Needle Cast of European Larch Caused by *Mycosphaerella laricina* in Wisconsin and Iowa

R. F. PATTON, Professor, and R. N. SPEAR, Specialist, Department of Plant Pathology, University of Wisconsin, Madison 53706

ABSTRACT

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A disease that results in premature defoliation has appeared recently on European larch (*Larix decidua*) in plantations in Wisconsin, Iowa, and one location in Michigan. The causal fungus is *Mycosphaerella laricina*, heretofore known only on larch in Europe. Symptoms are described, the life cycle is reviewed, and the morphology of the fungus is discussed, especially in comparison with species on larch from Japan and Europe.

In the spring of 1980, Allen Prey, Forest Pathologist for the Wisconsin Department of Natural Resources, called our attention to a needle cast disease in plantations of European larch (*Larix decidua* Mill.) in the Coulee Experimental Forest, La Crosse County, in southwestern Wisconsin. According to Prey (*personal communication*), European larch had been intermittently defoliated, presumably by the same disease, in this area for at least 10 yr. Recently, there has been considerable interest in the possible use of European larch for planting in the Lake States, especially as an alternate for red pine (*Pinus resinosa*). Because of this interest and the possible serious consequences of repeated defoliations by the disease, a Pest Alert (9) was issued by the U.S. Forest Service.

The fungus on collections of diseased needles from Wisconsin was compared with that on needle specimens from Japan and Switzerland through observations of its anatomy and morphology. Spore sizes, based on measurements of 25–50 spores in a lactophenol mount, were obtained for ascospores from single collections from Japan, Switzerland, and Wisconsin. Conidial measurements were made from at least three collections each from Switzerland and Wisconsin. From these examinations, we believe the causal fungus of the needle cast of European larch in Wisconsin and Iowa plantations is *Mycosphaerella laricina* (Hart.) Neg., which is reputed to cause severe defoliation of European and Japanese larch in Europe (8). This article, the preliminary report (7), and the abstract by Ostry et al (6) are the first reports of *M. laricina* on European larch in the United States.

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SYMPTOMS

Symptoms appear during summer (from about mid-June) and continue into autumn (as late as October) (Fig. 1). Areas of various size irregularly distributed from the base to the tip of the needle become yellow, then tinged with brown, and finally, entirely brown and necrotic. Borders of lesions, especially in the early stages, are not sharply defined, and discoloration gradually intergrades with normal green tissue. Several small, discolored areas may eventually coalesce into one larger area. The distal portion of the needle often becomes necrotic while the basal portion is still green. The infection causes premature defoliation, often while much of the needle remains green. As the season progresses, symptoms (both on individual needles and on the tree crown) intensify as a result of secondary cycles of infection. The lower portion of the crown is affected first, and if infection is intense, eventually all or most of the crown loses its needles as early as August. Some shoots that lose needles early in the season refoliate, and needles of this second crop also are subject to infection. Infection occurs both on single needles of long shoots and on needles clustered on the short shoots.

Black conidiomata begin to erupt through the epidermis of either the abaxial or adaxial surface in many of the affected portions of a needle soon after the first appearance of brownish discoloration. These usually occur in irregular groupings of a few to about 25 in a necrotic section (Fig. 2E) but occasionally may be scattered over the length of an entirely necrotic needle. The pustules are more or less globose but sometimes are somewhat elongate.

Hosts and occurrence. In a survey for this disease conducted by the U.S. Forest

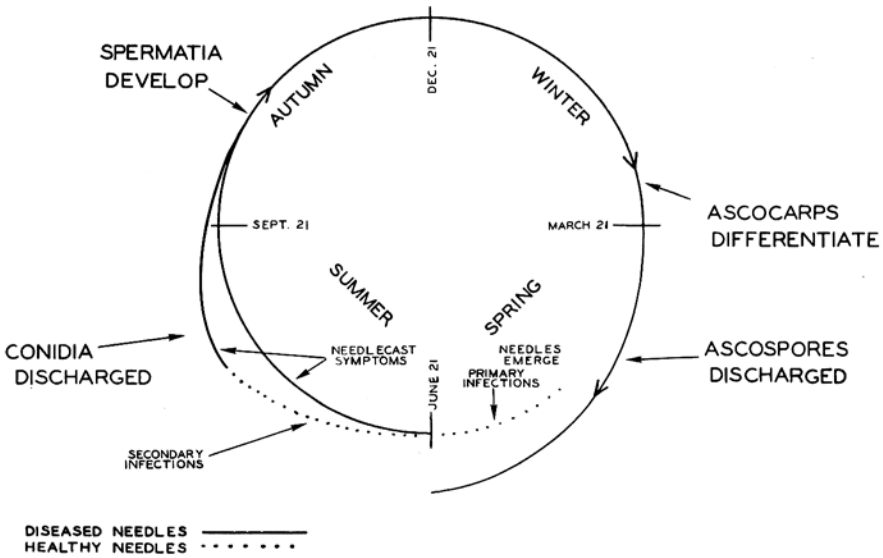

Fig. 1. Chart illustrating the approximate time for major stages in the disease cycle of larch needle cast and in the life cycle of the causal fungus, *Mycosphaerella laricina*

Service in the summer of 1981 (10), larch plantations in northeastern Iowa, southeastern Michigan, and western Wisconsin were examined. Of 63 stands, the disease was found in 28 of 42 stands of European larch, in 2 of 4 blocks of hybrid larch (*L.* × *eurolepis*), but in none of the 14 plantings of Japanese larch (*L. leptolepis* (Sieb. & Succ.) Gord.) or 2 stands of tamarack (*L. laricina* (Du Roi) K. Koch). In a mixed plantation, only European larch showed symptoms and the hybrid and Japanese larches were not affected. The disease was found in only one stand of 14 surveyed in four areas in Michigan.

In surveys by personnel of the Wisconsin Department of Natural Resources in 1981, the disease was also found on European larch stands in two additional counties in Wisconsin and in seedlings of European larch at Wilson State Nursery, Boscobel, WI (Wisconsin Forest Pest Situation Report, 1981, *unpublished*). In September 1982, we identified the fungus (from the conidial stage only) in a collection of needles made by Allen Prey from an ornamental European larch tree in Black River Falls, WI, and it was observed on *Larix* sp. (presumably European larch) in Oneida County, WI, by M. Palmer and K. Robbins of the U.S. Forest Service. These findings bring the number of Wisconsin counties in which the fungus was known to be present at the end of 1982 to six.

LIFE CYCLE AND MORPHOLOGY

The life cycle of the fungus and the disease cycle are illustrated in Figure 1 as modified from a chart originally prepared by Robbins (10). The primary infection is initiated by ascospores, which are released probably from April through June in Wisconsin from perithecia produced in overwintered fallen needles. Conidiomata (acervuli) appear in June and continue to develop throughout the season. Secondary cycles of infection develop throughout the summer and early autumn from conidial infections. After the first symptoms appear, all stages of symptom development can be seen until the normal period for needle-fall in autumn. Spermagonia develop in fallen needles during autumn and are mature by December or possibly sooner. Perithecia develop on fallen needles in late winter and early spring. In a collection from La Crosse County on 23 March 1981, ascocarps were immature but mature ascospores were seen in overwintered needles collected in May and June 1980. Ascospores have been collected on spore traps in Wisconsin plantations as early as April (T. Nicholls, U.S. Forest Service, *personal communication*).

Conidia. Acervuli emerged by breaking through the epidermis (Fig. 3A) of both abaxial and adaxial surfaces in necrotic portions of the needle at or adjacent to stomata. A layer of somewhat bottle-shaped conidiophores with truncate tips (Figs. 2D and 3A,B) developed on the surface of the cushionlike mass of hyphae that formed the base of an acervulus.

Conidia were bacillar to slightly curved or allantoid, sometimes aseptate when dislodged, but generally 1- to 4-septate with 3 septa most common (Fig. 2A–C). The spores were hyaline, rounded at the tip, and truncate at the base where they were cut off from the conidiophore. In a collection from July 1980, conidia measured 2–4 × 25–46 μm, with an average length of about 37 μm. The conidia were loosely attached, and when mature, they were easily dislodged in fluid mounts. We suggest that the conidial state fits well in the genus *Cercoseptoria* Petr. (1) but have made no attempt to assign a specific epithet to the anamorph.

Spermagonia. Spermagonia arose in stromatic pustules similar to those that produced conidia. It is possible that spermagonia developed from the same pustules that earlier produced conidia, but this conjecture is not based on developmental studies. The stromata in which the spermagonia arose varied from barely erumpent to almost entirely exposed on the surface of the needle, as in the case of acervuli. The cavity varied in shape from globose to oval or pyriform and occupied the bulk of the stroma. Occasionally, two spermagonial cavities occurred in a single stroma. Spores presumed because of their small size to be spermatia were discharged through an ostiole at the top. The spermatia were bacillar to pyriform, about 0.5 × 1–3 μm, and often filled the spermagonial cavity.

Spermagonia also formed readily in culture. They occurred at the margins of (and embedded in) the rather dense

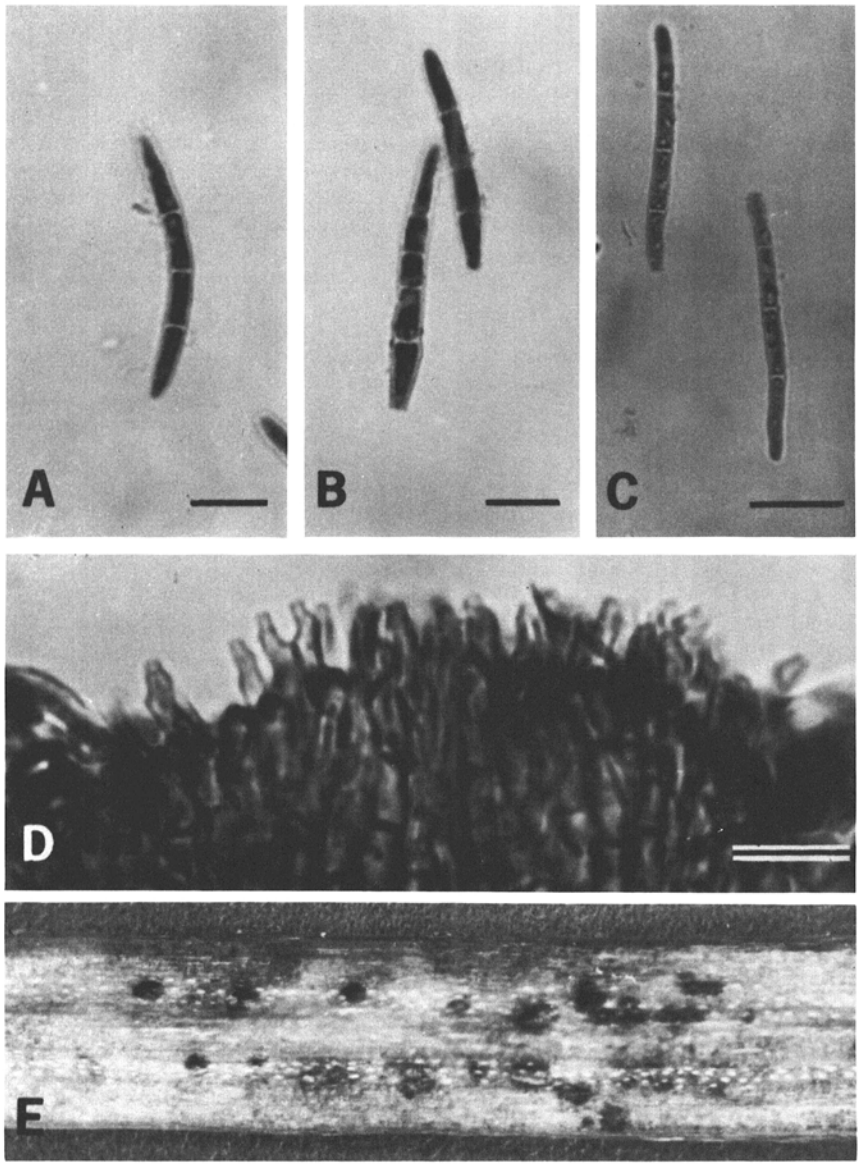

Fig. 2. Conidial stage of *Mycosphaerella laricina* on needles of European larch collected in Wisconsin. **(A–C)** Conidia, **(D)** longitudinal section of acervulus showing conidiophores with typical truncate tips, and **(E)** group of conidiomata on the abaxial surface of a necrotic portion of a diseased European larch needle. Scale bars = 10 μm.

mound of mycelium that typically formed in the slowly growing culture. They were similar in shape and size to those formed in stromata in the needles and were delimited by a definite wall-like layer of slightly darkened pseudoparenchyma-like hyphae.

Perithecia. During the winter, perithecia developed in black stromatic pustules, which may be the same as those that produced spermagonia. The stromata were erumpent, often in groups like the conidial pustules, more or less globose to slightly elongated or elliptical, almost entirely submerged to almost entirely superficial, and often in irregular lines on both halves of the needle. The perithecial cavities were globose to pyriform. In a March 1981 collection, several empty cavities were seen, presumably empty spermagonia. In the same collection, there were perithecial cavities with granular contents and occasional oblong saclike structures, presumably immature asci. In collections made in late May 1980, mature asci and ascospores were present in perithecia. On longitudinal sections of perithecial stromata, masses of ascospores were sometimes present on the surface around the ostiole. Asci in every perithecium were in various stages of development, from those containing mature ascospores to some that were merely sacs with nondifferentiated cytoplasm.

Ascospores were produced in asci that averaged about 46 μm long and ranged from about 36 to 58 μm. The spores were hyaline, 1-septate, not constricted at the septum, with cells of equal size, generally elliptical but ranging from fusiform-elliptical to elliptic-fusiform, straight or sometimes slightly curved, averaging 12.8 μm long and ranging from about 11 to 15 μm (Fig. 4).

Cultural characteristics. Cultures were made both from diseased needle tissue and from single ascospores cast from perithecia and grown under temperature (about 21 C) and light (diffuse daylight plus fluorescent illumination) conditions usually encountered in the laboratory. The fungus grew slowly and ceased radial growth on potato-dextrose agar or malt agar in a petri dish after reaching a colony size of 1–2 cm in about 3 wk. The culture formed a dense, compact mound with a very narrow margin of appressed mycelium. Aerial mycelium was greenish gray, sometimes containing white patches, in time becoming black with occasional patches of white fluffy aerial mycelium. The margins sometimes became greenish black to black and appressed.

Spermagonia were embedded in the marginal regions of the mound of mycelium but covered with aerial mycelium and not readily apparent on the surface of the colony. Conidia were formed on simple conidiophores throughout the aerial mycelium, but no stromatic masses similar to those on needles ever appeared in our cultures. Conidia were easily dislodged and satellite colonies sometimes became established around the parent colony.

Growth of single conidia from spore suspensions distributed over an agar surface was observed. Germination of the end cells was common and additional germ tubes often grew from other cells of the spore; as many as four germ tubes were common. Secondary conidia formed readily from the germ tubes or growing hyphae and these in turn sometimes germinated, most commonly by germ tube growth from the terminal cell of a conidium formed on the agar surface. Conidiophores were not distinctive and usually looked merely like hyphal branches of various lengths up to the length of a conidium. Some mounts from a mound of mycelium formed in a plate culture showed clumps or branching groups of swollen cells, some of which terminated in a simple conidiophore with one of the swollen cells as its basal portion. This type was not seen, however, in young conidial cultures forming secondary conidia on the agar surface.

Comparison with European and Japanese specimens. *Collection and culture of the Japanese species, M. larici-leptolepis.* A portion of the type specimen of *M. larici-leptolepis* K. Ito & K. Sato collected on 24 May 1954 at Kamabuchi, Yamagata, Japan, was examined from squash mounts of black stromata pustules on needles and from sections of needles embedded in plastic. No conidia were observed because this species has been reported to have no conidial form (5). This, or course, is one major difference between the Wisconsin fungus and the Japanese species. The

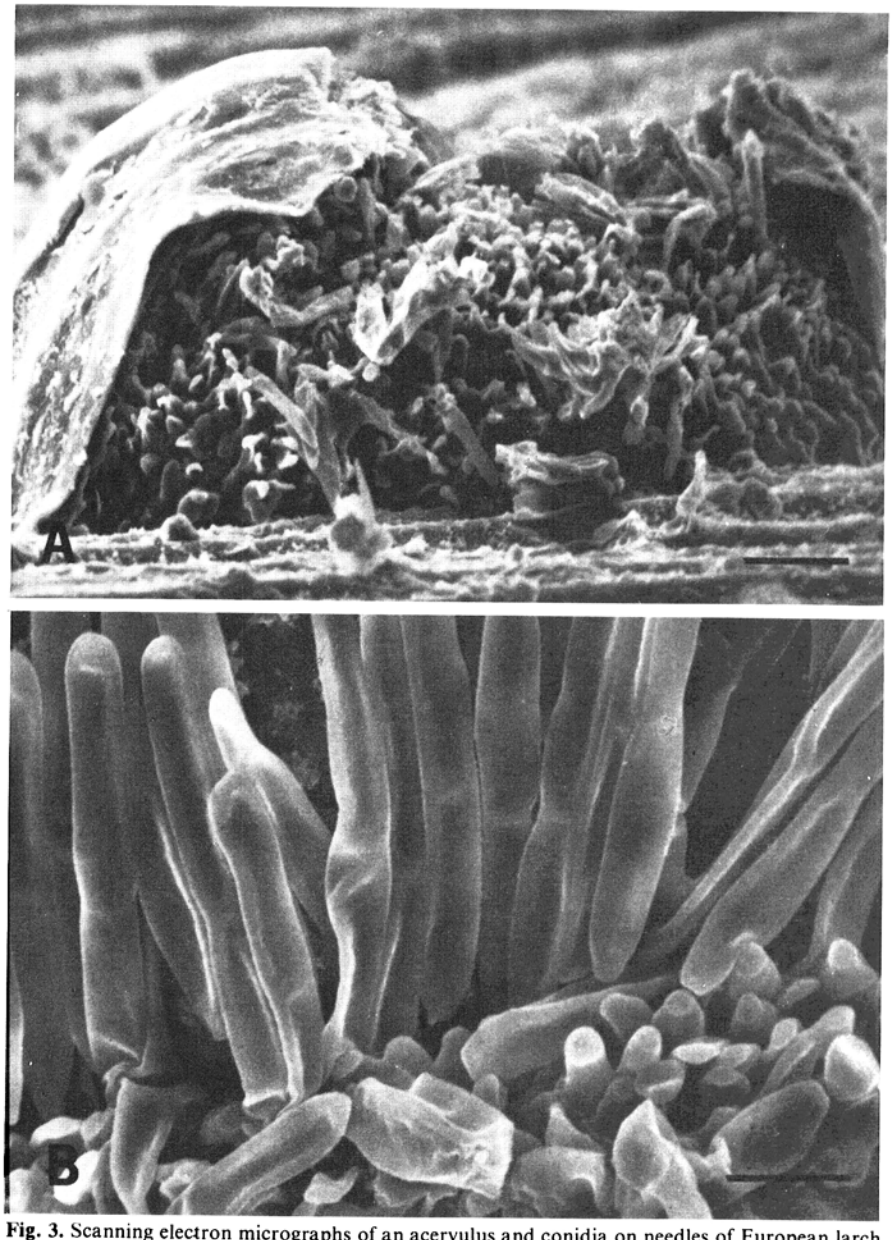

Fig. 3. Scanning electron micrographs of an acervulus and conidia on needles of European larch collected in Switzerland. **(A)** Acervulus erupting through the epidermis. Scale bar = 100 μm. **(B)** Conidia and tips of conidiophores. Scale bar = 5 μm.

other major difference is in the shape of the ascospores. The two-celled ascospores in the Japanese species were generally oblong-elliptical, often constricted at the septum, and the top cell often slightly wider than the bottom cell (Fig. 5C,D). They were less pointed at the ends than the spores in our collections. The spores in this sample averaged 13.8 μm and ranged from 12.5 to 15 μm long. Many spores in the perithecia were still immature and had not formed septa.

A culture of *M. larici-leptolepis* isolated 19 July 1968 in Japan was also compared with our isolates. In general, the appearance of the fungus in culture was similar to our cultures, and no special characteristics could be pinpointed that would consistently serve to differentiate between the two species. No conidia were formed by the Japanese isolate, but conidial formation was lacking to abundant in our isolates.

Collections and culture of the European species, M. laricina. Several collections of needles of European larch bearing *M. laricina* were examined. These represented collections from five areas in Switzerland made in May, July, September, October, and November from 1942 to 1952. Observations were made of squash mounts and sections of needles embedded in paraffin. Also, comparative observations of conidial pustules on Swiss and Wisconsin collections, both made in July, were made with the scanning electron microscope (SEM).

All stages in the life cycle of *M. laricina* were observed on the Swiss collections: acervuli and conidia, spermagonia containing spermatia, and perithecia with asci and ascospores. Conidia were similar to those from Wisconsin in shape and varied from 1- to 3-septate, although many had only one septum. We judged that the collections were perhaps less mature than ours from Wisconsin. The average length of conidia from several collections ranged from 27.2 to 34.6 μm, somewhat shorter than ours, but most spores with three septa were 35–37.5 μm long and more closely approximated the average length of the Wisconsin conidia. Conidia with fewer septa tended to be shorter. Conidial pustules and the associated spores of both Wisconsin and Swiss collections (Fig. 3) looked very similar under the SEM, and representatives of the two groups could not be distinguished from one another. Ascospores (Fig. 5A,B) averaged 12.4 μm long (range 9–15 μm) and could not be visually distinguished from Wisconsin ascospores.

A culture of *M. laricina* was obtained from the Centralbureau voor Schimmelcultures, Baarn. This had been isolated from a collection near Zurich, Switzerland, and had been sent to Baarn in 1952 by Dr. E. Müller. Because the fungus had been in culture so long, we doubted that it would produce conidia, and we found no conidia in any of our subcultures of this isolate. Otherwise, cultural characteristics were similar to those of Wisconsin isolates. Indeed, all of the cultures, including the Japanese isolate, were similar to one another, and there was enough variability among subcultures, even of a single isolate, that we were not confident about using cultural characteristics for differentiation of the isolates or species.

DISCUSSION

Practically all the information we have encountered on *M. laricina* goes back to Hartig's original paper (2) in which he first described the fungus (as *Sphaerella laricina*) and the disease it caused on European larch. On the basis of our comparisons with the collections from Switzerland and the close similarities of the disease and the fungus in the Wisconsin collections to the descriptions by Hartig, we believe our fungus is the European species, *M. laricina.*

When and how the fungus was introduced into the Midwest is unknown. Robbins (10) suggests that it was spread in this region on infected nursery stock, and this suggestion was strengthened when the fungus was found on seedlings

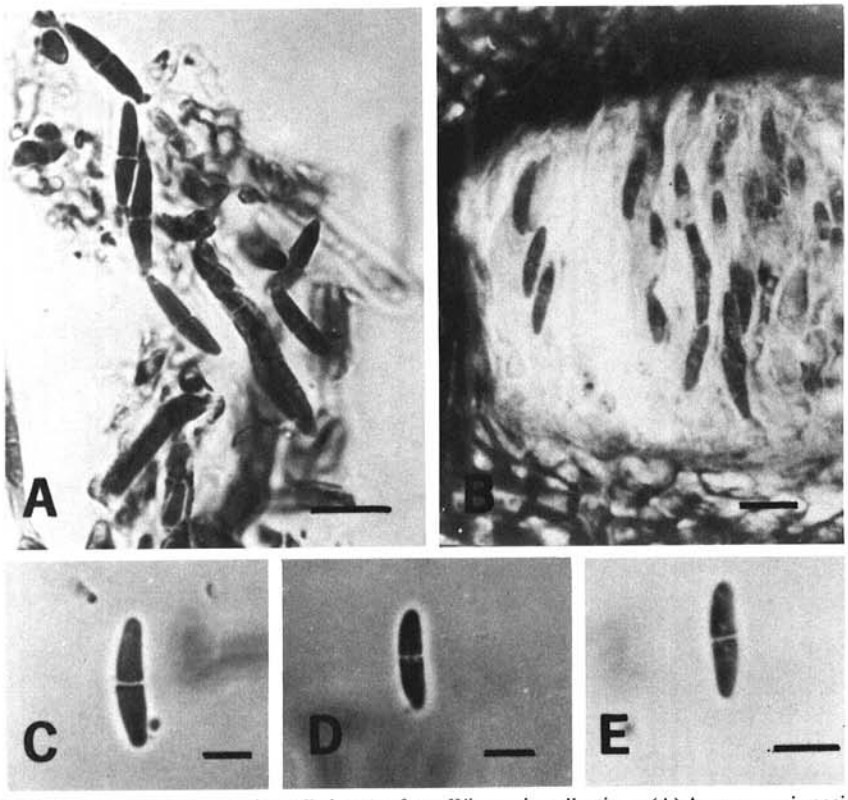

Fig. 4. Ascospores of *Mycosphaerella laricina* from Wisconsin collections. **(A)** Ascospores in asci in a mount of crushed perithecium. Scale bar = 10 μm. **(B)** Ascospores in asci in a longitudinal section of a perithecium. Scale bar = 10 μm. **(C–E)** Ascospores. Scale bar = 5 μm.

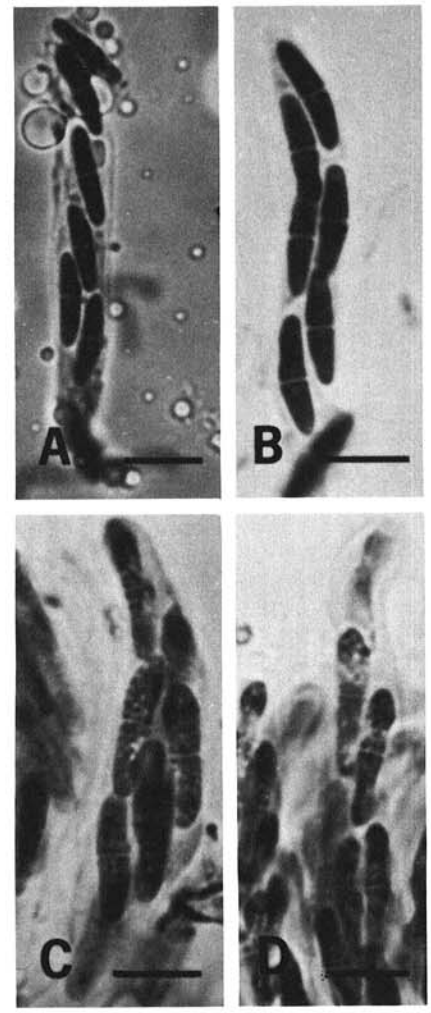

Fig. 5. (A and B) Ascospores of *Mycosphaerella laricina* in asci from perithecia in needles of European larch from Switzerland. **(C and D)** Ascospores of *M. larici-leptolepis* in asci from perithecia of the type specimen in needles of Japanese larch from Japan. Scale bars = 10 μm.

in the Wilson State Forest Nursery at Boscobel, WI. Moreover, plantations containing infected European larch in at least three Wisconsin counties were established from stock that originated at the Wilson Nursery. It seems likely that this pathogen was originally introduced into the United States on infected nursery stock, then became established and subsequently spread further on various outplantings of nursery stock.

European larch is the main host, and according to Robbins (10), severity of the disease in the plantations she surveyed varied with the seed source. Trees from Alpine sources (Austrian, French, and Italian) in her survey were most susceptible and trees from more northern provenances (Czech and Polish) were less susceptible.

Susceptibility of Japanese larch to *M. laricina* appears to be less than that of European larch. In the two areas in Iowa where the disease was present on both European larch and the hybrid, *L.* × *eurolepis*, the hybrid larch was more resistant (10). Trees in 14 Japanese larch stands did not show symptoms. Hartig (2) originally indicated that Japanese larch was not susceptible, even in artificial inoculation attempts. The next year, however, Hartig (3) did report success after artificial inoculation in a greenhouse.

Although none of the trees in two tamarack stands in Michigan in Robbins' survey (10) were infected, these stands were not near known sources of inoculum and the relative susceptibility of this species could not be judged. Thus, the major concern about the impact of this fungus has been primarily with its impact on European larch in plantations in the Midwest, but we must also be aware that we know essentially nothing about the susceptibility of either our native eastern larch or western larch to this pathogen.

Larch needle cast disease caused by *M. larici-leptolepis* is one of the most important diseases of larch forests in Japan, primarily on Japanese larch (4). In comparative tests (11), however, differences in susceptibility among several species were observed and both European larch (*L. decidua*) and tamarack (*L. laricina*) were highly susceptible. Perhaps, this serves as still another reason for caution in the planting of European larch, even though the Japanese fungus is not known to be present in this country.

The growth impact of the disease caused by *M. laricina* on European larch plantations in the United States remains to be determined. Preliminary indications reported by Robbins (10) that it could be severe were tempered by the possibility that growth differences among affected trees were also influenced by other pests and genetic characteristics. The importance accorded to larch needle cast disease in Japan (4) is an indication that the disease caused by *M. laricina* also might cause considerable growth loss in exotic plantations. The ability of *M. laricina* to produce conidia and cause secondary cycles of infection throughout the growing season gives it potential for causing even more damage than the Japanese fungus. If this has not happened in Europe, it may be because there are mitigating influences in its native habitat that make it less damaging than it might be on its native host in a new and different environment.

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