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STUDIES ON THE DIEBACK OF LACEBARKS.

Myxosporium Hoheria. n.f.sp.

By. "Assured".

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STUDIES ON THE DIEBACK OF LACEBARKS.

Myxosporium Hoheria. n.f.sp.

INTRODUCTION.

The Maori names Houi, Whauwhi and Houhere, or the settlers terms lacebark, and ribbon-wood, cover several species of flowering plants belonging to the order Malvales. These species, which are all indigenous to New Zealand, fall into the genera Hoheria, and Plagianthus, Laing and Blackwell (1927) list the following eight species:

Hoheria populnea.	Hoheria glabrata.
do sexstylosa.	do Allanii.
do angustifolia.	Plagianthus divaricatus.
do Lyallii.	do betulinus.

H. Populnea is found chiefly in the Auckland and North Auckland districts, as a member of the subtropical rain forest, but Laing has recently recorded its occurrence in Karamea. H. sexstylosa occurs throughout both islands as a member of the lowland bush communities. H. angustifolia is typically a South Island plant found in large numbers on Banks peninsula, but is also found in the south of the North Island. H. Lyallii is a deciduous shrub growing in the mountainous districts of the South Island. H. glabrata belongs to the subalpine flora, growing usually in situations where it can obtain abundant light, e.g. recent landslips. Cockayne (1928). H. Allanii is a small leaved shrub recorded from the Rakaia gorge, Canterbury.

Plagianthus divaricatus belongs to the coastal flora, growing as a dense bushy shrub on salt meadows and round tidal estuaries. P. betulinus occurs throughout the Dominion and outlying islands to the South. It is the largest of the ribbon-woods, growing into a canopy tree up to 60ft. in height. (Allan. 1928)

In many parts particularly the southern districts it assumes a deciduous habit.

Like many other New Zealand trees the lacebarks show a great difference between their juvenile and mature forms. Thus *P. betulinus* in its adolescent stage is a small divaricating shrub with small leaves only 1/3 - 3/4" long, while in its mature form it becomes a tall tree with leaves 1 - 4" long.

Economically the lacebarks are of no great importance. The Maori used the tough, lace-like inner bark for making mats, rope and twine, but today these arts have been almost lost. Two of the wild species (*H. populnea* and *H. sexstylosa*) and their hybrids are widely cultivated in the North Island as ornamental shrubs and fast growing shelter trees.

Some years ago it was observed that these cultigens were suffering from a form of dieback. Today the condition has become widespread, and large numbers of trees have been killed outright, so that the usefulness of these plants is seriously curtailed. Field observations indicated the presence of a pathogenic fungus, and the present work was undertaken in an attempt to determine the cause of the disease.

HISTORICAL.

Although no record has been made of a fungus causing dieback of lacebarks, it was observed about 1920 that fungous fructifications of the *Myxosporium* type often appeared on the bark immediately following the death of twigs and branches. During the years 1920 - 1923 specimens of lacebark bearing these acervuli were collected from Waikato, Southland, Canterbury and Wellington; in each case they were found only on the stems. Dr. Cunningham, Government Mycologist, New Zealand, identified this organism as a member of the form-genus *Myxosporium*, and later his decision was confirmed by Dr. Butler, Imperial Bureau of Mycology, Kew. Though it appeared probable that this fungus was causing dieback, no attempt was made to prove its pathogenicity.

Saccardo lists 101 form-species ascribed to the genus *Myxosporium*, but of these more than half are imperfectly described, and therefore of little value in diagnosis. Only three of those listed were found on hosts belonging to the same order as the lacebarks (Malvales). There is no record of a *Myxosporium* species occurring on a species of the family Malvaceae, to which the lacebarks belong.

A survey of the literature shows that the genus *Myxosporium* has received little attention. The few species with which experiments have been made have proved to be weak parasites of secondary importance or saprophytes associated with other diseases. Miss Gilchrist (1923), Briton-Jones (1925), and Zeller (1926), found that *M. corticolum* attacked only weak or unhealthy apple trees, and was not a serious pathogen. Day (1928) showed that *M. abietinum* (Rostrup) was commonly found in tissues of the Sitka Spruce, and Douglas Fir, that had been damaged by frost, but that it seldom spread beyond the injured areas. Fraulein Beck (1926) has shown that *M. cingulatum* is the cause of an anthracnose disease of privet (*Ligustrum vulgare*) seedlings, and also that *Gnomonia cingulata* (Beck) is the perfect stage of this fungus. Other workers have shown that certain *Myxosporium* form-species are imperfect forms of ascomycetous fungi. Miss Wilson (1928) demonstrated that *M. abietinum* is the conidial stage of *Dermatea livida* (B. et Br.)

DISTRIBUTION.

Specimens of lacebark showing *Myxosporium* fructifications have been collected from Waikato, Hawke's Bay, Manawatu, Wellington, Nelson, Canterbury and Southland. Collections have not been made from the remaining districts of the Dominion, but as the disease is known to occur from Waikato to Southland it is probable that the trouble is general throughout New Zealand.

TYPES OF INJURY PREVALENT ON THE HOSTS.

Dead wood on lacebarks in the field may be grouped on superficial appearances into four classes:-

1. Small isolated dead twigs.
2. Lesions on branches and trunks not bearing the acervuli of *Myxosporium*.
3. Lesions on branches and trunks bearing the acervuli of *Myxosporium*.
4. Insect injury common in the forks of the smaller branches.

1. Small isolated dead twigs appear to be common to many different trees, and are found on the three host species treated in this paper, *H. populnea*; *H. sexstylosa*; and *P. betulinus*. These twigs may be apparently free from fungous infection, or show fungous fructifications of various types. Inoculations with fungi obtained from such twigs have given only negative results, and it is probable that death was caused by mechanical injury or natural agents such as frost, or insufficient light.

2. The only fungous disease recorded among lacebarks is the rust, *Puccinia Plagianthi*. (Cunningham 1931). Excluding lesions caused by this pathogen, it is more common to find dead wood, without *Myxosporium* fructifications, on *H. sexstylosa* than on *H. populnea*. Mature trees of *H. sexstylosa* often show long narrow lesions on the trunk and branches. These are slightly depressed, and reddish-brown flecked with white, standing out quite clearly against the darker trunk. Fungous fructifications of varying types are found on such lesions. Whilst no pycnidia of the *Phoma* type have been observed on them, yet on making isolations from such areas a fungus, which forms pycnidia in culture, is obtained with fair regularity. Experiments with this fungus will be dealt with in a later section.

3. Dieback occurs commonly on *H. populnea* and to a lesser extent on *H. sexstylosa*, and *P. betulinus*. The first symptom of the disease is the wilting and death of the leaves above the point of infection. At this point the bark appears normal, but is soft and spongy to the touch, for the under-lying cells have collapsed.

Canker formation does not take place, and it is necessary to cut away the outer bark to find the edge of the lesion. The latter appears as a light brown zone quite distinct from the greenish white of healthy tissue. As the lesion becomes older the infected wood darkens, in some cases turning almost black,



Fig. 1. *H. populnea* hybrids showing severe dieback.

(Photo by author)

Shortly after the death of the infected branch small lumps appear on the bark. These are formed by the developing acervuli, which eventually rupture the bark, and push it aside until at maturity it appears as a wall bounding the central, pulvinate, salmon-pink spore-mass. The pink acervuli are a characteristic feature of dieback, and occur on diseased woody tissue of all ages, but they have not as yet been found on the leaves. The pustules are generally ellipsoid in shape varying in size from $2 \times \frac{1}{2}$ m.m. to $4 \times \frac{1}{2}$ m.m., and usually lie with their long axis parallel to the length of the branch. They may be thickly clustered or widely scattered over the infected area, and often occur in rows following the grain of the bark. Usually single, the acervuli occasionally merge into one another forming larger irregular masses.

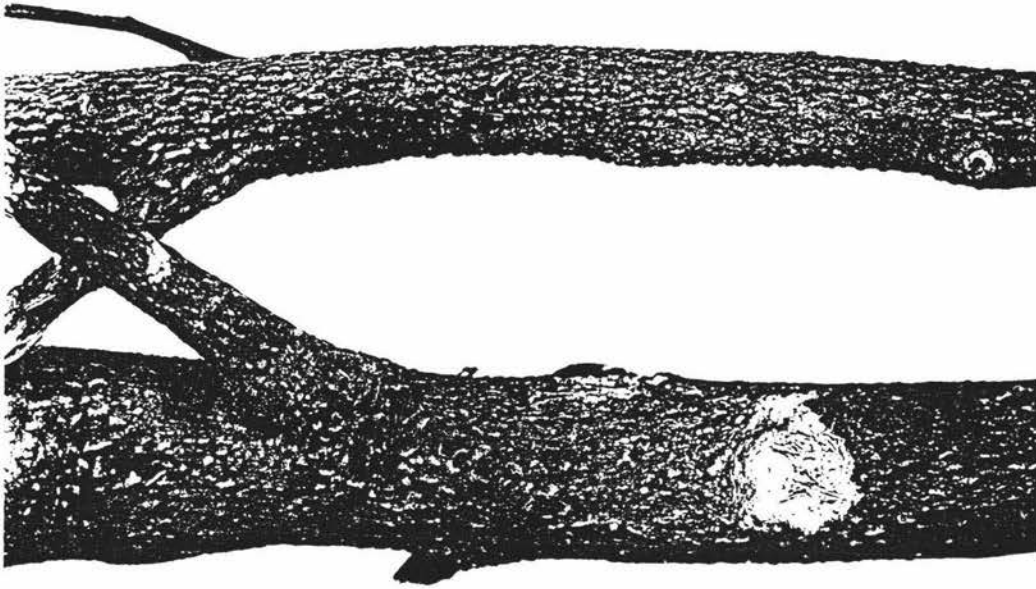


Fig. 2. *Myxosporium acervuli* on the bark of
H. populnea. (Natural size).

(Photo by H. Drake).

The spores developing from the acervulus are held together by a gelatinous matrix, which is hygroscopic in nature. In wet weather the spores are extruded from the acervulus, and the gelatinous material is dissolved away. Some of them may be carried down to the ground by the water, others left to dry on the bark and be blown away.

Once the fungus is established it grows rapidly, under favourable conditions, developing a copious intracellular mycelium. The hyphae spread in all directions, disorganising the cortex and cambium and penetrating into the wood vessels by way of the pits in their walls. The destruction of the cortex and cambium prevents further growth of the branch at the point of infection, and stops the downward passage of elaborated food materials. By girdling the branch and blocking the wood vessels with a mass of hyphae, the fungus cuts off supplies of food and water, and the branch rapidly

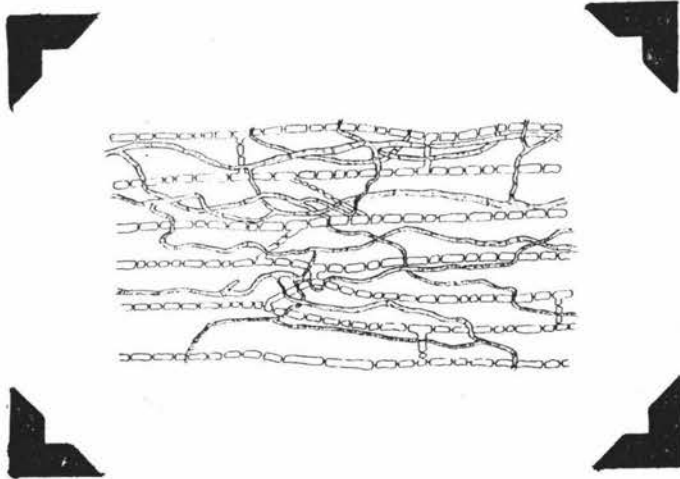


Fig. 3. Myxosporium hyphae in the wood vessels. x 370.

(original)

4. Apart from fungous attack considerable damage is caused by insects, particularly a species of beetle. The grubs of this beetle feed usually in the forks of the smaller branches, often killing them by eating away all the outer layers of tissue from their bases. Branches killed by their attacks rapidly become covered with all manner of fungous growths, but the characteristic, deep, irregular wounds are usually sufficient in themselves to account for the death of the part concerned. The insects have not yet been found feeding on dead wood, and the Myxosporium does not form cankers. Therefore if a dead branch has a canker-like wound at its base, it is reasonably safe to assume that death was caused by insects, and not by the dieback organism.

It would appear from field observations that *H. populnea* and closely related hybrids are more susceptible to dieback than *H. sexstylosa* or *P. betulinus*. No evidence regarding the incidence of the disease on other lacebark species is available. Over 300 *H. populnea* cultigens were examined, and of these fully 60% showed symptoms of dieback. On the other hand, acervuli could not be found on more than 10% of four hundred *H. sexstylosa* specimens.

Only a few plants of *P. betulinus* were examined but here again the percentage infection was low. *H. populnea*, however, shows few lesions other than those of dieback, while *H. sexstylosa* shows many lesions which do not bear *Myxosporium acervuli*.

CULTURAL STUDIES.

To obtain pure cultures of the causal organism, or organisms fifty specimens were collected from diseased larch barks. In this first series no discrimination was made between the types of lesion or the species of the host. Isolations were made from the specimens by the following method:-

The specimen was surface sterilised with 1:1000 acidulated Mercuric chloride and then introduced into the culture cabinet. With a sterile scalpel the outer layers of bark were cut away exposing the edges of the lesion. A second scalpel was used to remove small pieces of tissue from the edge of the lesion. Four such pieces were taken from each lesion and placed equidistant from one another towards the periphery of a petri-dish containing prune dextrose agar. (see appendix). The dishes were incubated at 21°C. until the fungus mycelium had grown well clear of the wood, usually 5 - 10 m.m., when a small section of the outer edge of the colony was transferred to a fresh dish of potato dextrose agar, (see appendix) and again incubated at 21°C. By this method clean colonies were obtained in the majority of cases.

As a check to these isolations single spore cultures were made by the poured plate method from *Myxosporium* spores, taken from acervuli on the host. By using prune dextrose agar as the pouring medium it was possible to obtain single spore colonies free from contamination. All contaminated cultures were discarded.

The isolants showed a distinct relation to the type of lesion from which they were obtained, and could be arranged into three groups :-

1. Isolations from 20 lesions showing pink acervuli yielded 12 cultures of *Myxosporium*, and three of *Nectria cinnabarina*, the remaining cultures being contaminated.

2. Isolations from 15 lesions showing no constant fungus fructifications gave 12 cultures of a fast growing fungus, which produced pyrenia and spores of the Phoma type in five days, and three unidentified cultures.

3. Isolations from the bases of 15 small twigs which had died back to their parent branches produced several different species of fungi. Of these a species of the genus *Fusarium* and an Ascomycete of the family Sphaeriaceae appeared in three, and four cultures respectively.

The regularity with which the first two types of lesions produced the fungi mentioned, indicated that these organisms might be pathogenic. In the third group no single organism appeared to be regularly associated with the dead twigs. Thus it seemed likely that the fungi concerned were merely saprophytes. Inoculations made with this group of fungi gave only negative results, and in view of these facts, small isolated dead twigs were set aside as having little, if any connection with the dieback condition.

A second collection of seventy specimens was made from diseased plants of the species, *H. populnea*, *H. sexstylosa*, and *P. betulinus* in Nelson, Wellington, Hawke's Bay, and Manawatu. Small dead twigs were excluded from this series. Isolations were made from each specimen by the method previously described. The results confirmed those obtained from the first series; where pink acervuli were present the lesion usually yielded the *Myxosporium*, occasionally *N. cinnabarina*; from the remaining lesions cultures of the Phoma-like fungus were generally obtained. All three of these fungi were obtained in culture from isolations made during the winter and spring months, showing that they can normally overwinter by means of an internal mycelium.

The *Myxosporium* was the only one of the fungi isolated from lacebarks, that inoculation experiments proved pathogenic. The following tests were carried out with this fungus to determine its physiological reactions to light, temperature, and certain synthetic media.

obtained by the poured plate method of single spore isolation, from spores produced in culture. These pure cultures were used for inoculations and cultural experiments. It was found that normal spores were produced in 15-18 days when the fungus was grown on potato dextrose agar at 21°C. in a dark incubator, and spore production was similar on autoclaved *H. sexstylosa* stems kept under the same conditions. This behaviour differed from that reported by Miss Gilchrist (1923) for *M. corticolum* (Edgert). She found that this species produced abnormally small spores on nutrient agar, yet Lewis (1912), working with the same fungus, records that normal spores were produced on sterilised bean pods.

To determine the effects of light and temperature on cultures of the *Myxosporium* the following tests were carried out. Twelve petri dishes of potato dextrose agar were sown from a single pure colony, and three dishes placed under each of the following conditions:-

1. 3° - 8°C. dark refrigerator.
2. 21°C. dark incubator.
3. 30°C. dark incubator.
4. 21°C. glass fronted incubator.

Examinations were made daily and records kept of the growth rate as measured by the colony diameter.

Brown (1925) has shown that environment has a direct and most marked effect upon the growth rate of certain strains of *Fusaria*. Unfavourable conditions produced staling, one feature of which was a reduction of sporulation. Spore production is desirable whether the culture is to be used as an aid to identification or as a source of inoculum. Therefore the conditions most suitable for routine cultural use would be those producing a freely sporulating, or non-staling colony. Brown found that the daily rate of increase in colony diameter was a convenient measure of staling, and therefore an indication of the worth of any particular medium. It is reasonable to assume that the *Myxosporium* under consideration would react similarly to changes of environment, and its growth rate has been taken as a measure of the relative value of any set of conditions.

No measurable differences could be found between the cultures grown in the dark and those subjected to the normal alternation of night and day. Temperature, however, had a marked effect upon growth. These results are shown graphically in Fig. 4.

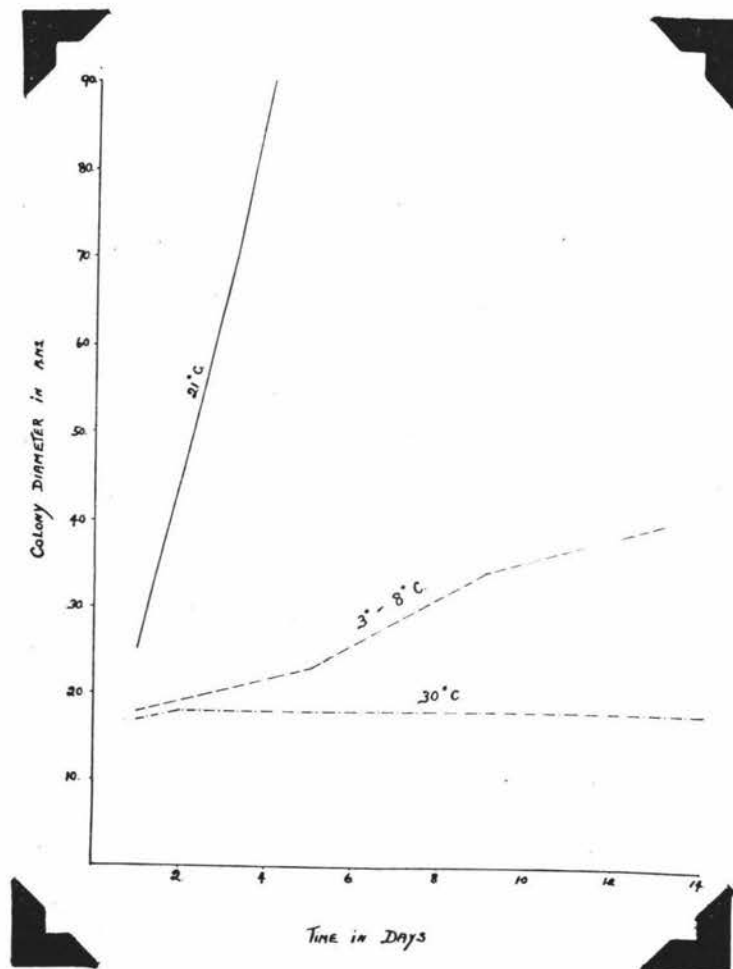


Fig. 4. Growth curves of *Myxosporium Hoheria* at varying temperatures.

These cultures to be incubated at 30°C. were kept at 21°C. for two days after sowing to allow growth a good start. On transfer to the 30°C. incubator the growth rate dropped rapidly until the colony became dormant, and it remained in this state for five days. At the end of this time the cultures were taken back to the 21°C. incubator, and within two days the normal

the fungus was killed. Replication of these tests produced no significant variation in results. As the fungus was apparently indifferent to light, and gave rapid, non-staling growth at 21°C., all later cultural work was carried out in a dark incubator at this temperature.

Brown found that his *Fusaria* would grow equally as well on a synthetic medium as on potato agar, and to determine whether the *Myxosporium* would behave in a similar manner, the following test was made. Two synthetic media were prepared, Brown's glucose medium, and a modified form of Brown's starch agar. (see appendix) Both these media approximate in some degree the food materials of potato agar which Brown found necessary for the growth of his *Fusarium* strains.

Four petri-dishes of each synthetic medium, and four of potato dextrose agar were inoculated from a single pure colony, and grown under conditions as nearly identical as possible. All the dishes were 90 m.m. in diameter, 15 c.c. of medium was added to each, and all were kept in the same incubator. The results are shown in graphical form. (Fig. 5.)

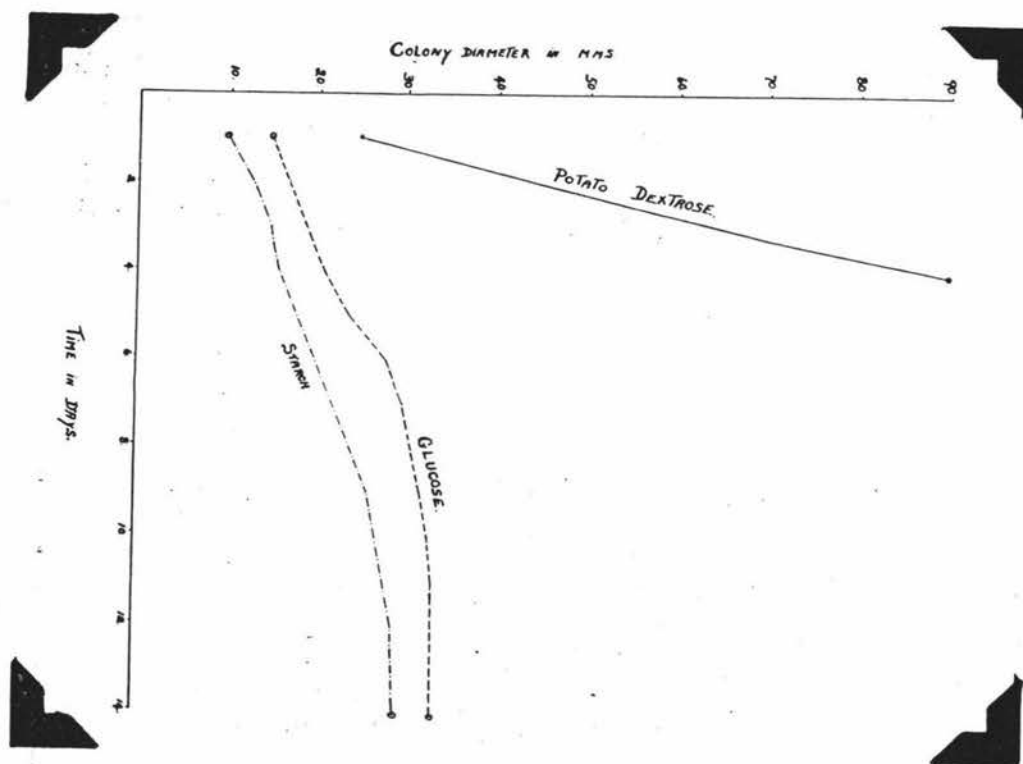


Fig. 5. Growth curves of *Myxosporium Hoheria* on different

The curves representing growth on the synthetic media show staling, and a definite inferiority to potato dextrose agar. A duplicate series gave similar results. Cellulose in the form of dessicated filter paper was added to each medium in a third series but produced no change in growth form.

No saltants appeared when the fungus was grown on potato dextrose agar, and different batches of this medium gave colonies that could not be distinguished from one another by any measurable, morphological, or physiological characters. Thus the potato dextrose agar was superior to the other media used, and apparently quite satisfactory as a medium for growing this fungus.

GROWTH AND APPEARANCE OF CULTURES.

Colony characters on potato dextrose agar at 21°C. in dark incubator, 90 m.m. petri-dish, 15 c.c. of medium per dish.

Colony fast growing, reaching periphery in five to seven days; margin entire. At first slimy when viewed from above; when 25 - 35 m.m. in diameter type of growth changes, and outer parts show small tufts of aerial hyphae. These increase in size and density until outer zone is grumose, while central ring becomes pubescent. Light pink, scattered masses of conidia can be seen when colony is one month old. At this age the mycelial mass is white from above; from below, medium is coloured light brown, with darker brown spots under spore clumps. On this medium a characteristic odour is produced, bearing a slight resemblance to the smell of esthers from apples in cool store.

SPORE GERMINATION.

Before making inoculation the germination capacity of the Myxosporium spores, and those of the Phoma type fungus, was tested by two methods:-

1. Spores were placed in a drop of sterile water hanging from the cover slip into the cavity of a hollow ground slide.

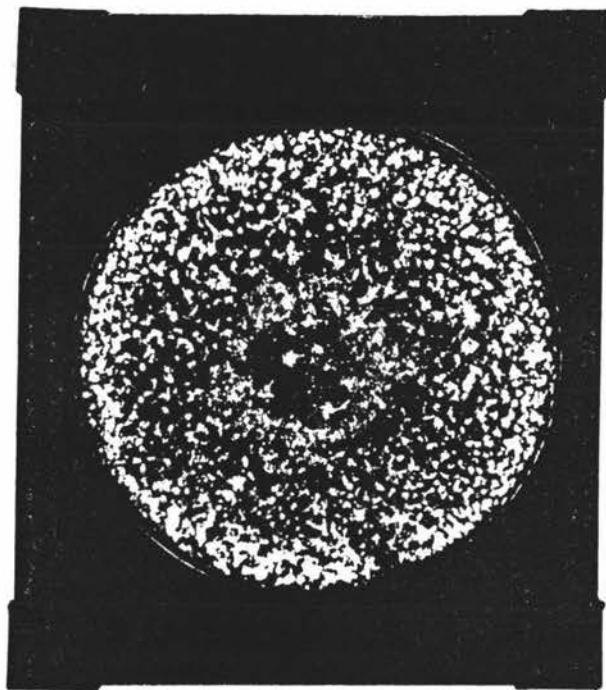


Fig. 6. One month old colony of *M. Hoheria* grown on potato dextrose agar at 21°C. in the dark. X5/6

(Photo by T. Gabriel)

2. Spores were sown on a thin flat film of potato dextrose agar, also suspended over the cavity of a hollow ground slide.

All the slides were incubated in petri-dishes at 21°C. The atmosphere was kept saturated by placing wet filter paper in the bottoms of the dishes.

The germination percentage was lower in water than on the nutrient medium, but no differences were observed in the manner of germination. *Myxosporium* spores taken from acervuli on the host gave 60 - 70% germination, those from pure cultures slightly less (50%) in 16 hours. The Phoma type spores could be found only in culture, and these showed a 50% germination in 24 hours. The *Myxosporium* spore puts out from one to three germ tubes. These usually appear towards the ends of the spore, but may grow out from any portion of the wall. If on a nutrient medium the hyphae grow rapidly, become septate, and

INOCULATION EXPERIMENTS.

Seedlings for inoculation were collected early in the year, and grown in pots, away from insects, for three months before use. As no sign of infection appeared during that period the plants were assumed to be free from disease and were not sterilised in any way. The collection represented three species of lacebark, *H. populnea*, *H. sexstylosa*, and *P. betulinus*, together with a number of horticultural varieties of these.

The inoculum was taken from pure lines of *Myxosporium*. Some of the first inoculations were made with mycelium alone, but these in every case showed negative results. Later inoculations were made with spores from agar, or sterilised twig cultures, a certain amount of mycelium being included incidentally. Four methods of inoculation were used; the details of which are as follows:-

1. With a sterile scalpel a small piece of tissue was lifted by cutting upwards along the stem. From below this flap a piece of wood was removed, the inoculum placed in the cavity, and the flap pressed back into place. To prevent drying out the wound was bound with moist cotton wool and finally cellophane.

2. As for No. 1. but some of the tissue under the flap was killed with a hot needle before inserting the inoculum.

3. Injecting a suspension of spores with a hypodermic needle, the method adopted by Neill and Brien (1931).

4. A portion of the stem was killed by ring-barking and the fungus established as a saprophyte on the dead parts.

One, or two uninoculated wounds were made on each plant to check the results. The results of these inoculations are shown in tabular form (Table 1.) Seedling plants were used throughout this series of tests.

Table 1. Myxosporium inoculations on various hosts.

Host.	Date of inoculation.	Method used.	No. of Points inoculated	No. of Plants inoculated	Results.
H.Sexstylosa	22-7-32	1	5	2	3 lesions
do	5-8-32	3	20	2	*no infection
do	6-8-32	1	8	2	5 lesions
do	13-8-32	2	4	1	2 lesions
do	29-8-32	2	6	2	5 lesions
do	2-9-32	1	24	7	16 lesions
<u>Totals.</u> 16 plants of H.sexstylosa were inoculated.					64.5% of inoculations showed infection.
					23 uninoculated wounds remained healthy.
H.populnea	22-7-32	1	5	2	2 lesions.
do	2-8-32	2	5	2	3 lesions
do	5-8-32	3	20	2	*no infection
do	6-8-32	1	7	2	6 lesions
do	13-8-32	1	15	4	10 lesions
do	14-8-32	4	6	2	4 lesions
do	29-8-32	1	5	2	3 lesions
do	2-9-32	1	4	2	2 lesions
<u>Totals.</u> 18 plants of H.populnea were inoculated.					61.3% of inoculations showed infection.
					20 uninoculated wounds remained healthy.
P.betulinus	22-8-32	1	6	2	4 lesions
<u>Totals.</u> 2 plants of P.betulinus were inoculated.					66.6% of inoculations showed infection.
					4 uninoculated wounds remained healthy.

* These figures were not included in the calculation of the percentage infection.

The check wounds healed over and showed no sign of infection. The lesions produced by inoculation were identical both macroscopically and microscopically with those found under natural conditions. In an unheated glasshouse acervuli formed on the bark in three to eight weeks from the time of inoculation.

each case cultures of Myxosporium were obtained.

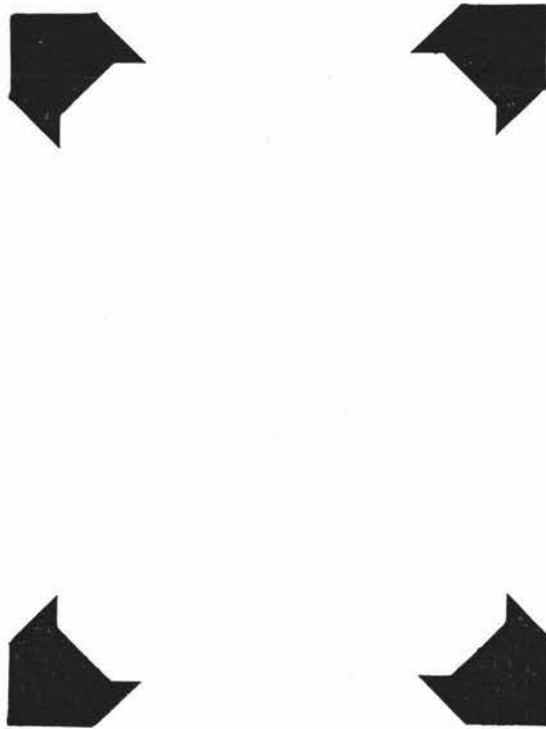


Fig.7. *H. populnea* seedling showing branch killed as a result of inoculation with *M. Hoheria*. The cross marks the point of inoculation.

(Photo by T. Gabriel).

From these results it appears that this *Myxosporium* is an active parasite on the three species into which it has been inoculated.

Further inoculations were made with the other fungi isolated from diseased lacebarks. Ninety inoculations on seedlings and thirty-two on mature tree in the field were made during the period January / July 1932, with the fungus of the *Phoma* type. The methods of inoculation were the same as those mentioned previously. On seedlings these have failed to show any positive results, but on old trees the fungus in some cases appears to have penetrated a short distance into the tissue. It has been reisolated from the extreme edge of the wound three months from the date of inoculation, but some of the original inoculum may have retained its vitality for this period. Until further evidence is obtained this fungus cannot be considered parasitic.

The cause of the original cankers has not yet been determined.

Seventy-five inoculations were made with the species of Sphaeriaceae; sixty on seedlings and fifteen on mature trees. No positive results were obtained. In cases where the fungus was established as a saprophyte on wood killed by heat it failed to spread to living tissue. Twenty-two inoculations made with the species of Fusarium also failed to show any infection. None of these three fungi have as yet been identified.

Both the ascigerous and conidial stages of *Nectaria cinnabarina* have been collected several times from *H. sexstylosa*, and the fungus has also been obtained in culture from the edges of lesions. Although this organism is a weak parasite on a wide range of hosts (Cunningham 1925), no experimental evidence can as yet be brought forward to show that it is parasitic on the lacebark.

The summarised results of this second series of inoculations are given in Table 2.

Table 2. Inoculations with various fungi.

Fungus Species.	Host plant.	No. of plants treated.	Total No. inoculations.	Period over which inoculations were made.	Results.
Species of the Phoma type.	<i>H. sexstylosa</i>	30	101	Jan. - July 1932	No Infection.
	<i>H. populnea</i>	7	21	Jan. - July 1932	No Infection.
Sphaeriaceae species.	<i>H. sexstylosa</i>	11	45	Mar. - July 1932	No Infection.
	<i>H. populnea</i>	8	30	Mar. - July 1932	No Infection.
Fusarium species.	<i>H. sexstylosa</i>	5	22	May 1932	No Infection.
<i>Nectaria cinnabarina</i> .	<i>H. sexstylosa</i>	4	16	August 1932	No Infection.

METHODS OF TRANSMISSION AND INFECTION.

It seemed possible that the gall producing mite, common on lacebarks, might play a part in spreading the disease, but field observation failed to show any correlation between gall formation and dieback infection. No experiments have been made in relation to dissemination but from the manner in which the spores are produced, and the widespread infection in the field it seems probable that wind plays a big part in dispersal.

Inoculation experiments have shown that infection hyphae from the spores can infect injured tissues. To determine whether the fungus could penetrate undamaged bark the following experiments were carried out:-

1. Seedlings kept in a water saturated atmosphere under a bell jar were sprayed with a suspension of spores.
2. Spores were smeared over the surface of the bark, and covered with moist cotton wool and cellophane.
3. Pieces of lacebark stem bearing pure cultures of the *Myxosporium* were tied to healthy branches and the whole wrapped with damp cotton wool and cellophane.

Twelve plants in all were inoculated, four by each method. Two direct wound inoculations were made in each plant in order to check the results.

No positive results were obtained from these tests, though the check inoculations showed 60% infection. Such negative evidence, though it does not prove that the fungus cannot enter an undamaged surface, at least indicates that wound infection is the more usual method of entry. Field observations bear out this indication, for in the large majority of lesions examined some form of wound was found, from which the fungus appeared to have spread. Infection was often found to be abundant where trees had been cut back; the fungus apparently entering through the cut surfaces.

OVERWINTERING.

The conidia of this *Myxosporium* have been found to

germination percentage of spores from specimens collected in June and July was 60 - 70% at the time of collecting, but fell below 1% by the end of September.

As mentioned previously several form-species originally assigned to the genus *Myxosporium* have since been connected with ascigerous forms. No perfect stage of the form-species under consideration has yet been found. However, the fungus is apparently able to overwinter without the aid of a special spore form. The conidia may retain their vitality through the winter, and isolations made during this period showed that the mycelium can overwinter within the host tissues.

LIFE HISTORY OF THE CAUSAL ORGANISM.

Dieback of lacebarks is caused by a species of *Myxosporium*, a fungus with only one known spore stage in its lifecycle. Conidia produced from acervuli on dead wood may be carried by wind or other agency to wounds on fresh hosts. Under favourable conditions of temperature and moisture the spores germinate and produce infection hyphae which penetrate the injured tissue. A copious internal mycelium develops, spreading rapidly from the point of entry. The hyphae break down the cortex and cambium, and penetrate into the xylem vessels, usually entering through the pits in their walls. Blockage of these vessels prevents the upward passage of water with food substances in solution, with the result that the branch wilts and dies.

After the death of the infected limb the fungus continues to live saprophytically, and from hyphae lying close to the periphery of the wood, produces stromata. A palisade layer of conidiophores grows up from each stroma. These conidiophores are of unequal length, and each bears apically a single ellipsoid conidium. The developing acervulus pushes its way through the now dead cortex, and appears on the surface as a salmon-pink, pulvinate pustule. The mature spores break away from the conidiophores, but are held together in a clump by a gelatinous matrix. This matrix is hygroscopic, and swells under humid

conditions forcing the spore mass upwards out of the acervulus. Rain dissolves away the gelatinous material liberating the spores for dispersal.

CONTROL.

No attempts have been made to control this disease but a study of the life history of the causal organism suggests certain remedial measures.

Spraying would obviously be impractical for the trees are seldom of sufficient value to warrant the expense. However, as the fungus appears to be largely a wound parasite, infection could be kept in check by cutting out, and burning all diseased branches, sterilising these cuts with acidulated Mercuric chloride, and finally painting all wounds with coal tar.

MORPHOLOGY.

A number of acervuli taken from natural, and artificially produced lesions on the host, were sectioned by the paraffin process, and the sections stained by the iron-alum, haematoxylin, and light green combination.

Comparison of the sections showed that the acervuli were constant in general form and structure though varying considerably in size. At the base of the acervulus is a stroma, from which rises the hymenial layer. The latter is a closely compacted palisade of filiform, septate, unbranched, conidiophores, which exhibit considerable variation in length.

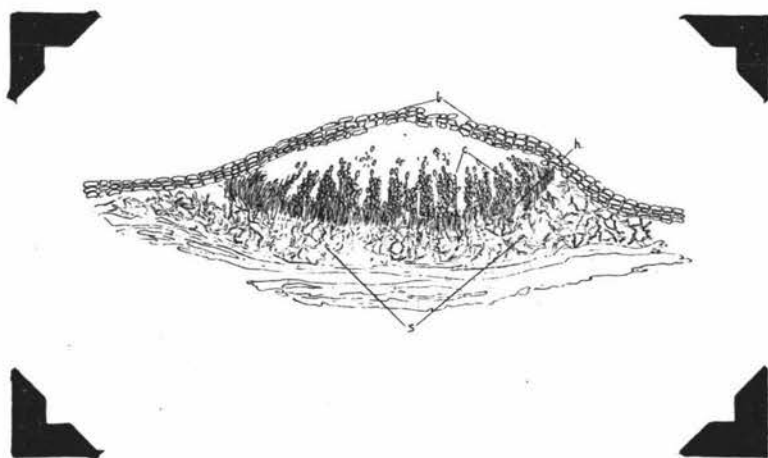


Fig.8. Longitudinal section of a young acervulus of *M. Hoheria*.

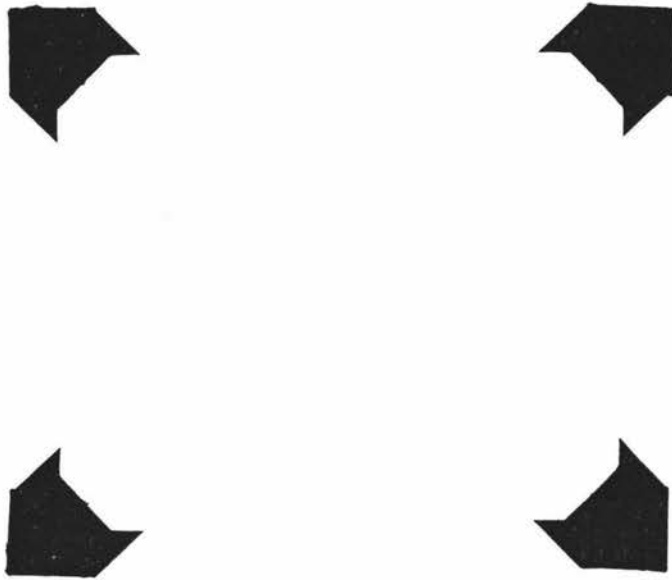
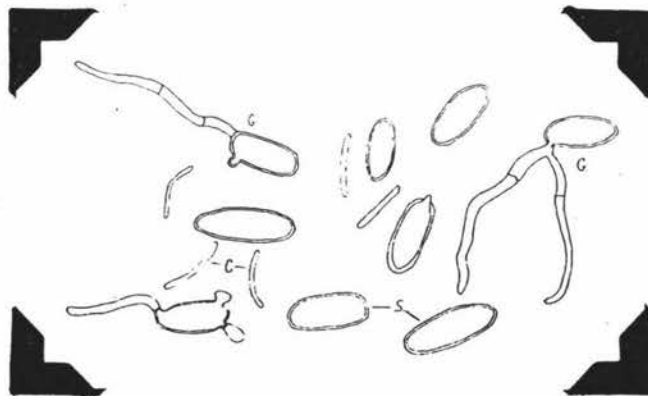


Fig. 9. Vertical section of the stroma and hymenium X 455.

(c) conidia (p) conidiophores (h) hymenium (s) stroma.

(Original)

Conidia are produced by simple abstriction of the swollen end of the conidiophores. Each conidiophore bears only a single spore, but in vertical section the spores appear to be in clumps or rows because of the unequal length of the conidiophores. Among the mature spores at the surface of the acervulus are slightly curved, filiform, vacuolate bodies which are bluntly curved at the ends. They show continuous variation in size between fairly well defined limits, and appear to be fragments of the conidiophores broken off at the septae. Whether they can be used as a diagnostic feature it is not possible to determine without studying the whole group.



The conidia themselves exhibit appreciable variation in length and diameter, and are very largely ellipsoid in shape. Mature spores of any one acervulus usually exhibit almost the entire range of variation, This character may be of some value to distinguish this species from others of the group, but cannot be used to separate the individual specimens examined from one another. The colour of the acervulus varies on different specimens from a dingy white, to a brownish red, the most common shade being salmon-pink. Spores from the different colour types all gave similar colonies and could not be distinguished morphologically.

As no constant morphological differences could be found among the specimens examined, it must be concluded that they were all members of the same form species.

Owing to the large number of alleged species listed by Saccardo under the genus *Myxosporium*, it was not considered feasible to construct a morphological key for the species of this genus, without access to specimens, and a much more complete knowledge of the group. Although by no means a general rule, it is reasonably safe to assume that members of one genus of fungi occurring on one order of host plants will resemble one another more closely, than members of the same genus on widely separated hosts. Working on this assumption, a comparison was made between the species under consideration and the three others recorded from the Malvales. One of these *M. pubescens*, is incompletely described, but the remaining two may be separated from the lacebark organism by distinct morphological differences as shown in the following key:-

A. Spores straight.

1. Conidiophores short, less than 12μ . *M. Mollerianum*

2. Conidiophores long, more than 20μ . *M. 'x'*

B. Spores curved.

M. fumosum.

Other differences are also recorded, thus, both *M. Mollerianum* and *M. fumosum* had definitely smaller spores than the lacebark *Myxosporium*. Further the spores of *M. fumosum* are pointed and those of *M. Mollerianum* are guttulate while those of

the lacebark species are bluntly curved at the ends and non-guttulate.

The practice of classifying by the host plant is convenient but open to serious criticism. However, no description covering this fungus could be found in Saccardo's "Sylloge Fungorum".

Saccardo in his arrangement of the fungi divides the Melanconieae into six sections, viz:

Hyalosporae; Scoléco - allantosporae; Phaeosporae;
Didymosporae; Phragmosporae; and Dictyosporae.

The subdivision is based on the shape, colour, and septation of the spores, which are morphological features usually found to be constant among the fungi. The Hyalosporae are further split on the structure of the acervulus, the shape of the spores and the manner in which they are borne. Thus the members of the Hyalosporae, which have muticulate, solitary conidia, borne in acervuli without a setose margin, are separated from the remaining forms and further subdivided into four form-genera:-

Hainesia; Melanstroma; Gloeosporium; and Myxosporium.

Hainesia is a small genus characterised by long filiform conidiophores, often bearing small branches in bundles at the tips and sides. Melanstroma, another small genus, is differentiated by very short conidiophores, and the fact that the spores are at first catenulate.

The two remaining genera appear to be similar morphologically. From the published descriptions the only separating feature appears to be a physiological one, namely the relative position on the host; Gloeosporium is recorded from leaves and fruits; Myxosporium from branches and stems. Such a criterion can have no value in a morphological classification, except perhaps for splitting a species into biotypes, and cannot be used for separating genera. Cunningham (1927) has clearly illustrated the confusion that may arise in taxonomy from the inclusion of physiological characters for anything higher than subspecific grouping. Thus the fungus causing dry-rot of swedes becomes *Phoma siliquastrum* on siliquas, *P. Napobrassicae* on bulbs, *Phyllosticta Brassicae* on leaves, and *Plenodomus lingam* on mummied bulbs; yet cultural and inoculation

experiments have shown these forms to be all members of the one species, *Phoma lingam*. Similar discrepancies have been noted in the genera under consideration.

Zeller (1925) dealing with a fungus of the *Hyalosporae* group found that it produced lesions on the branches and fruit of the apple. Yet he chose to include it in the form-genus *Gloeosporium*, rather than *Myxosporium*, for the sole reason that species of the latter genus are supposed to occur only on bark.

From descriptions of the species of the two genera no constant morphological differences could be found. The spores of both are of the same general shape and size. The conidiophores of either genus exhibit a wide variation in size, though all are of the bacillar type. Thus *M. Pruni-Mahaleb* has conidiophores of 8 - 16 x 4 μ ; *M. Cytisi* of 20 - 30 x 3 μ ; *G. Eucalypti* of 50 - 60 x 5 - 6 μ ; and *G. obtusipes* of 11 - 14 x 2 - 3.5 μ ..

Link erected the genus *Myxosporium* in 1825, while *Gloeosporium* was not proposed until 1849. As these two genera apparently cannot be separated on morphological characters, it appears that they should be grouped together under the prior name, *Myxosporium*. Whether this contention be substantiated or not, the species discussed in the present paper will fall into the genus *Myxosporium*. Therefore, as neither an ascigerous form nor a published description of this species has been found, the name *Myxosporium Hoheria* (n.f.sp.) is proposed.

The following diagnosis has been drawn up from the material discussed above.

MYXOSPORIUM HOHERIA. n.f.sp.

Acervuli variable in size, from 2 - 4 x $\frac{1}{4}$ - $\frac{1}{2}$ m.m. erumpent, arising sub - epidermally. Spore mass salmon-pink and held together by a gelatinous matrix.

Conidiophores cylindrical, septate, hyaline, straight or slightly curved, 20 - 100 x 2 - 3 μ . arranged in palisade layer. Spores borne apically. Conidia separate, hyaline, granular, continuous, ellipsoid, 14 - 23 . x 5 - 9 μ . mean 21 x 7 μ .

Known hosts. *Hoheria populnea*, *H. sexstylosa*, and *Plagianthus betulinus*.

Known distribution. New Zealand.

Waikato, Hawke's Bay, Manawatu, Wellington, Nelson, Canterbury, and Southland.

SUMMARY.

1. The botanical species included by the term lacebark, and their economic significance have been briefly outlined.
2. A survey of literature showed that the genus *Myxosporium* is not important pathogenically, and that some "species" have been connected with their ascigerous forms.
3. The dead wood found on lacebarks has been divided into four types.
4. The symptoms and occurrence of these types have been discussed.
5. Isolations showed a definite correlation between the type of lesion and the fungus isolated.
6. Cultural studies with *Myxosporium Hoheria* showed that it is apparently indifferent to light, and grew best at 21°C. on potato dextrose agar. Normal spores were produced in 15 - 18 days, and the colonies showed no sign of saltation.
7. The spores were found to germinate in 16 hours in water or on media.
8. Inoculation experiments have demonstrated that *M. Hoheria* is the cause of dieback on *H. populnea*, *H. sexstylosa*, and *P. betulinus*.
9. Inoculations with other fungi isolated from lacebarks gave negative results.
10. Infection appeared to take place only through wounds.
11. It has been shown that the fungus can overwinter in the mycelial stage within the host, and the spores may remain viable for three months.
12. Infected branches died following the blocking of the xylem vessels, and destruction of the cambium by the fungus. After the death of the limb the fungus grew saprophytically forming acervuli from which fresh conidia were produced.
13. It was suggested that the disease might be checked by cutting out and burning all infected wood, sterilising these cuts with acidulated Mercuric chloride, and finally painting with coal tar.
14. A description of the morphology of *M. Hoheria* has been given.

15. Morphological comparisons showed that recorded species of *Myxosporium* do not correspond with the fungus studied.
16. The genus *Gloeosporium* appeared to be synonymous with *Myxosporium* as no differences could be found in their morphology.
17. *Myxosporium Hoheria* n.f.sp. has been proposed as the name for the fungus causing dieback of lacebarks.

Brown's starch medium with increased asparagin content.

Water	1,000 c.c.
Agar	15 grs.
Glucose	2 grs.
Asparagin	2 grs.
K_3PO_4	1.25 grs.
$MgSO_4$	0.75 grs.
Potato starch	10 grs.

This medium gave results very similar to those obtained with Brown's glucose agar.

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