A NEEDLE BLIGHT OF PINUS STROBUS
IN ACADIA NATIONAL PARK:
ASSOCIATED FUNGI AND PATHOLOGICAL ANATOMY

A Thesis in
Plant Pathology

by
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of the Requirements
for the Degree of

Master of Science

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ABSTRACT

A needle blight of *Pinus strobus* L. has been observed in the northeastern United States for at least 80 years. This blight is characterized by a die-back of current-season needles, beginning in mid-summer. Although this blight has been observed and studied since 1908, no causal agent has ever been determined. This study addresses the fungi associated with this blight, particularly in regard to identity, time of infection, and pathological anatomy of needles in relation to infection.

A complex of fungi was found associated with this blight, including a previously undescribed hystereaceous fungus which infects current-year needles in June and July and fruits the following year, *Hendersonia pinicola*, *Truncatella truncata*, *Leptostroma* spp., a *Septoria* sp., a black yeast, and a white, nonsporulating hyphomycete. Injury at the anatomical level includes a collapse of mesophyll cells and differs from that which occurs on *P. strobus* needles exposed to ozone under greenhouse conditions.
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Mostly, I thank Frank Gecina for unfailing companionship and support through the past two years.
INTRODUCTION

For many years, reports have appeared in the scientific literature pertaining to a needle blight of eastern white pine, *Pinus strobus* L. This blight has been described as a die-back of current-season needles, giving a red or scorched appearance to affected trees. In 1908 Dana published the first report of this disease (10), noting it occurred from Maine to Pennsylvania. Campana (7) summarized the literature pertaining to a "red needle blight" dating from 1894 to 1952 and noted that during that period many causal agents had been suggested, including drought, root mortality, frost, adverse climate, industrial pollution, and parasites.

Campana (7) believed the root rot fungus, *Corticium galactinum* (Fr.) Burt, could be responsible for the blight. He inoculated *P. strobus* seedlings with this pathogen but was unable to reproduce the symptoms. He concluded that this fungus did not contribute to blight symptoms, and that the causal agent was abiotic. Baldwin (1) reported needle blight of *P. strobus* in New Hampshire and postulated that abundant rainfall followed by sudden drought was responsible.

In 1960 Linzon saw orange-red tips on current-year needles of eastern white pine in Ontario, Canada, and named the condition "semimature-tissue needle blight" (SNB) (20). Fungi fruiting 3 to 4 weeks after SNB outbreaks were considered saprophytes. In 1962 Linzon identified *Hypoderma desmazierii* Duby and *Cenangium acuum* Cook & Peck fruiting on one-year-old, attached necrotic needles, and a *Lophodermium* sp. (either *L. pinastri* [Schrad. ex Hook.] Chev. or *L. nitens* Darker) fruiting on fallen needles (21). Inoculation studies with *Lophodermium* sp. and *C. acuum* did not produce the SNB symptoms on current-year needles. However, *H. desmazierii* was not used for inoculations due to insufficient amounts of inoculum. Linzon continued to study SNB throughout the 1960s, including attempts to produce the symptoms with ozone fumigation,
but never identified a causal agent (22, 23). Also in the 1960s Banfield reported fungi to be responsible for the needle blight in the northeastern United States. He identified a \textit{Lophodermium} sp. that caused yellow-orange lesions that later developed into brown or reddish brown lesions on first-year needles, with casting of first-year needles from July throughout the year. Apothecia of a \textit{Lophodermium} sp. developed on fallen or dead needles, or occasionally developed the following spring on dead tips of attached needles (2). Later he again associated small yellow spots on white pine needles with a \textit{Lophodermium} sp. (3). Then in 1963 he described symptom progression over the course of a year which included yellow spots developing on current-year needles in early July, leading to yellowing and browning of needles with the imperfect stage of a needlecast fungus appearing in the fall. The following spring he identified \textit{Hypoderma desmazieri}i fruiting on the browned needles (4).

Since 1983 the blight has been noted to occur from West Virginia to Maine, and similar symptoms have been noted on \textit{P. strobus} in North Carolina and Wisconsin. In 1983 a needle blight of \textit{P. strobus} was observed in the Acadia National Park, Maine. Although it was not certain that this was the same blight mentioned in the literature, symptoms were strikingly similar. From a distance affected trees appeared brown when compared with unaffected trees. A variety of symptoms was observed on current-year needles, including chlorotic mottle, chlorotic spots, tip reddening, and tip necrosis. Needles in the same fascicle often showed different degrees of symptoms.

Participants at a workshop in Acadia National Park in August, 1986, concluded that the symptoms were due either to ozone damage or semimature-tissue needle blight (5). However, current-year needles collected at that time bore fruiting bodies of a \textit{Leptostroma} sp. (Skelly and Merrill, unpublished 1986). \textit{Leptostroma} spp. are the asexual stages of several needlecast fungi.
The symptoms generally caused by needlecast fungi are quite similar to the symptoms described for the blight, including yellow spots and red necrotic tips (6). This fact, along with the presence of the *Leptostroma* stage on current-year needles as early as August, suggested that needlecast fungi may have infected the needles during shoot elongation and thus could be playing a role in the blight.

The objectives of this study were to identify fungi associated with the observed blight of *P. strobus*, and to determine the time of infection by these fungi.
MATERIALS AND METHODS

To determine which fungi were present on needles, affected *P. strobus* trees were examined in May, July and October of 1987 and June and September of 1988 on sites in Pennsylvania, New Hampshire, Vermont, and Maine, including Acadia National Park (Figure 1, Appendix A). By direct observation of fruiting bodies and spores present on the needles, fungi were identified on the 1986, 1987, and 1988 needle complements.

In addition, intensive sampling of needles in Acadia National Park was conducted during the summer of 1988 to determine at what time the associated fungi were infecting. Four previously symptomatic trees were selected and observed for symptom development, and their needles were sampled for isolation and histological studies. Three permanently marked trees were located in Acadia National Park (C-1, C-6, C-11), and the fourth tree was located about 25 miles north near Dedham, Maine (E-1). In mid-June branches were tagged, in most cases one branch each on the southwest and northeast aspects. At this time needles were just beginning to emerge from the fascicle sheaths. Beginning June 13, 1988, these branches were sampled twice weekly until August 4, 1988, during the period of needle elongation, when it was thought that the previously observed fungi might be infecting. Needles with different symptom types as well as green, asymptomatic needles were sampled. In June the needles had barely emerged from the fascicle sheath, and thus entire fascicles were collected. After needle length reached 2.0 cm, individual needles were sampled. In the laboratory needle length was measured, then 1 cm sections were cut from symptomatic areas which were then cut again in half; one half was plated out on 2% acidified malt agar (19), and the other half was fixed in formalin-acetic acid-alcohol (FAA) (9) for later anatomical studies. Plates were examined weekly, and resulting growth was transferred to potato dextrose agar slants (15 g agar, 20 g dextrose, 1 L potato broth) and transported to Pennsylvania for further study at the end of the field season.
Figure 1. Location of sampling sites in Pennsylvania, Vermont, New Hampshire and Maine.
- = sites where the *Bifusella*-like ascomycete was found.
= sites where the *Bifusella*-like ascomycete was not found.
Sites are described in Appendix A, and fungi found fruiting on needles are listed in Appendix B.
For microscopy, needle pieces were dehydrated using a tertiary butyl alcohol schedule (Appendix C), vacuum embedded in Paraplast (melting point 55.5°C - 56.5°C), sectioned at 10 μm with a rotary microtome, mounted on glass slides, and stained with a modification of Jewell's Orseillin BB / Aniline blue staining schedule (17, Appendix C).

In related studies, cuttings taken from C-6 and two other trees in March 1988 were grafted to root stocks and maintained in a growth chamber. As 1988 needles developed, previous year's needles were removed from the plants. Necrotic needle tips developed on clones derived from one tree which were exposed to constant 40-70 parts per billion ozone with a 1 hour 90 parts per billion spike on June 9 and a 1 hour 160 parts per billion spike on June 30 (25). Selected symptomatic needle sections were fixed in FAA, dehydrated, sectioned, stained and examined in the same manner as field specimens.
RESULTS

1987 Survey

In the 1987 study, fungi were identified on both the 1986 and 1987 needle complements. One of the fungi most frequently found on 1986 needles was tentatively identified as a *Bifusella* sp. Usually only one or two needles per fascicle bore fruiting bodies. The long, shiny, black hysterothecia resembled the needlecast fungus *B. linearis* (Pk.) v. Höhn (Figure 2). However, the fungal fruiting body developed subepidermally (Figure 3), and asci measured 95 x 15.5 μm, each containing 8 rod-shaped ascospores, averaging 23 x 6 μm (Figure 4). Ascospores did not exhibit the distinctive bifusiform shape described for *B. linearis* but more closely resembled the size and shape of ascospores described for the needlecast fungus *Meloderma (=Hypoderma) desmazierii* (Duby) Darker (13). Dr. David Minter, International Mycological Institute, has determined that this *Bifusella*-like Ascomycete (BLA) is a previously undescribed species, and its genus is uncertain (D. Minter, pers. comm. July 29, 1988). Another Ascomycete fruiting on 1986 needles resembled a *Hemiphacidium* or *Phacidium* sp.; however, this fungus was not as prevalent in 1988 on the 1987 needles collected. Several Fungi Imperfecti were found on both 1986 and 1987 needles, including *Hendersonia pinicola* Wehmeyer, a *Septoria* sp., a *Leptostroma* spp. and *Truncatella truncata* (Lev.) Steyaert. In addition, *Lophodermium nitens* was ubiquitous on needles lying on the duff.

1988 Symptoms

During the 1988 study symptoms developed on three trees: C-6, C-11 and E-1. Three symptom types developed: chlorotic spots (CS), necrotic tips (NT), and chlorotic spots with necrotic centers (CS/NC). Table 1 shows the dates when each symptom was observed for each tree.
Figure 2. Hysterothecium of the Bifusella-like ascomycete (BLA) fruiting on a one-year-old needle of Pinus strobus.

Figure 3. Cross section through the hysterothecium of the Bifusella-like ascomycete (1750x). The fruiting body develops underneath the epidermis of the needle.
Figure 4. An ascus of the *Bifusella*-like ascomycete containing ascospores (6200x)

Table 1. *Pinus strobus* needle blight symptom types and the date of appearance on the 1988 needle complement.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Symptom type</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-1</td>
<td>chlorotic spot</td>
<td>June 28 - August 4</td>
</tr>
<tr>
<td></td>
<td>necrotic tip</td>
<td>July 8 - August 4</td>
</tr>
<tr>
<td></td>
<td>chlorotic spot/necrotic center</td>
<td>July 22 - August 4</td>
</tr>
<tr>
<td>C-1</td>
<td>chlorotic spot</td>
<td>July 1*</td>
</tr>
<tr>
<td>C-6</td>
<td>chlorotic spot</td>
<td>July 29 - August 4</td>
</tr>
<tr>
<td></td>
<td>necrotic tip</td>
<td>July 29 - August 4</td>
</tr>
<tr>
<td></td>
<td>chlorotic spot/necrotic center</td>
<td>August 1 - August 4</td>
</tr>
<tr>
<td>C-11</td>
<td>chlorotic spot</td>
<td>August 1 - August 4</td>
</tr>
<tr>
<td></td>
<td>necrotic tip</td>
<td>August 4</td>
</tr>
</tbody>
</table>

*one date only*
1988 Fungi

The fungi identified in 1988 on the 1987 complement were: the BLA on all four trees (but very sparse on C-1); *H. pinicola* on C-6, C-11, and E-1; *T. truncata* on C-6 and E-1; *Leptostroma* spp. on E-1, C-11, C-1; and a *Septoria* sp. and the *Hemiphacidium*-like Ascomycete on C-11. Appendix B lists fungi found fruiting on *P. strobus* needles during both 1987 and 1988.

Single spores of *H. pinicola*, *T. truncata* and the *Septoria* sp. were used to generate reference cultures for comparison with fungi isolated from current-year needles. When needles containing hysterothecia of the BLA were suspended over agar, ascospores were ejected and produced germ tubes on water agar, acidified malt agar, and potato dextrose agar. However, transfers were only successful when several spores were transferred together to potato dextrose agar. Even these cultures were very slow growing, mounding up and reaching a diameter of less then 1 cm. after 1 month. As these cultures aged, the medium became dark colored and cultures ceased growth. Subsequent transfers were unsuccessful.

1988 Isolations

Fungi isolated from 1988 needles were identified on the basis of conidia formation and by comparison of cultural characteristics with those of cultures obtained from spores of fungi fruiting on 1987 needles. Fungi identified from the 1988 needle isolations include: *H. pinicola*, *T. truncata*, and *Septoria* spp. Several *Leptostroma*-like cultures were obtained. Cultures resembling ascospore cultures of the BLA were also obtained. Two other fungi frequently isolated were a black, yeast-like fungus, and a white, appressed, nonsporulating fungus. Table 2 lists the identified fungi isolated from 1988 needles.
Table 2. Fungi isolated from the 1988 needle complement of *Pinus strobus*.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Symptom(^a)</th>
<th>Fungus</th>
<th>Date isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-1</td>
<td>A</td>
<td><em>Hendersonia pinicola</em></td>
<td>June 13 - August 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black yeast</td>
<td>June 13 - August 4</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td><em>Leptosphaeria</em> sp.</td>
<td>July 1 - August 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hendersonia pinicola</em></td>
<td>July 18 - August 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black yeast</td>
<td>August 4</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td><em>Bolbitospora</em> -like ascomycete</td>
<td>July 8 - July 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hendersonia pinicola</em></td>
<td>July 18 - August 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black yeast</td>
<td>July 22 - August 4</td>
</tr>
<tr>
<td>CS/NC</td>
<td></td>
<td>Black yeast</td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>A</td>
<td>Black yeast</td>
<td>June 24 - July 22</td>
</tr>
<tr>
<td>C-6</td>
<td>A</td>
<td>Black yeast</td>
<td>June 13 - July 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White nonsporulating</td>
<td>July 18 - August 4</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>White nonsporulating</td>
<td>July 29 - August 4</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td><em>Truncatella truncata</em></td>
<td>July 29 - August 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Septoria</em> sp.</td>
<td>July 5 - July 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hendersonia pinicola</em></td>
<td>August 1 - August 4</td>
</tr>
<tr>
<td>CS/NC</td>
<td></td>
<td>White nonsporulating</td>
<td>August 1</td>
</tr>
<tr>
<td>C-11</td>
<td>A</td>
<td>Black yeast</td>
<td>June 21 - June 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hendersonia pinicola</em></td>
<td>June 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Leptosphaeria</em> sp.</td>
<td>July 18</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td><em>Hendersonia pinicola</em></td>
<td>August 1 - August 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Truncatella truncata</em></td>
<td>August 1</td>
</tr>
</tbody>
</table>

\(^a\) = A: asymptomatic  
CS: chlorotic spot  
NT: necrotic tip  
CS/NC: chlorotic spot with necrotic center
This study established which fungi were present and when they may be infecting. Although the objectives of this study were qualitative in nature, quantitative values for percent recovery are provided for graphical presentation and comparison purposes. Values represent the percentage of needles from which a particular fungus was isolated on that date. Since the actual numbers of needles sampled often were small, as few as 6 needles of a given symptom type, it is likely that the values may be erroneous. Occasional isolates of numerous other fungi did not sporulate in culture and did not match the characteristics of reference cultures, and thus could not be identified. These isolates are designated "other."

From C-1 the black yeast was recovered on two occasions from asymptomatic needles, June 24 and July 22. In addition, unidentifiable, nonsporulating hyphomycetes were recovered on July 22 and July 25 (Figure 5). No fungi were recovered from the evanescent chlorotic spots appearing on July 1, 1988.

![Figure 5. Fungi recovered from the asymptomatic 1988 needles of tree C-1.](image)
From C-6 the black yeast was recovered from asymptomatic needles on June 13, 17, 21, and July 18 (Figure 6) and from NT on July 5 only (Figure 8). A white, nonsporulating hyphomycete was recovered July 18 to August 4 from asymptomatic needles (Figure 6), July 29 to August 4 from CS (Figure 7), on August 4 from NT (Figure 8) and CS/NC (Figure 9). *Hendersonia pinicola*, *T. truncata*, and the *Septoria* sp. were all recovered from NT July 29-August 4 (Figure 9). Other unidentifiable fungi were also recovered from asymptomatic needles during the month of June (Figure 6).

From C-11 the black yeast was recovered from asymptomatic needles on June 13, 21, and 24 (Figure 10). A *Leptostroma* sp. was recovered from asymptomatic needles on one sampling date, July 18 (Figure 10). *Hendersonia pinicola* was recovered from asymptomatic needles on June 24 (Figure 10) and from NT on August 4 (Figure 11). Other unidentifiable fungi were recovered from asymptomatic needles through the sampling period (Figure 10).

From E-1 the black yeast was recovered from asymptomatic needles June 13-28, July 25 and August 4 (Figure 12); from CS on July 18 (Figure 13); and from NT July 18-29 and August 4 (Figure 14). Cultures resembling ascospore cultures of the BLA were recovered from CS on July 22 (Figure 13) and from NT July 8-15 and July 18-22 (Figure 14). *Leptostroma* spp. were recovered from asymptomatic needles only on July 1, 8, 15, 18 (Figure 12). *Hendersonia pinicola* was recovered from asymptomatic needles June 13, 17 and August 4 (Figure 12), and from NT July 18 and July 25-August 4 (Figure 14). In addition, numerous unidentified fungi were recovered throughout the sampling period. No identifiable fungi were isolated from CS/NC (Figure 15).

**Microscopy**

The anatomy of *P. strobus* has several distinguishing features. On cross-section, needles are triangular-shaped with one adaxial and two abaxial faces. A thick-walled
Figure 6. Fungi recovered from the asymptomatic 1988 needles of tree C-6.

Isolation date

Figure 7. Fungi recovered from chlorotic spots on the 1988 needles of tree C-6. * indicates date symptom first detected.
Figure 8. Fungi recovered from necrotic tips on the 1988 needles of tree C-6.
* indicates date symptom first detected.

Figure 9. Fungi recovered from chlorotic spots with necrotic centers on the 1988 needles of tree C-6.
* indicates date symptom first detected.
Figure 10. Fungi recovered from the asymptomatic 1988 needles of tree C-11.

Figure 11. Fungi recovered from necrotic tips on the 1988 needles of tree C-11. * indicates date symptom first detected.
Figure 12. Fungi recovered from the asymptomatic 1988 needles of tree E-L.

Figure 13. Fungi recovered from chlorotic spots on the 1988 needles of tree E-L.

* indicates date symptom first detected.
Figure 14. Fungi recovered from necrotic tips on the 1988 needles of tree E-1. * indicates date symptom first detected.

Figure 15. Fungi recovered from chlorotic spots with necrotic centers on the 1988 needles of tree E-1. * indicates date symptom first detected.
hypodermis lies just underneath the epidermis on all faces. Stomata occur primarily on the abaxial faces. Two resin ducts occur on the adaxial side of the needle, just underneath the hypodermis. The vascular bundle is surrounded by transfusion tissue and elliptically-shaped, thick-walled hypodermal cells. Mesophyll cells are plicate with numerous air spaces throughout the needle (Figure 16).

No damage was noted in sections of either asymptomatic or symptomatic needles until July 12. From July 12 until August 4, mesophyll collapse and disintegration was noted in sections from necrotic tips of E-1, with thick hyphae in the resultant intercellular spaces (Figure 17, Figure 18). These hyphae were approximately 9 - 11 μm wide, and did not enter resin ducts or other cells. Endodermis and resin ducts showed no damage until July 22, when crushing and collapse of these cells was seen. On July 29 hyphae were noted in

Figure 16. Diagrammatic cross section of a Pinus strobus needle.
S = stomate, Epi = epidermis, H = hypodermis, M = mesophyll, Endo = endodermis, T = transfusion tissue, V = vascular bundle, RD = resin duct.
Figure 17. Cross section of the necrotic tip of a needle from tree E-1, July 12, 1988 (1182x).
Note collapsed mesophyll cells (M) and hyphae (H) in intercellular spaces.

Figure 18. Cross section of the necrotic tip of a needle from tree E-1, July 12, 1988 (4600x).
Note collapsed mesophyll cells (M) and unaffected resin duct (RD).
sections from necrotic tips of C-6 (Figure 19). At no time during the study were hyphae seen in sections from chlorotic spots or chlorotic spots with necrotic centers, although some slight collapse of the mesophyll was visible on July 29. By August 4 complete collapse of mesophyll cells was noted in sections from necrotic tips of E-1 (Figure 20, Figure 21).

Needle sections from ozone-exposed trees that incurred tip necrosis differed markedly from field materials. Mesophyll cells were not collapsed, but instead were spherical, rather than plicate. In addition, the epithelial parenchyma cells lining the resin ducts were greatly hypertrophied (Figure 22, Figure 23).

Figure 19. Cross section of the necrotic tip of a needle from tree C-6, July 29, 1988 (2680x). Note hyphae (H) closely associated with the mesophyll (M).
Figure 20. Cross section of the necrotic tip of a needle from tree E-1, August 4, 1988 (1280x). Note the remnants of the collapsed mesophyll cells.

Figure 21. Cross section of the necrotic tip of a needle from tree E-1, August 4, 1988 (3070x). Note the remnants of the collapsed mesophyll cells (M) and the unaffected resin duct (RD).
Figure 22. Cross section of a *Pinus strobus* needle damaged by ozone exposure in the growth chamber (1145x). Note the spherical mesophyll cells (M), hypertrophy and hyperplasia of vascular tissues (V), and hypertrophy of epithelial parenchyma cells lining the resin ducts (RD).

Figure 23. Cross section of a *Pinus strobus* needle damaged by ozone exposure in the growth chamber (3000x). Note the spherical mesophyll cells (M) and hypertrophy of epithelial parenchyma cells lining the resin ducts (RD).
DISCUSSION

Symptoms

Symptom development varied on each of the four trees observed in the summer of 1988. E-1 was the most symptomatic tree throughout the study, exhibiting all three symptom types. The same symptoms developed in the same order on C-6; however, symptom development did not begin until 4 weeks later than on E-1. On one date (July 5) C-6 exhibited necrotic tips which were the result of feeding by the pine chafer, *Pachystethus oblvia* (Horn). Necrotic tips were not evident again until July 29. Chlorotic spots and necrotic tips developed on C-6 toward the end of the study (August 1 - August 4). C-11 also developed chlorotic spots, and the necrotic tips; however, this did not occur until the last two sampling dates. C-1 was an asymptomatic tree in 1988, developing chlorotic spots on one day only (July 1), and not exhibiting symptoms thereafter. These evanescent chlorotic spots may have been due to insect feeding.

Needlecast Fungi

Six species of needlecast fungi have been reported on *P. strobus*: *Bifusella linearis*, *Meloderma (= Hypoderma) desmazierii*, *Lophodermium nitens*, *L. pinastri*, *L. pinicexcelsae* Ahmad, and *L. durilabrum* Darker (16, 24, 30). In this study *L. nitens* fruited on fallen needles, and a fungus resembling *B. linearis* fruited on necrotic areas on 1-year-old attached needles.

*Lophodermium nitens* was nearly always present in the duff of *P. strobus*, and thus could be the sexual stage of the *Leptostroma* spp. which were isolated from current-year needles. This cannot be confirmed, however, since descriptions of *Lophodermium* spp. in culture are lacking or inadequate (24), and cultures of *Leptostroma* spp. exhibit highly variable morphology, even among isolates derived from a single fruiting body (28).
*Lophodermium nitens* is believed to be a saprophyte of fallen or senescing needles (24), and thus one would not expect *L. nitens* to be present in green, attached needles, as the *Leptostroma* sp. isolated in this study. However, the fact that *L. nitens* sporulates only on fallen needles is not conclusive evidence that this fungus is not a pathogen. As for most needlecast fungi, the life history of *L. nitens* has never been thoroughly investigated. Furthermore, endophytic *Leptostroma* spp. have been isolated from *Pinus* spp. in the Pacific Northwest (8). Therefore, no conclusions can be drawn regarding the pathogenicity of the *Leptostroma* spp. isolated from *P. strobus* during this study.

The *Bifusella*-like ascomycete (BLA) occurred on all four trees during the 1988 study, and occurred throughout Vermont, New Hampshire, and Maine, as well as in Pennsylvania (Figure 1). It was also collected from *P. strobus* Christmas trees in central West Virginia (Merrill and Wenner, unpublished 1986). From field observations it appears the hysterothecium of this fungus develops subepidermally on 10- to 11-month- old needles during the early spring and is not noticeable until May. Ascospores mature in June and July and during wet periods the fruiting bodies split longitudinally, releasing the ascospores which presumably infect current-year needles during shoot elongation. After ascospore release, needles bearing fruiting bodies are cast, usually by late July or August. If an observer is not in the field during the short period of fruiting body maturation and sporulation, the signs of infection by this fungus could easily be overlooked. Furthermore, the similarities of the hysterothecia with those of *B. linearis* easily lead to misidentification. *Bifusella linearis* (=*Hypoderma linearis*) has been reported on *P. strobus* (14, 27), but has usually not been considered an aggressive pathogen. Since in some respects the BLA resembles *Meloderma (= Hypoderma) desmazierii*, this may be the fungus described by Banfield (4) as associated with and causing needle blight of *P. strobus* in Massachusetts and by Linzon in Ontario, Canada (21).
In our studies the BLA was isolated from necrotic tips of E-1 from July 8 to July 25. Since this was rather early during needle elongation, this fungus probably is pathogenic on *P. strobus*.

**Other Fungi**

During the two years of this study *Hendersonia pinicola* was found fruiting on one-year old *P. strobus* needles from Vermont, New Hampshire, and Maine, and was recovered from current-year needles of all three symptomatic trees during the 1988 isolation studies. *Hendersonia pinicola* was at first isolated from asymptomatic fascicles at the beginning of the 1988 isolations, from June 13 to June 24. However, this fungus probably had not infected the needles at that time. On those sampling dates the needles had barely emerged from the fascicle sheaths, and entire fascicles were sampled. It is possible that spores had lodged under the fascicle sheath and were not killed during surface sterilization. Further, when entire needles were sampled (June 28 and thereafter) *H. pinicola* was isolated only from necrotic tips near the end of the study (July 25 to August 4). Although *H. pinicola* has been considered a weak pathogen or saprophyte (14), some believe it may act as an antagonist of certain needlecast fungi (13) or act as a pathogen on *Pinus contorta* var. *latifolia* Engelm. (26). At the present time the role of this fungus on *P. strobus* is unknown.

Two fungi that were consistently isolated from 1988 needles remain unidentified. A fungus which grew as a black yeast in culture was isolated from asymptomatic needles of all trees, as well as from necrotic tips of E-1 and C-6, and on one occasion (August 4) from chlorotic spots with necrotic centers of E-1. As with *H. pinicola*, isolations from asymptomatic needles during the early days of the study (June 13 to June 24) may be the result of spores lodged in the fascicle sheath which escaped surface sterilization. Numerous species of approximately 30 fungal genera are known to grow in culture as
black yeasts (12). The majority of these fungi resemble species of *Aureobasidium* (= *Pullularia*), but can be differentiated by several morphological characteristics, chiefly by method of conidia formation (12). Virtually all references to species of *Aureobasidium* or *Pullularia* on conifer needles are suspect. If reference cultures are unavailable for confirmation of the identity of the fungus reports should be regarded as unproven.

A white nonsporulating hyphomycete was consistently recovered from all symptom types and from asymptomatic needles of C-6, beginning July 18. This culture did not match the morphological characteristics of any cultures derived from fruiting bodies present on 1-year-old needles. Until these two fungi are positively identified, no presumptions can be made as to their roles, if any, in the observed needle blight.

The *Septoria* sp. fruiting on 1-year-old needles was isolated only occasionally from necrotic tips of C-6. *Septoria spadicea* Patt & Charles has been associated with a needle blight of *P. strobus* and is considered a parasite (14). Two *Pestalozzia* spp. have been associated with blighted needles of *P. strobus* (14); however, this genus has since been segregated into five genera on the basis of conidial septation (13). In this study a species of one of those segregates, *Truncatella truncata*, was observed on 1-year-old needles and isolated from necrotic tips of current-year needles. Funk reports that this fungus is a secondary invader of *Tsuga heterophylla* (Raf.) Sarg. Presently the pathogenic capabilities of the *Septoria* sp. and *T. truncata* on *P. strobus* are unknown.

**Microscopy**

The main cellular damage observed in this study was a collapse of mesophyll cells in symptomatic areas. This is typical for many of the needlecast fungi (18). This injury differs from that described for ozone injury (12, 29). Although ozone exposure may cause needle tip necrosis which superficially resembles the blight symptoms that occur in the field, histological evidence indicates that the symptoms observed in the field are not caused
by ozone. The report that ozone exposure produced SNB symptoms (25) may have been premature, since the histology of the ozone-exposed needles was never compared to that described for SNB by Linzon (22). Furthermore, hyphae were present in the resultant spaces in damaged necrotic tips of samples from the field. Although hyphae were never seen in sections from chlorotic spots or chlorotic spots with necrotic centers, fungal infection cannot be discounted. The sparsely-distributed hyphae were intercellular and closely associated with mesophyll cell walls, and extremely difficult to distinguish by histological methods. Many needlecast fungi sparsely colonize needle tissue and are difficult to detect during the early period of colonization (18).
CONCLUSIONS

A complex of fungi has been found associated with a needle blight of *P. strobus* in the northeastern United States. A previously undescribed hystereaceous fungus fruits on one-year-old needles and can be isolated from symptomatic first-year needles as early as July. Other fungi associated with this blight include *Hendersonia pinicola, Truncatella truncata, Leptostroma* spp., a *Septoria* sp., a black yeast and a white, nonsporulating hyphomycete.

Koch’s postulates must be completed to determine which, if any, of these fungi is pathogenic. For some of the Fungi Imperfecti conidia can easily be produced in vitro. In this study *H. pinicola, T. truncata*, and the *Septoria* sp. sporulated readily on potato dextrose agar within several weeks. However, completion of Koch’s postulates with the BLA is impossible at this time. If the life cycle of this fungus is similar to those of other hystericaceous needlecast fungi, only ascospores are infectious. Since cultures of the BLA are short-lived and do not form ascomata, at present only ascomata from naturally infected needles can be used as a source of ascospores. However, these needles cannot be used to complete Koch’s postulates, since these infected needles often bear fruiting bodies of *H. pinicola* *T. truncata* and the *Septoria* sp. along with the BLA. Therefore, it would be impossible to separate out the effects of each individual fungus. A method must be devised to collect mature ascospores from the hysterothecia of needlecast fungi and to utilize these ascospores as inocula under controlled environmental conditions. Only when these methods are devised will we begin to understand the life histories of the various needlecast fungi and their interactions with other needle-inhabiting fungi and their hosts.
REFERENCES


## APPENDIX A
### STUDY SITES

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Elevation (m)</th>
<th>Dbh(^a) (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41°43'</td>
<td>77°7'32&quot;</td>
<td>600</td>
<td>56</td>
<td>Open-grown in old pasture, surrounded by young reproduction; hillside, south aspect</td>
</tr>
<tr>
<td>2</td>
<td>43°20'11&quot;</td>
<td>72°32'03&quot;</td>
<td>183</td>
<td>20</td>
<td>Pole-sized tree; sandy slope, south aspect.</td>
</tr>
<tr>
<td>3</td>
<td>43°15'30&quot;</td>
<td>72°27'40&quot;</td>
<td>287</td>
<td>-</td>
<td>Christmas tree plantation with 10% of trees affected; old hillside field, east aspect.</td>
</tr>
<tr>
<td>4</td>
<td>44°24'43&quot;</td>
<td>71°44'06&quot;</td>
<td>270</td>
<td>18</td>
<td>Pole-sized tree; moist, level, abandoned field.</td>
</tr>
<tr>
<td>5</td>
<td>44°24'51&quot;</td>
<td>71°46'02&quot;</td>
<td>378</td>
<td>46</td>
<td>Mature tree, fencerow; hillside, southeast aspect.</td>
</tr>
<tr>
<td>6</td>
<td>44°24'10&quot;</td>
<td>71°37'20&quot;</td>
<td>279</td>
<td>30</td>
<td>Fencerow; edge of level, gravelly abandoned field.</td>
</tr>
<tr>
<td>7</td>
<td>44°23'49&quot;</td>
<td>71°37'31&quot;</td>
<td>275</td>
<td>30</td>
<td>Open grown; marshy edge of abandoned field.</td>
</tr>
<tr>
<td>8</td>
<td>44°22'43&quot;</td>
<td>71°25'35&quot;</td>
<td>402</td>
<td>20</td>
<td>Edge of mixed conifer-hardwood forest, northwest aspect.</td>
</tr>
<tr>
<td>9</td>
<td>43°59'21&quot;</td>
<td>71°18'49&quot;</td>
<td>390</td>
<td>28</td>
<td>Mixed conifer-hardwood forest; level, gravelly site.</td>
</tr>
<tr>
<td>10</td>
<td>44°3'19&quot;</td>
<td>71°51'17&quot;</td>
<td>535</td>
<td>28</td>
<td>Mixed conifer-hardwood forest; moist hillside, east aspect; young reproduction seeding into previously logged area.</td>
</tr>
<tr>
<td>11</td>
<td>44°57'46&quot;</td>
<td>69°35'</td>
<td>177</td>
<td>47</td>
<td>On old fencerow along roadside; level gravelly site.</td>
</tr>
<tr>
<td></td>
<td>Latitude</td>
<td>Longitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>------------</td>
<td>------------</td>
<td>----</td>
<td>---</td>
<td>------------------</td>
</tr>
<tr>
<td>12</td>
<td>45°13'45&quot;</td>
<td>68°47'22&quot;</td>
<td>155</td>
<td>20</td>
<td>Mixed conifer-hardwood forest, level moist site.</td>
</tr>
<tr>
<td>13</td>
<td>44°40'59&quot;</td>
<td>68°35'</td>
<td>98</td>
<td>15</td>
<td>Young tree along roadside, gravelly soil, west aspect. Tree E-1.</td>
</tr>
<tr>
<td>14</td>
<td>44°23'47&quot;</td>
<td>68°14'58&quot;</td>
<td>58</td>
<td>30</td>
<td>Solitary on pond edge.</td>
</tr>
<tr>
<td>15</td>
<td>44°22'13&quot;</td>
<td>68°15'52&quot;</td>
<td>125</td>
<td>23</td>
<td>Mixed conifer hardwood forest. Tree C-1.</td>
</tr>
<tr>
<td>16</td>
<td>44°22'29&quot;</td>
<td>68°15'7&quot;</td>
<td>85</td>
<td>25</td>
<td>Edge of lake. Tree C-6.</td>
</tr>
<tr>
<td>17</td>
<td>44°21'55&quot;</td>
<td>68°16'24&quot;</td>
<td>98</td>
<td>18</td>
<td>Mixed conifer hardwood forest. Tree C-11.</td>
</tr>
<tr>
<td>18</td>
<td>44°21'49&quot;</td>
<td>68°14'46&quot;</td>
<td>107</td>
<td>25</td>
<td>Near roadside in mixed conifer hardwood forest.</td>
</tr>
</tbody>
</table>

^a = diameter at breast height
### APPENDIX B.
### FUNGI FRUITING ON P. STROBUS NEEDLES DURING 1987 and 1988

<table>
<thead>
<tr>
<th>Site</th>
<th>Fungi (on one-year-old needles, unless noted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bifusella</em>-like ascomycete</td>
</tr>
<tr>
<td>2</td>
<td><em>Hendersonia pinicola, Hemiphacidium</em>-like ascomycete, <em>Cryocaligula</em> sp.</td>
</tr>
<tr>
<td>3</td>
<td><em>Bifusella</em>-like ascomycete</td>
</tr>
<tr>
<td>4</td>
<td><em>Bifusella</em>-like ascomycete, <em>Lophodermium nitens</em> (on fallen needles in duff)</td>
</tr>
<tr>
<td>5</td>
<td><em>Leptostroma</em> sp.</td>
</tr>
<tr>
<td>6</td>
<td><em>Hemiphacidium</em>-like ascomycete, <em>Hendersonia pinicola, Cryocaligula</em> sp., <em>Septoria</em> sp.</td>
</tr>
<tr>
<td>7</td>
<td><em>Bifusella</em>-like ascomycete, <em>Septoria</em> sp.</td>
</tr>
<tr>
<td>8</td>
<td><em>Hemiphacidium</em>-like ascomycete, <em>Hendersonia pinicola, Lophodermium nitens</em> (on fallen needles in duff), <em>Leptostroma</em> sp., <em>Cryocaligula</em> sp.</td>
</tr>
<tr>
<td>9</td>
<td><em>Lophodermium nitens</em> (on fallen needles in duff)</td>
</tr>
<tr>
<td>10</td>
<td><em>Bifusella</em>-like ascomycete, <em>Meloderma desmazieri, Lophodermium nitens</em> (on fallen needles in duff)</td>
</tr>
<tr>
<td>11</td>
<td><em>Hendersonia pinicola, Cryocaligula</em> sp., <em>Septoria</em> sp.</td>
</tr>
<tr>
<td>12</td>
<td><em>Lophodermium nitens</em> (on fallen needles in duff), <em>Septoria</em> sp.</td>
</tr>
<tr>
<td>13</td>
<td><em>Bifusella</em>-like ascomycete, <em>Hendersonia pinicola, Leptostroma</em> sp., <em>Truncatella truncata</em></td>
</tr>
<tr>
<td>14</td>
<td><em>Bifusella</em>-like ascomycete</td>
</tr>
<tr>
<td>15</td>
<td><em>Bifusella</em>-like ascomycete, <em>Lophodermium nitens</em> (on fallen needles in duff), <em>Leptostroma</em> sp.</td>
</tr>
<tr>
<td>16</td>
<td><em>Bifusella</em>-like ascomycete, <em>Hendersonia pinicola, Septoria</em> sp., <em>Truncatella truncata</em></td>
</tr>
<tr>
<td>18</td>
<td><em>Bifusella</em>-like ascomycete, <em>Hendersonia pinicola, Leptostroma</em> sp., <em>Septoria</em> sp.</td>
</tr>
</tbody>
</table>
APPENDIX C.
SCHEDULES USED IN MICROSCOPY

Tertiary butyl alcohol dehydration schedule.

<table>
<thead>
<tr>
<th>Time</th>
<th>Solution (recipes to make 100 ml)</th>
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</thead>
<tbody>
<tr>
<td>&gt;2 hours</td>
<td>50% total alcohol (50 ml distilled water, 40 ml 95% alcohol, 10 ml tertiary butyl alcohol)</td>
</tr>
<tr>
<td>overnight</td>
<td>70% total alcohol (30 ml distilled water, 50 ml 95% alcohol, 20 ml tertiary butyl alcohol)</td>
</tr>
<tr>
<td>1 hour</td>
<td>85% total alcohol (15 ml distilled water, 50 ml 95% alcohol, 35 ml tertiary butyl alcohol)</td>
</tr>
<tr>
<td>1 hour</td>
<td>95% total alcohol (45 ml 95% alcohol, 55 ml tertiary butyl alcohol)</td>
</tr>
<tr>
<td>1 hour</td>
<td>100% total alcohol (75 ml tertiary butyl alcohol, 25 ml absolute alcohol)</td>
</tr>
<tr>
<td>1 hour</td>
<td>pure tertiary butyl alcohol</td>
</tr>
<tr>
<td>1 hour</td>
<td>pure tertiary butyl alcohol</td>
</tr>
<tr>
<td>overnight</td>
<td>pure tertiary butyl alcohol</td>
</tr>
<tr>
<td>&gt;1 hour</td>
<td>50 ml tertiary butyl alcohol, 50 ml paraffin oil</td>
</tr>
</tbody>
</table>

Orseillin BB and aniline blue staining schedule

<table>
<thead>
<tr>
<th>Time</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>xylene</td>
</tr>
<tr>
<td>5 min</td>
<td>xylene</td>
</tr>
<tr>
<td>5 min</td>
<td>xylene</td>
</tr>
<tr>
<td>5 min</td>
<td>50:50 Xylene:absolute alcohol</td>
</tr>
<tr>
<td>5 min</td>
<td>absolute alcohol</td>
</tr>
<tr>
<td>5 min</td>
<td>95% alcohol</td>
</tr>
<tr>
<td>5 min</td>
<td>70% alcohol</td>
</tr>
<tr>
<td>5 min</td>
<td>50% alcohol</td>
</tr>
<tr>
<td>24 hours</td>
<td>Orseillin BB: 1 g in 100 ml 3% aqueous acetic acid</td>
</tr>
<tr>
<td>rinse</td>
<td>50% alcohol</td>
</tr>
<tr>
<td>rinse</td>
<td>70% alcohol</td>
</tr>
<tr>
<td>10 min</td>
<td>Aniline blue: 0.5 g in 100 ml 90% alcohol</td>
</tr>
<tr>
<td>1 min</td>
<td>95% alcohol</td>
</tr>
<tr>
<td>1 min</td>
<td>acid alcohol: 1 ml conc. HCl in 50 ml 95% alcohol</td>
</tr>
<tr>
<td>10 min</td>
<td>absolute alcohol</td>
</tr>
<tr>
<td>30 min</td>
<td>clove oil</td>
</tr>
<tr>
<td>24 hours</td>
<td>xylene</td>
</tr>
</tbody>
</table>