were trapped and no noticeable changes were observed until late April, when a few hysterothecia were seen opening along a longitudinal slit (Fig. 3). Spores were first trapped in early May and sporadically thereafter until late June 1986. Between 2–7 May, more than 5 cm of rainfall was recorded at the Institute of Forest Genetics weather station, located about 13 km from the test site. During this interval, 1,170 ascospores were counted on 10 spore traps for an average density of about 50 spores per cm^2 of trap surface. The long, clavate ascospores of *L. abietis-concoloris* are readily seen at 100×, quite distinctive in shape and size, and easily distinguished from other spores or pollen.

With the exception of one spore each on three traps, no more spores were trapped and no rainfall was recorded for the remainder of May. During the period from 2 to 6 June when a portion of the plantation was irrigated, 1,524 spores were counted on 15 traps for an average density of about 44 spores per cm^2. No rainfall occurred thereafter, and very few spores were recorded on traps after 6 June. By July, the needles infected in 1984 had dried, and none of 10 hysterothecia examined bore asci with spores. Therefore, trapping was terminated. The dried, infected needles persisted on branches for several months after spore release.

Hysterothecia collected from Feburary through April and tested in the laboratory at various temperatures failed to open and release spores on agar in petri dishes, even though many asci and spores appeared to be mature. First spore release in the laboratory was noted for hysterothecia collected 2 May. After 5 days in dishes, nearly all fruiting bodies were open and had cast 16, 47, 7, 31, 2, and about 100 spores per cm^2 at 5, 10, 15, 18, 28 C, and room temperature, respectively. Hysterothecia collected on 13 May and tested at the same temperatures were dispersing more than 100 spores per cm^2 after 36 hr.

The test to determine the duration of spore release and number of spores released at intervals over time was begun on 27 May using needles with hysterothecia collected in mid-May (Table 1). At both temperatures (5 and 15 C), spore dispersal began within a few hours to a day after exposure to the moist conditions in a petri dish. At 15 C, spore release increased up to 103 hr then decreased, whereas at 5 C, dispersal of abundant numbers of spores continued over the 11-day duration of the test. How much longer spores would continue to be dispersed under these conditions was not determined.

Not all infected needles developed hysterothecia. In some, only partial development occurred (Fig. 4). Another fungus (*Phoma* sp.) was identified from portions of some needles bearing incomplete hysterothecia. It appeared that *Phoma* sp. invaded some infected needles, preventing complete development of the fruiting bodies of *L. abietis-concoloris*. Larvae of an insect (possibly hemlock looper) also were observed, reducing the numbers of hysterothecia by selectively feeding on the fruiting bodies without eating the needle itself (Fig. 5). Effects of this feeding on inoculum potential is unknown.

Infection of young 1986 foliage was markedly different for the trees irrigated and nonirrigated in June. On the nonirrigated area, only three of the 20 branches examined bore any infected needles, and on these branches only five of 87 needles were infected. On the irrigated area, all but one of the branches bore diseased foliage. On these branches, 191 out of 588 needles were infected for an average infection rate of about one needle in three.

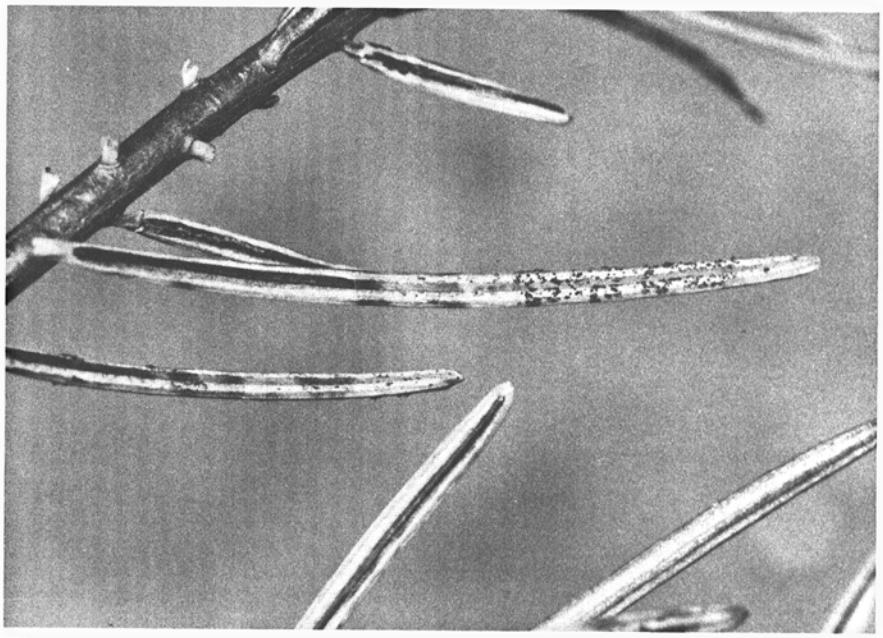

Fig. 4. Another fungus (*Phoma* sp.) also was associated with needles infected by *Lirula abietis-concoloris*. The occurrence of this fungus may inhibit the development of hysterothecia. The small, black spots on the outer portion of the needle are the asexual fruiting bodies of *Phoma* sp.

Fig. 5. The larva of an insect (possibly hemlock looper) feeding on the hysterothecium of *Lirula abietis-concoloris*.

DISCUSSION

Hysterothecia of *L. abietis-concoloris* developed and ascospores were released about 2 yr after infection of 1984 white fir foliage, 1 yr less than that reported by Hunt (R. S. Hunt, *personal communication*), and 2 yr less than that reported by Walla (12) for *L. macrospora* on spruce. A variable life cycle as reported by Darker (2), Funk (4), and Hartig (5) was not found.

I cannot explain why symptoms did not appear for at least a year after infection, or why fruiting bodies and asci that appeared to be mature in March 1986 failed to open and release spores until early May. Even fruiting bodies placed under varying conditions of temperature and moisture in the laboratory did not open and release spores. The maturation of fruiting bodies, spore dispersal, and length of incubation period has puzzled scientists working on other conifer needle diseases (12,13). With *L. macrospora*, Walla (12) reported mature asci with spores from April to September, but spores were trapped only from June to August. Possibly, spores and hysterothecia are not mature earlier in the season, even though they appeared to be. Also, physiological changes in host growth in the spring may in some way stimulate or trigger spore release. Spore dispersal appears closely syncronized with bud break and new foliage development when weather conditions are favorable.

Like *L. macrospora*, *L. abietis-concoloris* required rain for the opening of hysterothecia and release of spores (12). When wet again, *L. abietis-concoloris* was able to release large numbers of spores 1 mo after the first release, whereas subsequent spore release of *L. macrospora* was not correlated with amount of rain. Maturation of fruiting bodies of both species of fungi corresponded closely to early shoot growth.

One unexpected result of laboratory tests was the long duration of spore release from hysterothecia. It appears that new asci with spores develop and mature over time as the mature spores are being released. How long this process would take from single hysterothecia is not known.

Several secondary fungi are known to be associated with needle cast fungi on conifer needles (4). However, this is the first report of a *Phoma* sp. invading conifer foliage infected by a needle cast. Known biological control agents at present appear to have little effect on the epidemiology of this disease.

Results from the irrigated portion of the plantation indicate that infection occurs only on young foliage during periods of rainfall in the spring. If no rain occurs, spores are not released and no infection occurs.

Tests concurrent with this study were established in June 1986 to find a fungicide to protect young foliage from infection by *L. abietis-concoloris*. With the information provided here, and with the use of an effective fungicide, this sporadic, but serious, disease of white firs grown for Christmas trees may be controlled (6).

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