A NEEDLE BEAM MODEL FOR ORBITAL ELECTRONS

CAUSED BY DISTRIBUTED IMPULSES AND CONCENTRATED IMPULSES

By

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Bachelor of Science

Arizona State College

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A NEEDLE BLIGHT OF ORNAMENTAL PINES

CAUSED BY DOTHISTROMA PINI

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Introduction

For several years a needle blight disease has been known to be present and to be affecting ornamental pines in north central Oklahoma. During the 1950-51 season this disease was particularly serious, almost completely defoliating the trees. It has been found primarily affecting Western yellow pine (Pinus ponderosa Laws) and Austrian pine (P. nigra Arn. var. austriaca A. & G.). According to reliable reports and the diseased specimens received in our laboratories, the disease is common in Oklahoma and in several neighboring States. In Oklahoma diseased specimens from Ponca City, Bartlesville, Oklahoma City, Tulsa, and Stillwater were collected and examined.

The first signs of infection appear as small circular spots on the needle. The spots are yellow or tan in color, two to three millimeters in diameter, and are sharply delimited by the normal green of the needle. Gradually the spots turn brown and a girdling of the needle is effected. A single needle may have several such brown regions giving the needle a banded appearance. The distal portion of the infected needles become chlorotic and eventually necrotic. Throughout the winter, spring, and early summer the basal portions of infected, brown tipped needles remain green. Toward the end of August and extending through September and October these basal portions of infected needles become necrotic and the dead needles fall from the tree (Fig. 1).

Typically the disease first appears on the needles of the lower-most branches. Trees in this condition appear as though the lower needles had been scorched or burned. The disease appears to progress gradually upward through the trees as a result of repeated seasons of infection. The foliage of such trees is sparse because only the current seasons needles remain on the tree. Needles formed in previous seasons, that normally should remain

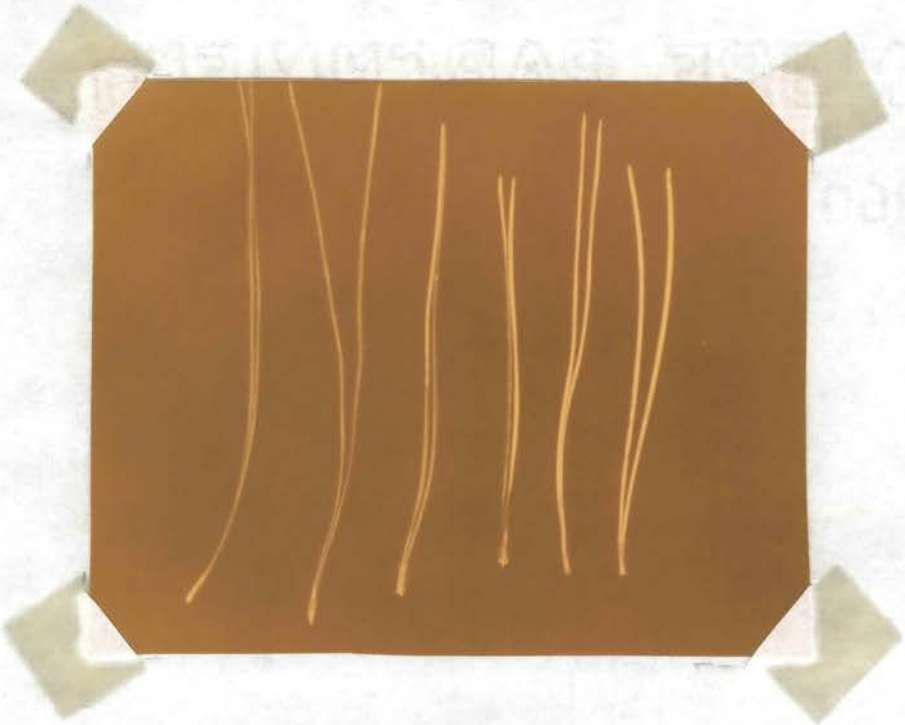

Fig. 1. Showing the extent of spotting and the stages in the development of the needle blight disease. At the left is a healthy needle followed to the right by a lightly spotted needle, a heavily spotted needle, a needle partially blighted and those which are completely blighted.

on the tree for about three years, drop during their second summer due to the effects of the disease. On many infected trees that have apparently been defoliated for several consecutive seasons, the needles are abnormally short. This is probably due to lack of food reserves resulting from reduced photosynthetic activity during the growing season.

Preliminary microscopic examinations of diseased needles showed fungus mycelium in the mesophyll tissue of the needles but no sporulating structures. Consequently the identification of the causal organism could not be definitely determined.

The present study was organized to: (1) Identify, if possible, the fungus or fungi causing the needle blighting, (2) to isolate and study these fungi in pure culture and (3) to attempt control measures in the field.

Literature Review

Since the diseased needles collected during the fall of 1950 lacked fruiting structures upon which to base identification, a literature review of all of the needle inhabiting fungi was instigated. Pine needle blights were found to be caused by several fungi as well as a variety of environmental conditions.

Spaulding (12) reported Septoria spadicea Patterson and Charles, occurring in the eastern United States and causing a white pine needle blight similar to the brown spot disease of longleaf pine.

Waterman (13) reported Sphaeropsis ellisi Sacc. as causing the stunting of new growth and a browning of pine needles. Small black pycnidia occurred at the base of the blighted needles, particularly within the leaf sheath. Mycelium grew down into the twigs which usually died back to the first nodes where cankers often formed. Various other names have been assigned to the causal fungus of this disease, most of which are in the genera Sphaeropsis or Diplodia.

Various needle cast diseases of conifers are caused by a number of different but closely related Ascomycetes. These fungi are easily recognized by their elongated, dark, sometimes glossy black hysterothecia.

The brown spot needle blight caused by Scirrhia acicola (Dearn.) Sig. is a destructive needle disease of longleaf pine (Pinus palustris Miel.). The disease also occurs on 23 other species of pines but is not considered important on these species. "Brown spot" occurs in the South throughout the general range of longleaf pine.

The imperfect stage of the brown spot fungus was first described by De Thumen in 1878 (11) as Cryptosporium acicolum (Thum.). Sydow in 1922 (11), unaware of earlier descriptions by De Thumen and Saccardo, described

Lecanosticta pini Syd. as the causal fungus. Later he changed the name to Lecanosticta acicola (Thum.) Syd.

In 1939, Siggers reported the relationship between the conidial stage Lecanosticta acicola and the ascomycete Oligostroma acicola Dearn. He then transferred the perfect stage to Scirrhia acicola (Dearn.) Sig. as one of the subepidermal, hyaline spored Phyllachoraceae.

Inconsistencies exist in the descriptive literature of the brown spot fungus. This fungus is characterized by olivaceous conidia produced from stromata which are subepidermal and covered at maturity except for a linear slit. The lesional needle area associated with a stroma is always brown at maturity. In several instances fungi which produced hyaline spores in highly erumpent stromata had been classified as the brown spot fungus. The lesional areas immediately surrounding these stromatic fruiting structures usually turned a distinctive reddish color at maturity.

Siggers (11) describes the probable reason for the confusion of these two separate fungi. A collection of infected Ponderosa pine needles made by Shattuck at Crofino, Idaho had hyaline conidia and reddish lesions. In 1920, this fungus was described by Saccardo as Actinothyrium marginatum Sacc. Later, Sydow examined specimens from Shattuck's collection and grouped this fungus with the brown spot fungus because both formed linear stromata, dothideaceous in structure, with acicular spores.

Hedgcock (5) referred to the "red spot" disease of pines as associated with Septoria acicola (Lecanosticta acicola). Later, Hedgcock published in favor of the common term "brown spot". Luttrel (7) described reddish brown spots associated with Scirrhia acicola on Ponderosa pine in Missouri.

Siggers (11) examined specimens from the mycological collections of the Bureau of Plant Industry, Soils, and Agricultural Engineering, which were

classified as Lecanosticta acicola or synonyms thereof. He found nine collections which were untenable as Lecanosticta acicola under the description originally established by Sydow. Most of these specimens were either on P. ponderosa or P. nigra var. austriaca and included the Sattuck collection as well as one of Hedgcock's collections. These specimens had highly erumpent stromata, hyaline spores and a reddish cast in the lesional areas.

Hulbary (6) described Dothistroma pini Hul. as the fungus causing a needle blight of Austrian pine (Pinus nigra Arn. var. austriaca A. & G.) in northern Illinois. This fungus was described as having a highly erumpent dothideaceous stroma and hyaline spores. Hulbary found spore development occurring only in the spring, the fungus existed the remainder of the season in the mycelial stage within the mesophyll of needle tissue.

Following Hulbary's description of D. pini, Siggers sent Hulbary six of the collections which he had found not fitting the description of Lecanosticta acicola. Hulbary identified these as being infected with D. pini, the fungus he had described on Austrian pine in northern Illinois. The six collections were on Pinus flexillis James, Waterloo State Forest, Ohio; P. nigra Arn. var. austriaca A. & G., Springfield, Ohio, Miami, Oklahoma, and Charles City, Iowa; P. nigra var. calabrica Schneid., Waterloo State Forest, Ohio; and P. resinosa Ait., Hocking County, Ohio.

As mentioned before, when the present study was instigated, no sporulating structures were found on the diseased needles. From the general appearance of the spots it appeared that the disease causing trouble in Oklahoma most closely resembled that described by Hulbary as a needle blight of pines caused by D. pini Hul.

Materials and Methods

Identification and developmental studies

Because sporulating fruiting structures were not present on the diseased needles when the investigation began, attempts were made to induce sporulation of the causal organism. Diseased needle collections from various parts of the State were set out to overwinter under natural conditions, but where they could be periodically examined.

Fifty needles from each collection were placed between two 5" X 6" pieces of window screen. The screen pieces were crisscrossed and the projecting ends bent back to hold the pieces together. Each "cage" was tagged with an aluminum label and pegged to the ground with heavy wires stuck through the screen and into the ground.

Isolations and growth studies

During the fall and winter periods, when conidia were not being produced on the needles, the fungus was isolated by tissue fragment isolations. Fresh material was always available from a number of sources. Needle sections two to three millimeters in length were placed in approximately one percent sodium hypochlorite solution for one to two minutes, then rinsed in sterile distilled water, and placed in sterile potato dextrose agar plates. Sections of tissue cut from diseased needles varied from pieces which involved only necrotic areas near the site of infection to those adjacent but below the infection points yet still green. The cutting and handling instruments used were frequently dipped in 95 percent alcohol and flamed.

While potato dextrose agar was used in most isolation studies, pine needle decoction agar, prune agar, nutrient agar and corn meal agar were also

included in the growth studies. The ingredients of these culture media were as follows:

Potato dextrose agar:

17 grams	agar
20 grams	dextrose
200 grams	sliced potatoes
1000 cc.	distilled water

Pine needle decoction agar:

17 grams	agar
20 grams	pine needle juice
1000 cc.	distilled water

Prune agar:

20 grams	agar
40 grams	prunes
1000 cc.	distilled water

Nutrient agar:

17 grams	agar
10 grams	peptone
3 grams	beef extract
1000 cc.	distilled water

Corn meal agar:

17 grams	agar
20 grams	corn meal
1000 cc.	distilled water

At the first signs of conidial development, diseased needles were placed in moist chambers consisting of damp filter paper in petri plates. These were kept in an incubator with a controlled temperature of 31° C. This procedure hastened the maturation of conidia which were used for single spore isolations. After four or five days in the moist chamber the larger stromatic structures containing mature conidia were picked from the infected needles, placed between sterile glass slides and crushed. The crushed fungus tissue was then rubbed gently over the surface of four percent water agar in petri plates. Single conidia were picked from the water agar surface using finely

drawn glass needles and a 90X stereoscopic microscope, and placed in sterile potato dextrose agar plates. The glass needles were kept sterile by quickly dipping in a beaker of boiling water. All isolations were made in an inoculation chamber.

The effect of temperature upon the growth of the fungus on various culture media were studied. To each petri plate containing one of the five culture media, a loopful of a conidial and mycelial suspension in sterile water was added. This suspension was prepared by breaking up, in a sterile water blank, pieces of culture that had been growing on potato dextrose agar. The tube of inoculum was well shaken between each transfer so as to give as nearly as possible an even distribution of conidia.

The temperatures at which the cultures were incubated for the growth studies were 16°, 21°, 26°, 31°, and 35° C. All pH determinations of the culture media were made on a Beckman pH meter.

Examination of diseased material

To follow the development of the sporulating structures of the fungus, diseased needles were sectioned at weekly intervals for microscopic examination. The sectioning was done by hand with a razor blade on a block of soft wood. The sections were placed in a drop of mounting fluid (two percent potassium acetate 300 ml., glycerin 120 ml., and 95 percent ethyl alcohol 180 ml.) on a slide and gently heated to evaporate the alcohol. A small piece of glycerin jelly (gelatin seven grams, distilled water 42 ml., glycerin 49 ml., and phenol crystals one gram) was placed on the drop and heated gently until it melted. As it melted a cover slip was placed on top so the melting materials spread out under the cover slip. After cooling the mounts were ringed with Canada balsam to prevent drying.

In culture Dothistroma pini forms a relatively hard, black, compact colony which does not spread out over the surface of the culture medium, but instead tends to rise above this surface by the formation of successive layers of stromatic tissue. These stromatic tissue colonies were sectioned and examined to study conidial development. To prepare the fungus culture material for sectioning, an F.A.A. solution (formalin, alcohol, and acetic acid) was used as the killing and fixing agent. The tissue was then run through a butyl alcohol-toluene series, and embedded in paraffin. Sectioning was done on a rotary microtome set at 15 microns. The tissue was not stained.

Inoculation studies

In preperation for inoculation experiments with Dothistroma pini two-year old Pinus nigra var. austriaca seedlings were obtained from the Oklahoma A. & M. College Forestry nursery and transplanted into four inch pots in the greenhouse. The temperature in the greenhouse was maintained between 60° and 70° F.

In preparing the conidial inoculum, one to two month old cultures were used. The fungus cultures were placed in a Waring blendor with 250 cc. of water and macerated for two minutes. By spraying the inoculum on a slide it was found to contain an average of 100 to 125 conidia per low power field of the microscope. The conidiel inoculum was sprayed on the healthy needles with a small atomizer. After the foliage was well covered with the inoculum, water-proof plastic bags containing moistened paper towels were slipped over the inoculated foliage and tied securely to the stem. Over this a brown peper bag was placed to try to avoid excessive heat build up within the plastic bag. After 48 hours the bags were removed.

Attempts at reproducing the needle blight disease were also made by

laying badly diseased needles, which were sporulating, among the healthy needles inside the plastic bag. The same procedure of covering the inoculated foliage with bags to maintain high moisture conditions was followed as with the conidial suspension inoculations. The bags in this experiment, however, were allowed to remain on the inoculated branches and seedlings for from one to three weeks before removal.

Inoculation trials using these methods were made both on the potted seedlings and on healthy branch terminals on larger trees growing in the field.

Control studies

In early April a microscopic examination of diseased needles revealed signs of conidial development, so the spray control program was started April 13, 1951. Five plots of trees consisting of either P. nigra var. austrica, P. ponderosa, or both, were sprayed at approximately two week intervals from April 13th to July 15th. Plot 1, located at the south end of the Hazen estate, one mile north of Stillwater, contained 45 mixed Ponderosa and Austrian pine trees ranging from five to 15 years of age. Plot 2, located near the center of the Hazen estate, contained 47 Ponderosa pine trees approximately ten to 15 years old. Plot 3, located at the north end of the Hazen estate, contained 72 Ponderosa and Austrian pine trees approximately five to 15 years old. Plot 4, located along the road through the west farm of Oklahoma A. & M. College, approximately one mile west of Stillwater, contained 76 Ponderosa pine trees approximatoly 15 to 18 feet tall. Plot 5, situated in a nursery row on the west farm of Oklahoma A. & M. College contained 18 Austrian pine trees 15 to 18 feet tall. All of the sprayed trees showed varying degrees of previous season infection with Dothistroma pini.

Within the plots, sections were set up by evaluating the severity of the preceding season disease on individual trees. Each tree was classified as to severity of disease by over-all sight estimations. The trees were then grouped into sections so as to include, as evenly as possible, the same amount of disease in each section.

The spray materials used were Fermate (76 percent ferric dimethyl dithiocarbamate) manufactured by E. I. du Pont de Nemours & Co., Welmington, Del., Puritized Agricultural Spray (5 percent Phenyl mercuri triethanol ammonium lactate) manufactured by Gallowhur Chemical Corp., New York, New York, and Bordeaux mixture (8-8-100). Fermate was applied at the rate of 1 1/2 lbs. per 100 gals. of water and Puratized at the rate of 1 1/2 pints per 100 gals. of water.

A 150 gal. capacity John Bean sprayer was used throughout the program. This equipment operated at 400 to 450 lbs. pressure, and the spray gun could be regulated to give a concentrated stream or a fine mist.

Results

Identification and developmental studies

Identification of the fungus causing this needle blight of ornamental pines was based on morphological characters of the developing fruiting structures as studied in cross sections through diseased needles. A weekly examination of diseased needles which had been set out to weather, and those which were still attached to the trees, was made starting February 15, 1951. Infected needles still partly green and attached to the trees revealed the first visible signs of fungus fruiting structures about March 1st. These appeared as small swellings usually in the center of discolored lesions. Cross sections through this area showed the swellings to be immature stromata originating in the hypodermal tissue of the needles; each stroma was composed of compact hyphae in a palisade-like arrangement. Growth of the stromata seemed to be by outward, uniform extension of the individual hyphae. By March 15th the stromatic tissue had increased in size until it forced the host epidermal tissue to rupture. This host tissue characteristically remained attached to the outer surface of the stroma, possibly indicating a sticky consistency of the stromatic tissue. By March 22nd the cells at the outer margins of many of the stromata had begun to darken and to develop thickened walls.

The first signs of locule formation within the body of the stromata were observed on April 5th. By April 12th the locules showed signs of conidial development. During this time, while the stromata were continually enlarging, the outer margins became progressively darker as though an uneven crust were forming as a covering for the inner locules. Conidial numbers increased progressively in the already formed locules. By May 3rd the outer stromatic

surfaces were found beginning to rupture, liberating the numerous conidia which lined the entire inner surface of the locules (Fig. 2).

Although the time of locule formation and conidial development and release was similar in most diseased needles, some stromata did not develop as fast as others. However, it is estimated that conidial numbers were at a high peak two or three months after the stromata were found rupturing through the epidermis. At this time the conidia were hyaline, straight to ellantoid, characteristically three septate, and 14 to 24 microns X 3 to 3.5 microns.

Abundant conidia were still present on July 5th but by July 20th had disappeared from most of the locules. Although the number of conidia in the locules declined progressively throughout July, a relatively few conidia remained or were newly produced in the locules. These remaining conidia were no longer hyaline but had turned a distinctive olivaceous color. This color change may be an indication of the physiological maturity of the conidia. Further evidence on this point is presented in the isolation studies.

With the further decline of conidial numbers through August, the needle lesions began to change from brown to a reddish color, and the palisade-like body of the stromata began to disintegrate into an unorganized mass of thick-walled, reddish-colored cells. Actively sporulating stromatic locules were found in some of the needles sectioned in late August. These instances were few and it is probable that these late maturing stromata were relatively unimportant in the over-all disease picture. These stromata could, however, produce enough conidia to force an extension of the spray period and thus complicate the control measures.

The important characters upon which Hulbary (6) based the description of Dothistroma pini Hul. were (1) the origin of the stromata in the hypodermal area of diseased needles; (2) their highly erumpent nature at maturity;

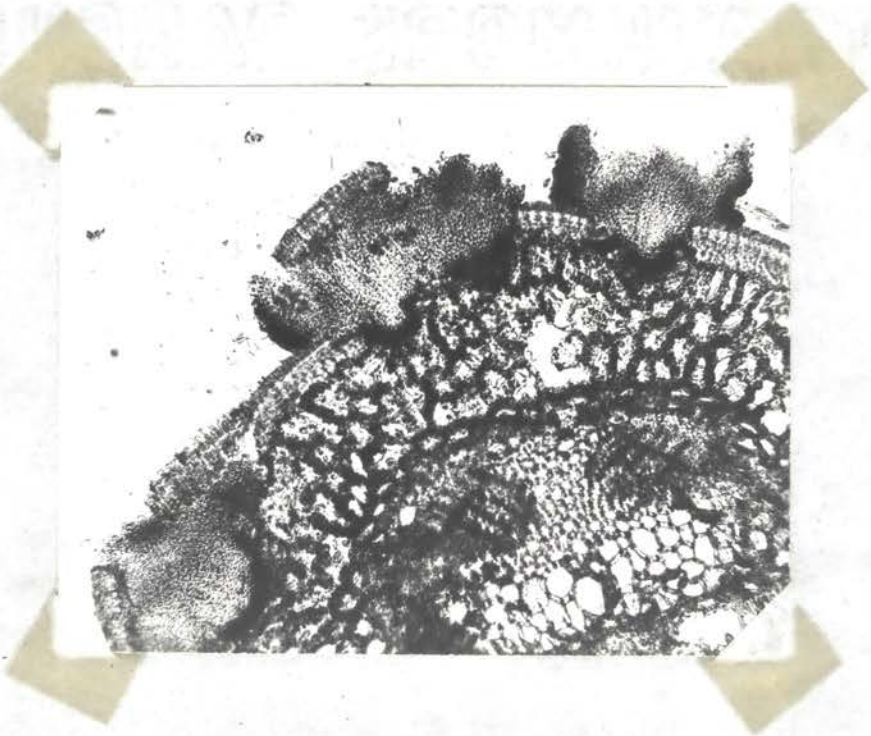

Fig. 2. A cross section through three stromata showing their highly erumpent nature, their sporiferous locules and the characteristic clinging of the host epidermal tissue to the outer surface of the stromata.

(3) the dothideaceous structure of the stromata with their light brown septate hyphae forming palisade-like bodies; (4) the darkening or "crusting" of the marginal cells of the stromata; (5) the formation of loculos which become lined over their entire inner surface with hyaline, allantoid conidia. Since these characteristics are identical with those found in this investigation, the present organism was identified as Dothistroma pini Hul.

Identification of D. pini was confirmed by P. V. Siggers from diseased needles which were sent to him. He also confirmed the identification of Scirrhia acicola (Dearn.) Sig. as the causal fungus of a needle spot found on one of the trees under observation in this study.

The development of Dothistroma pini in needles which were collected and set out to weather, as described in the materials and methods, was considerably different from that in diseased needles which remained attached to the tree. Diseased needles were collected in late September and October and "set out" in early November. By March 1st mature, highly erumpent stromata with reddish lesional areas had developed on the detached needles while only small immature stromata were found on all of the attached needles examined. The reddish cast of the lesional areas on attached needles did not become evident until after the peak of conidial production and during the period of apparent decline of the fruiting condition. Sections made through detached needles on March 22nd revealed numerous small locules near the outer margins of the highly erumpent stromata. These small locules were not conidial bearing.

Wolf and Barbour (15) described non-sporiferous locules imbedded in stromata of needles infected with Scirrhia acicola. These locules were of two types, spermogonial and carpogonial, the latter becoming transformed into mature perithecia within six to eight weeks after their formation.

The small, non-sporiferous locules observed in this investigation seemed to be of two morphologically distinct types. One type was spermogonial in character and contained small spermatia-like bodies. The contents of the other locules were never clearly determined. No perfect stage has ever been found in connection with D. pini.

Conidia bearing locules were not formed within the stromata on detached needles except in a few instances. When they were formed the development of these conidia closely paralleled that on attached needles. Toward the beginning of July the large highly erumpent stromata began partial disintegration. Often the inner body of the stroma will completely disappear leaving the outer rim of stroma and locules. By July 20th many of the stromata had almost completely disintegrated with only remnants of the basal portions remaining.

Isolations and growth studies

It was very difficult to isolate D. pini by surface sterilizing diseased needles and placing fragments of diseased tissue in agar plates. In almost every instance Alternaria spp. rapidly covered the culture media. A few attempts, however, were successful and the fungus was isolated in pure culture by this method. Potato dextrose agar was used in most of these isolations.

Except for color, the conidia produced in these cultures were morphologically similar to the conidia produced in the erumpent stromata on diseased needles. The conidia in the culture plates were olivaceous almost from the beginning while conidia produced on the diseased needles were hyaline for several weeks, later turning dark. Single hyaline conidia from diseased needles failed to grow on artificial culture media. Further these hyaline conidia suspended in sterile water in hanging drop slides did not germinate. It is probable that these early-produced conidia, while still hyaline, are

physiologically immature. For this reason diseased needles were incubated at 28° to 30° C. in moist chambers to encourage spore maturation. Four to five days of this treatment caused the erumpent stromatic fungus tissue to increase greatly in size, and the conidia within the inner locules to turn from hyaline to an olivaceous color. These colored conidia were isolated individually and placed in potato dextrose agar plates. After an incubation period of six to seven days at 28° to 30° C., minute mycelial colonies were produced. The small colonies were white to light grey in color and slightly raised. By 14 to 16 days after isolation, the colonies had turned greyish black and had increased to 13 or 14 mm. in diameter. If the young colonies are transferred to bottle slants, with air tight caps and a good supply of culture medium, individual colonies may grow to three or four centimeters in diameter and be raised above the agar surface a centimeter or more. Mature colonies of D. pini are dark black, hemispherical with a rough outer surface. No mycelial growth is visible around the colonies (Fig. 3).

Colonies three to eight weeks old were killed and fixed in F. A. A., run through a butyl alcohol-toluene series and imbedded in paraffin. Microtome sections showed the centers of the colonies to be made up of light colored mycelium covered by a layer of darker stromatic tissue containing numerous pockets of conidia. Conidia produced in culture are straight to allantoid, olivaceous and borne in locule-like depressions similar to the locules found within the stromata on diseased needles. Cultures which grew from single conidial isolations were identical with those colonies produced from diseased tissue isolations.

Hulbary (6), working with D. pini, stated that the cultures of this fungus on artificial media failed to produce spore bearing structures. No description of the fungus colonies or the methods used in isolations appeared in

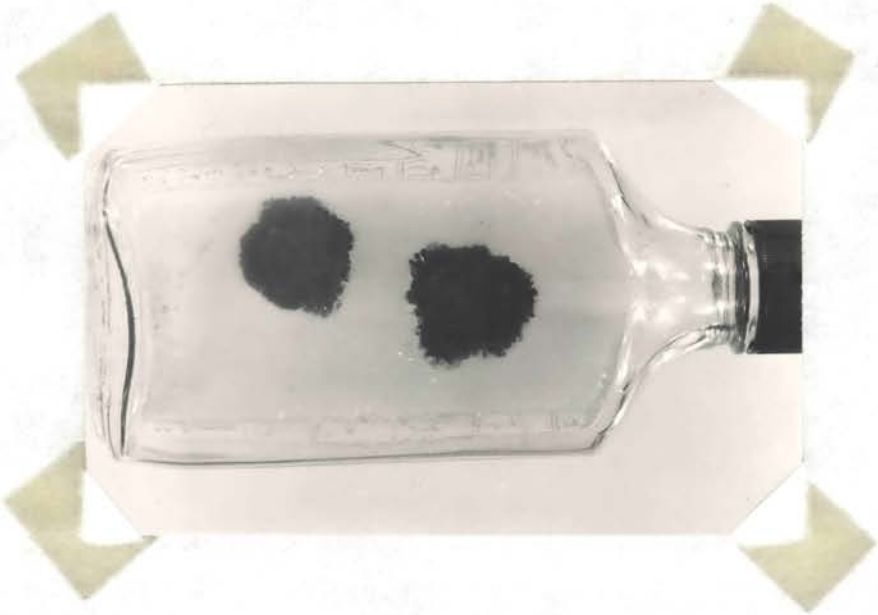

Fig. 3. Potato dextrose agar bottle slant with large, two month old colonies of D. pini. Note how high the upper surface of the colony is raised above the culture medium. No mycelium is visible on the culture medium around the colony.

his publication.

Growth of D. pini on several types of media was studied at temperatures of 16°, 21°, 26°, 31°, and 35° C. The culture media used were potato dextrose agar, prune agar, pine needle decoction agar, corn meal agar, and nutrient agar. These studies revealed an optimum growth at 26° C., satisfactory growth between 21° and 31° C., but no growth at the two extremes of 16° and 35° C. Table 1 shows the approximate temperature range over which D. pini grew on artificial media. Although 26° C. appeared to be near the optimum temperature for growth, there was better growth toward the higher 31° C. level than towards the lower 21° C. At 31° C. the diameter of the colonies, after 21 days incubation, were from 1 1/2 to twice the diameter at 21° C.

Of the culture media used in this experiment, potato dextrose agar, prune agar, and nutrient agar proved to be best for growth of D. pini. According to the diameter measurements of the colonies in table 1, pine needle decoction agar appeared to be equally as good as either prune agar or nutrient agar. However, the colonies produced on this agar were grey in color, closely appressed to the surface of the culture media, and produced few conidia, a condition not at all typical of the colonies on the other media. Growth on corn meal agar was relatively unsatisfactory. The colony diameter never approached that of the colonies on potato dextrose agar, prune agar, or nutrient agar (Table 1). Diameter measurements of the colonies after 21 days incubation indicated that growth was better on potato dextrose agar than on either prune agar or nutrient agar. At the near optimum temperature of 26° C. the average diameter of colonies on potato dextrose agar was 23 mm., approximately twice the growth on prune or nutrient agar which showed 10.5 mm. and 14.5 mm. colonies respectively.

Table 1. Effects of temperature and media on colony size of D. pini after 21 days incubation. Shown as average diameter of colonies in millimeters. Each figure is an average of two trials.

Incubation temperature	Average colony size in millimeters on each of the following media:*				
	P.D.A.	P.A.	P.N.A.	C.M.A.	N.A.
	mm.	mm.	mm.	mm.	mm.
16° C.	0	0	0	0	0
21° C.	10.5	7	4.5	1.75	2
26° C.	23	11	10.5	4**	14.5
31° C.	15.5	11	10**	3**	13
35° C.	0	0	0	0	0

* P.D.A. Potato dextrose agar pH 5.6
P.A. Prune agar pH 4.2
P.N.A. Pine needle decoction agar pH 4.3
C.M.A. Corn meal agar pH 6.2
N.A. Nutrient agar pH 6.6

** One replication contaminated--figures are for only one trial.

Hanging drop preperations of conidia suspended in sterile water were placed in incubators at temperatures of 16°, 21°, 26°, 31°, and 35° C. The conidial suspensions were prepared by crushing small pieces of D. pini cultures in a sterile water blank. This experiment was repeated twice with duplicate slides at each temperature. The slides were checked for germination at approximately 12 hour intervals.

The first evidence of spore germination was seen with a swelling of the conidia (Fig. 4). At 26° to 31° C. swelling conidia were very numerous after 24 hours incubation. Germination did not take place however, until approximately 48 hours after the conidia were placed in the incubation chambers. At 16° and 21° C. some swelling was observed after about 36 hours, but germination did not occur until after approximately 72 hours. At 35° C. no swelling or germination was observed.

The results from the germination studies correlated nicely with the growth studies. Both germination and growth were best at 26° and 31° C. Germination at 21° C. was approximately 24 hours slower than at 26° C., but an equal percentage of spores germinated. At 35° C. no growth occurred, probably due to lack of spore germination. At 16° C. germination of some of the conidia was observed after 72 hours. However, no growth occurred on the agar plates at this temperature, indicating that, while the spores might germinate, growth of the mycelium would be inhibited.

Artificial Inoculation

The first attempts to artificially inoculate healthy pine needles were initiated on April 17th. Needles badly infected with Dothistroma pini were used as the source of fungus inoculum. The diseased needles were laid among the healthy needles of the branch terminals and these terminals were then

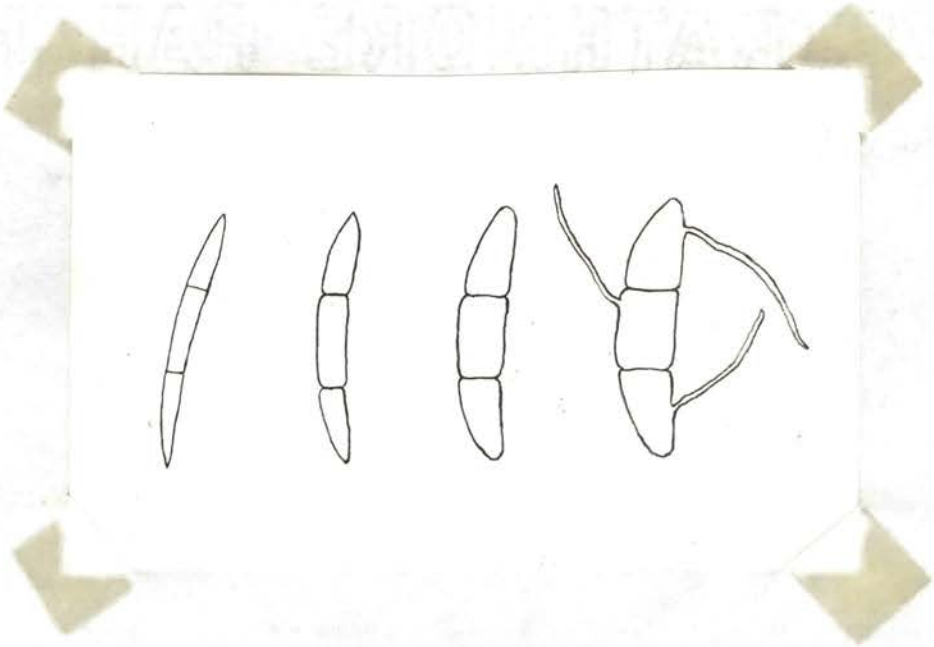

Fig. 4. Stages in the germination of conidia of D. pini. The spore at the left has not yet begun to swell. The two center spores show successive stages in the swelling process and at the right is a germinated conidium.

covered with plastic bags containing moist paper towels. Control terminals were treated similarly except that the diseased needles were not applied.

At the beginning of this experiment the conidia within the locules of the diseased needles were hyaline. With the high humidity within the plastic bags, these conidia matured within four or five days as they did when needles were put in moist chambers. The old diseased needles and the bags were removed after 21 days and all of the needles on the inoculated branch terminals which showed signs of spotting or blighting were counted. Needle infection counts were made on May 8th, 15th, 22nd, July 15th, and August 20th to determine whether or not there would be any development of spot lesions.

Of the seven terminals inoculated five became partially infected. All five were on Ponderosa pine and included terminals 3, 4, 5, 6, and 7 in table 2. The needles which became infected appeared to be only those in contact with the diseased needles. No infection occurred on either of the inoculated Austrian pine terminals. It is possible that some strains of Austrian pine are to some extent resistant to D. pini. In the field it has been observed on several occasions that, where two Austrian pines are growing close together one may be badly infected while the other tree develops few or no diseased needles. The control terminals numbered 8, 9, and 10 showed no signs of infection.

Table 2 shows the number of infected needles per inoculated terminal. In no case did all of the needles on a single terminal become infected, only those which were in contact with the diseased needles. Whether infection on new needles results from direct mycelial contact or by germinating conidia is not known. No increase in the number of spotted needles occurred after the first record was taken on May 8th.

The second set of artificial inoculation trials using diseased needles

Table 2. Showing the number of infected needles per terminal which were inoculated by placing diseased needles among healthy needles. Moist chamber bags were left on for 21 days.

Terminal number	Species of tree	Infected needles per terminal at each of stated dates:				
		5-8-51	5-15-51	5-22-51	7-15-51	8-20-51
		No.	No.	No.	No.	No.
1.	Austrian	0	0	0	0	0
2.	do	0	0	0	0	0
3.	Ponderosa	27	27	27	25	25
4.	do	28	28	28	28	28
5.	do	35	35	35	35	35
6.	do	24	24	24	24	24
7.	do	47	47	47	47	47
8.*	Austrian	0	0	0	0	0
9.*	Ponderosa	0	0	0	0	0
10.*	do	0	0	0	0	0

*Controls

included 12 Ponderosa pine terminals, three of which served as controls. The healthy terminals were inoculated as previously described. The inoculations were made May 8th and the bags were removed at intervals varying from one to three weeks after application. Table 3 shows the number of needles which became infected on each inoculated terminal. As much infection occurred on those terminals on which the bags were allowed to remain for one week as on those which were covered for two or three weeks. This indicates that an exposure period of one week or less is sufficient to produce infection of healthy needles inoculated by this method. Longer periods of enclosure within the water-proof bags only increases the possibility of injury to the terminals. No increase in number of infected needles per terminal occurred after the bags were removed, nor did any of the control terminals show any signs of infection.

Attempts were made to inoculate small Austrian pine seedlings by spreading diseased needles over their foliage. Water-proof plastic bags containing moist paper towels were also used to cover these plants. These potted seedlings were bagged May 8th, nine trees being inoculated and three serving as controls. The bags were removed from four of the potted trees at successive weekly intervals on May 15th, 22nd, and 30th. There was no sign of infection on any of the needles when the bags were removed nor did any develop later.

Conidial and mycelial suspension inoculation trials were made June 1st and June 15th on healthy, mature Ponderosa pine terminals and on potted Austrian pine seedlings. One to two month old cultures of D. pini were macerated in a Waring blendor for two minutes. The resulting suspension was sprayed on the healthy foliage with a small atomizer. The sprayed terminals and seedlings were then covered with the plastic and brown paper bags. The conidia in the suspension were olivaceous and capable of germination as

Table 3. *Pinus ponderosa* terminals inoculated by placing diseased needles on healthy needles inside water-proof plastic bags. Showing the number of infected needles per inoculated terminal. Terminals were inoculated May 8th and bags removed from four terminals at successive weekly intervals on May 15th, 22nd, and 30th.

Date bags removed	Terminal number	Infected needles per terminal at each of stated dates:				
		5-15-51	5-22-51	5-30-51	7-15-51	8-20-51
		No.	No.	No.	No.	No.
5-15-51	11	12	12	12	12	12
5-15-51	14	21	21	21	21	21
5-15-51	17	9	9	9	9	9
5-15-51	22*	0	0	0	0	0
5-22-51	12		11	11	11	11
5-22-51	16		29	29	29	29
5-22-51	18		8	8	8	8
5-22-51	20*		0	0	0	0
5-30-51	13			8	8	8
5-30-51	15			14	14	14
5-30-51	19			10	10	10
5-30-51	21*			0	0	0

*Controls

indicated in hanging drop germination slides. All bags were removed after 48 hours. Of the 20 terminals and 12 seedlings inoculated, none ever showed any positive signs of infection.

Considerable injury to the needles of the Ponderosa pine terminals occurred in all of these later inoculation trials. This injury made infection readings difficult or impossible in most cases and may have been due to excessive heat during the warm days of June. This type of injury did not occur during the earlier inoculation trials in April and May.

Control studies

Because this needle blight disease appeared to be caused by D. pini, fungicide applications for control were started before the study of the identification and seasonal development of the organism were completed. The first signs of conidial development on diseased needles were observed in early April and the spray control program was started April 13th. Bordeaux mixture, Fermate, and Puratized Agricultural Spray were chosen for the first season, as representative types of fungicides. These were applied at the following concentrations: Bordeaux mixture 8-8-100, Fermate 1.5 lbs. per 100 gals. of water, and Puratized 1.5 pints per 100 gals. of water. Du Pont Sticker and Spreader was used with all spray materials at the rate of 1/3 of a pint per 100 gals. of spray. Spray applications were made when possible, at approximately two week intervals.

This spray control work was divided into five plots. Plots 1, 2, and 3 were located on the Hazen estate one mile north of Stillwater and contained 159 trees, mostly Ponderosa pine approximately ten feet tall, but with some scattered Austrian pine seedlings one to three feet tall. Plot 4 was located one mile west of Stillwater on the A. & M. farm and consisted of 76 Ponderosa

pine trees approximately 20 feet tall. Plot 5 was also located on the A. & M. farm but in a nursery row and contained 18 Austrian pine approximately 20 feet tall. Each plot was divided into four sections, one section for each of the spray materials and one for control. Sections were set up by evaluating the severity of the disease from the preceding season as described earlier. As shown in table 4, in plot 1 the trees sprayed with Bordeaux mixture averaged one percent of the new needles spotted, those with Fermate four percent, Puratized Agricultural Spray seven percent, and the unsprayed controls 26 percent. This plot consisted of 45 trees, most of which were Ponderosa pine but with some scattered small Austrian pines. The amount of infection was usually high on these small trees where their foliage was near the ground. The controls in this plot were situated on a hill which was open to prevailing winds which would have considerable drying effect. The trees sprayed with Fermate, Puratized, and Bordeaux mixture were each growing on lower terraces in that order. As a result these trees were less exposed to the drying winds, consequently conditions for infection were probably better.

Table 5 for plot 2 shows that trees sprayed with Bordeaux mixture had four percent infection of the new needles, those sprayed with Fermate two percent, Puratized four percent and the unsprayed controls 40 percent. The 41 trees in this plot were located in a grassy area which was shielded by hardwood trees. The trees in the center of this plot were so enclosed that the area would remain wet for several days after a rain.

The trees in plot 3 (Table 6) sprayed with Bordeaux mixture avereged two percent of the new needles spotted, those sprayed with Fermate 12 percent, with Puratized five percent, and the unsprayed controls 62 percent. This plot was located in an enclosed area and contained both small Austrian pine seedlings and ten feet Ponderosa pines. There was considerable undergrowth

Table 4. Disease control results on spray plot 1. The individual figures show the number of newly infected needles out of 500 needles counted on ten random terminals on each tree. Except for those marked * the trees in this plot were Ponderosa pines.

Tree number	Treatment			
	Bordeaux mixture	Fermate	Puratized	Control
	Number of infected needles in the 500 counted on each tree			
	No.	No.	No.	No.
1.	13	19	22	13
2.	5	4	175*	7
3.	3	11*	5	269*
4.	2	2	4	270*
5.	0*	1	2	206*
6.	12*	1	58*	9
7.	5	0	13	250*
8.	16*	3	30*	180
9.	3	30*	50*	7
10.	5	4	3	205*
11.	0	138*	1	14
12.		2		
Total	54	215	363	1430
Percent Infected	.98%	3.58%	6.60%	26.00%

* Denotes small Austrian pine seedlings.

Table 5. Disease control results on spray plot 2. The individual figures show the number of newly infected needles out of 500 needles counted on ten random terminals on each tree. All of the trees in this plot were Ponderosa pines.

Tree number	Treatment			
	Bordeaux mixture	Fermate	Puratized	Control
	Number of infected needles in the 500 counted on each tree			
	No.	No.	No.	No.
1.	3	3	17	423
2.	64	3	16	328
3.	71	1	14	330
4.	26	3	5	500
5.	24	7	1	252
6.	13	16	101	114
7.	2	10	13	9
8.	2	14	4	11
9.	0	19	5	1
10.	12	8	8	15
11.	3			
Total	220	84	184	1983
Percent Infected	4.00%	1.68%	3.68%	39.66%

of grass which often was as tall as the small trees themselves. Both the section sprayed with Bordeaux mixture and the one sprayed with Fermate contained several of the small Austrian pines. The percentage of spotted needles on the seedlings sprayed with Bordeaux mixture was considerably less than those sprayed with Fermate (Table 6). The controls averaged 62 percent of the needles infected even though all these trees were tall Ponderosa pines. Fermate appeared the least effective of the fungicides in this plot, but it should be noted that eight of the 17 trees in the Fermate sprayed section were small Austrian pines which normally showed a greater degree of infection than the larger Ponderosa pine trees.

Plots 1, 2, and 3 received eight spray applications. The first spray was applied April 14th and the others on each of the following dates: April 28th, May 5th, 12th, 26th, June 9th, 23rd, and July 21st.

Plot 4 was located in a different area and all of these trees were exposed to nearly the same environmental conditions. These trees were sprayed six times: April 26th, May 3rd, 19th, 28th, June 9th, and July 7th. The Puratized was not applied on May 19th because of rain. The results of this plot (Table 7) show that the trees sprayed with Bordeaux mixture developed approximately two percent infection on the new needles. Those sprayed with Fermate four percent, Puratized four percent and the unsprayed controls had 58 percent infected.

Spray plot 5 was adjacent to plot 4 but in a nursery row where wet weather made it impossible to spray during the month of June. Table 8 gives the results from this plot. Bordeaux mixture gave good control, apparently adhering over the wet period of June, in spite of continued washing. Control with Fermate and Puratized was less effective in this plot. This is attributed to their poorer adhesive qualities. The unsprayed controls showed 64

Table 6. Disease control results on spray plot 3. The individual figures show the number of newly infected needles out of 500 needles counted on ten random terminals on each tree. Except for those marked * the trees in this plot were Ponderosa pines.

Tree number	Treatment			
	Bordeaux mixture	Fermate	Puratized	Control
	Number of infected needles in the 500 counted on each tree			
	No.	No.	No.	No.
1.	3	5	1	206
2.	8	6	23	452
3.	4	244*	2	227
4.	2	2	10	169
5.	2	1	4	388
6.	2	10	0	224
7.	1	1	41	253
8.	0	5	7	403
9.	1	17*	2	267
10.	4	35*	13	281
11.	22*	30*	49	178
12.	9*	90*	11	496
13.	3*	148*	4	388
14.	10*	270*	18	335
15.	14*	100*	102	215
16.	5*	22	154	348
17.	24*	20	5	284
18.	17*		5	500
Total	131	1006	451	5616
Percent Infected	1.45%	11.83%	5.01%	62.40%

* Denotes small Austrian pine seedlings.

Table 7. Disease control results on spray plot 4. The individual figures show the number of newly infected needles out of 500 needles counted on ten random terminals on each tree. All of the trees in this plot were Ponderosa pines.

Tree number	Treatment			
	Bordeaux mixture	Fermate	Puratized	Control
	Number of infected needles in the 500 counted on each tree			
	No.	No.	No.	No.
1.	29	12	7	147
2.	29	3	11	34
3.	6	38	25	56
4.	2	10	11	71
5.	4	7	10	28
6.	5	9	32	56
7.	22	3	6	65
8.	8	9	3	56
9.	4	8	12	217
10.	3	13	28	40
11.	4	18	5	70
12.	3	19	141	309
13.	0	8	16	104
14.	10	21	5	232
15.	15	30	18	491
16.	8	36	46	411
17.	6	48	28	461
18.	16	21	2	455
19.	14	69	8	286
Total	188	382	414	3599
Percent Infected	1.97%	4.02%	4.35%	37.88%

Table 8. Disease control results on spray plot 5. The individual figures show the number of newly infected needles out of 500 needles counted on ten random terminals on each tree. All of the trees in this plot were Austrian pines.

Tree number	Treatment			
	Bordeaux mixture	Fermate	Puratized	Control
	Number of infected needles in the 500 counted on each tree			
	No.	No.	No.	No.
1.	11	114	46	500
2.	6	36	121	34
3.	28	43	26	248
4.	18	207	8	500
5.	23		285	
Total	86	400	486	1282
Percent Infected	3.44%	20.0%	19.45%	64.10%

percent of the new needles developing spots.

The control data are summarized in table 9. In all of the plots each of the fungicides used gave some degree of control. In general Bordeaux mixture gave the best control results but both Fermate and Puratized gave sufficient control to recommend their use. There was an abundance of new infection in 1951. The unsprayed controls in the plots ranging from 26 to 64 percent of the new needles showing disease lesions (Table 9).

Table 9. Summary of the spray control data from all of the spray plots. Summarized as the percent of new needles which become infected in each plot. Each figure represents counts of 5000 or more needles on Ponderosa and Austrian pine trees of varying ages.

Plot number	Treatment			
	Bordeaux mixture	Fermate	Puratized	Control
	Percent of new needles infected for each plot			
	percent	percent	percent	percent
1.	.98	3.58	6.60	26.00
2.	4.00	1.68	3.68	39.66
3.	1.45	11.83	5.01	62.40
4.	1.97	4.02	4.35	37.88
5.	3.44	20.00	19.45	64.10
Average percent	2.39	8.22	9.02	46.01

Discussion

In 1941 Hulbary found a needle blight fungus on *P. nigra* var. *austriaca* which he believed to be different from all previously described forms of needle blight fungi. For this fungus Hulbary proposed the name *Dothistroma pini* Hul. Hulbary also identified six collections, sent to him by Siggers, as being infected with *D. pini* not *Scirrhia acicola*.

Siggers (11) states that more than one fungus has been grouped under the common name "brown spot" needle blight of pines. He found several needle collections which had been identified as being infected with the brown spot fungus (*Scirrhia acicola*) to differ in important fundamental characteristics. Since there is a great deal of similarity in the type of symptoms produced by the two fungi, it is understandable why such confusion exists, particularly when they are identified by superficial examination. Although these similarities do exist, there are, however, several outstanding characters by which these organisms may be separated and individually identified.

The basic structures in which the conidial stages of the two fungi are produced is perhaps the most outstanding separable feature. Conidia of *S. acicola* are borne in acervulus-like structures which originate from sub-epidermal stromatic tissue which is covered at maturity except for a linear slit. The epidermal tissue of the host is often bent back exposing the conidial surface. Conidia of *D. pini* are borne in locules of various shapes which originate as lighter areas within an already highly erumpent stroma. Within 20 to 30 days after the first signs of locule formation, the entire inner surface of the locules become sporiferous. It is very common for the host epidermal tissue to remain attached to the outer margins of the stroma. The stroma may be raised 200 to 300 microns above the surrounding host tissue. (Figs. 5 and 6.)

Fig. 5. Cross section of a pine needle infected with D. pini showing the characteristic highly erumpent stromata containing conidial locules and with portions of the host epidermal tissue still adhering to the surface.

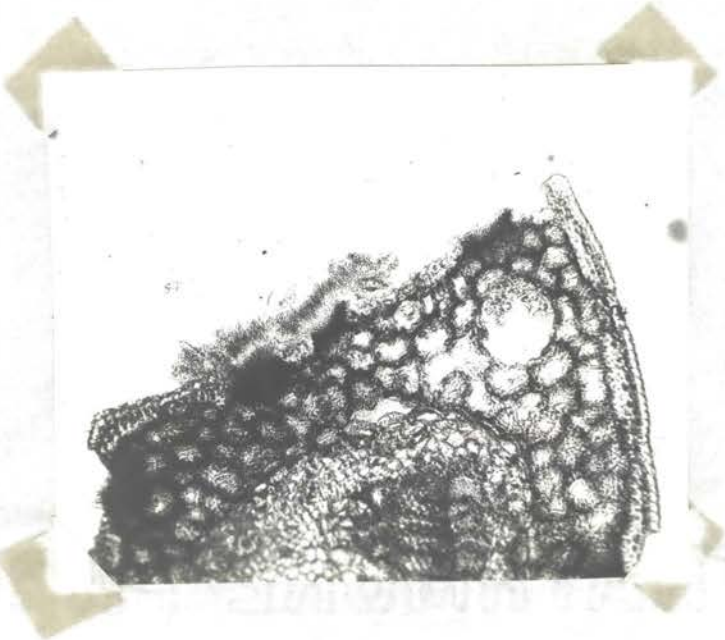

Fig. 6. Cross section of a pine needle infected with S. acicola showing a characteristic subepidermal stroma with an acervulus containing conidia and a portion of the host epidermal tissue bent back.

The conidia produced by Scirrhia acicola are olivaceous in color throughout the period of their development. After dissemination of most of the conidia the stromata may increase in size, become very compact, and turn black or carbonaceous in appearance. Spermogonial and carpogonial initials may be formed within these stromata, the latter eventually becoming mature perithecia. Conidia produced by Dothistroma pini are at first hyaline, but may become olivaceous with age. Color change is especially apparent when conditions of moisture and temperature are favorable. Hyaline conidia on infected needles became olivaceous after four to five days incubation in a moist chamber at 28° to 30° C. As the conidial population declines, the highly erumpent stromata begin to disintegrate. Although non-sporiferous locules have been observed within the stromata of diseased needles, no perfect or ascigerous stage of D. pini has ever been found or described.

Another character which can be used to distinguish between the two fungi is the color of the lesional area immediately surrounding a fructification on a diseased needle. Lesional areas of S. acicola are, as the common name of the disease implies, "brown spots". Lesional areas on needles infected with D. pini are at first brown but acquire a distinct reddish cast with age of the fructification.

The periods over which conidia are produced may also serve to differentiate the two fungi. Conidia of S. acicola may be found present on infected needles at any time during the year. When conditions are favorable new infections occur which may soon result in the formation of other sporulating fructifications. The conidia of D. pini appear to be produced only during a certain season of the year. Under the 1950-51 conditions in Oklahoma this period ranged from April 20th to August 15th. New infections during this period resulted in the appearance of spotting and blighting symptoms about

November 1st, but not in the formation of new fructifications. Under natural conditions an incubation or overwintering period of the fungus mycelium within the needles seems to be necessary. The following spring, as conditions became favorable, stromata developed and subsequent sporulation resulted.

In culture the colonies produced by the two fungi are very similar in appearance. Both form dark, compact, stromatic colonies which tend to rise above the surface of the culture media. No mycelium is visible on the surface of the agar around the colonies. One slight difference was observed, however, in the surface color of the colonies. All colonies of D. pini were completely black, while the surface of S. acicola colonies were consistantly speckled with light colored areas. (Figs. 7 and 8.)

Microscopic examination of sectioned fungus colonies also revealed differences between the two fungi. Conidia in the stromatic culture of S. acicola were characteristically borne on a flattened or slightly cup-shaped hymenium. Conidia borne within the stromatic cultures of D. pini seemed to arise within depressions or locules. The sporiferous layers in culture correspond very closely to those produced in nature by the respective fungi. (Figs. 9 and 10.)

Hulbary (6), working with D. pini, stated that the fungus failed to produce spore bearing structures when grown on artificial media. Contrary to Hulbary's findings, it was found by both single spore and tissue isolations that the colonies produced by D. pini are made up of successive layers of dense, light colored mycelium and dark stromatic tissue in which numerous conidial locules are imbedded.

Single spore isolations made at the first signs of conidial production failed to produce colonies on culture media. At this early stage the conidia were hyaline. When diseased needles were placed in a moist chamber at 28°

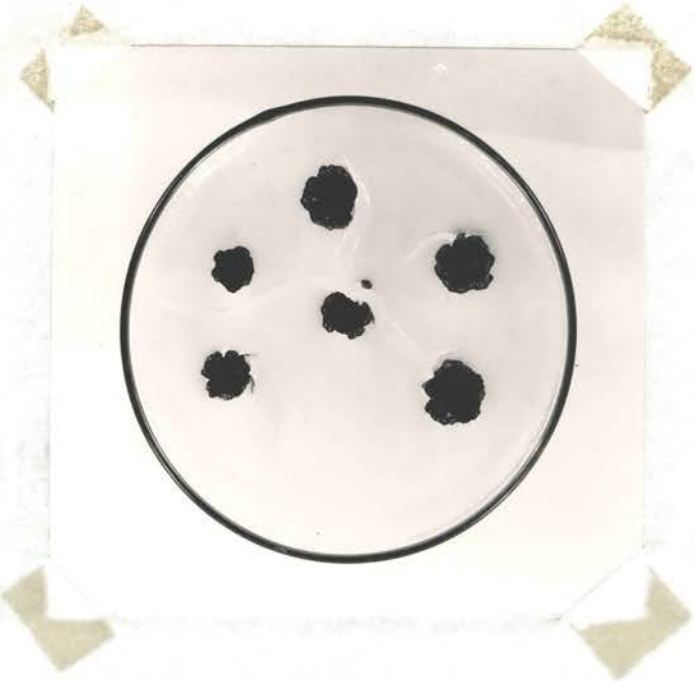

Fig. 7. Culture plate colonies of D. pini resulting from single spore isolations, showing the uninterrupted black color of each colony.

Fig. 8. Culture plate colonies of S. acicola resulting from single spore isolations, showing the lighter speckled surface of each colony.

Fig. 9. Cross section of a stromatic colony of D. pini grown in culture showing the depressions or locular-like openings filled with conidia.

Fig. 10. Cross section of a colony of S. acicola grown in culture showing the slightly cup-shaped sporiferous layers.

to 30° C. the stromata increased in size and the conidia changed from hyaline to an olivaceous color. These conidia, when isolated individually, germinated and produced colonies on artificial media. From the behavior of the conidia, it is probable that at the hyaline stage they are physiologically immature and a period of moist conditions at a temperature between 21° to 31° C. is necessary for their maturation.

Infection by artificial inoculation of healthy needles on branch terminals of P. ponderosa was accomplished by placing diseased needles among the healthy needles and covering with water-proof plastic bags. Infection and typical diseased symptoms were produced on only those needles which were in direct contact with the diseased needles. A period of one week with the healthy needles in contact with the diseased needles, was sufficient to cause infection and bring out the typical spotted symptoms characteristic of the disease. Most of the conidia from the diseased needles were hyaline and probably immature when placed over the healthy foliage and covered with the bags. It is probable that maturation, as indicated by the color change from hyaline to olivaceous, resulted in a very short time due to the high temperature and humidity within the bags. Transmission of the fungus from diseased to healthy needles may have been from actual mycelial contact. The rapid development of diseased symptoms on these inoculated needles, as compared with the relatively long period required in nature, may have been due to the more favorable growth conditions within the inoculating bags.

No positive signs of infection resulted from the spore suspension inoculations on either the Ponderosa pine branch terminals or the Austrian pine seedlings. These inoculations were made in June and so much injury resulted that it was impossible to determine whether or not any infection had actually occurred. This injury may have been due to excessive heat

within the plastic bags during the warm days of June.

The three materials used in the spray control program were fairly representative fungicides. Bordeaux mixture, an old but effective fungicide, Fermate, one of the new widely used dithiocarbamates, and Puratized, a good fungicide but limited in its use on any product that is to be eaten, however, not a problem in our case. Only three fungicides were used at this time because of limitations in setting up adequately replicated spray blocks.

The spray control results show that, at least for this one season, all of the fungicides used gave good control. Bordeaux mixture appeared to be slightly more effective than Fermate or Puratized, probably due to its better adhesive qualities.

The relatively high percent of new needles becoming infected, as shown by the unsprayed controls, indicates that a fungicide spray program is necessary if certain types of ornamental pines are to be successfully grown in this area of Oklahoma. Successive defoliations weaken the trees and the browning of the infected needles causes them to appear unsightly.

From six to eight spray applications were made in these trials. From the evidence accumulated it appears that, in a normal season, four applications would probably give good control.

An important factor effecting control is that Dothistroma pini does not sporulate throughout the year like Scirrhia acicola. Consequently, sprays need only be applied during a relatively short season.

The results of the present control studies indicate that spray applications of Bordeaux mixture made about May 1st, June 1st, June 16th, and July 1st should give adequate control. Fermate or Puratized might have to be applied at less than monthly intervals over the same period of time. Excessive, heavy rainfall during the active sporulating period of the fungus would increase the number of spray applications required.

Summary

Dothistroma pini Hul. is identified as the causal fungus of a serious needle blight disease of ornamental pines in north central Oklahoma. The disease primarily affects Pinus ponderosa and P. nigra var. austriaca. Identification was based on the morphological characters of developing fruiting structures as studied in cross sections through diseased needles.

Young, hyaline conidia failed to germinate when single spore isolated. Mature, olivaceous conidia produced raised, dark, stromatic colonies. Growth in culture was favorable on several media with the optimum being near 26° C.

Typical disease symptoms were produced on healthy pine needles, inoculated by placing diseased needles on branch terminals inside plastic moist chamber bags for one week. Only those needles in contact with the diseased needles became infected.

Spray applications of Bordeaux mixture, Fermate, and Puratized Agricultural Spray all gave good control of the disease. However, Bordeaux mixture was consistently better than the other two fungicides.

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