Some studies on Rhabdocline pseudotsugae Syd. in western Montana

Davis Andrew Weistaner

The University of Montana

1955

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1955
MONTANA STATE UNIVERSITY

Masters of Science
The degree of
Presented in partial fulfillment of the requirements for

B.S., Montana State University, 1947

DAVIES A. WEINSTAHL

Approved by

IN WESTERN MONTANA

SOME STUDIES ON RHABDOIDINE PSEUDOSIS AND
D. V. A.

...who made the surprising experiments possible.

I am grateful to Ranger Bos, Ben Draper and to the

Read and in the laboratory.

certified out. He gave freely of the time both in the
under whose direction the project was undertaken and
I am especially indebted to Dr. Charles W. Watere.

may be mentioned here.

ence was received from many persons, only a key of whom
during the course of these studies invaluable assist-

ACKNOWLEDGMENT
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As shown on the map in Fig. 1, work pertaining to these studies was done in western Montana, which are located in Montana's forest and conservation experiment station. The work was initiated by the Montana State University Forestry School and the Forest and Conservation Experiment Station.

This paper is a part of a long series of Christmas tree problems that can be attempted to develop a better understanding of this theme. These studies were conducted by the writer in an attempt to determine the ultimate death of the tree. Several attacks by this fungus may cause death of Christmas tree. Several successful years corresponding delay the mortality, severely retard the growth of a young Douglas fir tree, and the nation's Christmas tree shortage (15). This disease may also since the forests of Montana produce about one-seventh of Christmas trees. Christmas tree plant is of major importance can ruin the appearance of an otherwise marketable Christmas tree. Premature neede drop, the partial death, premature death of the trees, and eventually result in a drop in the need for the trees. Christmas trees are commonly known in western Montana as Christmas tree plants, the Douglas fir, which cause a disease of the needles or buds. This disease, which attacks the needled trees, is an important

STATEMENT OF PROBLEM
INTRODUCTION AND HISTORICAL BACKGROUND

*Rhabdocline pseudotsugae* was first observed by J. R. Weir in 1911. In 1916 he reported that the fungus in northwestern United States was so aggressive in its attacks that a strenuous effort should be made to prevent serious injury to Douglas fir seedlings in the nurseries (23). Foresters of that time were quite concerned since the fungus was widely distributed over the forests of northwestern United States and was damaging the young Douglas fir trees. Although Weir described this fungus in 1917, he did not propose a name, and it was not until 1922 that the fungus was described and named by H. Sydow (15,22).

The disease is particularly prevalent in Montana, Idaho, Oregon, Washington and British Columbia, where it becomes epidemic in certain years. Although native to the West, *Rhabdocline pseudotsugae* has recently been found on ornamental Douglas fir in the northeastern states (2). Now widely distributed in Great Britain, it first appeared in Scotland where apparently it was brought directly on trees shipped from western North America prior to 1914 (3,19). From Great Britain it entered Germany about 1926, presumably on nursery stock, and reached Czechoslovakia in 1938.

It also occurs in Holland, Switzerland, Denmark, Norway,
and Sweden (4,6). The Christmas tree blight* has so far proved far less serious in North America than in Europe where it has been extensively investigated and is serious enough to cause much anxiety as to the future of Douglas fir (4,20).

Only one species of Douglas fir, *Pseudotsuga taxifolia* (Poir.) Britt., is recognized in western United States. In Europe this American species is considered to include two distinct species and one variety, that is, *P. douglasii* Carr., commonly called coast, Pacific coast, Oregon, or green Douglas fir; *P. glauca* Mayr., known as mountain, Rocky mountain, Colorado or blue Douglas fir; and *P. douglasii* Carr. var. *Caesia* Schwen., termed Fraser River, or intermountain Douglas fir (3,4,20).

In America all forms of *Pseudotsuga taxifolia* are apparently attacked. In Europe intermountain Douglas fir is most heavily attacked, followed by blue Douglas fir; the green Douglas fir is only lightly infected. One German investigator attributed the relative resistance of green Douglas fir in Germany to the fact that the buds of this form do not open until after the spores of the fungus have been disseminated, whereas the buds of the other two forms open during the period of maximum spore discharge (4,6).

---

*In this paper the term Christmas tree blight will refer to infection caused by both *Rhabdocline* and a fungus which appears to be associated with it called *Rhabdocloesum*. 
In North America there is great individual variation in susceptibility to the parasite. Many trees in a stand remain completely or nearly free from infection, while others are severely attacked. Two Douglas fir trees, apparently similar in all respects, may be growing side by side with their branches interlaced and one of them may appear heavily infected while the other is completely healthy.

*Ehabsocline pseudotsugae* infects trees from the seedling size to about the thirty year age class (4). Trees subject to heavy infection for consecutive seasons are almost entirely defoliated and as a result either die or merely exist for an indefinite period without making perceptible growth. As a consequence, there is a marked check to the yearly increments in height, and the amount of secondary wood in the annual rings of affected trees becomes less and less every year (4, 6, 23). Damage is particularly severe in dense pure stands and in forest nurseries surrounded by infected stands. The first evidence that this fungus might cause a serious disease of seedlings in the forest nursery was obtained from a study made prior to 1915 of all age classes on a south slope in the Bitterroot National Forest, near Missoula, Montana. Practically all reproduction up to twenty-five or thirty years of age was heavily infected, and in numerous instances the seedlings were dead (23). There is danger of introducing the disease into plantations where the shock of planting combined with the attack of the parasite would cause
the death of many trees. Butler and Jones (6) report that it occurs in Britain and in other parts of Europe only on trees ten to sixteen years old. Older trees are generally immune and in mixed stands with broad-leaved trees, Douglas fir is not so frequently attacked (23).

In 1915 Weir (23) found Christmas tree blight on every two to four year old Douglas fir seedling in the Forest Service Nursery at Boulder, Montana. The pure stands of Douglas fir in the same canyon where the nursery was located were severely infected and exhibited the most serious injury so far discovered anywhere in the Northwest prior to 1915. From the observation of field plantings, it was known that Douglas fir seedlings previously infected in the nursery by this fungus would succumb in a very short time. Weir planted some infected seedlings from the Boulder nursery in pots in a greenhouse in December and by May all of the infected needles had fallen causing death to the seedlings, while the controls remained healthy (23).

In Britain the fungus was more virulent than in America and showed no loss of activity during successive years after the initial infection (3,6). In the United States epidemics of Christmas blight last about two years and then subside. Examples of this occurred in 1916 and 1917, 1923 and 1924, and 1947 and 1948. The epidemic in 1947 and 1948 nearly resulted in disaster for Montana's Christmas tree industry. In the late Fall of 1947 the Christmas trees which had been
recently bought by the concentrators and shippers either had turned color or had shed their needles, as a direct result of Rhabdocline infection. As soon as the concentrators realized what was happening, purchases were curtailed in certain areas and consequently the number of bales shipped from Montana dropped twenty-four per cent from the 1946 peak year of production (16). Rhabdocline pseudotsugae was mainly responsible for this needle condition, although several insects capable of causing similar damage were present also. The weather conditions were ideal for the spread of the fungus since the springs of both 1947 and 1948 were unusually wet. The weather conditions immediately before and after cutting in the fall of 1947 also had an important bearing on the behavior of the blighted tree (16).
MACROSCOPIC APPEARANCE OF THE DISEASE

Symptoms of *Rhabdocline*

The first symptoms of a Douglas fir needle infected with *Rhabdocline pseudotsugae* usually appear in the winter as slightly yellow colored spots on the needle’s undersurface. (Fig. 2a). Weir (23) stated that if a wet summer follows the infection, the needles begin to show signs of being diseased before October; otherwise they will remain apparently healthy until December. A short time later the tissues on the upper surface of the needle opposite the original spots also turn yellow. By April or May these spots have changed to a yellow-brown color with a narrow yellow margin surrounding the original spot. (Fig. 2b). By the first of June the spots have assumed a uniform darker brown color (Fig. 2c) after which no other color changes take place. Each of these brown areas represents a single infection, although in a heavily infected needle the spots may run together and appear to be a larger single infection.

During the winter and early spring the brown areas often have a slightly sunken appearance on both sides of the needle. Soon after the first of June small elongated blisters resembling cushions begin to form at the dark brown spots on the undersurface of the needle. The cushions may form on either side of the middle nerve. About the middle of June the epidermis covering these cushions ruptures with an irregular
A nutrient jar to the tree is often all that is necessary to extract or remain attached to the tree for several years (4, 6). Usually the droppings are dropped by the detritivores, but the droppings may drop entirely from the tree. On trees over thirteen feet tall the detritus over the tree may be found and collected. On small trees the detritus may be found at the base of the tree in the fork as shown in Fig. 3a.

Various communities of insects and fungi may be associated with the detritus, but the detritus is most commonly found nearest to the edge of the tree to the other, communities covering a portion of the tree. In this type the brown area forms a band from one band.
cause the needles to fall in a shower. The needles usually fall after the apothecia have discharged their spores, but infected needles may be lost spontaneously during the autumn and winter months before there are any signs of apothecia on them (6). According to Brown (5) premature fall seems to be connected with a marked decrease in water content of the infected needles, but another investigator finds that needle fall is not the result of water loss nor does it depend on the degree of infection (4). Some leaves containing the fungus may remain on the tree without forming any fructification at all. In case of a very severe infection, owing to the rapid drying out of the twigs, the needles may remain indefinitely attached and the apothecia may become black and shrunken. The defoliated fir trees have a moth eaten appearance as shown by the branchlet in Fig. 21 and the heavily defoliated trees soon die.

Other Defoliating Agencies

There are several other defoliating agencies that are often confused with Christmas tree blight. They are sometimes called Christmas tree blight by some Christmas tree cutters and contractors who want to buy stumpage more cheaply from an unsuspecting landowner. Cooley’s gall (Chermes cooleyii Gill.) is very common throughout the Christmas tree areas of western Montana. The primary host for Cooley’s gall is Colorado blue spruce, Engelmann spruce, or sitka spruce, and the secondary host is Douglas fir (10). Small cottony
patches with adjacent minute black spots appear on the Douglas fir needles and these symptoms are often accompanied by a twisting or bending of the needle. (Fig. 4). Another insect that causes a partial browning of the needles is a Douglas fir needle miner. This insect causes a purple-brown band to form around the needle (Fig. 5a) and in time browning may occur all the way to the needle's apex. (Fig. 5b). If the needle is broken at the purple-brown band a small orange colored larva is visible. (Fig. 5c). This condition is most commonly found in autumn and early winter, and was very common in the fall of 1953. Frost damage may also be confused with Christmas tree blight. This type of damage is identified by all of the needles and terminal buds of the new growth turning entirely brown. Frost damage is found in the spring and summer. There are several other diseases of less importance on Douglas fir needles caused by fungi in western Montana. The most common of these are a rust caused by Melampsora albertensis Arth., a snow blight caused by Phacidium infestans Korst., and a gray mold twig blight caused by Botrytis cinerea Aust. (1,23).
MICROSCOPIC STUDIES OF CHRISTMAS TREE BLIGHT

Structure and Development of *Rhabdocline*

About the middle of June the epidermis covering the brown cushions of the *Rhabdocline* infected needles rupture with an irregular slit exposing the fruiting layer. (Fig. 6). These pustules occur on the underside of the needle at the same time that the new buds are opening. By the first of July the asci are fully mature and sporulation is active. The ascospores are discharged into the air or are liberated by the force of the wind and rain on the leaf. When these spores fall on the tender young needles of the host, it is observed that infection takes place shortly afterwards, provided sufficient moisture is present. It is the belief of most investigators that the ascospores are the means by which the infection is spread (4,15,23). The exact mode of infection of the leaf is not certain but is believed to be cuticular at either surface of the leaf (6). Hubert (15) states that the fungus is actively parasitic and apparently penetrates the needle tissue of the host through the stomatal openings.

The elongated apothecia of *Rhabdocline pseudotaugae* are embedded in the epidermal layer of the substratum on the underside of the needle. The fructification has a hyaline
basal layer 40-65μ thick and is covered by the epidermis (24). No fungal tissue is developed above the hymenium. These fruiting bodies are not included within a stroma, but open along a median ridge to expose an orange-brown colored hymenium. The apothecia, seldom found on the midrib, are found in one row on either side of the midrib (Fig. 7) or in two rows separated by the midrib. When the epidermal covering ruptures by the irregular longitudinal slit to expose the brownish convex disk, the line of rupture is more frequently toward one side. It may also rupture from the center in lobes to form single or isolated apothecia (23). Sydow (22) reports the fruiting bodies to be 3 mm. long, 300μ broad, and 200μ high. In western Montana these measurements are similar, but pustules 4.5 mm. long have been observed.

In August and September microscopic examinations of cross sections of the infected current year’s needles reveal the presence of very few hyphae. By October the hyphae appear more developed as they begin to branch. In the winter and spring, coarse intracellular hyphae 2.5-5μ in thickness are found in the cells of the mesophyll. At this time the hyphal cells are spherical to ovoid with cross walls that are scarcely noticeable (19). Large oil droplets almost fill the hyphal cells. During April and May fine intercellular hyphae 1.5-2μ in thickness are found in the mesophyll cells and the coarser intracellular hyphae is found in the chlorenchyma cells (4).
After accumulating in the mesophyll, concentrations of mycelium give rise to the apothecia by collecting at the lower surface of the leaf just below the two bands of stomata. During the spring and summer the hyphae are colorless, septate, branched, and have a full appearance. (Fig. 8). During the winter months the intercellular hyphae appear dormant and shrivelled. In May the shrivelled hyphae begin to fill out and become active. The stele and epidermal cells remain free from invasion by the hyphae. The mycelium is confined to the lamina of the needle and in no case has hyphae ever been found in the petiolar region (5).

The fruiting layer is composed of cylindrical to clavate asci with very short pedicels interspersed with paraphyses that are slightly swollen at the tip. (Fig. 9a). The parallel asci are 115-125 x 17-21 μ (24) or, 113.9-153.3 x 15.7-19.4 μ (23) or, 120-130 x 18-20 μ (22). The paraphyses are about the same length as the asci, 3 μ in thickness, filiform, hyaline, and unbranched. There are eight ascospores within each ascus and are usually obliquely uniseriate but may be irregularly bisporiate. (Fig. 9a). The ascospores are characteristically constricted in the middle and rounded at the ends. When shot out of the ascus, most of the spores are unicellular and are surrounded by a gelatinous sheath which expands on the wet surface of the leaf prior to germination. Immediately after discharge the spores become two-celled when a cross wall forms at the constriction. When germinating
usually one cell becomes thick walled and dark colored to produce a single germ tube (24). The other cell remains hyaline, thin walled, and does not germinate. (Fig. 9b). Wilson and Wilson (24) have found that a certain number of spores become two-celled within the ascus but do not develop further until after ejection. These same authors state that this later development varies considerably under artificial conditions, and it is difficult to ascertain which is the normal method of germination. The ascospores measure from 17-21 x 7-10 μ (24) or, 18.2-19.3 x 6.6-7.4 μ (23) or, 15-20 x 6-9 μ (23). Weir (23) reports that the pore of the ascus is colored blue by iodine. All of the measurements of asci, paraphyses, and spores from needles collected in western Montana by the writer lie within the ranges given above.

The young needles are infected in the summer at the time of ascospore discharge and the apothecia on these newly infected needles develop the following spring. After the spores have been shed the needles usually drop from the tree but form no absciss layer or cork layer at their base prior to falling (24). Therefore the needles usually persist for a little over a year after initial infection instead of normally persisting for eight years. However, some of the brown spotted needles may remain on the tree for several years without forming any fructification. Under some circumstances it may take two years for the fruiting bodies to form (19). After the infected needles have dropped there
is no further development of the fruiting bodies.

Brown (5) reported that infected needles are somewhat thinner than healthy ones because of the destruction to the protoplasm of the infected leaf cells and their consequent loss of turgidity. Since the stomata are no longer functional, transpiration is not controlled and the presence of mycelium in the sub-stomatal spaces greatly increases the surface area of evaporation. Consequently, the water content of infected needles is less than that of normal leaves of the same age. Brown (5) also discovered that there is a marked decrease in the starch content of infected needles.

**Taxonomy of Rhabdocline**

At first there were some differences of opinion regarding the classification of *Rhabdocline pseudotsugae*. All of the early investigators of this fungus agreed that it belonged to the *Ascomycetes* under the order *Phacidiaceae*. When Weir (23) first described *Rhabdocline* in 1917, he and several other pathologists stated that it plainly belonged to the family *Stictidaceae*. In 1922 Sydow (10) described the fungus and placed it in the *Phacidiaceae*. Most of the more recent taxonomists now agree that it belongs in the *Phacidiaceae*.

The order *Phacidiaceae* is characterized by having the asci borne in apothecia that are at first sunken and later become erumpent. The apothecia usually open by lobes but may sometimes open by a cleft. The asci are typically cylindric, eight-spored, and interspersed with paraphyses.
In describing the Phacidiaceae, Clements and Shear (7) state:

"Apothecia innate, often concretes with the epiderm and splitting with it into lobes or a cleft, or free and then more or less crumulent and splitting separately, discoid or elongate, black, membraneous to carbonous, separate or gregarious, or crowded in black stroma-like areas of the leaf; hypotecium poorly developed as a rule; asci mostly cylindric and 8-spored, occasionally stalked and clavate; paraphyses usually numerous, often hooked or branched at the tip, sometimes sparse but very rarely absent; spores various."

The members of the Stictidaceae are characterized by the fact that the apothecia are never concretes with the epiderm. These apothecia are white, bright colored, or very rarely dark colored, but never black (7).

The writer agrees with the majority of taxonomists and places Rhabdocline in the Phacidiaceae. The apothecia of Rhabdocline are concretes with the epiderm at the terminal side of the asci prior to the splitting of the epiderm. Although the apothecia of Rhabdocline are not black, they are dark colored instead of being white or bright colored as in the Stictidaceae. In Rhabdocline, the asci are cylindric, clavate, and stalked, interspersed with numerous swollen tipped paraphyses. In view of these characteristics it seems that Rhabdocline undoubtedly belongs to the Phacidiaceae.

Structure and Development of Rhabdogleum

When Sydow (22) described Rhabdocline pseudotsugae Syd. on Douglas fir needles from Montana in 1922, he also described a conidial fungus on these needles and named it Rhabdogleum
pseudotsugae Syd. He suggested that the latter is the imperfect stage of the former. Prior to 1945 there was very little literature available concerning Rhabdogleum. Dearnness (8) reported the presence of *Rhabdogleum pseudotsugae* in a collection from Colorado and at the same time he described and named *Rhabdogleum abietinum* Dearnness on *Abies Fraseri* (Pursh.) Poir. from North Carolina. In 1926 Wilson and Wilson (24) reported that in America conidia are developed on the fir needles in July on the upper surface only and this stage had not been found in Scotland. Ellis and Gill (9) stated that Van Vloten considered the Wilsons' fungus to be *Rhabdogleum pseudotsugae* but did not believe it was linked in any way with *Rhabdogleum*. In 1945 Ellis and Gill (9) described and named *Rhabdogleum hypophyllum* sp. nov. and strongly suggested that it is a stage in the life history of *Rhabdogleum*.

*Rhabdogleum* is classified as a Deuteromyxete in the order Melanconiales and family Melanconiacaeae. Clements and Shear (7) describe the *Melanconiacaeae* as follows:

"Pyconidia lacking, represented by a stroma-like stratum; strata typically bearing simple or ramose basidia upon which the conidia arise, forming acervuli or masses, which are immersed or erumpent, black, gray or light colored, waxy, horny or gelatinous; conidia various."

In *Rhabdogleum* the conidia are one celled, hyaline, globose, and somewhat constricted near the middle. The acervuli raise the epiderm into circular or elongate concolorous blisters that later rupture to release the conidia.
Rhabdocloeum pseudotsugae

In western Montana this imperfect fungus produces conidia during the latter part of June and most of July. The fruiting bodies are most commonly found on the upper surface of the needle, but they sometimes appear on the lower surface. Prior to fruiting, the infected areas are brown spots located on both surfaces of the needle. These spots are indistinguishable from the ones caused by the Rhabdocline organism.

The fructifications are in the form of small rounded or elongated blisters, up to 2 mm in length, that rupture with an irregular longitudinal slit similar to the apothecia of Rhabdocline. Sydow (22) reported that these fruiting bodies are about 2 mm long, 250u broad, and 50u high. At first they develop subepidermally with a flat brown stroma-like basal layer of filamentous hyaline small-celled hyphae. This layer forms a hymenium from which septate branched hyphae 4-5u broad arise (22).

The conidia are cylindrical, straight or sometimes slightly curved, and constricted in the middle. (Fig. 10). They are hyaline, one-celled, and vary in size from 11-20 x 3-5u (average 14 x 3.5u) in western Montana. In the original description Sydow (22) reported the spores to be 15-21 x 4-5u. Fig. 11 shows several of the conidiospores germinating. The germ tubes appear to originate from a dark portion of the spores which is somewhat similar to the germination of ascospores of Rhabdocline. The conidiophores are rod-shaped
and quite indistinct because of their small size. They vary in length from 7-14μ and are only 1.5μ wide. The spores apparently are not borne in chains. After one spore matures it apparently is released from the conidiophore soon after the initial formation of the new underlying spore. Clements and Shear (7) state that the spores are not borne in chains and the masses are linear. Fig. 12 depicts the cross section of a typical acervulüus prior to the rupture of the epidermis, and Fig. 13 illustrates a more mature fruiting body whose epidermis has ruptured. Both of these pustules were found on the upper surface of the needle. Before the rupture of the epidermis occurs the conidia are more or less embedded in a mucilaginous material which apparently builds up a concentration large enough to force the epidermis to rupture. This mucilaginous substance is also a means by which the newly liberated spores may adhere to the needles of the new host.

**Rhabdocloma hypophyllum**

*Rhabdocloma hypophyllum* sp. nov. was described in detail by Ellis and Gill (9) as follows:

"Fruiting bodies hypophyllous, rarely epiphyllous, in reddish-brown conspicuous spots, scattered or confluent, often in parallel series on either side of the mid rib, 130 to 300μ wide by 35 to 150μ high, averaging about 300 x 90μ, raising the epidermis into elongate pustules 0.5 to 4 mm long, at first covered, later erumpent. Conidia hyaline, continuous, oblong, straight or slightly curved, often somewhat constricted near the middle, 6.7-11.1 x 2.2-3.7μ, average 9.1 x 3μ."
Conidiophores slender, simple, continuous or septate, 10-36 x 0.9μ, average 25.3 x 1.8μ."

This imperfect fungus in Montana is found alone in May and increases in abundance until June when it appears with *Rhabdocline pseudotuscae*. In quite a few instances ascii have been noted to originate beneath the conidial layer of *Rhabdocline hyophyllum* in the same fruiting structure and replace this imperfect fungus by apparently forcing it out. Ellis and Gill (9) have made similar observations. In some instances cross sections of the infected needles revealed the conidial fungus on the edge of a *Rhabdocline* apothecium. The original investigators (9) state that *Rhabdocline* in the Southwest is rarely epiphyllous. In Montana during the spring of 1955 it was quite often found on either surface of the needle. (Figs. 14 and 15). In the Blackfoot River area near Marco Flat the fructification invariably was found on the underside of the needle, while material collected on the east shore of Flathead Lake revealed the fruiting bodies to be on the upper side of the needle. (Figs. 16 and 17).

Comparison of Both Conidial Stages

Although these two imperfect forms seem to be quite similar in many respects, there are several characteristics that may be used for identification. *Rhabdocline hyophyllum* appears earlier in the season than *Rhabdocline pseudotuscae*. *R. hyophyllum* has smaller spores, longer and more prominent conidiophores, and the underlying hyphal layer from which the conidiophores arise is thicker and more conspicuous than that
of *R. pseudotsugae*. At first it was believed that the surface of the needle that produced the fruiting body was an identifying characteristic. This may be true in some areas, but not in western Montana. The hyphae in *Rhabdocline pseudotsugae*, *Rhabdocloeum pseudotsugae*, and *Rhabdocloeum hypophyllum* appear to be exactly the same and not characteristic to any one species. The fruiting bodies may be longer in *R. hypophyllum*, but obviously this is not a positive characteristic that may be used in identification.
LABORATORY EXPERIMENTAL

Culture Methods

Ellis and Gill (9) stated that Van Vloten (1932) considered *Rhabdocline* to be an obligate parasite and was not able to grow it artificially. Ellis and Gill have successfully grown the conidial fungus on malt, corn meal, and Douglas fir needle decoction agar. They report that the mycelial growth was sparse, but the conidia were produced in abundance and accumulated in masses that formed the most conspicuous part of the colony. On malt agar the colonies were at first white, but later passed through deepening shades of gray and finally turned dark olive green after one or two months (9).

The conidia produced in culture by Ellis and Gill were similar to those produced in nature, but in cultures more than three weeks old the spores were more constricted in the middle. They often became two-celled at maturity and resembled the ascospores of *Rhabdocline* to a certain extent. In old cultures the conidia frequently became thick walled and dark colored (9).

The method most commonly used by the writer in the attempts to isolate *Rhabdocline hypophysium* in culture was the placement with sterile forceps of an infected fir needle upon the medium in Petri dishes. Prior to the placement of
were placed in most chambers to hasten the putative formation.

The anticipated condition of the hyphal interface barriers at this time, no outpite results were obtained. To overcome this problem, we present, since the hyphale appeared dormant.

The anterior to improve their hyphate from hyphate of these needes were first chosen to be placed upon the medium, these needes were then placed in the culture buttons with brown spots or bands were made when the hyphal buttons of the injected needes were writer was in the early summer of 1993. These results were

The above aspect to cultivate this organism by the

from chambers.

No effort was made to control the light in any of the culture

Hunt (7/1) several plates were part in a refererence at

an leasess refererence similar to the one described by

introduction chamber in the laboratory at 9Z°C to 16°C. In

petri dishes were kept either at room temperature in an

after the placement of the needes on the medium, the

the boufe (7/12).

containing one cc of propylene oxide per letter of the

needes for twenty-four hours in a tightly covered bottle

face sterilization was accommpanied by leaching the injected

- three changes of sterilize water. In several instances sur-

two minutes in 1:1000 mercuric chloride and then washed in

the needes on the medium, they were surface sterilized for

-27-
Ellis and Gill (9) reported that needles collected in New Mexico in March and kept in a moist chamber at room temperature would form conidia of *Rhodocollybia hypophyllum* in one week. When these same needles were kept in moist chambers for a month, mature fruits of *Rhodocollybia* were formed. Small branchlets collected in western Montana in March and kept in moist chambers in the laboratory produced conidial fruiting bodies in approximately six weeks. Placement of infected needles upon the various media was accomplished throughout the stages of development of the fungus. The stages of development were from the time the hyphae within the infected needles lost its shrunken appearance to the time of fruiting.

Various media were used in attempting to culture this organism, namely, Bonar's Modification of Leonian's Agar Medium (21), Malt Agar, Douglas Fir Needle Decoction, Douglas Fir Needle Infusion, and Douglas Fir Dextrose Agar Medium. The three Douglas Fir Media were made as follows:

**Douglas Fir Needle Decoction**

- Douglas fir needles: 200.0g
- Agar: 20.0g
- Distilled Water: 1000.0cc

Fresh Douglas fir needles (taken from trees that were known to be susceptible to *Rhodocollybia* and *Rhodocollybicium*) were put through a coarse grinder, then steamed in 500cc of distilled water for 30 minutes. Agar was then dissolved in the remaining water which had been heated. Both solutions were then mixed, filtered, and autoclaved at 8 pounds pressure for 30 minutes.
Douglas Fir Needle Infusion

Douglas fir needles . . . . . 5.0g/Petri dish
Agar . . . . . 20.0g
Distilled water . . . . 1000.0cc

Fresh Douglas fir needles (taken from trees that were known to be susceptible to Rhabdocline and Rhabdoclosus) were put through a coarse grinder. The agar was mixed with the distilled water and autoclaved at 8 pounds pressure for 30 minutes. The ground needles were sterilized by propylene oxide and sprinkled in the Petri dishes prior to being covered with lukewarm agar. When Propylene oxide was not used, the hot agar solution was poured over the ground needles, thus sterilizing them by heat. Approximately 5 grams of ground needles were used in each Petri dish of media.

Douglas Fir Dextrose Agar Medium

Douglas fir needles . . . . . 5.0g/Petri dish
Agar . . . . . 20.0g
Dextrose . . . . . 20.0g
Distilled water . . . . 1000.0cc

The fresh Douglas fir needles were treated by the same methods as in the Douglas Fir Needle Infusion. The agar and dextrose were mixed with the distilled water and autoclaved at 8 pounds pressure for 30 minutes. Approximately 5 grams of ground needles were used for each Petri dish of media.

When sterilizing by the propylene oxide treatment, the ground needles were kept for twenty-four hours in a tightly covered bottle containing one cc of propylene oxide per liter capacity of the bottle. This method was similar to the one used by Hansen and Snyder (11,12). A moist filter paper was also kept in the bottle during the twenty-four hour treatment to prevent the needles from drying out. When the propylene oxide treatment was not used, the ground needles were sterilized by pouring the hot medium over them.
Culture Results

The first attempts to culture <i>Abdioscopem hypophyllum</i> in the summer of 1953 were successful. Conidia were produced in abundance on the Douglas Fir Needle Decoction, and to a somewhat lesser extent on the Malt Agar. The conidial fungus was undoubtedly the same as that grown by Ellis and Gill (9). After four or five weeks the original gray-colored colonies turned an olive green color. The cultured conidia were constricted in the middle and often became two-celled as they grew older. The cells of the conidia eventually became thick walled and dark colored. In several instances one cell of the two-celled spore was noted to be much darker and thicker walled than the other cell. In the spring of 1954 the cultures were accidentally contaminated and consequently destroyed as a result of an unexpected rebuilding program within the laboratory.

Since the destruction of the original culture, many subsequent attempts have been made to culture the fungus, but without success. These trials were made using infected fir needles in all stages of development but no conidial growth resulted upon any of the artificial media. It is the hope of the writer that conidial growth can be isolated again upon the artificial media. At the present time (April, 1955) infected needles on branchlets that have been kept in moist chambers for one month are beginning to show symptoms of producing conidiospores. As soon as the conidiospores appear
mature, more culture work will be attempted.

Host Inoculations

In the fall of 1953 and early spring of 1954 about fifty *Rhabdocline* infected trees less than two feet high were collected at various locations in the Blackfoot Valley and on the east shore of Flathead Lake. These trees were planted in pots and kept in the greenhouse for observation and inoculation tests. During the middle of April most of the buds on twenty-five of these trees were opening and two weeks later there was enough new growth to begin the inoculations.

Twenty-five of these potted trees were inoculated using cultures of *Rhabdocline hypophyllus* growing on the Douglas Fir Needle Decoctions. The inoculum was mashed in a small amount of distilled water and poured into a flask. A small camel's-hair brush was dipped in the suspension, smeared on a microscope slide, and viewed through the microscope to show the relative amount of spores in the suspension. This suspension was applied to the new growth of each branchlet by means of the camel's-hair brush. A non-toxic wetting agent was added to the suspension to make the liquid adhere to the new needles. Most of the inoculated potted trees were placed in a large inoculation chamber that maintained a high relative humidity. Several of the inoculated trees were placed in the "iceless refrigerator." The new growth of several other trees were brushed with distilled water and
placed in the inoculation chamber to act as controls.

In June six potted trees were inoculated with a suspension of fresh ascospores secured from *Phadecline* infected trees in a shelterbelt five miles north of Kalispell, Montana. The suspension was prepared by scraping apothecia from the infected needles with a scalpel and crushing them in water. This suspension was applied in the same manner as the cultured conidia suspension. All of these trees and their controls were placed in the large inoculation chamber.

Host Inoculation Results

The inoculated branchlets were examined regularly for almost a year, but no positive results were obtained from any of the inoculations. Other potted trees placed in contact with diseased trees failed to produce any symptoms of Christmas tree blight.
FIELD EXPERIMENTAL

Survey of Lubrecht Forest

In the early summer of 1953 a survey was made to study Christmas tree blight on the better Christmas tree areas of the Lubrecht Experimental Forest. The purpose of this study was to determine the relative numbers of Douglas fir trees that were infected with Christmas tree blight. It was hoped also that the study would reveal certain factors which might aid in the determination of the infection requirements of this fungus. Data on aspect, slope, position in stand, vigor of the individual tree, size of tree, and damage caused by agencies other than Christmas tree blight was compiled in an effort to determine their effects on the "holdover" capacity of this disease as well as the susceptibility of the trees.

Field maps were prepared from a Forestry School "Christmas Tree Inventory Map of the Lubrecht Forest." Four distinct areas are delineated on this map: those areas containing 80+ Christmas trees per acre; those containing thirty-one to seventy-nine Christmas trees per acre; those

*The Lubrecht Forest is an experimental forest 20,000 acres in size owned by the Montana State University School of Forestry. This forest is located thirty-two miles northeast of Missoula in the Blackfoot Valley. (Fig. 1).
containing five to thirty Christmas trees per acre; and those containing less than five such trees per acre.

An attempt was made to survey all areas containing more than thirty trees per acre. However, in the allotted time it was only possible to complete the survey of all the 80+ trees per acre and approximately half of the thirty-one to seventy-nine trees per acre areas. Approximately 4,000 acres were surveyed.

At first it was thought that a ten percent cruise would be suitable consisting of eight strips one chain wide for each section. It soon became apparent, however, that the disease could not be recognized at a distance greater than four feet. Even when browning of the needles appeared densely enough to be seen at a greater distance, the trees had to be checked closely to ascertain if this was the result of Rhabdocline, Cooley's gall, or needle miners. To examine all Douglas fir trees within a strip one chain wide was a time consuming job. As a result it was decided to use the plot system and reduce the survey to a five percent sample. This was accomplished by examining twenty one-fifth acre plots in each of the eight strips per section.

A two man crew was used on this survey. One man paced, ran the compass, and kept notes, while the other wandered over the strip or plot examining the trees and informing the notekeeper of the infection found. Some typical information recorded by the notekeeper may be found on a survey form as
shown in Fig. 13. One page of notes was used for each plot that had some infection in it. Complete information was recorded concerning the first infected tree found in each plot. Any other infection found in the same plot was listed under "other infected trees."

The terrain varied from level ground to very steep slopes. The entire survey was run north and south to cross the major drainages on the Lubrecht Forest. The stands ranged from pure stands of reproduction and pole-size fir to scattered reproduction overtopped by mature fir and larch. The trees examined in the survey varied in size from seedlings four inches tall to trees six inches in diameter and thirty feet tall.

Lubrecht Forest Survey Results

A total number of 1,063 blighted trees were tabulated for the entire survey and complete information was recorded on 763 of them. Approximately twenty-eight percent or 237 plots out of a total of 834 plots contained some *Rhabdocline* infected trees. Table No. I illustrates the variation in the number of infected trees that were in these 237 plots.

Since the stands surveyed were not homogeneous, the information recorded on the position of the infected trees within the stand may not be characteristic in all cases. Forty-three percent or 327 infected trees were dominant and codominant; and forty percent or 308 infected trees were intermediate. The remainder, or seventeen percent of the
Table No. I. Number of infected trees found in the plots containing infection.

<table>
<thead>
<tr>
<th>No. of infected trees in plot</th>
<th>Number of Plots</th>
<th>Percentage of infected plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>38.8</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>14.4</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>11.0</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>5.1</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>6.3</td>
</tr>
<tr>
<td>6-10</td>
<td>32</td>
<td>13.3</td>
</tr>
<tr>
<td>11-15</td>
<td>13</td>
<td>5.5</td>
</tr>
<tr>
<td>16-20</td>
<td>8</td>
<td>3.4</td>
</tr>
<tr>
<td>21-25</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>26-30</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>31-35</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>36-40</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>41-46</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Infected trees were classified as suppressed. It has been claimed that *Rhabdocline pseudotsugae* attacks the needles of the most vigorously growing trees as readily as those of suppressed or unhealthy trees (23). Infection was apparently due to the chance of the fungus spores reaching the individual tree rather than the actual effect of the dominance or vigor of the tree in the stand. Normally there would be a greater chance of the wind disseminated fungus spores contacting an open grown tree than reaching a suppressed tree.

The aspect of all plots having some infection was recorded. (Table No. II). The major drainage of the area is the Big Blackfoot River which flows from east to west in this particular area and is located near the northern boundary of the forest. Therefore, most of the Lubrecht Forest has
Table No. II. The aspect of plots containing infection.

<table>
<thead>
<tr>
<th>Aspect</th>
<th>No. of Plots</th>
<th>Breakdown of Plots</th>
<th>Percentage of total infected plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northerly</td>
<td>137</td>
<td>66 N</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 NE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 NW</td>
<td></td>
</tr>
<tr>
<td>Southerly</td>
<td>14</td>
<td>3 S</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 SE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 SW</td>
<td></td>
</tr>
<tr>
<td>Eastern</td>
<td>36</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Western</td>
<td>25</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Levelground</td>
<td>25</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

either a northerly or a southerly exposure. Rather than designate each of the eight directions that were recorded a separate aspect, northeast and northwest were included in the term northerly aspect, while southeast and southwest were included in the term southerly aspect. Most of the Christmas tree areas were located on the north slopes of the forest. As a result, fifty-seven percent of the plots having some infection were found on slopes with a northerly aspect while only six percent were found on southerly slopes. The greatest concentration of infected trees were found in small draws and stream bottoms. In many of the plots the only infection found was on Douglas fir reproduction less than four feet high growing on open areas where there was evidence of the soil having been disturbed. These disturbances were generally old logging roads or skid trails.

In this survey the side of the tree infected was called
the infection aspect. Fifty-one percent of the 763 infected
trees had Christmas tree blight on all sides of the tree.
(Table No. III).

Table No. III. Infection aspect of blight infected fir trees.

<table>
<thead>
<tr>
<th>Side of tree</th>
<th>No. of Trees</th>
<th>Percentage of total number of infected trees</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>175</td>
<td>23</td>
</tr>
<tr>
<td>South</td>
<td>46</td>
<td>6</td>
</tr>
<tr>
<td>East</td>
<td>69</td>
<td>9</td>
</tr>
<tr>
<td>West</td>
<td>84</td>
<td>11</td>
</tr>
<tr>
<td>All Sides</td>
<td>389</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>763</td>
<td>100</td>
</tr>
</tbody>
</table>

Twenty-three percent of the trees were infected on the north
side while only six percent occurred on the south side of
the trees.

Although approximately twenty-eight percent of the plots
examined were found to contain blighted trees, this figure
did not indicate what percentage of the Douglas fir popula-
tion on the Lubrecht Forest was actually blighted. It was
apparent that the percentage of blighted trees in the
population was much less than this figure. Thirty-nine per-
cent of the plots contained but one infected tree, and fifty
percent contained only two to ten such trees. Therefore,
approximately ninety percent of the plots contained less than
ten infected trees per plot. Time did not permit the counting
of all fir trees within the plots. This figure was approxi-
mated as follows: 80 trees/acre + 55 trees/acre (average of
31-79 trees/acre) x 1/5 acre plots + 2 acres = 13.5 trees/plot. Therefore, 834 plots x 13.5 trees/plot, or an approximate total of 11,259 fir trees were examined. Since 1,063 trees out of the 11,259 examined trees were blighted, roughly nine percent of the fir population was infected with **Ehdocline.** This infection usually was not severe enough to render the Christmas tree unmarketable. In many cases only one or two of the lower branches had some infection on them. Probably much less than one percent of the total population was not marketable as a Christmas tree because of Christmas tree blight.

Cooley's gall (**Ghormes cooley**i) was found to be more prevalent than Christmas tree blight on the Lubrecht Forest. No correlation was noted between the appearance of this insect and infection caused by the **Ehdocline** organism. Cooley's gall was found abundantly on both blighted and blight-free trees.

**Experimental Spraying**

Although several investigators (2,4,23,24) recommend spraying blighted Douglas fir trees with various fungicides, only one writer (6) has written about any results. Butler and Jones (6) stated that good control has been obtained in nurseries in Germany by spraying with one percent bordeaux mixture using alum as the adhesive. The first application of this spray was made at the beginning of May and subsequent applications were made at ten day intervals for a month.
Boyce (4) suggested spraying with bordeaux mixture or lime sulfur at the time of ascospore discharge. In the nursery at Boulder, Montana, an attempt was made to control the disease by means of a soap-bordeaux spray, followed by spraying with a kerosene emulsion. The kerosene emulsion was found effective in controlling the infestation of plant lice and Cooley's gall which were present in large numbers on the Douglas fir seedlings. The nursery was soon afterward abandoned and no check tests were made to determine the effectiveness of the spray program (15).

Several different locations were picked for the spraying tests. Ideal areas were found in two shelter belts five miles north of Kalispell, Montana. (Fig. 1). These belts were located on two farms owned by Bauer Bros. and Ben Bruyer, respectively. The Douglas fir trees within these windbreaks were approximately fifteen feet tall and twenty years old. (Fig. 19). *Rhabdocline* infection was heavy within these belts and was probably a result of the high winds and moist conditions of the belt during the period of infection. Two other areas of heavy infection were located along the highway on the east shore of Flathead Lake and were chosen for spraying plots because the trees were under four feet in height. (Fig. 1). One of these areas was located east of Gravel Bay and the other was located one-quarter mile east of the A. W. Hollensteiner residence northeast of Skidoo Bay. The trees in these areas were between the highway right-of-
way and the mature fir trees on the edge of the forest. An area of trees less than two feet tall was selected east of Maroo Flat along the Blackfoot River highway about fifteen miles east of Missoula. (Fig. 1). These trees were located on the highway right-of-way within thirty feet of the edge of the road's surface. All of the fir trees in these five areas were heavily infected with Christmas tree blight.

The fungicides used in the spraying experiments were bordeaux mixture, lime sulfur, and Phygon. Two types of bordeaux mixture were used. One was a commercial product in the form of a powder called "Acme Bordeaux Mixture" and was manufactured by Acme White Lead and Color Works of Detroit, Michigan. The other was homemade bordeaux mixture. The commercial preparation was used at a concentration of eight tablespoons per gallon of water to make a 4-4-50 solution. Rough and Mason (14) stated that no commercially prepared bordeaux mixture or copper spray has been superior to homemade bordeaux mixture in fungicidal efficiency.

When making homemade bordeaux mixture, stock solutions of each ingredient were prepared separately and not mixed together until just before the actual spraying. The stock solution of copper sulfate was prepared by dissolving one pound of copper sulfate in one gallon of water and the stock solution of lime was prepared by dissolving one pound of slaked lime in one gallon of water. The required amount of each stock for making any formula of bordeaux mixture was
The first preparation took place on May 12, 1949, in the
trees were two teaspoons per gallon of water.
The concentration used on Christmas tree picture included the
in the trade name for 12' diameter 1/4 mphonaphone 0.125
which lower well below 20% pH of European pancake powder. Phospho-
which made by the same company that was used in these tests.
Chamber of Commerce, another commercial
concentrate was manufactured by Robeco by-products and
and seventy-four percent content in categories. This impact
either by weight were twenty-six percent calcium phosphates
per gallon of water. The effective ingredient of this paint-
with spreader was used at a concentration of three teaspoons
v commercial preparation, which I call "Cold Lime Solution Spots"
Time Concentrate.

The procedure so some precautions must be observed to protect the
water very slowly. A great deal of heat is generated in this
If untested time is needed, it must not be added by adding
oxide or untested time or hydrated time (cottonwood hydrate)
needed in the stock solution may be either calculated (cottonwood
standing or when subjected to high temperatures. The time
just before applying because the mixture breaks down very
water. The two stock solutions are not mixed together until
of each stock solution to three and one-third gallons of
solution were made by adding approximately one-third gallon
pound of the concentrated one or gallon of stock containing one

---3---
the previous area and only the lower three feet of each tree shatterproof received fur spraying on the same dates as commercial fumigant spray was used. The segment of the

In another row or double the fur in the same windbreak the

the wind hampered the spraying to a certain extent.

weather was generally warm during each of the four days, but

so subsequent sprays were not considered necessary. The

so subsequent sprays were not considered necessary. The

June 19

June 19 took place on May 21, June 1, June 4, and June 19. All of the

out the plants

carrot. Subsequent sprays up with home-made Bordeaux mixture

twenty feet required about three gallons of spray per plot.

Those served as controls and the un sprayed adjacent trees. Those

heavily injured trees, the time above the three foot level

regardless of how high into the tree the injection ran. In

small sprayer. Only the lower three feet was sprayed

lower part of the tree and the trees were too tall for our

lower part of the tree and the trees were too tall for our

spray the whole tree. Since the area of injection was in the

with the commercial Bordeaux mixture. It was decided not to

inoculated, and the lower three feet of each tree was sprayed

were treated with small meat teas numberling from 250 to 269. These

with 25° F. Even heavily infected trees

a three foot section applied and were manifested by this.

the sprayer was used for all of the spraying experiments. It had

double the trees. A match "beneath open top compressed air

and south and contiguous two rows of quite heavily infected

methotrefacts on the farm. farm. This part ten north
was sprayed. A total of sixteen trees were tagged and then sprayed with lime sulfur.

Commercial bordeaux mixture was used exclusively in another shelterbelt which ran east and west. This shelterbelt was owned by Ben Bruyser and was located about one mile south of the other shelterbelts. Only seven trees were sprayed because the snow and wind of the previous winter broke many branches and covered most of the lower branches with mud. Four applications were sprayed on the lower four feet of these trees.

The two areas along the east shore of Flathead Lake received one application of commercial bordeaux mixture and three applications of homemade bordeaux mixture. Since these trees were under four feet high, the entire trees were sprayed. These two areas were sprayed on the same days as the previous areas.

Sixteen of the trees in the Blackfoot area were sprayed with Phygon and thirteen were sprayed with lime sulfur. Each group of trees received three applications of its respective fungicide. The spraying dates were May 14, May 28, and June 14.

Experimental Spraying Results

All of the spraying plots were checked during the first week in October of 1954. The results of the bordeaux mixture applications are shown in Table No. IV. Seventy percent of the trees that were sprayed showed favorable results. This
Table No. IV. Results of spraying bordeaux mixture on the lower three feet of twenty blight infected Douglas fir trees in a shelter-belt.

<table>
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<tr>
<td>269</td>
<td>x</td>
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</table>

meant that the lower three feet of each tree that was sprayed had less blight on the current year's growth than did the portion of the tree above the three foot level. In most cases prior to spraying, the blight appeared to a much greater extent on the lower three feet than on the remainder of the tree so that the fungus was given a considerable advantage.

Figure 20a shows a typical branchlet of an unsprayed portion of the tree while Figure 20b shows a typical sprayed branchlet taken from the same tree. Only ten percent of these trees showed unfavorable results, which was probably a result of
poor spraying techniques. It was impossible to determine the results of twenty percent of the sprayed trees because no new infection was found on either the sprayed or unsprayed portion.

In several instances the current year's needles had already fallen by the first week in October. Figure 21 shows this defoliation on an unsprayed portion of a severely infected tree while Figure 22 shows how the bordeaux mixture saved the current season's growth of the same tree. Some of the needles were 'dwarfed as a result of the serious defoliation in previous years.

On several of the trees a sharp demarkation line could be seen between the sprayed and unsprayed portion of the tree. This was impossible to photograph because of the denseness of the various trees and shrubs within this windbreak. Bordeaux mixture was very effective in preventing the spores from producing new infection on the current season's growth of branchlets.

Of the sixteen trees sprayed with lime sulfur in the same shelterbelt, none showed any favorable results. Not one instance could be found where infection had been inhibited by the spray. Since the lime sulfur apparently did not harm any of the trees, it might be possible to increase the concentration in future studies.

The seven trees sprayed with commercial bordeaux mixture in the other shelterbelt showed some favorable results.
The new growth of many of the branchlets on the sprayed portion of each tree was *Rhabdosline* free while the remainder of the growth on the other branches was quite heavily infected. The mud on the lower branches prevented the spray from reaching the young needles and consequently, these needles were still vulnerable to attack by the infecting spores.

The new growth of the sprayed trees at Gravel Bay was remarkably free from the blight. Since previous years’ infection was found, this appeared to be the results of the bordeaux mixture. Closer study revealed, however, that no new infection could be found on any of the unsprayed trees in the immediate area. Therefore, no conclusive results were available for these ten trees.

An error was made when making one batch of the bordeaux mixture that was used on the other area on the east shore of Flathead Lake. Not enough lime was put into the solution and the excess of copper sulfate caused most of the needles to turn brown. In many cases the trees were killed. Only a few trees remained undamaged and all of the new growth on these trees were free from new *Rhabdosline* infection.

No results were obtained from any of the trees sprayed in the Blackfoot River area. During the latter part of August highway crews removed all of the fir trees that were growing along the right-of-way. Since Phygon did not appear to harm the foliage of the trees, future studies should include the use of this fungicide.
PERMANENT CHRISTMAS TREE BLIGHT PLOTS

In the fall of 1954 five 1/40 acre permanent plots were established for the purpose of keeping known infected trees under observation for several years. Each plot was in the form of a square and the corner posts were 1" x 2" stakes extending four feet out of the ground with the top eight inches of each stake painted a bright blue color. Each tree within the plot was tagged with a numbered metal tag breast high on the larger trees and knee high on the smaller ones. A rough map was made of each plot to show the number of each tagged tree and its relative position within the plot. When Christmas tree blight was found on a tagged tree, it was listed as an infected tree on the rough map.

Plot B-1 and Plot B-2 were located two miles east of Bigfork, Montana (Fig. 1) on land owned by Art Whitney and managed exclusively for the production of Christmas trees. Apparently the best formed Christmas trees were removed from the stand when this area was cut over in the fall of 1953. Both plots were located adjacent to an old logging road and contained trees averaging twenty feet in height. Plot B-1, located on level ground, had a small clearing in one corner. This plot contained twenty-nine trees, of which nine trees or thirty-one percent were infected. (Fig. 2). Plot B-2 was
established on a gentle northwest slope and supported ten infected trees out of a total of fifty-eight or seventeen percent. (Fig. 23).

The remaining three plots were established on the Lubrecht Experimental Forest (Fig. 1) and all of the trees within these plots were less than six feet tall. Plot L-1 was located in section 12 on a gentle east slope 7.6 chs. south and 8.3 chs. west of 1 6 12 7 (T. 13 N., R. 14 W., 15 W.). Forty-six percent or eleven trees out of twenty-four were infected with Rhabdocline. (Fig. 24). Plot L-2 was established on an east slope within thirty feet of a small stream in section 7. It was 27.7 chs. south and 7.7 chs. east of 1 6 12 7 (T. 13 N., R. 14 W., 15 W.). Fifty-six percent or fourteen of the twenty-five trees within the plot were infected. (Fig. 24). Plot L-3 was located on a very gentle northwest slope 11.6 chs. west and 3 chs. north of 25 24 (T. 13 N., R. 15 W.). Twelve of the twenty-eight trees or forty-three percent of this population were infected. (Fig. 25). A thorough examination of these plots should be made at least once each year to study further the rate of spread and other characteristics of the disease.
DISCUSSION

Macroscopic Symptoms

In the early spring it is comparatively easy to distinguish between Christmas tree blight and other needle damage, but it is usually impossible to predict whether the brown spots will result in fructifications of *Rhabdoscelis* or *Rhabdoscelium*. In June after the fruiting has begun it is almost an impossibility to differentiate between *Rhabdoscelis pseudotsugae* and *Rhabdoscelium hypophyllum* without the aid of a microscope. The *Rhabdoscelis* apothecia assume a characteristic appearance, but there is always the possibility that both the perfect and imperfect stages may be in the same pustule.

During seasons of heavy infection the infected fir trees may have an overall yellowish or brownish cast that can be seen for several hundred feet. It requires much closer scrutiny to determine positively whether this yellowing or browning is caused entirely by Christmas tree blight. Other damaging factors that will cause similar browning effects are frost damage, needle miners, and other insects. As a result, a careful examination of the browned needles is necessary to determine accurately the cause of any brownish cast.

In June and July it is usually possible to identify
Rhabdospora pseudotauvae since it occurs on the upper side of the needles as small blisters. By this time most of the Rhabdocline pustules are fully open and are characterized by their irregular longitudinal slits on the underside of the needle. It is not uncommon to observe an imperfect fructification on the upper side of a needle and an apothecium on the lower side situated on the same necrotic brown spot. Although the differentiation between perfect and imperfect forms may be difficult, they are quite easily distinguished from other types of needle damage throughout most of the year.

Infection Requirements

Although the complete pattern of infection has not been definitely established, it seems that one manner of infection is by the ascospores. It is also possible that the conidiospores of the imperfect stages might be another means by which the current year's needles are infected. The first outward appearance of infection is visible in September or October when yellow spots appear on the lower surfaces of the needles. Since this infection does not show up before autumn, it is possible that the ascospores and both types of conidiospores might be able to cause new infection. It is believed that the spores infect only the current season's tender needles, but it may be possible that one-year-old needles might also be susceptible to new infection during years of heavy infection.

Dominance or vigor of an individual tree does not seem
to influence completely the susceptibility of that tree. Consequently, the chance of a tree becoming infected with Christmas tree blight must depend upon (1) the viability of the spore, (2) the chance of a spore reaching a susceptible part of a needle of that tree, (3) the microclimatic conditions surrounding the particular needle, and (4) the overall climatic conditions of that season. The climatic conditions for a particular season probably influence the susceptibility of an individual tree the most. Moist growing seasons and days of high relative humidity are conducive to the spread of the fungus, as was the case during the epidemic in 1947 and 1948.

Conidial Stages

The biggest problem in the study of *Rhabdocline pseudotaxus* is the determination of the life cycle. Many investigators very simply state that the ascospores infect new needles which, in turn, produce more ascospores, etc. When this theory is used there is no mention of either conidial stage. It has been suggested that the imperfect stages are on their way out of the picture. This is based on the assumption that at one time they were a part of the life cycle, but various environmental changes are bringing about their elimination. If this theory is true it seems to the writer that the conidial stages should not appear in nature as often as they do.

Ellis and Gill (9) state that *Rhabdocline pseudotaxus*
is probably not the imperfect stage of *Rhabdocline pseudotsugae* because the former is collected so seldom and has only been found in nature after the fruiting bodies of the latter are mature. Although this is another plausible theory, it seems strange that the hyphae of both forms should be so similar in size and appearance. The spores have some similarities in shape and in their manner of germination. Therefore, the writer believes that there may be some definite connection between these two forms.

*Rhabdocline hypophyllum* is probably more common than *Rhabdocline pseudotsugae* and therefore seems more apt to be an imperfect stage of *Rhabdocline pseudotsugae*. An argument in favor of this theory is the appearance of mature fruiting bodies of *Rhabdocline hypophyllum* usually before the fruits of *Rhabdocline*. Since this conidial fungus has been known to be forced out of a pustule by the asci originating under it in the same pustule, a close relationship seems to exist between these two forms. Perhaps it is possible for both ascospores and conidiospores to infect the host.

Another possible explanation is that *Rhabdocline pseudotsugae* might be a heterothallic fungus. If this is the case, conjugation would be dependent upon the presence of mycelia which have arisen from two inherently different strains. Therefore, a "plus" strain and a "minus" strain might be necessary to cause the fungus to fruit. If conjugation did not occur perhaps neither the perfect or imperfect
pustules would be formed during that same year. There is also the possibility that conidiospores would be produced, but conjugation might be necessary to produce the perfect fructifications.

The life cycle of this fungus appears to be very complex and much more research is necessary before any positive conclusions can be determined. A good deal of culture and inoculation research is needed to prove or disprove the heterothallic theory.

Control

Since bordeaux mixture has been proven effective in controlling Christmas tree blight, cost studies should be initiated to determine how practical a spray program would be. It seems that it would be economically feasible to spray trees in nurseries, plantations, and in young stands intensively managed for Christmas tree production. If the blight ever built up to epidemic proportions for several years in succession, it would be imperative for the Christmas tree farmers to spray. Western Montana has not yet seen any intensively managed land used exclusively for Christmas tree production. If this intensive management should ever be initiated, disease and insects would have to be controlled to produce the better grades of Christmas trees. Since years of severe blight cannot be predicted, a study should be carried on to determine how much money could be spent for disease and insect control on Christmas trees.
If Christmas tree blight ever became serious enough to completely ruin the Christmas tree industry of Montana there would be two alternatives for the Christmas tree industry. The first would be to utilize other species of conifers for Christmas trees as is being done in many of the eastern states. The other alternative would be to breed blight resistant trees. Since some Douglas fir trees in an infected stand appear resistant to infection by Rhabdocline, it might be comparatively simple to grow a resistant variety. Some of these resistant trees might be similar to the green Douglas fir which is only lightly infected in Europe.

Suggested Future Studies

The most important work remaining in these studies is to determine the life cycle of Rhabdocline pseudotsugae and to establish the role of Rhabdocloeum hypophyllum and Rhabdocloeum pseudotsugae. To accomplish this, much research is needed in culture and inoculation work. Some culture work should be attempted with spores from both conidial stages. Other culture work should include spores from different needles in the same dish to check for "plus" and "minus" strains. Inoculations are necessary to prove Koch's pustulates and also aid in the determination of the mode of infection. The permanent plots at Bigfork and at the Lubrecht Experimental Forest should be examined each year to study rate of spread and the influence of the blight upon each infected tree.

There is still more research needed in the control of
this fungus. As was previously mentioned, cost analyses should be made regarding the spraying of bordeaux mixture on a large scale to Christmas tree stands managed intensively and to young fir trees in a nursery. More spraying experiments should be made using lime sulfur, Phygon and other commercial products at various concentrations. Probably the most important phase of control would be to develop a blight resistant variety of Douglas fir. A control measure of this nature would undoubtedly be the most satisfactory to Christmas tree farmers.
SUMMARY

*Rhabdocline pseudotsugae* Syd. is a fungus (*Ascomycota, Phacidiaceae*) that causes a defoliating disease of young Douglas fir trees. This disease is quite common to the fir of the northwestern states and was shipped to Europe on planting stock prior to 1914. Serious epidemics have occurred in the United States in 1916 and 1917, 1923 and 1924, and 1947 and 1948.

The fungus is recognized by the brown spots and bands that are produced on Douglas fir needles. These brown areas develop into the fruiting bodies of either *Rhabdocline pseudotsugae*, *Rhabdocleaeum hypophyllum*, or *Rhabdocleaeum pseudotsugae*. Usually trees having Christmas tree blight lose their infected needles after one or two years instead of the normal eight years for healthy trees. The destruction caused by frost and several insects is often confused with the damage caused by *Rhabdocline*.

The apothecia of *Rhabdocline pseudotsugae* are elongated and embedded in the epidermal layer of the substratum on the underside of the needle. The epidermal covering ruptures by an irregular slit to expose the fruiting layer which is composed of eight-spored asci and swollen tipped paraphyses. The ascospores become two celled after discharge; one cell
turns brown and is usually the one that produces the germ tube.

There are two conidial stages of *Rhabdosloeum* that often appear with *Rhabdoolineae pseudotauruaa*, but their role in the life cycle has not been determined. *Rhabdosloeum hypophyllum* appears earlier in the season than *Rhabdosloeum pseudotauruaa*. The former has smaller spores, longer and more prominent conidiophores, and the underlying hyphal layer is thicker and more conspicuous than that of the latter. There are many speculations as to the role of these two conidial stages, but definite proof is lacking for all of these theories.

Attempts to culture this organism were at first successful, but later unsuccessful. The conidia produced in culture were somewhat constricted in the middle and in old cultures became two-celled, thick walled, and dark colored. All attempts to inoculate young Douglas fir trees failed.

A survey of the Lubrecht Experimental Forest was made in the early summer of 1953 to learn more about the disease and its relation to the environment. The vigor of the individual tree apparently did not have any effect upon susceptibility, but infection seemed due to the chance of the spore reaching a susceptible needle of that tree. It seems possible that the disease "holds over" on young trees growing on open areas such as old logging roads and skid trails where there is evidence that the soil had been disturbed.
many years before.

A good means of control was found by spraying the infected trees with 4-4-50 bordeaux mixture during the time of ascospore discharge. Lime sulfur was also tried, but the concentration that was used did not seem strong enough to give any positive results. Two permanent plots were established at Bigfork, Montana and three plots were established on the Lubrecht Experimental Forest for the purpose of further studying the blight.
BIBLIOGRAPHY


FIGURES

Photography

A 35 mm Leica camera and Plus-X film was used in all of the photography except Figs. 19, 21 and 22. A press camera was used for these three pictures. The Kodacolor pictures in Fig. 20 were taken with the 35 mm Leica camera, using a closeup attachment. All photomicrographs, as well as the ones taken through binoculars, were taken with the aid of a Leitz Micro Ibo attachment.
Fig. 1
Map Showing Location of Field Work

Key
- Spraying plots
- Boundary of Lubrecht Forest Survey
- Permanent plots
Fig. 2. Color changes on the lower surface of a Douglas fir needle infected with *Rhabdocline pseudotsugae*. 

a. Yellow spots forming at infected areas. 
b. Brown spots with yellow margins. 
c. Brown spots. 
d. Pustules showing white ridges and the darker valleys from which the ascospores are discharged.
Fig. 3. The usual appearance of Douglas fir needles infected with *Rhabdocline pseudotsugae*.

a. Spotting only. b. Banding only. c. Combination of spotting and banding.
Fig. 4. Cooley's gall (Chermes cooleyii) on Douglas fir needles. a. Cottony mass with accompanying adjacent black spots. b. Bending of needle. c. Twisting and bending of needle.
Fig. 5. Purple-brown banding caused by a Douglas fir needle miner. 

a. Purple-brown band in middle of needle. 
b. Purple-brown band at tip of needle. 
c. Needle broken at band to expose the orange colored larva.
Fig. 6. Infected Douglas fir needle showing apothecia of *Rhabdocline pseudotsugae*. Note the manner in which the epidermis ruptures by longitudinal slits to expose the fruiting layer. (x 15).
Fig. 7. Cross section of a Douglas fir needle infected with *Rhabdocline pseudotsugae*. In this particular needle the apothecium is on one side of the midrib. (× 150).
Fig. 8. Hyphae of *Rhabdocline pseudotsugae* in the cells of the mesophyll. These hyphae have just lost their shrivelled appearance. This photomicrograph was taken in the early spring of 1955. (x 850).
Fig. 9. a. Arrangement of ascospores within asci and accompanying swollen tipped paraphyses. (x 520).
b. (After Wilson and Wilson) Germination of an ascospore in water. (x 1400).
1. Mature spore after being shed.
2. Spore that has become two-celled.
3. Germ tube arising from the dark colored cell.
Fig. 10. Conidiospores of *Rhabdoclorum pseudotsugae*.
(x 2300).
Fig. 11. Germinating conidiospores of *Rhabdogloeum pseudotsugae*. (x 640).
Fig. 12. Cross section of a fruiting body of Rhabdocloea pseudotuscae prior to rupture of the epidermis. (x 360).
Fig. 13. Cross section of mature fruiting body of Rhabdocordum pseudotsugae after rupture of the epidermis. (x 500).
Fig. 14. Cross section of a Douglas fir needle with an immature fruiting body of Rhabdosloeum hypophysllum on the lower surface. (x 120).
Fig. 15. Cross section of a Douglas fir needle with an immature fruiting body of *Rhabdoclosum hypophyllum* on the upper surface. Note the dense underlying hyphal layer. (x 180).
Fig. 16. Cross section of a Douglas fir needle with an epiphyllous fruiting body of *Rhabdocolus hypophyllum* shortly after the rupture of the epidermis. (x 150).
Fig. 17. Cross section of Douglas fir needle with two epiphyllous fruiting bodies of *Rhabdoglecum hypophyllum* shortly after the rupture of the epidermis. (x 150).
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**Fig. 18.** Typical information as recorded by the notkeeper in the Christmas tree blight survey of the Lubrecht Experimental Forest.
Fig 19. Portion of shelterbelt on Ben Bruyer's farm five miles north of Kalispell, Montana. The fir trees were approximately fifteen feet tall and twenty years old. This belt was very similar to the one on Bauer Bros.' farm. Both belts were heavily infected with Rhabdocline and served as ideal plots for the spraying tests.
Fig. 20. Comparison of sprayed and unsprayed branchlets of the same tree. a. Typical branchlet from an unsprayed portion of a fir tree infected with *Rhabdocline pseudotsugae*. Note the heavy infection on the current season's growth. b. Typical branchlet from a sprayed portion of the same tree. Note the absence of infection on the current season's growth as a result of spraying with bordeaux mixture.
Fig. 21. Typical branch taken from a Douglas fir tree that has been severely infected with Christmas tree blight for three successive years. This picture was taken in October. Note that most of the current year's needles have already been dropped.
Fig. 22. Branchlet from a sprayed portion of a heavily infected tree. The bordeaux mixture saved the current season's growth. The stunted needles are a result of the defoliation of the previous years.
Fig. 23. Diagrams showing permanent Christmas tree blight plots two miles east of Bigfork, Montana. The green dots denote healthy trees while the brown dots represent trees infected with *Phytophthora cinnamomi*.
Fig. 24. Diagrams showing two of the permanent Christmas tree blight plots on the Lubrecht Experimental Forest. The green dots denote healthy trees while the brown dots represent trees infected with *Rhabdocline*.
Fig. 25. A diagram showing the third permanent Christmas tree blight plot on the Lubrecht Experimental Forest. The green dots denote healthy trees while the brown dots represent trees infected with *Rhabdocline*.