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# A Needle Blight of Austrian Pine

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Blighted needles of *Pinus nigra* var. *austriaca* with areas of original infection apparent in various parts of the needles. In this, the fall, stage, there often is a droplet of resin near the base of the lesion, and only the entostroma of the fungus is present.

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# A Needle Blight of Austrian Pine

ROBERT L. HULBARY*†

OVER a period of years, blighted needles of ornamental pines have constituted an important part of the diseased evergreen samples received by our laboratory for diagnosis. Needle blight is a well-known and widespread type of pine injury. It has been attributed to such environmental factors as frost, heat, intense sunlight, wind burning and drought, alone and in various combinations. Also, it has been attributed to the attack of certain fungi which have been classified chiefly in the genus *Septoria*. In the case of many of the samples submitted to us, however, it has been impossible to attribute the injury to any obvious cause or, when a fungus was present, to assign this fungus accurately to any described form.

Late in the fall of 1938, badly blighted needles of *Pinus nigra* Arn. var. *austriaca* Aschers. & Graebn. were obtained in northern Illinois. Superficially these needles appeared to have been injured by insects, for some of them seemed to have been punctured and there was oozing of resin near the bases of the blighted regions. Microscopic examination of transverse sections of these needles revealed, however, that the punctures were small epidermal ruptures due to the development of young fungous stromata. Since none of these stromata were mature, a method of wintering the infected material was devised, so that the needles could be examined periodically and the development of the fungus studied in detail.

## The Method

Twelve lots of infected needles, each lot consisting of 50 fascicles and including at least 10 badly blighted fascicles, were placed in as many test tubes. The open ends of the test tubes were covered with cheesecloth, and each tube was labeled according to a predetermined order of examination. The test tubes were then laid on the ground, in a well-drained grassy spot, so as to be subject to out-of-door winter weather. They were tilted slightly to allow water to drain out of them rather than collect in them.

Beginning with December 23, 1938, one test tube of needles was examined every 15 days. Beginning with March 10, 1939, the interval between examinations was shortened to 10 days and, beginning with April 5, to 5 days, in order to follow in detail the rapid development of the fungus, which accompanied warm spring weather.

Also, cultures of the fungus were made on artificial media, but they failed to produce spore-bearing structures.

For the purpose of hastening development at certain times, diseased needles which had been weathered in test tubes for various periods were placed over moistened filter paper in sterile Petri dishes and kept at laboratory temperature. Conidiospores were produced by well-developed stromata in 4 to 5 days under these conditions.

## Development of the Fungus

The first evidence of infection visible on the needle is a small brown or tan region up to 10 mm. long, which in a few days involves all sides of the needle, is sharply delimited basally and fades distally into the normal green of the leaf (frontispiece). Such a region may develop anywhere on the needle; usually, however, it develops on the distal half. Often, if an infected needle is held before a light, the

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Fig. 1.—Cross sectional view in the mesophyll in the region of a lesion, showing how the mycelium of the causal fungus grows within the mesophyll cells and brings partial disintegration of these cells.

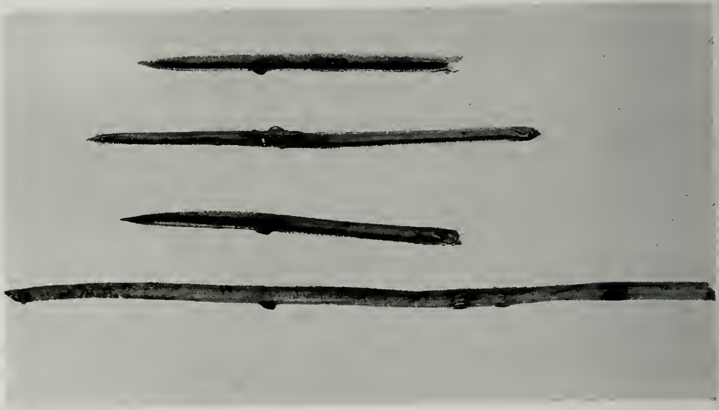

Fig. 2.—Fragments of infected needles after overwintering. Stromata appear in various stages of development. Not until the ectostroma is fully developed, as on the needle at the bottom of the illustration, do locules form and spores begin to be produced.

infected region will appear translucent.

The effect of the mycelium within a needle is evidently to cut off the flow of nutrients, or at least to weaken it, for the part of the needle distal to the infection soon dies. In the blighted part of a needle, hyphae can be found in abundance in and between the mesophyll cells, fig. 1; but they occur only rarely in the transfusion tissue and have not been found in the vascular bundles. Although a fruiting body may appear at any point in the dead portion of a needle, it usually develops near the site of infection.

Under the out-of-door conditions described above, rapid enlargement of stromata began to take place on infected leaves between February 15 and 28, fig. 2. Between April 10 and 15, differentiated regions appeared in the upper parts of these stromata, in which the hyphae were lighter amber than elsewhere in the stromata. A month later these lighter regions had been transformed into locules, contained conidiophores and were producing conidia. Overwintered needles brought into the laboratory from time to time during May had mature, sporulating, ruptured stromata.

## Morphology of the Fungus

The stroma of this needle-blighting fungus arises between the mesophyll and the hypodermis, fig. 3, and is seated on the mesophyll. The basal part of it is a pseudoparenchyma, from which hyphae push up between the hypodermal cells, usually dissolving them at the same time. These hyphae spread out laterally below the epidermis and form a compact, pseudoparenchymatic entostroma. Often the entostroma becomes extensive and has attachments to the mycelium in the mesophyll at several points. This multiple anchorage is suggestive of the dothideaceous family Polystomellaceae (Stevens 1925). Growth of the stroma, produced by the elongation of the stromatic hyphae, causes the epidermis above the stroma to split longitudinally, usually along a row of stomates. As the stroma emerges, it may

**Fig. 3.**—Section through two mature stromata. Origin of the stroma at the periphery of the mesophyll, dissolution of the hypodermis, variable extent of the entostroma, palisadelike arrangement of ectostromatic hyphae, poorly defined but mature and sporiferous locules, and adherent fragments of epidermis are evident.

rip off sections of epidermis lying between rows of stomates; and these epidermal fragments usually remain attached to the top of the stroma.

The size and shape of the ectostroma are correlated with the vigor and extent of the hypostromatic mycelium, the thickness of the host cuticle and epidermis, and the ratio, as to volume of stromatic tissue, of ectostroma to entostroma. There naturally is, therefore, much variation.

The ectostroma ranges from 125 to 1,500 $\mu$ long, from 50 to 450 $\mu$ wide and up to 600 $\mu$ high. Usually it is elongated and loaf shaped, and its height often exceeds its width. It is nearly always oriented parallel to the longitudinal axis of the needle, fig. 2. The tips of stromatic hyphae, as they grow outward to form the ectostroma, secrete a gelatinous fluid. On the surface of the mature stroma this fluid hardens into a crusty, dark brown coating; within the stroma it cements together the walls of adjacent filaments.

The ectostroma consists of brown hyphae with numerous cross-walls. These hyphae are vertical, parallel and closely applied to each other. In microscopic view, they present the palisadelike appearance characteristic of the Dothideaceae. Cells of the stromatic hyphae are somewhat smaller than the cells of the hyphae in the mesophyll and transfusion tissues and are also darker.

The first indication of the development of sporiferous locules is a localized change in the color of hyphae in the upper part of the ectostroma. This change, which may occur in one to several places in each stroma, fig. 3, results in the formation of an ovate to tubular chamber which lies parallel to the longitudinal axis of the ectostroma. The tissue surrounding the locule is continuous with that of the stroma, and no distinct locular wall is developed. The continuity of tissue from stroma to locule wall also indicates relationship with the Dothideaceae. The locule is sporiferous around its entire inner surface.

Conidiophores arise directly as branches from the palisadelike hyphae forming the body of the stroma, and their bases form the poorly defined boundary of the locule. They are light amber to hyaline, unbranched, very numerous, densely packed and almost as long as the conidia but slightly narrower. They cut off from their tips hyaline, septate, scoleciform spores which are blunt at the ends, straight or slightly curved and, in ratio of length to width, never greater than 10 to 1.

When the stroma is mature, a rift occurs along the top of each locule, which provides for the emission of spores. Spore dissemination is evidently aided by wind and by spattering raindrops.

## Taxonomy

A careful study has been made of all descriptions and, when possible, of exsiccati types or authentic specimens of the species which might resemble the Austrian pine fungus.

The majority of fungi associated with needle blight in various parts of the country have been placed in the genus *Septoria.* Spaulding (1909) reported *Septoria spadicea* Patterson & Charles as the cause, in the East, of a blight of white pine needles similar to the brown-spot of longleaf pine. Graves (1914) associated *Ascochyta piniperda* Lind. (*Septoria parasitica* Hartig) with a blight occurring on young shoots of red and Norway spruce in North Carolina. Dearness (1928) described *Septoria pinicola* on blighted needles of the lower limbs of *Pinus virginiana* Mill. in Virginia. Hedgecock (1929) and Siggers (1932, 1934) studied *Septoria acicola* (Thüm.) Sacc. in connection with brown-spot on longleaf pine needles in the southeastern part of the United States and considered it a distinct threat to seedling plantations in that region. Boyce (1938, p. 98) has pointed out that brown-spot ranges westward to Kansas and Texas and occurs in Idaho on ponderosa pine and in Oregon on knobcone pine.

The taxonomy of *Septoria acicola,* the cause of brown-spot, has been given attention by several men; but it still is a moot question.* At least two diseases, perhaps more, have been included under the term Septoria needle blight and attributed

*After the manuscript of this article was submitted for publication, Paul V. Siggers defined the brown-spot disease, confirming the binomial *Lecanosticta acicola* (Thüm.) Syd. for the imperfect stage of the fungus associated with the disease and proposing the binomial *Scirrhia acicola* (Dearn.) Siggers for the perfect stage (Phytopath. 29(12): 1076–7. 1939.). Later still, Frederick A. Wolf and W. J. Barbour also confirmed the binomial *Lecanosticta acicola* but considered the perfect state a *Systremma* and proposed for it the binomial *S. acicola* (Dearn.) Wolf & Barbour (Phytopath. 31(1):61–74. 1941.).

to the brown-spot fungus. Sporiferous chambers contained in the top of a highly developed stroma such as that produced by the Austrian pine fungus are, however, conformable neither with the estromatic pycnidium of a typical *Septoria* nor with the non-septorioid structure of the brown-spot fungus.

Of Sydow's genus *Hemidothis* there are two species; both occur in Venezuela on the leaves of shrubby species of the melastomaceous genus *Miconia* Ruiz & Pav. From Sydows' (1916) original description and the exsiccati it is clear that the Austrian pine needle fungus cannot be assigned to *Hemidothis*, for in that genus the locules are partially liberated at the vertex, the stromata are arranged in concentric rings in the infected areas, and the conidia are much longer and narrower than in the pine fungus and are non-septate. Despite these points of difference, the dothideaceous structure of the stroma in both forms suggests that they may be related.

Clements & Shear (1931, p. 367) list *Septocyta* Petrak as a synonym of *Hemidothis* Sydow. Petrak's (1927) description of *Septocyta* indicates distinct similarities with the Austrian pine fungus; but the spores of *Septocyta* are long, threadlike and continuous. In *Septocyta* a dothideaceous relationship is suggested by the structure of the stroma and conidiferous chambers.

Sydow & Petrak (1922) described a fungus associated with brown spots on pine needles in Arkansas and Oregon, which they later (1924) called *Lecanosticta acicola* (Thüm.) Sydow. According to Sydow's description, this fungus has a rim of hairlike appendages on its stroma, dark spores and branched conidiophores—characters markedly different from those of the Austrian pine fungus.

Hansborough (1936), in his paper on the Tympanis canker of red pine, has illustrations of the imperfect stage of *Tympanis*, which in arrangement of locules and in internal structure somewhat resemble the stromata of the Austrian pine fungus. He reports, however, that in *Tympanis* the conidiophores are branched and that the spores measure 2–4 × 1–2 μ and are borne at the tips and on lateral branches of the conidiophores. The fact that the needle blight fungus on Austrian pine, which is distinct generically as well as specifically from all previously described needle-blighting fungi, cannot be placed in any recognized genus warrants the proposal of a suitable genus. This genus is placed in the scolecosporous division of the Phomaceae and is closely associated therein with genera such as *Hemidothis* Sydow and *Septocyta* Petrak, which exhibit dothideaceous characteristics.

### *Dothistroma* gen. nov.

Stroma dark, elongated, endogenous, becoming prominently erumpent and swollen, dothideoid, with a stalk extending into the substratum; composed internally of densely arranged, vertical, parallel, septate hyphae. Locules separate, one to several in the upper part of the stroma, ovate to tubular, not distinguished from the surrounding stroma, the entire inner face sporiferous. Conidiophores simple, arising directly from stromatic hyphae. Conidia hyaline, scoleciform, several-septate.

Stroma atratum, elongatum, endogenum, mox multo exsertum et bullatum, dothideoideum, cum pede in substrato extante, intus hypharum compactarum, verticalium, parallelarum compositum. Loculi sejuncti, unus vel plures in parte superiore stromatis, ovati usque tubulosi, non stromate circumdante distincti, in facie interna omnino sporiferi. Conidiophora simplicia, ex hyphis stromatum directe orienta. Conidia hyalina, scoleciforma, nonnulla-septata.

### *Dothistroma Pini* sp. nov.

Stromata seated on the mesophyll, forming more or less extensive hypostromata between mesophyll and hypodermis, erumpent through rents in the epidermis, oriented parallel to the longitudinal axis of the needle, dull dark brown, 125–1500 μ long, 50–450 μ wide, up to 600 μ high. Locules oriented parallel to the longitudinal axis of the stroma, without a distinct wall. Conidia hyaline, scoleciform, 1- to 5- but usually 3-septate, blunt at the ends, straight or slightly curved, 16.5–29 × 3.5 μ. Conidiophores numerous, approximately the same size as the conidia, hyaline or amber, dense, unbranched, producing conidia at their tips.

Stromatibus in mesophyllo stantibus, inter mesophyllum et hypodermidem hypostromatem plus minus magnum formantibus, per scissos in epidermide erumpentibus, cum axe longitudinali foliorum parallelis, atrofuscis, 125–1500 μ longis, usque 600 μ altis; loculis cum axe longitudinali stromatum parallelis; conidiis hyalinis, scoleciformibus, 1– usque 5– frequentissime 3–septatis, apicibus rotundatis, rectis vel curvulis, 16.5–29 x 3.5 μ; conidiophoris numerosis, conidiis subaequantibus, hyalinis vel succineis, compactis, simplicibus, conidios in apicibus generantibus.

Type specimen: Collected by J. C. Carter, De Kalb County, Illinois, November 29, 1938, on *Pinus nigra* Arn. var. *austriaca* Aschers. & Graebn., Ill. Nat. Hist. Surv. Acc. No. 27,093.

Through the courtesy of Dr. Paul V. Siggers, the following specimens have been examined and found conspecific with the above: (1) on *Pinus flexilis* James, Waterloo State Forest, Ohio, February 26, 1936, C. C. Green col.; (2) on *P. nigra* Arn. var. *austriaca* Aschers. & Graebn., Springfield, Ohio, May 8, 1932, R. L. Beard col.; (3) Miami, Oklahoma, August, 1934, Moore col.; (4) Sherman Nursery, Charles City, Iowa, January, 1934, B. C. Helmick col.; (5) on *P. nigra* var. *calabrica* Schneid., Waterloo State Forest, Ohio, January 11, 1936, C. C. Green col.; (6) on *P. resinosa* Ait., Old Man's Grove, Hocking County, Ohio, February 26, 1936, C. C. Green col.

## Summary

In blighted needles of Austrian pine collected in northern Illinois in the fall of 1938, immature stromata indicated the cause of the blight. Infected needles were wintered out-of-doors and examined periodically. The stromata remained quiescent through the winter but very early in the spring began to develop and by March 1 had emerged as strongly erumpent, loaf-shaped structures. A month and a half later, pycnidial locules were becoming differentiated, and by May 15 conidia were being produced.

The distinctive dothideaceous structure of the stroma distinguished the fungus from every described group. For it the new genus *Dothistroma* is proposed.

The well-marked dothideaceous structure of the stroma and the spore characters place the new fungus in the scolecosporous group of the Phomaceae close to *Hemidothis* Sydow and *Septocyta* Petrak.

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