SOME ASPECTS OF THE ASSOCIATION BETWEEN RHABDOGLOEUM PSEUDOTSUGAE SYDOW AND RHABDOCLINE PSEUDOTSUGAE SYDOW

bу

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ABSTRACT

Rhabdogloeum pseudotsugae Sydow has been suspected of being the imperfect stage of Rhabdocline pseudotsugae Sydow since their original description by Sydow in 1922. Because Rhabdogloeum has been reported so seldom in North America, and never in Europe, this possibility has been considered slight by most investigators. Investigation of the problem of Rhabdocline pseudotsugae in British Columbia revealed several new aspects of the association between the two leaf-cast diseases.

Inspection of <u>Rhabdocline</u> and <u>Rhabdogloeum</u> collections from the interior of British Columbia indicated that the association of <u>Rhabdogloeum</u> pseudotsugae and <u>Rhabdocline</u> pseudotsugae is more prevalent than was formerly believed. Collections from the coast, and on Vancouver Island in particular, indicated that the association is probably less prevalent there than in the interior. In relation to the frequent and widespread occurrence of <u>Rhabdocline</u> throughout the Douglas fir range however, <u>Rhabdogloeum</u> is seldom found. If <u>Rhabdogloeum</u> pseudotsugae is the imperfect stage of <u>Rhabdocline</u> pseudotsugae, then it appears that frequently

the Rhabdogloeum stage and occasionally the Rhabdocline stage is completely suppressed, as both stages have been found dissociated from each other.

Frequent observation of a group of tagged Rhabdocline-infected trees on Vancouver Island over a period of two years did not reveal the presence of a Rhabdogloeum stage. Tissue cultures of lesions from these tagged trees, however, resulted in the growth of a fungus quite similar in appearance to that produced in tissue cultures by lesions typical of those produced by Rhabdogloeum pseudotsugae. This supports the view that Rhabdogloeum pseudotsugae is the imperfect stage of Rhabdocline pseudotsugae. Observation of these trees indicated that Rhabdocline pseudotsugae is capable of vegetating two years before producing apothecia. Apparently it is also capable of vegetating more than two years or of infecting leaves other than those of the current year.

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SOME ASPECTS OF THE ASSOCIATION BETWEEN RHABDOGLOEUM PSEUDOTSUGAE SYDOW AND RHABDOCLINE PSEUDOTSUGAE SYDOW

INTRODUCTION

The problem of <u>Rhabdocline pseudotsugae</u> Sydow, the fungus organism causing a needle cast disease of Douglas fir, is becoming of increasing importance in North America (9, 11, 22, 29, 32). This is attributed to the increasing demand for second-growth Douglas fir for timber and for Christmas tree stock. Considerable concern has been shown in Europe since the introduction of the disease into Scotland from North America about 1913 (33) and its introduction into Germany from Scotland about 1926 (2). <u>Rhabdogloeum pseudotsugae</u> Sydow has been found associated with <u>Rhabdocline pseudotsugae</u> in North America only rarely and has never been reported from Europe (2, 8, 28).

Both pathogens were named and described by Sydow in 1922 (26), Rhabdocline pseudotsugae being placed in the Ascomycetes, Phacidales and Rhabdogloeum pseudotsugae in the Fungi Imperfecti, Melanconiales. He suggested at the time that Rhabdogloeum pseudotsugae was the imperfect stage of Rhabdocline pseudotsugae. In 1926 Wilson and Wilson (33) reported that, in America, conidia of Rhabdocline pseudotsugae developed on the upper surface of leaves during July. In referring to this statement by Wilson and Wilson,

Van Vloten (28) pointed out that an association of imperfect with perfect fruiting bodies did not constitute proof of their relationship. In 1945 Ellis and Gill (13) named and described a new species of Rhabdogloeum, R. hypophyllum, associated with Rhabdocline pseudotsugae in the southwestern United States. Rhabdogloeum hypophyllum differs from R. pseudotsugae in having considerably smaller conidia, longer conidiophores and thicker underlying hyphal layers, from which the conidiophores arise.

Rhabdogloeum pseudotsugae has been reported so infrequently in North America that the possibility of it
being the imperfect stage of Rhabdocline pseudotsugae has
been considered slight by most investigators (2).

Because of the increasing importance of Rhabdocline pseudotsugae in this country the Dominion Laboratory of Forest Pathology, Victoria, British Columbia, assigned the author to investigate the disease. This thesis constitutes a report on a portion of the investigation and presents some of the results of studies aimed at clarifying the association between Rhabdocline pseudotsugae and Rhabdogloeum pseudotsugae.

<u>METHODS</u>

All herbarium specimens of the two fungi deposited with the Dominion Laboratory of Forest Pathology, Victoria,

British Columbia were examined. Examinations were made also of portions of all collections of these fungi deposited with the United States Department of Agriculture, Division of Forest Pathology, Albuquerque, New Mexico, and some of those deposited with the United States Department of Agriculture, Division of Forest Pathology, Portland, Oregon. Freehand sections were made of lesions on most of these specimens. The sections were mounted in 5 per cent potassium hydroxide and measurements made with a Filar micrometer. Each spore size recorded represents the average measurement of fifty or more spores.

All material for culturing was obtained from tagged trees in the British Columbia Forest Experimental Station at Cowichan Lake on Vancouver Island. One tree was infected with Rhabdogloeum pseudotsugae and the remaining trees, one-half mile distant were infected with Rhabdocline pseudotsugae. The trees have been examined periodically by the author since May, 1949.

In making tissue cultures of lesions whole leaves were dipped in 95 per cent alcohol, surface sterilized in 1:1000 mercuric chloride for two minutes and washed in three separate dishes of sterile water. The lesions were then cut out, planted on Difco malt agar and kept at room temperature (60 - 80°F).

In March 1950, using a method described by Griffith

(14), two hundred cuttings of the previous year's growth of Douglas fir trees were made for use in inoculation experiments. The cuttings were from the tagged trees having necrotic lesions typical of <u>Rhabdocline pseudotsugae</u> on two-year-old and older needles (Fig. 1).

OBSERVATIONS

Study of Herbarium Specimens

There were twenty-eight different localities throughout the Douglas fir range in British Columbia represented by herbarium collections. Examination revealed Rhabdogloeum pseudotsugae to be present in nine widely scattered localities at the time the collections were made (Table I). At Cranbrook inspection of 206 trees on a one-tenth acre plot of Christmas tree stock in July, 1950 yielded thirteen trees infected with R. pseudotsugae. Intensive scouting in the Cowichan Lake Forest Experimental Station has yielded only one infected tree with the pathogen (30). Collections made from the other areas do not indicate a systematic search for the pathogen.

An association of Rhabdogloeum pseudotsugae with Rhabdocline pseudotsugae (Fig. 2) was noted in collections from five of the nine localities. In the Lemon Creek area a tree was found with an infection of Rhabdogloeum unassociated with Rhabdocline, whereas other trees a few yards

away had only a <u>Rhabdocline</u> infection. In the Sooke area also a tree was found infected with only <u>Rhabdogloeum</u> and further inspections made in July, 1949 and May, 1950 still revealed no <u>Rhabdocline</u> infection. The remaining two collections infected with <u>Rhabdogloeum</u> but showing no evidence of <u>Rhabdocline</u>, one from Langford, the other from Kamloops, were made early in May, too early in the year for an association to be evident.

Acervuli of <u>Rhabdogloeum</u> were found on needles twoyears old and older in the collections from Sooke and Kamloops and on needles one-year-old and older on the remainder.

The spore measurements obtained from the collections and tabulated in Table I, did not vary appreciably from one another or from those described by Sydow (26).

Study of Fresh Material

In May of 1949 and in May, 1950 all Douglas fir trees within a 132 foot (two chain) radius of the tagged Rhab-docline-infected trees failed to show any sign of Rhab-dogloeum. No sign of Rhabdogloeum was found on fresh material collected from the tagged trees in this area over a period of twenty-two months. Collections represented every month of the year except April. The three separate crops of Rhabdocline apothecia observed during

this period occurred only on two-year-old, and older needles. All but a very few leaves with lesions were cast by the end of August each year. As the disease was not severe during the observed period, not all the leaves of any one year on a branch were infected. New apothecia appeared each year on some of these previously uninfected two-year-old, and older needles.

Signs of disease appeared on one-year-old, and older leaves of the tagged Rhabdogloeum-infected tree. Chlorotic lesions appeared in the period September - January and were the first indications of new infection. Acervuli occurred on the upper surface of leaves and apothecia of Rhabdocline pseudotsugae occasionally appeared directly below the acervuli on the lower surface of the leaves.

EXPERIMENTS AND RESULTS

Tissue Cultures

Over the two-year period six sets of tissue cultures were made of the lesions on tagged trees. Each set was made during a different late fall and winter month. Of the 163 tissue cultures obtained from four Rhabdocline-infected trees, forty-five per cent resulted in macroscopic growth similar to that obtained from a polysporous culture of Rhabdogloeum pseudotsugae (Figs. 4, 5), thirty per cent produced no growth and the remaining twenty-five per cent

produced a number of different organisms. Seventy-four per cent of the thirty-one cultures made from lesions occurring on the Rhabdogloeum-infected tree resulted in growth similar to the polysporous isolate, ten per cent produced no growth and sixteen per cent produced a number of different organisms.

Mycelial growth from these tissue cultures appeared on the agar in from one to six weeks. Subsequent growth, which was white and irregular in pattern, reached a diameter of about 10 mm. within two to three weeks. After three to four weeks of visible growth a yellow-green colour appeared at the centre of the mats and extended over most of the mat within six to eight weeks. Light-grey and light-brown patches of mycelium were often interspersed with the yellow-green. The malt agar under the mats turned an olive-green to a dark-brown colour in about two months (Fig. 3).

Microconidia and macroconidia were produced in both tissue cultures and polysporous culture (Fig. 6, 7).

Microconidia from the three sources were pale green to hyaline and oblong to oval. Microconidia from five tissue cultures of the Rhabdogloeum-infected tree measured $3.1 - 5.5 \mu x$ $1.5 - 2.4 \mu$ (mean $2.1 \times 4.2 \mu$). Ten spores from each of four tissue cultures and twenty from the remaining tissue culture were measured. Microconidia from four

tissue cultures of Rhabdocline-infected trees measured 2.1 - 4.3 μ x 1.2 - 2.3 μ (mean 1.7 x 3.3 μ). Ten spores from each of two tissue cultures and twenty from the remainder were measured. Macroconidia from six tissue cultures of the Rhabdogloeum-infected tree measured 8.3 - 14.3 μ x 3.4 - 5.8 μ (mean 4.7 x 11.4 μ). Ten spores from each tissue culture were measured. Macroconidia from four tissue cultures of Rhabdocline-infected trees measured 9.1 - 13.7 x 3.3 - 5.4 μ (mean 4.3 x 10.5 μ). Ten spores from each of two tissue cultures and twenty from the remainder were measured. Chi-square values of the means of these spore measurements were computed. A value of 0.26 was obtained for the lengths and 0.11 for the widths of microconidia. probability of obtaining greater Chi-square values for these dimensions were between 0.6 and 0.7 for the lengths and between 0.7 and 0.8 for the widths. A Chi-square value of 0.07 was obtained for lengths and 0.05 for the widths of macroconidia. The probability of obtaining greater Chi-square values for these dimensions were between 0.7 and 0.8 for the lengths and between 0.8 and 0.9 for the widths. To have ninety-five per cent confidence, a Chi-square value of 3.84 would have to be obtained with the mean values of the spore measurements, from the different tissue cultures, to indicate a significant difference. Microconidia from the polysporous culture measured

3.8 - 6.2 μ x 1.7 - 3.2 μ (mean 2.3 x 5.0 μ). Macroconidia from this culture measured 10.5 - 16.0 μ x 3.8 - 6.5 μ (mean 5.2 x 13.6 μ). These spore sizes were based on the measurement of twenty spores of each type.

Macroconidia from all cultures were similar in shape to those occurring in nature. When produced in abundance they occurred as olive-green masses which often covered a considerable portion of the mycelial mat. Macroconidia were one or two-celled and were generally light-green in colour although occasionally hyaline. Germination on malt agar took place at room temperature within about twenty-four hours. Both one and two-celled spores germinated. In some of the older cultures one cell of the septate spores became dark-walled. A similar condition was noted by Wilson and Wilson (33) in mature ascospores of Rhabdocline pseudotsugae.

Microconidiophores appeared similar in shape to those producing macroconidia (Fig. 8, 9, 10, 11), and measured $8.0 - 40.0\mu$ in length by $2.0 - 3.2\mu$ in width (mean $2.6 \times 12.5\mu$). Macroconidiophores measured $8.5 - 39.5\mu$ in length by $2 - 3.2\mu$ in width (mean $2.6 \times 12.0\mu$). These conidiophore sizes were based on the measurement of ten microconidiophores and ten macroconidiophores.

Subsequent single spore isolations of macroconidia produced in culture from the three sources gave similar

results. Repeated attempts to germinate and grow microconidia failed.

In January 1950, thirty-six leaves were taken from four tagged Rhabdocline-infected trees for tissue culture purposes. Only those leaves with a lesion similar in appearance to one occurring on an adjacent leaf were taken. The positions of the control leaves were marked and observations were made to see if there was any indication of Rhabdogloeum pseudotsugae, in the latter part of March and May and in the first week of August. At the time of observation in August, fourteen of the leaves had been cast.

No evidence of Rhabdogloeum pseudotsugae was observed on the remaining needles.

Thirty-one per cent of the tissue cultures made from these leaves produced growth similar to that described above; thirty-six per cent failed to produce growth of any kind and the remaining thirty-three per cent gave rise to a number of different organisms.

During the months of January, February, March and May, leaves from tagged trees having necrotic lesions typical of those caused by <u>Rhabdocline pseudotsugae</u> were placed in moist chambers and inspected periodically. No evidence of <u>Rhabdogloeum</u> or apothecia of <u>Rhabdocline</u> appeared. Growth Rates

A comparison of growth rates was made of isolates

from a needle lesion on a Rhabdocline-infected tree (isolated August 1950), a needle lesion from a Rhabdogloeuminfected tree (isolated August 1950) and a polysporous culture of Rhabdogloeum pseudotsugae (isolated September 1949). The isolates were grown on three per cent Difco malt agar at room temperature for four weeks. Sections were then cut from the edge of the culture using a sterile 4 mm. cork borer and were transferred to fresh plates containing three per cent Difco malt agar. The plates were then divided and placed in six constant temperature chambers with temperatures ranging from 5 to 30°C. Growth was measured weekly for eight weeks. After five weeks the growth rate in all chambers declined considerably. All three isolates grew best at 15°C and those at 30°C failed to grow. At 1500 the tissue cultures originating from the Rhabdogloeum-infected tree attained a maximum diameter of 4.1 cm., the polysporous culture of Rhabdogloeum pseudotsugae 3.2 cm. and the isolate originating from the Rhabdocline-infected tree 2.9 cm.

These isolates were grown also at 15°C for eight weeks on prune, corn-meal, dextrose and potatoe-dextrose agars. The media, in the order of greatest growth attained by the three isolates were: potatoe-dextrose, dextrose, malt, prune and corn-meal. The growth rates of the three isolates decreased after the fifth week on all media.

The tissue culture from the Rhabdogloeum-infected tree showed more growth on all media than did the remaining two isolates which grew at approximately equal rates.

The growth of a tissue culture made from a Rhabdog-loeum-infected leaf was observed on separate plates containing three per cent Difco malt agar adjusted to various pH values. The organism was grown in Petri dishes at 15°C and measurements of growth were made weekly for eight weeks. A Beckman pH meter was used to obtain the readings. The average diameter-growth of the organism increased with increasing pH up to 7.7, and declined sharply at the next highest pH, which was 8.3. There was only a slight increase in growth between the three cultures whose pH readings were 4.15, 5.45, and 6.00.

Fruiting in Culture

Modifications of the methods described by GwynneVaughan and Barnes (15), and Zentmyer et al (35), to obtain
perfect fruiting bodies in culture were tried, using
isolates from leaf lesions of Rhabdocline-infected trees
and the Rhabdogloeum-infected tree. The experiments were
started in November. Claussen's medium was prepared and
poured into Petri dishes as described by Gwynne-Vaughan
and Barnes. Eight of these poured plates were then inoculated with isolates from the Rhabdocline-infected tree

and eight were inoculated with the isolate from the Rhabdogloeum-infected tree. From each of those groups of inoculated plates, four were placed in the greenhouse, two outdoors and the remaining two were left at room temperature. No evidence of perfect fruiting bodies was noted within three months.

Douglas fir leaves were sterilized using propylene oxide after the method described by Zentmyer. The sterilized leaves were placed in test tubes and Petri dishes containing warm liquid, two per cent Bacto-agar which held the leaves firmly in position on hardening. Fourteen test tubes and fourteen plates were then inoculated with isolates from the Rhabdocline-infected trees and an equal number of tubes and plates were inoculated with isolates from the Rhabdogloeum-infected tree. Eight plates from each group were placed outdoors, four were placed in a greenhouse and the remaining two were left at room temperature. The test tubes were dispersed in a similar manner. No evidence of perfect fruiting bodies was noted within three months.

Inoculations

Early in August 1950, eighty of the cuttings described on page 4 were inoculated with a spore suspension made from tissue cultures obtained from Rhabdocline-

infected trees and forty were inoculated with a spore suspension made from tissue cultures obtained from Rhabdogloeum-infected trees. Spores were suspended in a sterile five per cent glycerine solution and were sprayed on the cuttings with an atomizer. Thirty cuttings sprayed with a five per cent glycerine in water solution were used The cuttings were placed in a moist chamber as a control. for sixty hours after inoculations were completed and were then removed to a greenhouse. Most of the buds on the cuttings failed to open and in no case was growth of the current year vigorous. The cuttings were inspected in October, 1950 and were found to have chlorotic lesions on the previous year's growth similar to those occurring on trees naturally infected by Rhabdocline pseudotsugae at that time of year. Fifty-four per cent of both sets of cuttings inoculated with spores from tissue culture sources and sixty-nine per cent of the cuttings used in the control had lesions. Subsequent tissue cultures from the three groups of cuttings produced cultures similar to those obtained from Rhabdogloeum and Rhabdocline-infected trees.

DISCUSSION

Inspection of herbarium collections from the interior (1) of British Columbia indicated that the association of Rhabdogloeum and Rhabdocline is more prevalent than was formerly believed. Collections from the coast, and on Vancouver Island in particular, indicated that the association is probably less prevalent there than in the interior. In relation to the frequent and widespread occurrence of Rhabdocline throughout the Douglas fir range however, Rhabdogloeum is seldom found. The collections from Sooke and Lemon Creek indicated that Rhabdogloeum does occur unassociated with Rhabdocline. If Rhabdogloeum pseudotsugae is the imperfect stage of Rhabdocline pseudotsugae, it is apparent that, frequently, the Rhabdogloeum stage is completely suppressed and that, occasionally, the Rhabdocline stage is suppressed.

In Scotland, Wilson (34) occasionally found one-yearold leaves on Douglas fir showing no sign of <u>Rhabdocline</u>
infection while those of the previous year were diseased.
He stated that "either the infected needles have remained
on the tree for two years or they have become infected
during their second year, but both these conditions appear
abnormal". Boyce (2) has suggested that the disease is
capable of vegetating for nearly two years. Observations
made during the past two years in an area where only the

⁽¹⁾ That portion of B. C. east of the Cascade Mountains.

two-year-old and older leaves bore apothecia in early summer favour Boyce's suggestion. The appearance of Rhabdocline-like lesions on cuttings used as a control for inoculation experiments supplies further evidence for this suggestion. The time of the appearance of these chlorotic lesions on the one-year-old leaves of the cuttings coincides with the time of their appearance on the one-yearold leaves of trees naturally infected with Rhabdocline pseudotsugae. As the cuttings were kept in a greenhouse away from sources of infection it is very unlikely that they became infected after collection. As in the same year, apothecia of Rhabdocline appeared on the two-yearold and older leaves of naturally infected trees, the fungus must either be capable of vegetating for two or more years, or, it is able to vegetate for only two years and of infecting leaves of both current and previous years. Rhabdogloeum pseudotsugae, occurring on one-year-old and older needles, would appear capable of either vegetating one or more years or of infecting leaves of both current and previous years.

The tissue cultures from Rhabdocline-infected trees were similar in nearly all respects tested to those from Rhabdogloeum-infected trees. While the average growth rate of the tissue culture from the Rhabdogloeum-infected tree was greater than that of the tissue culture from the

Rhabdocline-infected tree, the type of growth and the size and shape of spores occurring in all cultures would indicate that they were the same organism. Chi-square values of average spore measurements indicated that there was no significant difference in size between the spores from tissue cultures of the Rhabdogloeum-infected tree and those from tissue cultures of the Rhabdocline-infected tree. A fair comparison of growth rate between the polysporous culture and the tissue cultures cannot be made because of the difference in time between isolations and difference in the type of inoculum used. As final proof that Rhabdogloeum pseudotsugae is the imperfect stage of Rhabdocline pseudotsugae awaits results of successful inoculation experiments, a new series has been designed.

Microconidia have not been found associated in nature with Rhabdocline pseudotsugae, Rhabdogloeum pseudotsugae, or Rhabdogloeum hypophyllum, nor did Ellis and Gill (13) report finding them in cultures of Rhabdogloeum hypophyllum. In accordance with the findings of Drayton (10), their presence in cultures of Rhabdogloeum pseudotsugae would probably indicate the occurrence of a perfect stage in the life-cycle of this organism.

SUMMARY

Inspection of collections of <u>Rhabdocline pseudotsugae</u> and <u>Rhabdogloeum pseudotsugae</u> revealed the two organisms to be associated on Douglas fir in the interior and coastal regions of British Columbia. The association appeared to be more common in the interior than on the coast. In relation to the frequent and widespread occurrence of <u>Rhabdocline</u>, <u>Rhabdogloeum</u> is seldom found. <u>Rhabdogloeum</u> pseudotsugae appeared to be capable of occurring unassociated with <u>Rhabdocline</u> pseudotsugae.

Observation of <u>Rhabdocline pseudotsugae</u> on Vancouver Island indicated its capability of vegetating two years before producing apothecia. It is also capable of either vegetating more than two years or of infecting leaves other than those of the current year.

Tissue cultures of lesions on Rhabdocline pseudotsugae-infected trees that occurred on two-year-old and
older leaves, produced cultures of a fungus quite similar
in appearance to that obtained from a polysporous culture
and tissue cultures of lesions similar in appearance to
those caused by <u>Rhabdogloeum pseudotsugae</u>. This supports
the view that the imperfect stage of <u>Rhabdocline pseudot-</u>
sugae is <u>Rhabdogloeum pseudotsugae</u>.

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TABLE I

LIST OF RHABDOGLOEUM PSEUDOTSUGAE COLLECTIONS IN BRITISH COLUMBIA WITH AVERAGE SPORE SIZES OBTAINED FROM EACH, AND A RECORD OF THOSE COLLECTIONS SHOWING AN ASSOCIATION WITH RHABDOCLINE PSEUDOTSUGAE

T 7	Dat	. 0	Occurrence of Rhabdocline in	Average Conidial (1)	
Locality of Collections	Date Colle	or ection	Association with Rhabdogloeum	Measurements u	
VANCOUVER ISLAND					
Lake Cowichan	May	6,1944	Present	5.3 x 16.7	
Sooke			Absent	4.9 x 14.5	
Langford			Absent	5.4 x 15.1	
INTERIOR					
Spences Bridge	e June	23,1949	Present	6.6 x 14.4	
Kamloops	May	5,1950	Absent	5.7×15.6	
Winfield	May	16,1950	Present	5.8 x 14.3	
Lemon Creek	June	5,1950	Absent	6.3 x 16.4	
Cranbrook	July	6,1950	Present	6.3 x 15.3	
Invemere	July	7,1950	Present	6.1 x 15.4	

⁽¹⁾ The spore sizes recorded by Sydow in the original description of Rhabdogloeum pseudotsugae were $15-21_u$ x $4-5_u$.

The spore sizes recorded by Ellis and Gill for Rhabdogloeum hypophyllum were 2.2-3.7 $_{\rm u}$ x 6.7-11.1 $_{\rm u}$.

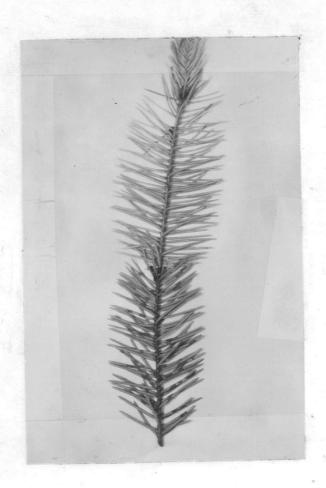


Figure 1. Lesions of Rhabdocline pseudotsugae on two-year-old leaves of Douglas fir.

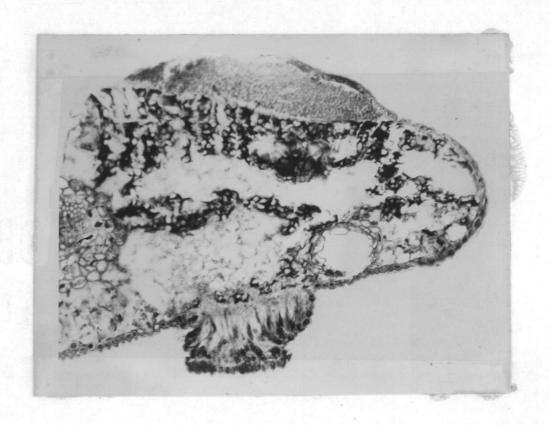


Figure 2. Photomicrograph of a cross-section of a Douglas fir leaf with an acervulus of Rhabdogloeum pseudotsugae on the upper surface and an apothecium of Rhabdocline pseudotsugae on the lower surface.

X approx. 40

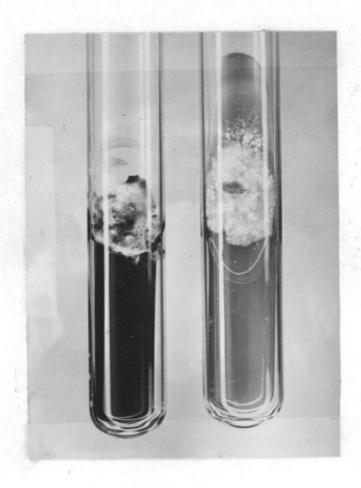


Figure 3. Tissue cultures of lesions typical of those caused by Rhabdogloeum pseudotsugae on malt agar at the end of eight weeks (left) and five weeks (right). The agar has turned an olive-green colour in the eight week old culture and has remained unchanged in the five week old culture.



Figure 4. Mycelial growth at room temperature from a lesion typical of those caused by Rhabdogloeum pseudotsugae on malt agar at the end of four weeks. X 2/3

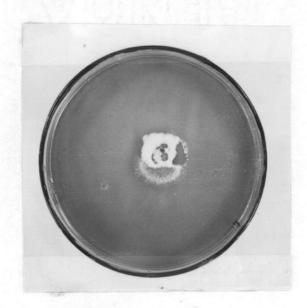


Figure 5. Mycelial growth at room temperature from a lesion typical of those caused by Rhabdocline pseudotsugae on malt agar at the end of four weeks. X 2/3

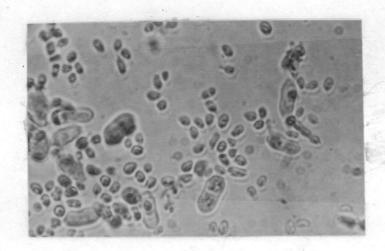


Figure 6. Photomicrograph of microconidia and macroconidia from a tissue culture of a lesion typical of those caused by Rhab-docline pseudotsugae. X approx. 1050

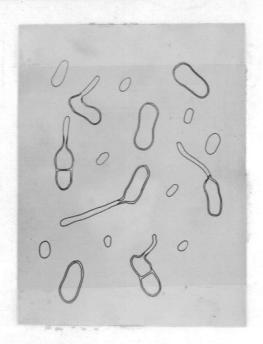


Figure 7. A camera-lucida drawing of spores occurring in tissue cultures of lesions typical of those caused by Rhabdocline pseudotsugae.

X approx. 1065



Figure 8. A photomicrograph of microconidiophores from a tissue culture of a lesion typical of those caused by Rhabdocline pseudotsugae. X approx. 1050

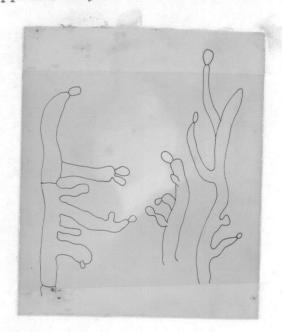


Figure 9. A camera-lucida drawing of microconidiophores from tissue cultures of lesions typical of those caused by Rhabdocline pseudotsugae. X approx. 1050

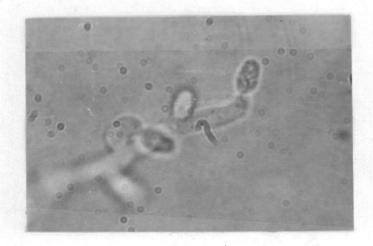


Figure 10. A photomicrograph of macroconidiophores from a tissue culture of a lesion typical of those caused by Rhabdocline pseudot-sugae. X approx. 1050

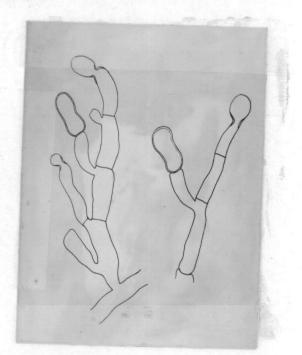


Figure 11. A camera-lucida drawing of macroconidio-phores from tissue cultures of lesions typical of those caused by Rhabdocline pseudotsugae. X approx. 1050