STUDIES ON THE DIEBACK OF LACEBARKS.

Myxosporium Hoheria. n.f.sp.

By."Assured".

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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 penninsula, 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 costal flora, growing as a dense bushy shrub on salt meadows and round tidal esturies. 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 differance 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.

HSITORICAL.

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 occuring 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 x ¼ m.m. to 4 x ½ 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.

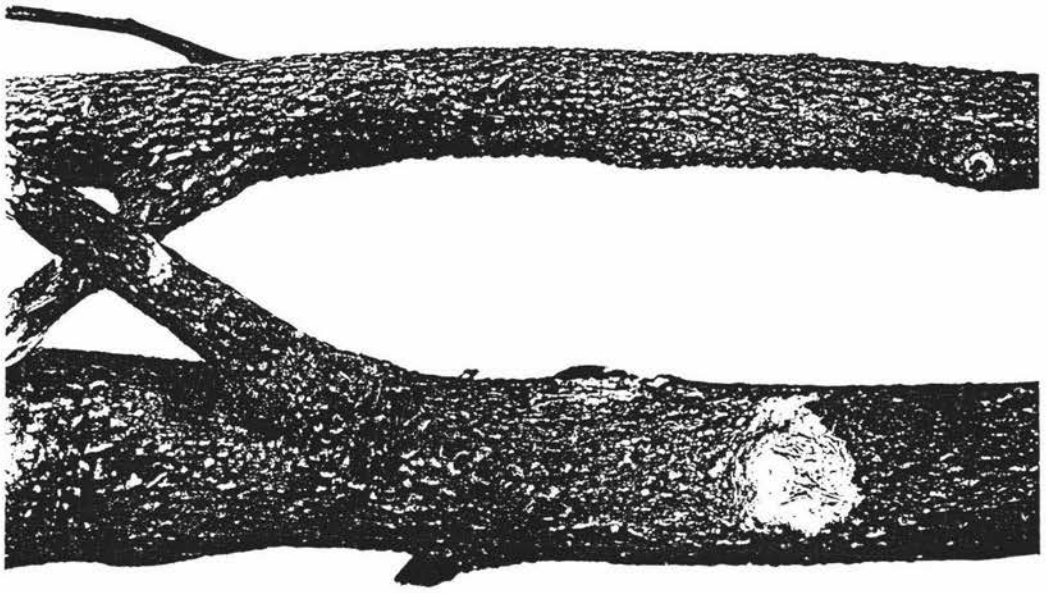

Fig. 2. Myxosporium acervuli on the bark of H. populnea. (Natural size).
(Photo by H.Drake).

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 disolved 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 distruction 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

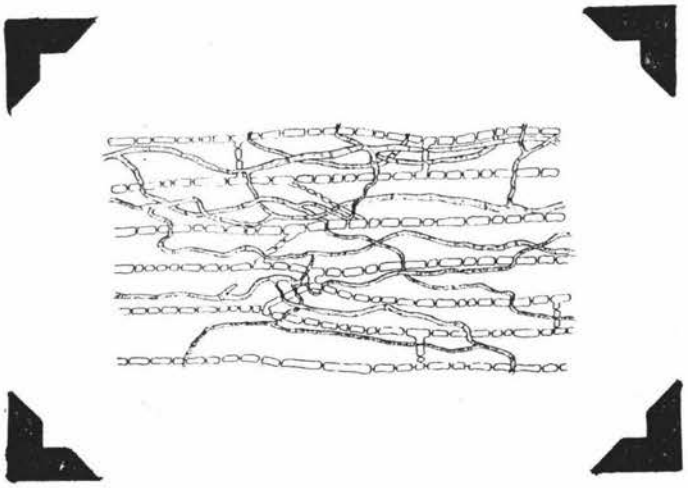

Fig. 3. Myxosporium hyphae in the wood vessels. x 370.
(original)

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.sexstylnsa 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 lacebarks. 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 Mecuric 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 pycnida 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.

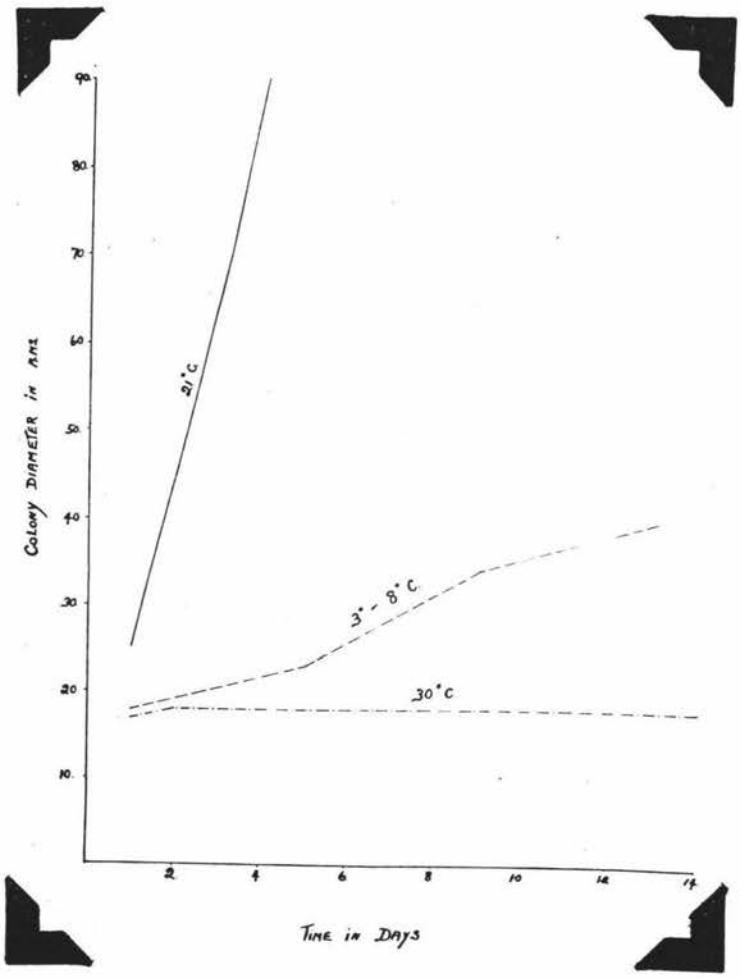

Fig. 4. Growth curves of Myxosporium Hoheria at varying temperatures.

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 Myxsoporium 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 apendix) 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.)

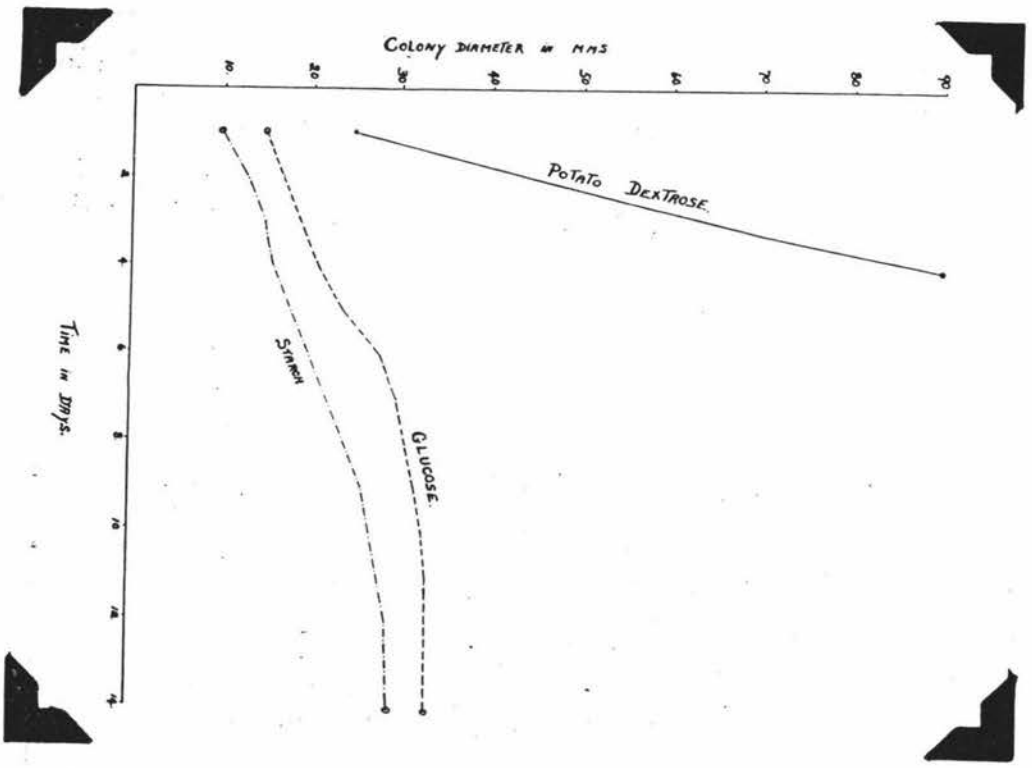

Fig. 5. Growth curves of Myxosporium Hoheria on different

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.

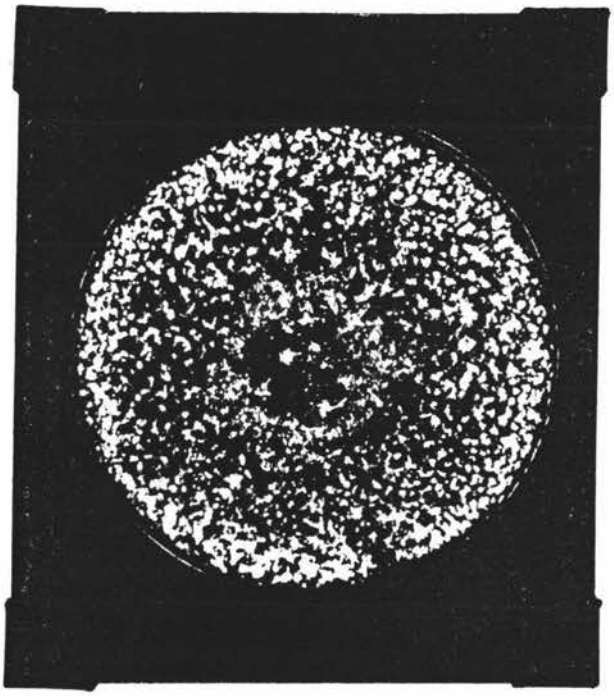

Fig. 6. One month old colony of M.Hoheria grown on potato dextrose agar at 21°C. in the dark. X5/6

(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