[Proc. Roy. Soc. Victoria **47** (N.S.), Pt. II., 1935.]

Art. XXI.—*An Account of Sclerote-forming Fungi causing Diseases in Matthiola, Primula, and Delphinium in Victoria.*

By ILMA G. BALFE, M.Sc.

(Government Research Scholar).

[Read 8th November, 1934; issued separately, 8th May, 1935.]

I. RHIZOCTONIA SOLANI ON MATTHIOLA SEEDLINGS.

Introduction.

Duggar (4) and Peltier (9) reviewed the early literature concerning diseases caused by *Rhizoctonia,* and the former made an extensive study of the genus, the outcome of which was the reduction of a large number of species to be included under either *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.) or *R. crocorum* (Pers.) DC. He also listed the synonyms of the two species. Different types of growth may occur on the same media even when the isolations are made from the same host. Thus macroscopic differences exist in culture, but microscopic differences are so slight as not to warrant the formation of new species.

Since 1915 several other species of parasitic Rhizoctonias have been described. J. Matz (5) reported several from Porto Rico. These are *R. alba, R. dimorpha, R. ferruginea, R. grisea, R. macrosclerotia, R. melongena, R. microsclerotia,* and *R. pallida.* K. S. Thomas (13) came to the conclusion that *R. microsclerotia* was synonymous with *R. solani,* and Briton-Jones (2) considered that the variations in different strains of *Rhizoctonia solani* and also in a single strain on different media were no greater than the differences between the species of *Rhizoctonia* created by Matz (5). As noted by Braun (1), in strains belonging to the species *R. solani* it is especially difficult to distinguish what we have to take as species, because in most cases they are sterile mycelia. Their classification is really more or less based on their vegetative growth, and it is possible that on the discovery of the perfect stage, closely related forms may be given far distant positions in the system. The disputed question as to whether we have to do with different species or only races therefore seems useless as long as the perfect fruiting stage is not obtained.

Recently a trinomial system of nomenclature in which the host name is indicated, e.g., *R. solani* Kühn *Brassicae* II, has been suggested by K. S. Thomas (13).

Records of Rhizoctonia solani in Australia.

In Victoria *R. solani* was first described on potato in 1903, and on turnips in 1911. In 1911 McAlpine (6) reported the fungus as occurring on potato in all the States of Australia. In an unpublished census of Australian fungi compiled by Mr. C. C. Brittlebank, formerly Biologist to the Department of Agriculture, Victoria, there are records of *R. solani* occurring on various hosts in Victoria. Among these are two Cruciferous hosts, Wallflower and Cabbage. In Western Australia W. M. Carne (3) records foot rot of *Lathyrus odoratus* due to *Corticium vagum*, also *Rhizoctonia* scab of potato. In South Australia Samuel and Garrett (12) record the attack of cereals in the Mallee district by *R. solani*.

Rhizoctonia solani from Matthiola incana in Victoria.

Stock seedlings in the seedling beds of the Footscray Gardens showed "damping off" symptoms in 1932 and 1933. The diseased plants appeared in patches which gradually spread out to larger circles. The young stock seeds germinated quite normally at first and showed no pathological symptoms until the first pair of true leaves were produced. At this stage "damping off" occurred, as the pathogen caused a collar rot, and the plants collapsed and died.

In some cases, usually in older and stronger plants, lesions were formed higher up on the stem but below the cotyledons. Microscopic examination showed the presence of numerous thin brown distributive hyphae in the lower stem and upper root regions of the diseased stocks, occurring as a web on the outside of the host tissue. Sections indicated that the fungus grew first in the cortical tissue of the stem near the ground and killed the cells as it proceeded. The cortical tissue was invaded and destroyed by the hyphae, the vascular strand remaining free from the fungus. Hyphae were not found extending into the roots. The weakening of the stem finally caused collapse, and when the soil was kept very moist the fungus formed a mat over the surface of the ground and covered the whole seedling. Lesions were brown in colour and in section were seen to be pocket-like cavities in the cortex, containing a compact mass of hyphae approximating to the density of a sclerote.

Malt agar plates were inoculated with portions of diseased tissue and *R. solani* was repeatedly isolated. *R. solani* has been recorded by Peltier (9) as causing "damping off" of greenhouse seedlings of *Matthiola incana* in Illinois in 1914.

Eight stock seedlings were grown in sterilized soil until the first pair of true leaves appeared. The fungus grown on agar was then introduced into the soil. After a few days "damping

off" symptoms were noticeable and re-isolation of the fungus from diseased tissue was possible. Some weeks later fresh seedlings were transplanted to these same pots and showed disease symptoms in a few days.

Many seeds were also planted in soil obtained from the Footscray Garden seedling beds. The young plants were quite healthy until the first true leaves appeared, when many began to show "damping off" symptoms. The stronger plants all showed brown lesions on the lower stem region.

The discovery by Rolfs (11) of the perfect sporing stage shows that *R. solani* is a Basidiomycete, but what position the fungus occupies within this group is still a disputed question. The form found by Rolfs agreed with the fruiting layer of *Corticium vagum* B. and C., but owing to the appearance of the sporing mass and the parasitic life habit, he held it advisable to make it a variety of this fungus—*Corticium vagum* var. *solani*. Rolfs further estimated that it approached *Hypochnus solani* P. and D. and may prove to be identical with it. The *Corticium* stage, though common in nature, seems strictly parasitic, and has never been produced in artificial culture except perhaps by K. O. Müller (8).

The strain isolated from stock seedlings, and three others which will be referred to later, were subjected to the methods used by him to produce the basidial stage, but without success.

Comparison of Four Strains of Rhizoctonia solani K.

A culture of the strain parasitic on cereals in South Australia was obtained, so that it was possible for a comparison to be made between that form and the strain isolated from *Matthiola* in Victoria. Two cultures were also obtained from Baarn, Holland; *Rhizoctonia solani* Kühn from *Solanum tuberosum* sent to Baarn by Porte, and *R. solani* Kühn *Brassicae II* sent by K. S. Thomas.

The four strains were grown in triplicate on malt agar plates at various temperatures. The depth of the agar in the plates was equal, and care was taken to try to obtain similar sizes in the amount of inoculum used in each case. The cultures used were all of the same age and had been grown under the same conditions. For the sake of convenience the four strains will be referred to as follows:—

R1 .. From cereal, South Australia.
R2 .. *R. solani* Kühn, Holland.
R3 .. *R. solani* Kühn *Brassicae II*, Holland.
S11 .. From *Matthiola incana*, Victoria.

The experiments were conducted at 19°C., 22-26°C.*, 26-28°C.*, 30-32°C., and increases in the radii of the colonies were measured daily.

The figures in Table I. represent the area (in sq. cm.) covered by three days' growth. The first day's growth was not used because in all cases the fungus was slow in starting to grow.

Table I.—Area of Growth of the Four Strains in Square cm.

——	19°C.	23°C.–25°C.	26°C.–28°C.	30·5°C.–31°C.
S11	33·0	30·2	10·4	1·2
R1	11·9	12·6	17·1	2·0
R2	22·4	28·3	34·2	30·2
R3	13·3	8·0	5·0	0·14

The relation of temperature to increase in radius of the culture is represented graphically in Figs. 1–4. The optimum growth of S11 and R3 occurs at about 19°C., and that of R1 and R2 at 26–28°C. The growth of R3 at 30.5°–32°C. practically ceased.

From these results it appears that the isolations of *R. solani* from Cruciferous hosts have a lower optimum temperature than those from cereals and potato. At all temperatures tried the strain from potato had a greater growth rate than that from cereals. This is contrary to the results recorded by Monteith and Dahl (7), who dealt with strains from grasses and potato.

Variations in the Growth Characters of the Forms Studied.

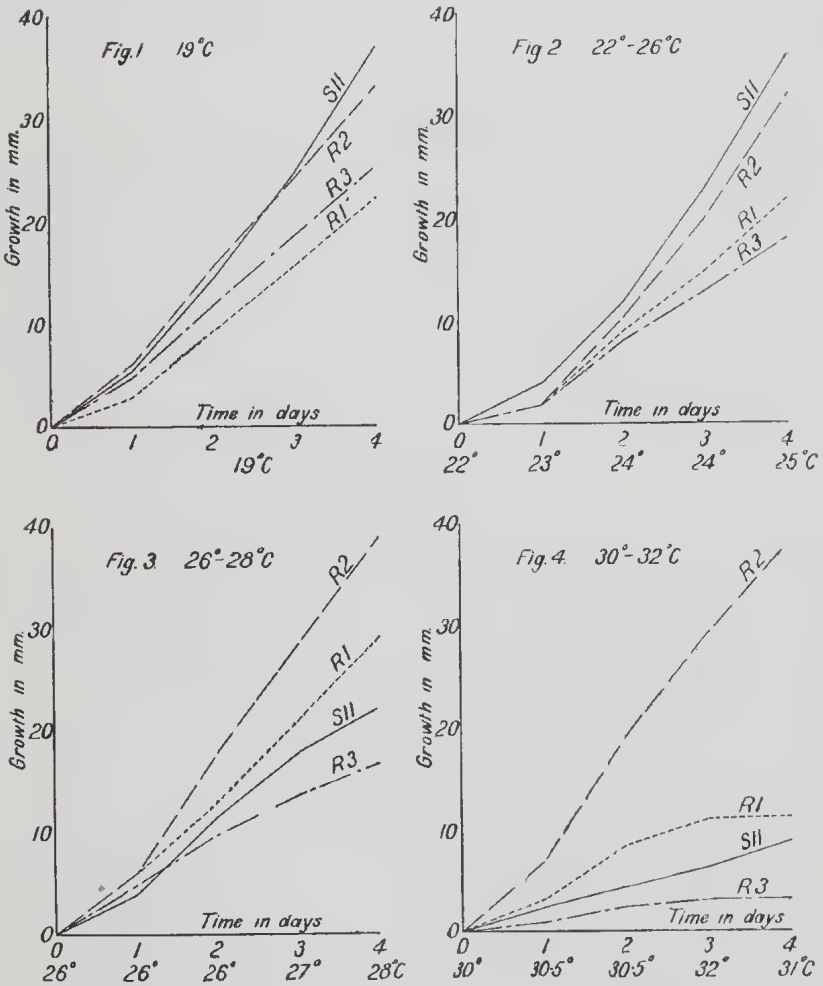
S11.—A thick fluffy aerial mycelium with distinct concentric zonations was formed. At all temperatures tried except 30–32°C. a compact ring of sclerotes developed towards the outside of the plate, and sometimes an inner circle. The agar was discoloured to yellow ochre (Ridgway (10)) below the sclerotes. Scattered sclerotes also occurred mostly towards the centre, and these turned sudan brown and stained the agar beneath them.

R1.—The aerial mycelium was not as dense as in S11 but concentric zonations were very distinct. Scattered sclerotes were

* These experiments were conducted in an incubating room where the temperature was not absolutely constant. The temperature recorded each morning has been indicated on the graphs.

formed, and after three weeks the agar showed a sudan brown discolouration with graduations to paler shades, giving a zoned effect.

R2.—The aerial mycelium was thick and fluffy but with no distinct concentric zonations. At 26–28°C. and 30–32°C. numerous scattered sclerotes were formed, but not at lower temperatures. The agar later showed a yellow ochre discolouration.



R3.—The aerial mycelium differs in being more compact and is mostly short and appressed to the surface of the medium. Concentric zonation is absent and the agar becomes discoloured a uniform sudan brown. Sclerotes were not formed at any temperature tried, and in this respect it differs markedly from

the other three strains. This distinct macroscopic appearance is coupled with a difference in the width of the hyphae, which is 2μ less in R3 than in the other strains (Table II.).

TABLE II.

Strain.	Average width of Hyphae on Malt Agar.
S11	$9{\cdot}3\mu$
R1	$9{\cdot}0$
R2	$9{\cdot}2$
R3	$7{\cdot}3$

Summary.

Although *R. solani* is an extremely widespread parasite, published accounts of its occurrence in Victoria are restricted to records on potato and turnip (McAlpine (6)).

The fungus flourishes with excess of moisture and has proved troublesome by causing "damping off" of *Matthiola* seedlings in the seedling beds at the Footscray Gardens.

Comparisons are made between the strain isolated from stocks with the cereal form from South Australia and *R. solani* Kühn and *R. solani* Kühn *Brassicae II* from Holland. Growth rates at different temperatures are represented graphically.

References.

1. BRAUN, H. Der Wurzeltöter der Kartoffel *Rhizoctonia solani* Kühn. Monographie zum Pflanzenschutz. 1930.
2. BRITON-JONES, H. R. Strains of *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.). *Trans. Brit. Myc. Soc.*, ix., 1924.
3. CARNE, W. M. Foot-rot of *Lathyrus odoratus.* (*Corticium vagum*). *Journ. Roy. Soc. W. Aust.*, xiv., 1927.
4. DUGGAR, B. M. *Rhizoctonia crocorum* (Pers.) DC. and *R. solani* Kühn (*Corticium vagum* B. and C.) with notes on other species. *Ann. Missouri Bot. Gard.* ii. (2), 1915.
5. MATZ, J. Rhizoctonias of Porto Rico. Dept. Agr. Porto Rico, 1921. (Ref. *Rev. App. Myc.*, i., 1922).
6. MCALPINE, D. *Potato diseases in Australia.* Dept. Agr. Vic., p. 60, 1911.
7. MONTEITH, J., and DAHL, A. A comparison of some strains of *Rhizoctonia solani* in culture. *Journ. Agr. Res.*, xxxvi., 1928.
8. MÜLLER, K. O. Uber die Beziehungen zwischen *Rhizoctonia solani* Kühn and *Hypochnus solani* P. and D. *Arb. Biol. Reichanst. für Land und Forstwirtsch*, xi. (4), 1923. (Abstracted in *Rev. App. Myc.*, i., 1923).
9. PELTIER, G. L. Parasitic Rhizoctonias in America. *Univ. Ill. Agr. Exper. Stat. Bull.* 189, 1916.
10. RIDGWAY, R. *Colour standards and Colour Nomenclature.* Washington, D.C. 1912.
11. ROLFS, F. M. *Corticium vagum* B. and C. var. *solani* Burt. A fruiting stage of *Rhizoctonia solani. Science* (n.s.), xviii., 1903, p. 729.
12. SAMUEL, G., and GARRETT, S. D. *Rhizoctonia solani* on cereals in South Australia. *Phytopath.* xxii., 1932.
13. THOMAS, K. S. Onderzoekingen over Rhizoctonia. Electr-Druk. "*De Industrie*," J. van Druten, Utrecht. 1925 (not seen).

II. SCLEROTINIA MINOR ON PRIMULA.

Introduction.

Sclerotinia minor Jagg., a fungus similar to *Sclerotinia libertiana* Fuckel but with smaller sclerotes, was first separated from the latter as a distinct species by Jagger (3) in 1920.

The fungus was isolated by him from lettuce in America, but has since been found on other hosts and in other localities, although not recorded for Australia.

Sclerotina minor from Primula in Victoria.

Primula plants in the Footscray Gardens, Victoria, were attacked in the collar region and soon collapsed and died. Small black sclerotes were found to be present at the bases of diseased plants and the pathogen was isolated and brought into pure culture on malt agar.

The appearance of the fungus in culture conforms with the description of *S. minor* given by Jagger (3). In the early stages of growth, abundant Roman green (Ridgway (6)) appressoria are formed where the mycelium comes into contact with the

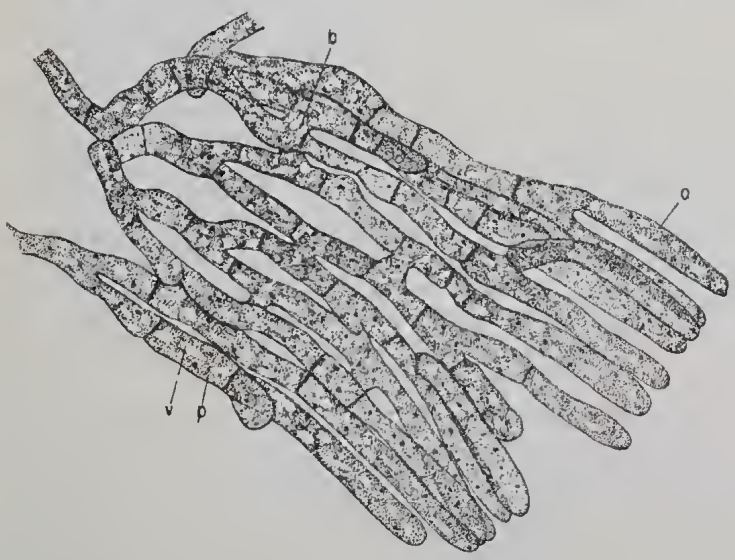


Fig. 5. Roman green appressoria of *S. minor* (× 300). (a) Palisade arrangement of outer cells of the hyphae. (b) Anastomosis between hyphae. (v) Vacuole. (p) Granular protoplasm. From culture on malt agar.

glass. These are very conspicuous and fan-like in appearance. Under the microscope the outer cells show a palisade arrangement (Fig. 5). Appressoria are only rarely seen on oatmeal agar.

Jagger (3) in his original description of the species mentions the presence of abundant appressoria, but does not mention their conspicuous green colour, whereas Ramsey in his description of *Sclerotinia intermedia,* n.sp., a form intermediate in characters between *S. libertiana* and *S. minor,* describes the fungus as forming Roman green appressoria when coming in contact with foreign bodies.

The size of the sclerotes varies, decreasing with lower temperatures, which is in contrast to *Sclerotinia intermedia* which Ramsey (4) and Chivers (1) describe as showing an increase in size of sclerotes with decreasing temperatures.

The fungus was grown in sterile distilled water and on plain agar to induce the formation of microconidia which were not seen on malt nor oatmeal agars. After ten days a few microconidia were produced and after a month they became more abundant on plain agar. They were similar to those described by Jagger (3) for *S. minor,* being spherical and hyaline, and varying from 3–4.2μ. They were borne apically in series or clumps on short obclavate conidiophores (Fig. 6).

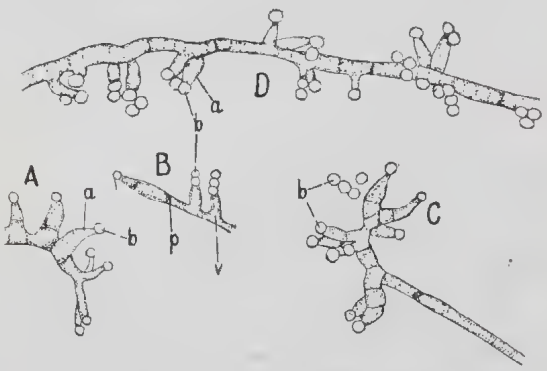


Fig. 6. Microconidia of *Sclerotinia minor* (× 300). A, B, C. Microconidia produced in distilled water. D. Microconidia produced on plain agar after oatmeal agar. (a) conidiophores, (b) conidia, (v) vacuole, (p) protoplasm.

As experiments for the development of apothecia were being conducted, large numbers of sclerotes were needed, and for this purpose the fungus was often grown on oatmeal agar, where sclerotial development was most prolific. It was observed that a culture on an oatmeal plate at 19°C. showed no development of sclerotes even after six weeks, the surface of the medium being covered with appressed white powdery patches, which is the characteristic appearance of the cultures just before sclerotes are developed. The powdery mycelium was examined under the microscope, and seen to bear numerous microconidia varying from 3–4.5μ, with an average diameter slightly higher than was found in water and on plain agar, which is to be expected on a richer medium. The inoculum used in this culture was a

month-old sclerote taken from oatmeal agar, the previous transfer having been from malt agar. This appearance of abundant microconidia on oatmeal agar was unusual, as previous to this no microconidia had been observed on the oatmeal and malt plates, where only sclerotes were formed.

Transfers from this plate were made to malt, oatmeal and plain plates and slopes, and kept at 19°C. After five days characteristic green appressoria and numerous white clumps appeared. These powdery patches of appressed mycelium, in contrast to those produced on cultures where the transfers were from a colony bearing selerotes, were more concentrated around the inoculum itself at first, and later spread out from that centre.

These tufts showed few microconidia after five days, but they increased in number and by the tenth day were very numerous, while no selerotes developed even after three months.

Again three months later, a culture on an oatmeal slope at room temperature (about 23°C.) was seen to have produced no sclerotes. The inoculum was again a sclerote from a culture which had been on oatmeal agar for a considerable time. In this case the myeelium was short, light buff in colour and much denser in texture. Under the microscope the microconidia were seen to be extremely abundant.

Several malt and oatmeal slopes were inoculated from this tube, and after five days showed cottony aerial myeelium which soon disappeared. Green appressoria were present and powdery tufts around the inoculum. The whole surface of the medium was soon densely covered with powdery appressed myeelium.

A soil agar plate was also inoculated, and after seven days showed a rather sparse cottony aerial myeelium with no powdery white patches but fairly numerous microconidia around the inoculum. After two weeks the aerial myeelium bore numerous microconidia. A colony from a sclerotial culture on soil agar had a similar macroscopic appearance and the myeelia in both cases were the same, but after a week tiny black sclerotes were formed and no microconidia were present.

Ramsey (5) noticed that the formation of microconidia seemed to depend on a poor nutrient supply. He conducted experiments with *S. libertiana* and *S. intermedia* to determine whether there was any tendency to change from the normal. No change in the number of selerotes nor in microconidial production was, however, observed.

Godfrey (2) working with *Sclerotina ricini* found that sometimes a culture produced numerous conidia (Botrytis stage) but no sclerotes. There was, however, a normal development of selerotes if transfers from such cultures were put on oatmeal agar.

In the case of *Sclerotinia minor* saltation evidently occurs on rich media, and some inherent differences in the sclerotes cause some to produce mycenum with sclerotes, others with microconidia only. Transfers for several generations on to various media from these microconidial cultures have so far yielded no colonies producing sclerotes. Many workers have experimented with the microconidia of Sclerotinias and various functions have been suggested.

Unsuccessful attempts were made to germinate the microconidia of *S. minor* in sterile water and turnip juice.

Experiments for Apothecial Production.

An attempt was made to produce apothecia from the small sclerotes of *S. minor*. The methods employed were those used by Ramsey (5). Small porous pots were filled with pure sterile sand and placed in halves of petri dishes containing sterile distilled water. Some were placed under bell jars in strong diffuse light, others were covered with circular glass plates.

Sclerotes were also planted in pots containing sterilized garden soil. Various methods were used to try and induce germination. Sclerotes of ages varying between twelve months and two weeks were used, and planted during different months of the year. Some were from cultures on malt, others from oatmeal agar. They were planted at different levels in the sand from the surface to as deep as 1 cm.

Some sclerotes were specially treated before planting. They were subjected to:—

(1) Ether vapour for various periods between a half and two hours.

(2) Cold. Sclerotes in refrigerator for one week before planting.

(3) Water. Sclerotes soaked in sterile distilled water for one week.

(4) Drying. Sclerotes in desiccator from four days to one and a half months. Sclerotes were also allowed to remain on dry sand for two and three months before watering. This latter method was used by Jagger (3) for developing apothecia from sclerotes of *S. minor*.

(5) Heat. Sclerotes kept dry at 30°C. for three months.

Results.

Only two results have so far been obtained from these experiments. One of the month-old sclerotes from malt agar which was subjected to ether vapour for one hour before putting on

the surface of sterile sand, germinated, producing a pale yellowish, slightly curved, slender cylindrical body about $\frac{1}{4}$-in. long. When examined with a lens it was seen that the surface was not smooth but slightly roughened and had a frosted appearance.

The pot had been well watered and covered with a ground glass plate. The sclerote was planted in November, and germination occurred three months later in January, after three days of hot weather when the temperature had been over 100°F. During the next four weeks this structure was watched closely to see if a disc-shaped apothecium would develop. The tip, which was at first attenuated, broadened a little and seemed to indicate that an apothecium was forming. There was also a slight elongation in length and a deepening of colour, especially near the base. As light is necessary for the expansion of the apothecium, this was increased by replacing the ground glass with a bell jar, which also provided a greater humidity.

Godfrey (2) in his experiments with germinating sclerotes of *S. ricini* remarked on the necessity for high humidity and a warm temperature for the development of apothecia. Ramsey (5), however, found that more apothecia were produced between January and April, the summer months being unfavorable. Germination occurred between 18° and 22°C. but not between 22° and 30°C.

In February, four months after planting, a sclerote from a similar culture, which had been subjected to the same conditions, but in a different pot and with a plain glass cover, germinated, producing a short greyish cylindrical body with a lighter-coloured translucent tip. Germination again occurred after a spell of very hot weather. The glass plate was replaced with a bell jar and growth in length was noticeable within a few days.

From these results it seems that a high temperature is necessary to induce germination of sclerotes and as in both cases they had been subjected for one hour to ether vapour, the latter may have had some stimulatory effect. Both of the sclerotes which germinated were relatively large and anastomosing with others.

Summary.

(1) *Sclerotinia minor* Jagg. is recorded as causing a collar rot of *Primula malacoides* in Victoria.

(2) Saltation is found to occur on oatmeal agar so that sometimes a sclerote may produce a colony bearing microconidia only, instead of the usual development of abundant sclerotia.

Transfers were made from such cultures (and these were again transferred for several generations) on to various agars but no production of sclerotia followed.

(3) Experiments were carried out for the production of apothecia, and in two cases sclerotes germinated producing horn-like outgrowths. Development proceeded to a swollen tip.

References.

1. Chivers, A. H. A comparison of *Sclerotinia minor* and *S. intermedia*. *Phytopath*, xix., 1929.
2. Godfrey, G. H. Gray mold of Castor Bean. *Journ. Agr. Res.*, xxiii., 1923.
3. Jagger, I. C. *Sclerotinia minor*, n.sp., the cause of a decay of lettuce, celery and other crops. *Ibid.*, xx., 1920.
4. Ramsey, G. B. *Sclerotinia intermedia*, n. sp. A cause of decay of salsify and carrots. *Phytopath*, xiv., 1924.
5. Ramsey, G. B. Sclerotinia species causing decay of vegetables under transit and market conditions. *Journ. Agr. Res.*, xxxi., 1925.
6. Ridgway, R. *Colour standards and colour nomenclature*. Washington, D.C., 1912.

III. CORTICIUM CENTRIFUGUM ATTACKING DELPHINIUMS.

Introduction.

Sclerotium rolfsii has a wide distribution, occurring in most tropical and sub-tropical countries and causing the greatest damage during the wet seasons. The fungus is capable of attacking a large number of plants, the usual hosts being succulent annuals, although sometimes, under favorable conditions, it may cause disease in woody perennials. Weber (6) listed 189 host plants, including two species of Delphinium. Fajardo (4) reported a stem and root disease of annual Delphiniums due to *S. rolfsii* in the Philippine Islands. Birmingham (1) reported *S. rolfsii* from several hosts in New South Wales including a Delphinium plant from Goulburn.

Delphinium plants at Essendon, Victoria, were attacked by a fungus which caused yellowing and wilting of the leaves and finally drying up and death due to the rotting of root and collar regions. White mycelium with small brown sclerotia encircled these parts.

The pathogen was isolated by placing pieces of diseased tissue, mycelium and sclerotia on malt agar, and pure cultures were obtained.

Description of the Pathogen in Culture.

On malt agar the fungus grows in a fan-like manner and produces a dense fluffy mass of aerial mycelium. After about four days sclerotes begin to form, usually at the margin of the plate, and gradually extend inwards towards the inoculum. They generally arise singly in the aerial mycelium and appear first as irregular white knots of hyphae (Fig. 7c), which soon become compact and rounded, and a definite cortical layer develops (Fig. 8). They are uniform in size and almost quite globose. The colour gradually changes from white, through light buff, warm buff, cinnamon brown, and finally when old and dry, to clove brown (Ridgway (5)). Liquid is often exuded in droplets from the surface of the sclerotes and at first is colourless but later amber-coloured.

As the sclerotes mature the aerial mycelium collapses, and the sclerotes, which were formerly suspended, are left on the surface of the agar. Sclerotes produced by the mycelium on oatmeal, potato dextrose and malt agars show no significant differences in size but are more numerous and massed together on oatmeal. They vary in size from 0.5 to 2 mm. but are usually about 1.2 mm.

If an abundance of agar is not present the size and number of sclerotes are appreciably less. On malt agar the width of the septated hyphae varies from 2 to 12μ (generally 3 or 7μ), the narrower threads being more crooked. Anastomoses between hyphae are numerous, and clamp connexions are present (Fig. 7).

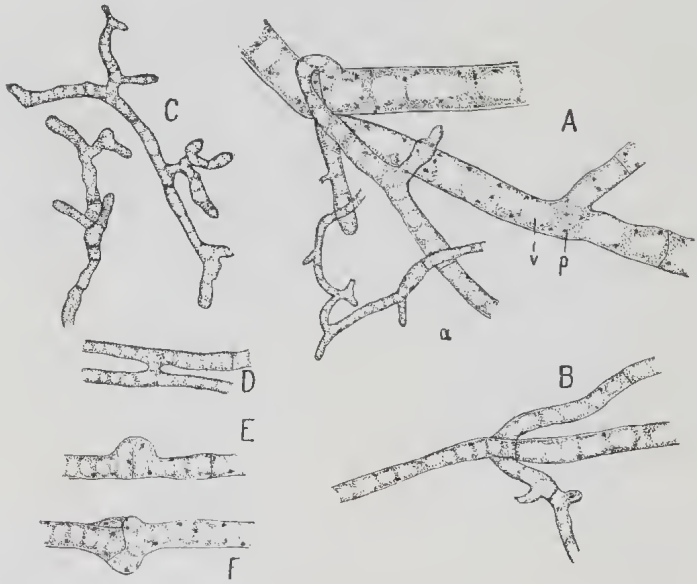


Fig. 7. Mycelium produced on malt agar. × 300. A, B. Hyphae of *C. centrifugum* showing variation in width. (a) Narrow crooked threads. C. Irregular hyphae from very young sclerote. D. Anastomosis between 2 hyphae. E, F. Clamp connections in aerial hyphae. (v) vacuole. (p) granular protoplasm.

Comparison with two other sclerotial forms.

The fungus isolated from Delphiniums in Victoria (D2) was compared with Whetzel's strain of *Sclerotium Delphinii* (SCI) obtained from Centraal-Bureau voor Schimmelcultures, Baarn, Holland. Comparison was made on malt agar, and microscopically the mycelium was quite similar except that in SCI there was sometimes a development of rounded irregular bodies occurring in an intercalary position on the hyphae (Fig. 9).

Macroscopic differences were, however, much greater. In D2 a dense fluffy aerial mycelium was produced. The numerous small sclerotes (0.5–2 mm. average 1.2 mm.) were formed first near the margin of the plate and gradually spread inwards. The liquid exuded from the sclerotes was amber-coloured. The final colour of the sclerotes was clove brown, and in section the outer brownish cortex (up to 50μ wide) was composed of compacted hyphae giving a parenchymatous appearance. The inner medullary region is composed of short and elongated colourless hyphae with numerous intercellular spaces (Fig. 8).

In SCI the aerial mycelium showed a more dendritic type of growth but greatest distinction was seen in the size, shape, number and colour of the sclerotes. The sclerotes were fewer and larger (1–6 mm. average 3.4 mm.) and in contrast to those

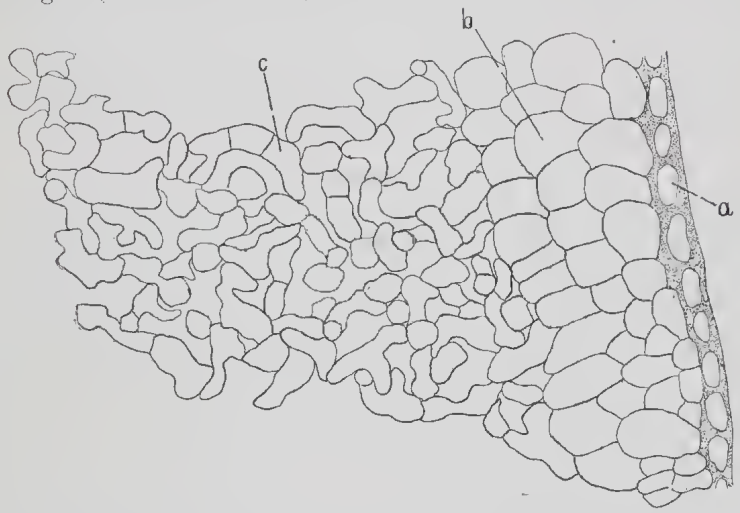


Fig. 8. Hand cut T.S. of portion of a mature sclerote of *Corticium centrifugum* (× 200) from malt agar. (a) Outer rind of compact cortex. Deep brown in colour. (b) Cortical tissue composed of false parenchyma and light brown in colour. (c) Inner lacunar medullary region composed of long and short irregular hyphae.

of D2 were often found coalescing. They were irregular in shape, but convex or flattened above, and usually concave on the lower surface, where they were often attenuated into a stalk-like region. Sclerotes one week old were a conspicuous

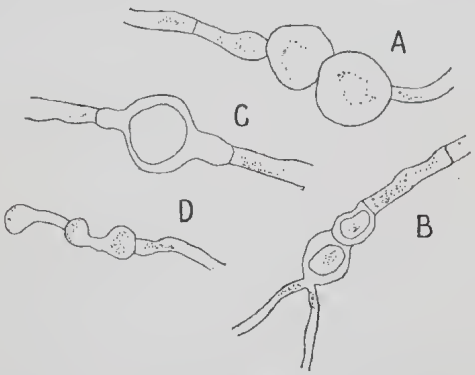


Fig. 9. A, B, C. Rounded chlamydospore-like bodies on the hyphae at the junction of SCI and D2 colonies. (× 300.) D. Irregular swellings on hyphae in the same region. (× 300.) Culture on malt agar.

orange cinnamon colour and the final stage was warm sepia. At all stages they showed a more reddish tinge than those of D2. The colourless liquid was exuded from darker depressed

spots. Sections of sclerotes showed that the cortex was if anything slightly wider but composed of smaller hyphae, while hyphae of the medullary region were distinctly wider than in D2. SCI also showed a development of felted white appressed mycelium around the inoculum.

This comparison indicates that the fungus isolated from Delphiniums in Victoria cannot be considered identical with *Sclerotium Delphinii.*

Comparison was also made with Wolf's strain of *Sclerotium rolfsii* which is known now as *Corticium centrifugum* (CI) obtained from Baarn, Holland.

CI on malt agar produced mycelium similar to that of D2 and the sclerotes were numerous and small (0.3–2 mm. average 1 mm.) but more irregular in shape and varied more in size than those of D2. Also in contrast to the initial marginal formation of sclerotes in the latter, they formed at first near the central inoculum (where masses of sclerotes were often found), and gradually spread out to the margin of the plate. The liquid exuded was colourless, but the colour changes of the developing sclerotes was practically the same as in D2, and the final colour again clove brown. Sections of sclerotes were very similar to those of D2. A felted white appressed mycelium was often developed around the inoculum. This was not seen in D2. The microscopic appearance of the hyphae of D2 and CI was identical.

D2, SCI and CI were plated against each other to determine whether any antagonising action existed between them. In each case there was a distinct line at the junction of the two colonies, and it was found that D2 was the fastest and SCI the slowest grower. If two colonies of one strain were started on the same plate there was free intermingling of the hyphae.

It was evident that a greater antagonistic action existed between SCI and D2, and between SCI and CI, than between D2 and CI, the agar in the two former cases being slightly stained along the junction. SCI developed numerous sclerotes along the line of junction, but this was not apparent with CI and D2. On microscopic examination it was found that there was a considerable development of rounded or irregular bodies up to 25μ diameter occurring in an intercalary position on the hyphae of SCI near the junction with CI and D2 (Fig. 9). It was evident that D2 was much more closely related to CI and the slight differences present could not be considered as specific.

Attempts were made to produce the fruiting stage by growing the three forms on various media (potato dextrose, malt, oatmeal, soil and plain agars) and on a solution of 1% Witte peptone + .5% potato starch + 50 cc. distilled water (C1 and D2 formed thick conglomerated masses of sclerotes on the latter medium, but it was unsuitable for SC1) at 30°C., 19°C. and room temperature. No basidia or spores were observed although they were examined constantly.

Discussion.

Curzi (2) in Italy compared a sclerotial fungus isolated from aster with one from potato. Differences were found to exist in vegetative characters, the former producing a flocculent mycelium while in the latter the mycelium was arranged in strands and numerous stromata were present. The two were mutually antagonistic.

The aster fungus produced a loose aerial hymenium showing affinities with *Corticium centrifugum,* while the potato fungus had a dense crust-like hymenium which he considered was not identical with any known Basidiomycete. He named it *Corticium rolfsii,* n. comb., as he considered this latter fungus to correspond with that from which Saccardo described *Sclerotium rolfsii.* Wolf's strain, which is usually regarded as a typical strain of *Sclerotium rolfsii,* also has a loose hymenial stage similar to the aster *Corticium* and undoubtedly corresponds with it. Since *Corticium centrifugum* cannot be regarded as the perfect stage of the true *S. rolfsii,* it should be considered as a distinct species.

The study of various isolations of *S. rolfsii* therefore indicates that the name includes more than one species whose systematic positions should be determined by attempts to discover the perfect stage, or an accurate examination of the vegetative organs under various conditions.

Curzi (3) made three distinct groups for the strains examined by him:—

(1) Sclerotial stage of *Corticium rolfsii* (Sacc.) *Curzi.*

(2) Sclerotial stage of *Corticium centrifugum* (= *S. centrifugum* n. comb.).

(3) *Sclerotium Delphinii* Welch.

The perfect stage of the strain isolated from Delphinium was not obtained, but the vegetative characters under various external conditions showed a close resemblance to Wolf's *Corticum centrifugum* and Curzi's description of his aster strain, while the distinctive characters of his potato strain were not present.

Summary.

(1) *Corticium centrifugum* is recorded as causing a root and collar rot of Delphiniums in Victoria.

(2) A description of the pathogen in culture is given.

(3) Comparisons are made between the strain isolated from Delphinium, Wolf's strain of *Corticium centrifugum* and Whetzel's *Sclerotium Delphinii* (Welch).

Acknowledgments.

The writer wishes to express her gratitude for the helpful interest shown by Professor Ewart and also to thank Dr. McLennan, under whose direction the work was conducted, for her helpful criticism and suggestions.

References.

1. Birmingham, W. A. A fungus and some of its host plants. *Agric. Gaz., N.S.W.*, xxxv. (6), 1924.

2. Curzi, M. Stûdi sur lo *Sclerotium rolfsii*. *Boll. R. Staz. Pat. Veg.*, N.S. 11.4.1932.

3. Curzi, M. Contributo alla conoscenza della biologia e della sistematica degli stipiti dello *Sclerotium rolfsii*.. *Rendic. R. Acad. Linnei* (6), xv., 1932.

4. Fajardo, T. G. Sclerotium stem rot of Delphinium and other ornamental plants in Trinidad Valley, Mountain Province, Philippine Islands. *Philip. Journ. of Sci.*, li. (4), 1933, pp. 447-453.

5. Ridgway, R. *Colour standards and colour nomenclature*. Washington, D.C., 1912.

6. Weber, G. F. Blight of carrots caused by *Sclerotium rolfsii* with geographical distribution and host range of the fungus. *Phytopath.* xxi., 1931.