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A STUDY OF CERTAIN MEMBERS OF THE SOUTH AFRICAN XYLARIACEAE,
WITH REFERENCE TO THE USE OF CULTURAL CHARACTERS
IN CLASSIFICATION.

A thesis submitted to Rhodes University
for the Degree of Master of Science

by

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S U M M A R Y.

Sixty-three species, drawn from the genera Rosellinia Hypoxyton, Nummularia, Daldinia, Penzigia and Xylaria, are studied in order to determine whether any correlation exists between various characters of the perfect stage and characters connected with the mycelium in artificial culture that might be used to supplement the existing classification of the Xylariaceae. An examination is made of the morphological and anatomical characters of several stromal types and a statistical method of evaluating differences in ascospore dimension between samples of closely related species is described. Cultural work shows that:-

- a) Each species group, based primarily on similarity of stromal form, has a characteristic set of cultural characters.
- b) Members within a Species group can usually be distinguished clearly in culture.

The use of cultural characters as an additional criterion in classification is therefore recommended.

INTRODUCTION.

The present work deals with South African species of selected stromatic genera of the Xylariaceae, Rosellinia, Hypoxylon, Nummularia, Daldinia, Penzigia and Xylaria, in an attempt to determine firstly whether the commonly accepted distinctions between these genera are valid and secondly whether there is any correlation between variations in morphology and structure of the perfect stages and their characters in artificial culture.

The features studied were:

1. The morphology and anatomy of the stroma and the manner of its development in selected species.
2. The germination of the spores and the external characters and growth rate of the mycelia.
3. The types of conidiophore and the manner and place of origin of the conidia.

Very little work appears to have been done on these aspects.

This study has inevitably raised the problem of which characters should be selected for classification since it is always difficult to decide which are of real phylogenetic significance. Many of the criteria chosen by the early workers, particularly those employed for subgeneric division, are recognised today as inappropriate while others are retained only for their convenience. In a group such as the Xylariaceae the wide range of stromal form encountered even in the same species may justifiably result in the placing of extreme forms in different genera. Shear (1945) writes:-

"There is no general agreement among mycologists as to the generic limits of Hypoxylon and its relatives (based on structure of the perfect stage). The material available in our herbaria shows conclusively that there are no distinct boundary lines to be found between Rosellinia, Hypoxylon, Nummularia, Penzigia, and Xylaria. Generic segregation must be a matter of individual judgement, based entirely on considerations of convenience and conservation. Too much splitting of genera serves no useful purpose."

It is difficult, in this connection, to determine how much weight should be placed on the use of mycelial and conidial characters in classification. Up to the present, in mycological taxonomy, they have played a minor or subordinate role in relation to the characters of the perfect stage. Their value, however, in confirming or minimising specific differences based on stromal characters has nevertheless been recognised by such authorities as Shear (1928, 1945) and Miller (1928).

Tulasne (1863) appears to have been the first to illustrate and describe the imperfect stages of members of the Xylariaceae, Rosellinia aquila, Hypoxyton coccineum, Ustulina deusta, Xylaria polymorpha and X. hypoxyton. Subsequently Saccardo (1882 et seq.) and Miller (1928 et seq.) made passing reference in specific descriptions to the presence of conidia on young stromata but no comparative work was undertaken. The only noteworthy advance in this respect has been the discovery by Barnett (1957) of a Basidiobotrys imperfect stage produced by H. punctulatum in culture.

This investigation, involving 63 species in all, seeks to answer some of the problems just discussed and to establish a broader and sounder basis for the separation and classification of species groups than has been employed hitherto.

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CHAPTER I.

THE STROMA IN THE XYLARIACEAE.

The family Xylariaceae was established by Winter (1887) when he grouped under this title all the stromatic members of the Sphaeriales that had single-celled dark coloured spores. These genera comprised Hypoxylon, Nummularia, Ustutina, Poronia, and Xylaria. Today the concept of the family has changed and the latest system, proposed by Arx and Muller (1954) rates stromal organization of secondary importance to spore characters. In the Xylariaceae are placed practically all the genera in the Pyrenomycetes that possess dark unicellular spores and develop true ostiolate perithecia with dark carbonous or leathery walls. This would appear to be a natural arrangement, since it recognises a distinct series in evolution from a condition where the perithecia are naked and seated either singly or aggregated on the moist substrate, to one where they become enclosed, singly or severally by a hard stromal covering, and finally to a state where the stromal covering becomes partly fleshy in texture. Unfortunately it is much easier to point out trends in stromal organization than to define unambiguously the limits between the individual genera.

It is generally recognised that there are two main series or groups of affinity within the stromatic Xylariaceae. In one group of genera the stromata are almost entirely carbonaceous or leathery in texture while in the other carbonization, if present, is restricted to the outermost part of the stroma and is predominantly fleshy.

Predominantly carbonous
or leathery.

Arthostoma de Not.
Camarops Bull.
Daldinia Ces. and de Not.
Hypoxylon Bull.
Nummularia Tul.
Rosellinia de Not.

Predominantly fleshy.

Camillea Fries.
Kretschmaria Fries.
Penzigia Sacc.
Phylacia Lév.
Poronia Will. ex Fries.
Sarawakus Lloyd.
Sarcoxylon Cooke.
Thamnomycetes Ehrenberg.
Xylaria Hill. ex Fries.

The present work has been confirmed to the genera Rosellinia, Hypoxyton, Nummularia, Daldinia, Penzigia and, Xylaria. They have been separated by Saccardo (1882 et seq.) and later by Miller (1928) and Clements and Shear (1931), on the following characters:-

1. Rosellinia:

Stromata uniperitheciate, carbonous, usually solitary but sometimes aggregated; immersed or developing superficially on the host wood. Established by de Notaris (1847).

2. Hypoxyton:

Stromata multiperitheciate with a darkly coloured carbonaceous, woody or leathery interior; immersed or superficial on wood. Established by Bulliard (1791), amended by Miller (1928).

3. Nummularia:

Stromata multiperitheciate, heavily carbonized, always deep seated in the substrate and erumpent through the bark at maturity. The genus was separated from Hypoxyton by Tulasne in 1863 because of the current belief that the conidia were produced in a layer inside the developing stroma and only reached the surface by disappearance of the outer tissues. Other minor features included the characteristic indefinite effuse nature of the stroma and the metallic sheen of the surface which gives the genus its name. Miller's emendation of the genus (1932) introduces a different concept and the taxon is restricted to certain species only that have a circular form and concave surface.

4. Daldinia:

Stromata always pulvinate or globose, with the perithecia developing in the periphery; interior of the stroma dark and conspicuously zonate, and cited by various authors as being corky, membranous or fleshy in texture. Established by Cesati and de Notaris in 1863.

5. Penzigia:

Stromata variable in form, effuse, pulvinate or clavate but usually attached to the substrate at a central point only. The outermost part of the stroma is carbonous while the interior is fleshy. The genus, established by Saccardo in 1888, and emended by Petch (1924) is an intermediate group between Hypoxyton and the next genus Xylaria; it can be separated

from the former by its white or yellow interior and from the latter by the shape of the fertile part of the stroma, which is flat or umbrella-shaped instead of conic or cylindrical.

6. Xylaria:

Stromata conic or cylindrical and usually with a distinct sterile setose base; interior white or fleshy, exterior grey or black. Established by Fries in 1849.

The reproductive organs inside the stroma of Hypoxylon and its allies were also used by early mycologists as minor characters in classification:

i. The perithecium:

The structure of the perithecium is less variable than that of the stroma as a whole in the Xylariaceae, but nevertheless may show interesting differences. Basically it consists of a wall of tightly packed dark cells surrounding an inner "centrum" comprising the asci and periphyses. Though always dark in colour at maturity, the wall has been shown to differ by Miller (1928) according to the type of stroma. When the stroma is non-carbonous the perithecia are uniformly membranous but if carbonization is present in the stroma it usually extends to the upper parts of the perithecia as well.

The ostiole, here defined as that part of the stroma and perithecium forming the canal which leads from the perithecium to the outside, is characteristically papillate though occasionally somewhat depressed or level in outline when developed by species with entirely carbonous stromata and characteristically umbilicate in one species group of Hypoxylon (Miller's Group I, discussed later). In the latter group the ostiole never projects from the surface of the stroma but are apparent simply as pits. The significance of the type of ostiole in classification will be discussed more fully with the experimental work at the end of this chapter.

ii. Asci and Spores:

The asci in the Xylariaceae are clavate or cylindrical and always contain 8 spores. These are, with few exceptions, in monostichous series. Usually the length of the ascus and that of its sterile base or stalk can be used in classification but intraspecific variation in this respect is probably greatly underestimated and seriously reduces its value.

The spores in the Xylariaceae are characteristically oval-elliptic, but are sometimes crescent-shaped, cylindric or subglobose, light or dark brown to black and often inequilateral due to one side being concave. The size, shape and colour of the spores are generally recognised as useful in taxonomy so long as they are taken in conjunction with other stromal characters.

The old and the new systems of classification:

The early mycologists, Fries (1849), Nitscke (1867) and Saccardo (1882 et seq) relied primarily on the shape, size and degree of immersion of the stroma in the substrate to separate the genera and species known in their time. As further material was described however, it became evident that these characters could be influenced at least partly by environmental factors as well as differences in hereditary constitution. True assessment of generic differences was, however, prevented by lack of sufficient comparative work.

The position was clarified by Miller's work on the Xylariaceae (1928) in which he discussed the concept and various definitions of the stroma. He suggested that classification of the group should be based rather on the relative proportions and consistency of the layers composing the stroma than merely on the external form. Two separate layers of the stroma were clearly defined:-

1. the ectostroma:

which "is that part of the stroma first formed in or on the periderm, or on the bark when the wood has been removed, and functions in rupturing the bark when the latter is present and which normally functions in producing the conidia".

2. the entostroma:

which "is that portion of the stroma that develops under the ectostroma and bears perithecia in its periphery".

Miller's emendation (1928) of the genus Hypoxyton was based on the appearance and relative proportions of these 2 structures. The genus Hypoxyton had been created in 1791 by Bulliard and because of its heterogeneity was reconstructed by Fries in 1849 who excluded unrelated species and introduced others of closer affinity from the original genus Sphaeria. His and subsequent divisions into species groups were based entirely on stromal form.

Miller divided the genus according to internal characters of the stroma.

These included:-

- a. the quantity of stroma developed outside the perithecia.
- b. the depth in the wood to which the stromata were sunken.
- c. the colour of the stromal surface and that part immediately below.
- d. the type of ostiole.

His classification was as follows:-

- Group I : Species with the mature stroma of woody texture; with red to purple ectostroma and dark entostroma; ostioles umbilicate (not protruding) (Plate VI.)
- Group II : Species with carbonaceous stromata and annular depressions encircling the papillate ostioles.
- Group III : Species with little or no entostroma, and perithecia sunken in the substrate; ostioles papillate (Plate VII).
- Group IV : Species with constantly effused carbonaceous stromata; ostioles usually papillate. This group contained many species formerly placed in the genus Nummularia.

The genera Rosellinia, Penzigia and Xylaria were recognised as separate from Hypoxyton on account of the characters listed at the beginning of this chapter but sufficiently closely related to be placed in the same family.

Miller's conclusions regarding the genus Rosellinia are also interesting. Rosellinia had been created in 1847 by de Notaris with R. aquila as the type. The genus was not included by early mycologists in the Xylariaceae because it was thought that a stromal covering was absent and that the perithecia were formed naked, partly embedded in the substrate or superficial. Miller, however, clearly saw that there was a carbonous layer surrounding the perithecia and that the true affinities of the genus lay with Hypoxyton. The old concept has still persisted, however, and as recently as 1954 Rosellinia aquila was illustrated by Arx and Muller showing naked perithecium seated on a "hypostroma" of uncertain origin. (See Plate IX).

Another problem of a different nature was also discussed by Miller in relation to the genus Nummularia.

Tulasne (1863) and later Saccardo (1882), stated that the conidia of the species placed in Nummularia arose as a layer within the stroma and were thus partially enclosed instead of purely superficial as in Hypoxylon. This feature and the characteristic effuse form of the stroma were taken as the chief characters to separate the two taxa. Miller (1928 b) however, with reference to N. discreta and other forms, stated that the conidia arose superficially in both genera and then transferred several species of Nummularia to Hypoxylon on account of their basic similarity in form. These species included, inter alia, the type, Nummularia bulliardii, and N. mediterranea. Later (1932) Miller amended the genus Nummularia taking N. discreta for the type, to include those species of the original genus which had small circular erumpent stromata with concave surfaces. Moreover, in such species he stated that "the perithecia are not borne in the periphery of the stroma but are developed in the base and reach the surface by more or less elongate ostiolar necks". The validity of these criteria will be discussed later in this chapter.

The differences between the old and the recent systems of classification can be summarised best by the following table:-

Classification adopted by Saccardo. (1882 et seq.)	Miller's classification (1928 et seq.)
<u>Rosellinia de Notaris (1847)</u> Perithecia single, thought to be without stromal covering.	Perithecia single but a definite stromal covering was shown to exist.
<u>Hypoxylon Fries (1849)</u> Stromata multiperitheciate, variable in form. Divided by Saccardo into:- <u>Eu-Hypoxylon</u> . Stroma globose, pulvinate, or effuse, superficial. a. exterior coloured b. exterior black or initially brown then black. <u>Placoxylon</u> . Stroma broadly effuse, superficial with indistinct outline. a. exterior coloured b. exterior brown or black.	Group I. Species with the interior woody or leathery in texture; ectostroma purple or red; ostioles umbilicate. Group II. Species with carbonaceous stromata and annular depressions surrounding the papillate ostioles. (N.B. Both Saccardo's groups fall into either of Miller's categories.)

Classification adopted by Saccardo
(1882 et seq.)

Miller's classification
(1928 et seq.)

<u>Endoxylon</u> . Stromata more or less immersed, black.	<u>Group III</u> . Species with little or no entostroma, and perithecia sunken in the substrate.
<u>Coenopus</u> . Stromata superficial above the bark; underneath subfruticose or tuberculous. Includes species later placed in <u>Penzigia</u> and <u>Kretschmaria</u> by Saccardo himself.	Placed by Miller in <u>Penzigia</u> and <u>Kretschmaria</u> on account of the white entostroma.
<u>Species Incertae sedis</u> .	Dispersed throughout Miller's groups.
<u>Nummularia Tulasne (1863)</u>	
Stromata multiperitheciate, variable in form but characteristically <u>effuse</u> , immersed and distinguished from <u>Hypoxylon</u> on presumed internal origin of the conidia.	Placed in <u>Hypoxylon</u> , Group IV; stromata not found to produce conidia internally and therefore not considered to be essentially different from <u>Hypoxylon</u> .
Later (1932) Miller retained the name <u>Nummularia</u> for some species of the original genus which have small circular concave immersed stromata. Hence his definition of the genus differs considerably from the old.	
<u>Daldinia Ces. and de Notaris (1863)</u> .	Accepted by Miller as a distinct genus on account of the zonate entostroma.
Stromata multiperitheciate, usually large, with carbonous crust and dark zonate interior.	
<u>Penzigia Saccardo (1888)</u> .	Accepted by Miller as a distinct genus.
Stromata with white fleshy interior and carbonous exterior.	
<u>Xylaria Hill ex Fries (1847)</u> .	Accepted by Miller as a distinct genus but he implies later (1942) that the same species may have variants that are placed in both <u>Penzigia</u> and <u>Xylaria</u> .
Stromata similar to <u>Penzigia</u> but differing in cylindrical form.	

Experimental work on the stromata of the Xylariaceae:

The study of prepared sections of many species has confirmed the basic tenets of Miller's classification. On the other hand it has appeared necessary to qualify some of his findings in the light of further evidence. The following methods were used to investigate the problem:

1. Sections were cut of the mature stromata of 61 of the 63 species collected. (See Appendix I, §§ 2 and 3). These comprised representatives of Rosellinia, Miller's 5 groups of Hypoxylon, Penzigia and Xylaria, and also a species N.kalchbrennera, of the genus Nummularia as redefined by Miller. These taxa have already been illustrated diagrammatically in Diagram I. Further diagrammatic drawings of each stroma type are to be found with the illustrations for each species in Volume II.
2. 58 species, drawn from the species groups listed under 1, were used in an attempt to follow out the development of as many kinds of stroma as possible by culturing them in the laboratory.

Eight species of host wood were chosen from the list of hosts recorded on which the fungi had originally been collected. These were: Acacia karoo, Cassine croceum, Olea capensis, Tarchonanthus camphoratus, Aloe pluridens, Gonioma kamassi, Sideroxylon inerme and Trichocladus crinitus. The first four of these were inoculated by all species. The latter four were used only in selected cases, notably when the species concerned appeared to be host specific.

Dead logs or twigs, 4-24 months old after separation from the tree, were cut into lengths 4-6" long and 2" wide. In some the bark was retained, while in others the wood was decorticated. The lengths of wood were boiled in water for an hour to ensure penetration of water. Four of these wood lengths, one of each species, were placed together in each of 210 ball-jars in which a little wet sand was placed previously. 90 smaller bottles were treated in a similar way, each bottle containing one or two twigs. The bottles were closed tightly and sterilized by autoclaving. After cooling the wood was inoculated speedily under sterile conditions with one fungal strain. Duplicate and often triplicate cultures were made. The cultures were then left to stand in a well-lit room, but out of the sun. After 3 months, one set of cultures were deposited on the ground under a forest by the side of a waterfall. Both these and the remaining sets of cultures were observed at fortnightly periods.

In all cases the fungi developed a luxuriant growth of mycelium, but only one species, H. vividum, produced stromata. The cultures retained in the laboratory ceased to grow further after about 9 months but remained viable. The culture placed in the open degenerated soon after although it is impossible to say whether they died entirely.

The experiment was thus largely a failure as far as the production of stromata was concerned. However, imperfect stages were produced on wood in the great majority of cases, one of which (Rosellinia aquila) was not produced on agar culture. Much useful information was therefore obtained.

The stromata of the successful species, H. vividum, were removed from the culture jars while in all stages of development and sectioned in the manner outlined in Appendix I § 3. The results are discussed in this chapter.

3. Stromata collected in the field in various stages of development were also sectioned. The species concerned were:

Rosellinia mammoidea

Daldinia concentrica

Rosellinia corticalis

Penzigia berteri

Hypoxylon glomeratum

Hypoxylon truncatum

Hypoxylon microcarpum

Hypoxylon deustum

Hypoxylon piceum

Hypoxylon 18 B, (unidentified).

Results:

The results will be discussed under the following headings:-

1. The number of perithecia in the mature stroma:

The number of perithecia in the mature stroma is of importance because this is the main feature that now separates Rosellinia from Hypoxylon. In 2 out of 9 species of Rosellinia investigated, only uniperitheciate stromata were found. These were R. pulveracea and R. moroides. In the other 7 species, R. protuberans, R. obtrissima, R. apiculata, R. mammoidea, R. corticalis, R. thelena and R. aquila, the majority of the stromata were found to be

uniperitheciate in any sample collected but bi- or tri-peritheciate stromata also occurred to a greater or lesser extent. Furthermore many bi- or uniperitheciate stromata were often found associated with the normal multiperitheciate stromata of most species of Hypoxylon, the only notable exceptions being those transferred from the genus Nummularia. Uniperitheciate stromata are common in the following species: H. rubiginosum, H. truncatum, H. stygium, and H. glomeratum. Usually a whole range was found in the sample collected from uniperitheciate to multiperitheciate stromata. One species (Rosellinia 3A), unfortunately not identified with certainty, formed uniperitheciate and multiperitheciate stromata in approximately equal proportions so that it was difficult to decide whether to place it in Rosellinia or Hypoxylon. A similar range of uniperitheciate and multiperitheciate stromata was produced by Hypoxylon vividum on dead wood in culture. The parent strain was derived from multiperitheciate stromata only.

Another interesting point, which should be mentioned here is the anatomical similarity between the stromata of Rosellinia and those of Miller's Groups III and IV of Hypoxylon. Both possess a granulate carbonaceous crust surrounding the upper halves of the perithecia and a small quantity of lighter tissue below. (For relevant figures please consult Vol. II especially diagrams A, B & C).

The question now arises as to whether there is a valid difference between the 2 genera. Miller (1928) considered that Rosellinia was sufficiently distinct because the majority of stromata were uniperitheciate. He points out in connection with R. aquila and other species however, that "aggregation" may occur, presumably by the development of perithecia some distance away from each other but under a common stromal covering. This considerably weakens his own definition.

Shear (1945) clearly recognised that the boundary lines between 2 genera often become obsolete once a large quantity of material has been collected. This conclusion is borne out by the material studied for the present work. There seems to be good evidence to justify the sinking of Rosellinia with Hypoxylon but further examination involving examination of type material is clearly required to settle the matter finally.

2. Variation in form of the stroma as related to the Nummularia question.

The South African species N. kalchbrennera described by Miller (1942 p.260) falls under the definition of the genus cited previously (Miller 1932). Material collected recently in South Africa and sent to Kew was compared with the European N. discreta (Schw.) Tul., and found to be closely similar in general morphology (Booth, private communication). Sections of the stromata of N. kalchbrennera confirmed Miller's account of the perithecial and ostiolar features (see fig.76B). Unfortunately, however, some of the other species earlier transferred from Nummularia into Hypoxylon were found to be so closely similar in these respects as to make the distinction cited on p.8 worthless. Sections cut of the stromata of H. asarcodes, H. nummularium and H. merrillii (figs 66,70) show that the perithecia also arise at the base of the stroma and that the ostioles are more or less attenuated.

The other distinguishing feature of Nummularia Tulasne emended Miller is the circular form and concave surface of the stroma and its erumpent development. While these characters taken in combination were found to distinguish N. kalchbrennera satisfactorily from other species of Hypoxylon, it is worth noting that none of them will do so when taken singly. This can be seen by examination of figs. 61-76. The species transferred by Miller originally from Nummularia are nearly all erumpent and vary greatly in stroma shape and surface level. The problem is confused further by the fact that many species of the original genus e.g. Nummularia uni-apiculata that are similar to H. mediterraneum (de Not.) Mill. were not included in Hypoxylon by Miller in 1928 or subsequently. Clearly a new emendation of the genus is a logical necessity.

3. The differentiation of the stromata: (Refer to Diagram A).

A. Evidence from mature stromata. The following classification is based on the nature of the stromal tissues as well as on the quantity in which they are present:

I. Stromata homogeneous, concolorous or nearly so (Plate VI).

a. Texture leathery, without carbonization; ostioles umbilicate.

Some Hypoxylon species of Miller's Group I,

e.g., H. plumbeum

H. 18B (unidentified)

b. Texture carbonous and extremely hard; perithecial vertices emerging through the stromal covering; ostioles papillate
Hypoxyton, Group II.

e.g., H. truncatum

H. stygium

II. Stromata in which the tissue above the perithecia is different in texture and colouration to that below. (Plates VII, VIII).

a. Tissue black and carbonous above and round the upper halves of the perithecia; that below the perithecia lighter, uncarbonized and usually slight in quantity. Ostioles papillate. Rosellinia, Hypoxyton Groups III and IV, and Nummularia.

b. Carbonization restricted to a relatively narrow zone above the perithecia; tissue beneath the perithecia well developed and soft at least up to maturity. Ostioles papillate.

i. Fleshy tissue prominently large celled, dark in colour and zonate.

Daldinia.

ii. Fleshy tissue yellow or white, not zonate.

(i) fertile part of stroma pulvinate or effuse.

Penzigia.

(ii) Fertile part of stroma clavate or cylindrical.

Xylaria.

c. Carbonization absent; tissue usually darkly coloured, sometimes red or olive green, and lighter in hue above the perithecia than below.

Hypoxyton Group I (except those mentioned under Ia).

This key serves to show that the variation in characters accepted at present in classification of the group is often as great between the species making up one single genus as between the genera themselves.

B. Evidence from immature stromata.

a. Rosellinia mammoidea and R. corticalis.

Material collected in all stages of development on Cassine croceum and wood unidentified in December and April respectively

In these two species a white mealy hyphal layer bearing pale grey conidiophores is the first visible sign of infection of the wood. The globose stromata develop at intervals beneath this layer. Prior to development of perithecia, deposition of a carbonous substance takes place in the upper part of the soft tissue that develops beneath the conidial layer. When the perithecia begin to grow and to occupy the central portion of each stroma, the tissue below and on their sides turns dull brown. As maturity approaches, the conidial layer scales off the surface of the carbonous matrix, and the small globose stromata are revealed. (See Diagram B and figs 16,17, 19-22).

b. Hypoxylong glomeratum.

Material collected intermittently on Passerina falcifolia and wood unidentified. Development proceeds as above except that carbonization of the matrix below the conidial layer is more continuous, so that there is less likelihood of the occurrence of uniperitheciate stromata. Uniperitheciate stromata have, however, been observed to develop by the side of those which are multiperitheciate.

c. Hypoxylon deustum.

This account is based on successive collections and observations of material over a $2\frac{1}{2}$ year period at the Garden of Eden, Knysna where the group stromata develop abundantly each year on old trunks of Olea capensis and Ocotea bullata.

The stroma, first appearing in September, is very variable in form (See Chapter VI. p.182) and is unique in its tendency to develop a fleshy branching system beneath decaying bark. The pattern of development of the aplanata fertile portion above the bark is always the same however. A thin white plate of tissue grows radially over the bark until several cms. in diameter. This is accompanied by progressive increase in thickness of up to 3-5 mms. At a very early stage while the plate is only 4-5 mms. diameter, a palisade of tightly packed parallel pale grey conidiophores develop in the centre, leaving a sterile margin of 2-3 mms.

Carbonization takes place as in H. glomeratum, between December and January, just below the conidial layer which gradually scales off. As the perithecium develops below this, the white matrix surrounding them first becomes hard and then gradually darkens in colour. The final hue is attained only when the asci are mature. Maturity reached during April - May, after which the tissue beneath the perithecia starts to disintegrate.

The development and final structure of the stroma differs from that of the two Rosellinia species and H. glomeratum in the predominantly fleshy nature of the stroma up to maturity and the partial disintegration of the basal part after it. These features are typical of the species of Penzigia studied. For these reasons Hypoxylon deustum has been included with the Penzigia group (Chapter VI).

d. Penzigia berteri.

Material was collected intermittently on Olea capensis and Rhus legati. The development of the stroma follows the same pattern as the preceding species except for the greater development of the white fleshy tissue beneath the perithecia and the absence of a conidial layer. This is replaced by a sterile layer of dense white mycelin which eventually seals off the carbonous layer beneath. The exact duration of growth is unknown but is probably a few months only.

e. Hypoxylon truncatum and H. microcarpum.

Material was collected on Olea capensis in January and September respectively.

The young stromata differ from the preceding species in that they are coloured uniformly dark yellow or dark purple red respectively from a very early stage. There is never any differentiation of the stroma. The conidial layer which develops on the surface is the same colour as the stroma beneath in both cases, and is shed as in the other species, just before maturation of the perithecia. Carbonization of the entire stroma begins well before perithecial initiation and the stromata are usually dry and hard before the perithecia are mature.

f. Hypoxylon vividum.

The following account is based on stromata reared in the laboratory on Acacia karoo.

The first stromata appeared 6 weeks after inoculation, irrespective of cultural treatment, and reached maturity 2 weeks later. Other stromata developed intermittently after this time. Stromata of all ages were embedded in wax and sectioned.

The stroma is first recognizable as a small orange cushion of pseudoparenchymatous tissue on the surface of the wood. (Plate I). The texture of the stroma is uniformly soft and light coloured except for a thin superficial dark crust but not fleshy as in Penzigia because the individual hyphae are coarser. The perithecia develop near the base of or towards the periphery of the stroma depending on the initial depth of mycelium (Plates III, IV, VIII). No differentiation of tissue was found in stromata where the first perithecia had not attained a late stage of ascus formation. When older stromata were examined, however, in which the first perithecia had matured and other perithecia had started to form in between them, a dark basal layer of chestnut-brown mycelium was observed that contrasted markedly with the pale orange tissue above (Plate VIII). This dark layer was composed of hyphae of rather broader diameter than elsewhere but must clearly have originated from pale coloured tissue already in existence. The light and dark layers of the stroma must correspond to Miller's ecto- and entostroma respectively although no conidial layer was formed at any stage.

Clearly then, the ectostroma is different in formation when compared to the species discussed above, is not clearly recognizable until a late stage of stromal development, and is independent of perithecial formation. The findings for this species do not agree with Miller's conclusions in 1928 for the general pattern of development in Hypoxylon.

g. Hypoxylon piceum. (Plate II).

Material was collected intermittently on Passerina falcifolia, Stromal development corresponds with the species just described except that the colour of the stroma is yellow and the entostroma is not clearly defined.

Darkening of the stroma appears at the base of the mature perithecia but grades imperceptibly into the paler tissue above.

h. Hypoxylon 18B (Plate VI).

Material was collected in September on Tarchonanthus camphoratus.

This is a species closely related to the two just described but has not been identified with certainty. The interior of the young stroma is uniformly pale yellow-grey. Development is similar to that in the above 2 species but no darkening of the stroma occurs.

4. The formation of the ostiole.

The manner of penetration and opening of the developing perithecial wall to the outside of the stroma is a subject that has not received a great deal of attention up to the present day. Many authors such as de Bary (1887) and Miller (1928) have implied that the apex of the perithecium expands and bores its way through to the exterior. The definite regular form of the ostioles observed in so many spp. of the Xylariaceae itself indicates, however, that more is involved than the rupture of the outer layers to form a pore.

The formation of the umbilicate ostiole in 3 species of Miller's Group I of Hypoxylon was studied. These were H. piceum, H. 18B and H. vividum. The process was the same in all of them. While the perithecium is still small, consisting of a dark wall surrounding a space that is nearly hyaline, a hollow spherical swelling nearly equal to it in size and similar to it in appearance develops in the stroma immediately above. This is the future "ostiolar cap" (Plate III). The development of Woronin hyphae in the perithecium alone distinguishes the two structures. Enlargement of the perithecium gradually pushes the ostiolar cap towards the exterior crust of the stroma (Plate IV). The latter does not enlarge greatly but changes shape, becoming bluntly triangular with the narrow end pointing outwards. Thickening and darkening of the wall occurs to a varying extent.

The next stage takes place any time beyond the initial phase of development but usually when the asci inside the perithecia have begun to form. The perithecial wall and the adjacent wall of the ostiolar cap becomes attenuated and ruptured so that the two cavities become continuous.

The two sides of the perithecial wall lie against those of the ostiolar cap and may partly fuse with the dark tissue composing them, so that it becomes difficult to distinguish between the structures clearly. Short hyaline periphyses now appear from the upper part of the perithecial walls. (See Plate III).

The final stage is reached when the ostiolar cap breaks through the outer crust of the stroma just when the asci become mature. The lumen of the ostiole becomes continuous with the exterior due to the disintegration and disappearance of the outer part of the ostiolar cap. Normally, therefore, only the wall of the perithecium, and possibly some carbonized tissue surrounding it, can be seen at maturity. The perithecial wall does not quite reach the surface of the stroma, so the ostiole appears as a pore. (See diagram for H. 18B, Plate VI).

There is as yet no evidence concerning the development of the umbilicate ostioles where these occur in certain species of the Nummularium group, e.g. H. punctulatum, N. succenturiata and N. uni-apiculata (original classification). No immature material was available of the last two species which are described in the present work. Barnett (1957) in describing the development of H. punctulatum clearly regards the ectostroma as equivalent to the conidial layer. Objections to this idea have already been discussed. He states, however, that "the freshly exposed surface of the ectostroma bears numerous papillae while the surface of the entostroma is punctulate. Before separation the papillae of the ectostroma fitted into the shallow pits of the entostroma. Ostioles of the perithecia are located directly beneath the pits." Although no precise evidence or illustration is presented, this statement would seem to indicate that the mechanism is essentially the same as that recorded above.

The formation of the papillate ostiole, which is characteristic of all the other species studied, appears to be a much simpler process than that of the umbilicate type. Unfortunately it has not been possible to rear species with this type of ostiole in the laboratory. Serial sections of young material of the species described above show that the apex of the young perithecium enlarges and pushes its way through the carbonous tissue above. The latter is obviously still plastic in spite of its dark colour.

The triangular channel forced by the perithecium through the stroma is reinforced by deposition of carbonous material on both sides. This is particularly conspicuous in Penzigia berteri and H. glomeratum but less so in Daldinia concentrica and the 2 species of Rosellinia. This reinforcement, surrounding the protruding perithecial wall, results in a conical protuberance from the surface of the stroma. In no case were any structures found that were similar to the ostiolar caps of H. vividum and associated species (See Plates VI, Diagrams B & C).

In Hypoxylon truncatum and its allies the shape of the perithecium differs markedly from those in the other species described in that the upper half is broader and flattened in outline. The walls are considerably thickened beneath the ostiole to form a distinct "shoulder" on each side. Furthermore, the carbonous matrix above the shoulder is shed just before maturity so that a large circular area of the perithecial surface becomes exposed. The ostiole, which is papillate in outline, thus consists of perithecium only. Unfortunately many questions concerning the development of H. truncatum, H. stygium and other species with annulate papillate ostioles still remain unanswered. Due to the paucity of immature stromata and the great difficulty experienced in sectioning the hard carbonous material, it has not been possible firstly to discover why the outer part of the stroma should be shed above the perithecium or whether the shoulder of the perithecial wall is a single or double structure involving part of the stroma in its construction. Further work is required to elucidate these problems.

Discussions and conclusions:

It appears to be a basic assumption of Miller that there are two layers only in the stroma and that the outer (ectostroma) functions both for production of conidia and as the outer covering of the stroma beneath. In the species investigated the conidial layer was found to be independent of the stromal layers beneath and to have a separate function. Usually the conidial layer disappeared at maturity. In Daldinia it was obviously retained in part. It is worth noting here that in each of the 5 Xylaria species studied the carbonous layer of the mature stroma was also covered partially by a dense layer of stout brown hyphae that were obviously derived from the base of the superficial conidial layer of the young stroma. It is not known, however, how common this feature is throughout the genus.

The developing stroma beneath the conidial layer may differentiate or remain uniform, and there is great variation in the degree of carbonization. The application of the terms ecto- and entostroma to stromata where there is no or scarcely any differentiation is clearly inappropriate. Only when the mature stroma is differentiated into two layers of different texture is it possible to speak of these terms precisely, otherwise they must have only a regional or topographic connotation.

The variation in stromal anatomy and the differences in ostiole formation indicate that some revision of generic concepts may be required. A logical classification would recognise each division of the genus Hypoxylon as a taxon of equal value with other species groups now recognised as genera in the Xylariaceae. Whether or not the genera are retained as such or are merged, one or two with another, is a matter of individual interpretation.

The grouping adopted in the present work is based mainly on stromal differentiation and only secondarily on general morphology and the number and degree of aggregation of the perithecia. The generic names, as defined by Miller in 1928, will, however, be retained for convenience. Nummularia will be retained as a generic name for all species of the original genus not placed by Miller in Hypoxylon but the term Nummularium group will comprise both species transferred and the original species.

GROUP I. (p.29).	<u>Rosellinia</u> , <u>Hypoxylon glomeratum</u> (5 varieties) and <u>Nummularium</u> <u>group</u> . (includes Miller's Group III <u>Endoxylon</u> , and Group IV).	Conidial layer disappearing before maturity; stroma differentiated into carbonous ectostroma and paler brittle entostroma; ostioles usually normal papillate, occasionally umbilicate; perithecia sometimes 1 per stroma, but always with carbonous vertices.
<hr/>		
GROUP II. (p.98).	<u>Annulatum</u> group of <u>Hypoxylon</u> (= Miller's Group II).	Conidial layer disappearing before maturity; stroma uniformly carbonous; ostioles annulate papillate; perithecia sometimes 1 per stroma, also uniformly carbonous.
<hr/>		
GROUP III. (p.118).	<u>Rubiginosum</u> group of <u>Hypoxylon</u> (= Miller's Group I).	Conidial layer not formed or disappearing before maturity; stromata uniform or differentiated but never carbonous; ostioles umbilicate; perithecia occasionally 1 per stroma.

GROUP IV. (p.173).	<u>Daldinia</u>	Conidial layer partly persistent; stroma differentiated into carbonous ectostroma and corky zonate entostroma; ostioles papillate; perithecia several per stroma.
GROUP V. (p.178).	<u>Penzigia</u> and <u>Hypoxylon deustum</u> .	Conidial layer not retained; fertile part of stroma aplanate and differentiated into carbonous ectostroma and fleshy entostroma.
GROUP VI. (p.197).	<u>Xylaria</u>	Conidial layer partly persistent (in 5 spp. studied); stroma differentiated as in <u>Penzigia</u> but conic or cylindrical in form.

These groups will now be dealt with in detail in the following chapters.

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(with particular reference to pp. 98
et seq.)

CHAPTER II.

A DISCUSSION OF CERTAIN QUANTITATIVE METHODSOF INVESTIGATION.

The various features of the perfect stage in the Xylariaceae and their value in classification were discussed in the last chapter. An account of the characters and the methods employed in the qualitative study of cultural characters is given in Appendix I §§ 6-12. This chapter outlines some of the quantitative methods of investigating the differences between species:

A. Spore Size:

Up to the present, most descriptions of species in the Xylariaceae, given in Saccardo's *Sylloge Fungorum* and elsewhere, simply record a range of spore dimension rarely accompanied by an average measurement to the nearest micron. This lack of precision was the main stumbling block in identification of material for the present work. When the observed range of dimension exceeded or overlapped the one recorded it was impossible to know whether the identification was correct or not. Confirmation by overseas authorities only partly solved the problem.

In recording spore dimensions, two items were therefore clearly determined:-

- a. the range of width and length to the nearest 0.5μ
- b. the average value of width and length to the nearest 0.1μ

These were based on measurements of at least 60 spores per species to the nearest 0.75μ .

Statistical analysis was carried out to determine how far the measurements of each species differed from the others significantly. Recordings from different samples were pooled, provided that the means did not differ among themselves by more than 0.4μ and furthermore, that the stromata from which they were derived corresponded in morphology and anatomy. Samples which behaved differently were treated separately.

A table of analysis of variance was then drawn up for the breadths and lengths of each species group according to the method outlined by Snedecor (1946, p.232). A common fiducial limit or least significant interval between the means of the dimensions of any 2 species could then be calculated. Because the sample sizes were unequal a slight modification of the usual formula was drawn in the following way from the principles outlined by Cochran and Cox

(1957, p. 76.) and Snedecor (1946, pp. 42 and 81):

Pooled variance, s^2 = Within Sample Sum of Squares / Degrees of Freedom
or "error mean square"

$$\text{Standard deviation of means, } s_{\bar{x}} = \sqrt{s \frac{n_1 + n_2}{n_1 n_2}} = s \sqrt{\frac{n_1 + n_2}{n_1 n_2}}$$

Where n_1 & n_2 = number of items respectively of any 2 species considered.

$$\begin{aligned} \text{Least significant interval } \Delta \bar{x} &= t_{05} \times s_{\bar{x}} \\ &= t_{05} \times \sqrt{\frac{\text{error}}{\text{mean square}}} \times \sqrt{\frac{n_1 + n_2}{n_1 \times n_2}} \end{aligned}$$

The value of t at the 5% level of significance (t_{05}), for the appropriate number of degrees of freedom, was obtained from tables (Snedecor, 1946).

When the difference between any two species means does not exceed the least significant interval calculated, the means cannot be regarded as significantly different. This information can be used to test the reliability of spore measurements as a criterion in classification. Furthermore, when two samples are considered which differ slightly anatomically, the similarity of their spore measurements may support the hypothesis that the samples are drawn from the same species. (See Tables IV, VI, VIII, X, XI; Appendix III).

Differences in spore dimension were also illustrated graphically. An important and interesting feature is that the breadth and length of spores do not appear to be correlated so that the relation between them can be quite simply expressed. This was first discovered by Corner (1946) in his work on basidiospores and cystidia of the Basidiomycetes. Measurements of the spores of 4 species, Rosellinia aquila, Hypoxyylon truncatum, Hypoxyylon mediterraneum, and Penzigia discolor now confirm this fact. The respective lengths and breadths of each of a large number of spores from these species were measured to the nearest 0.75μ , and tables were constructed to show the number of spores that fell within each interval of breadth and length. (See Table IA-D, Appendix III). The columns show the range and number of spore breadths for each interval of spore length. Values of χ^2 were calculated to show that the values of spore breadths obtained for each length interval did not fluctuate significantly.

The correlation coefficient r was also calculated (see Moroney, 1951) and found to be extremely low, which indicated little or no correlation between the two dimensions (see Table II, Appendix III). Thus the average breadth of the spores for each species plotted against the range in length results in a straight-line graph that is a fairly accurate estimate of dimension. The % number of spores per length interval (0.75μ) was also plotted against range in length when this information was found to be of interest.

B. Growth Rates of the Species in Culture:

Species often differ from each other in having different temperature optima and different growth rates at various temperatures. Information about this type of behaviour can, therefore, be of value. The method and general procedure outlined here is based on that of Fawcett (1921), Snell et al (1928), and Brancato and Golding (1953).

Method:

Since it was not practicable in most cases to test all the strains available of each species, only approximate measurements of their growth rates were made. A representative strain was then selected for testing. When two or more strains of the same species were found to differ markedly, all of these were tested and treated individually in the statistical analysis.

Discs of agar 1 cm. in diameter were cut by sterilized cork borer from a plate culture 1-2 weeks old of the strain to be investigated. The discs were taken from a locus 1-2 cms. behind the margin of the parent colony. Each one was transferred, mycelium downwards, to the middle of a plate containing 10 cc. of malt agar prepared in the manner described in Appendix I, § 6. The plates were incubated in triplicate for 1-3 weeks depending on the growth of the fungal colony, at 15° , 20° , 25° , 28° , and 31° C. Four diameters at 45° to one another were marked in black ink on the reverse side of the plates.

8 test species were used to determine how the rate of growth varied with time. These species were Hypoxyylon glomeratum, H. mediterraneum, H. truncatum, H. stygium, H. 18B (species unidentified), H. rubiginosum, H. hypomiltum and Nummularia uni-apiculata. The diameters of each of these species were measured every day after inoculation until the margin of the colony had reached the edge of the plate in every case. The daily radial increments of growth were obtained by subtraction of consecutive diameter readings from one another, and by division of this quantity by 2.

Graphs were drawn to show the increase in radial growth with time, and as can be seen from Graph I, Appendix III, this relationship was approximately linear. This fact was confirmed statistically by calculation of χ^2 . For the results and calculations see Appendix III, Table III.

The growth rates of the rest of the 63 spp. investigated were assumed to be linear. Only two sets of readings were therefore recorded, the first soon after inoculation and the second after an interval which varied according to the growth rate of the individual species. The test species showed an initial lag period of 2-4 days, clearly reflected on the graphs, during which time the growth rate rose to the normal. For this reason the first set of readings was also taken 4 days after inoculation. Each set comprised 8 radii, from which the radius of the inoculation disc was later subtracted. The average daily radial increment of growth at each temperature was calculated by subtracting the initial readings from the final and dividing by the number of days interval between the two. These results were plotted against their respective temperatures. In this way differences in growth rates and optimum temperatures could be shown clearly (see Chapters III - VI).

Statistical analysis to show whether the species differed significantly in growth rate was carried out on the same plan as the preceding one for spore dimensions. For each species group a simple analysis of variance table was calculated for the growth rates observed at each temperature. Multiple analysis involving both temperature and growth rate interaction was not undertaken because the variation between the sample items of each species differed from one temperature to another. Hence no single fiducial interval could be applied to a set of readings involving all 5 temperatures. The influence of temperature on growth rate moreover, did not really require statistical verification.

The mean of the 8 radial readings obtained from each plate culture and not the readings individually were used in the calculations. Close inspection of the radial readings showed in many cases that for a given temperature they varied about a point above or below the common mean obtained for all the plate cultures of the species concerned, rather than about the common mean itself.

In other words, the growth of each radius was influenced by that of the colony as a whole. The final number of sample items for each strain cultured varied from 2 to 7. In some cases more than one strain per species was cultured so that intraspecific variation could be studied as well.

Clearly a greater number of sample items drawn from several strains would be required for a broader comparison between any two species. The experiment was, however, designed primarily to investigate the range in growth rates in the various species groups, which would provide a background from which other explorations could be made in future. For this purpose a small number of samples is adequate.

Finally, for descriptive purposes, the following standards of growth rate were arbitrarily defined:-

Very slow	< 1.5 mm. per day
Slow	1.5 - 2.5 mm. per day
Moderate	2.5 - 4.0 mm. per day
Fast	> 4.0 mm. per day

Utilization of Results:

The results of the experiment were treated with caution since they were not based on a large number of strains of each species. Moreover, it was obvious that, due to chance, quite unrelated species could have a similar growth rate. Personal judgement and subjectivity therefore, had to enter into the interpretations to some extent.

The main value of the results lay in the confirmation of predetermined specific limits by the association of a set of distinctive cultural or stromal features with a growth-temperature reaction that was characteristic, and not easily confused with those obtained for different combinations.

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CHAPTER III.
ROSELLINIA, AND THE ENDOXYLON AND NUMMULARIUM
SPECIES GROUPS.

A. The Perfect Stage:

As stated in Chapter I, these genera all appear to be closely related since they all possess the following characters:-

1. Carbonous stromata, with the ectostroma predominant and dark in colour; entostroma scanty and of lighter hue. The two regions of the stroma are not clearly separable.
2. Perithecia large in relation to the stroma and seated near the base of it; ostioles normally papillate.

Key to Species:-

1. Stromata containing one to several perithecia; outlines of the perithecia clearly evident above the general level of the stroma - 2.
- 1'. Stromata usually multiperitheciate; perithecia not evident in outline so that stromal surface is smooth and flat or nearly so -12.
2. Stromata immersed partly in the substrate ----- 3.
- 2'. Stromata superficial ----- 4.
3. Spores $4.5-7.5 \times 8 - 13.5 \mu$, av. $5.5 \times 10.7 \mu$.
Rosellinia protuberans (1,p.32).
- 3'. Spores $6.0-10.0 \times 10.5-16.5 \mu$, av. $7.4 \times 13.3 \mu$.
Rosellinia obtussissima (2,p.32).
- 4². Stromata very small, < 1 mm. in diameter ----- 5.
- 4'. Stromata larger, at least 2 mms. diameter ----- 6.
5. Spores subglobose to almost spherical $5.0-8.0 \times 6.0-10.5 \mu$,
av. $6.2 \times 8.5 \mu$. Rosellinia pulveracea (3,p.33).
- 5'. Spores oval-elliptic, $6.0-9.0 \times 10.5-15.0 \mu$
av. $7.6 \times 12.2 \mu$. Rosellinia moroides (4,p.33).
- 6⁴. Stromal covering scanty; uniperitheciate stromata globose ----- 7.
- 6'. Stromal covering well developed; uniperitheciate stromata semi-circular or broadly coniform ----- 8.

7. Spores oval or oval-elliptic equilateral with rounded ends ----- 8.
- 7'. Spores elliptic or navicular, inequilateral,
with ends bluntly pointed -----10.
- 8.(6) Spores $3.0-6.0 \times 6.0-11.0 \mu$, av. $4.5 \times 9.1 \mu$
Rosellinia apiculata (5,p.33).
- 8'. Spores $5.0-10.5 \times 10.0 - 18.0 \mu$ ----- 9.
9. Stromata normally uniperitheciate, occasionally biperitheciate
Spores $6.0-10.5 \times 10.0 - 19.0 \mu$, av. $7.6 \times 15.0 \mu$
Rosellinia mammoidea (6,p.34).
- 9'. Stromata variable, either uniperitheciate or with several perithecia
aggregated under a common thin stromal covering.
Spores $5.0 - 9.0 \times 13.5 - 18.0 \mu$, av. $6.7 \times 15.7 \mu$
Rosellinia corticalis (7,p.34).
10. Spores elliptic, inequilateral, $4.5 - 9.0 \times 14.0 - 24.0 \mu$
av. $6.8 \times 18.7 \mu$ Rosellinia thelena (9,p.35).
- 10'. Spores navicular, $6.0 - 9.0 \times 17.0 - 39.0 \mu$
av. $7.7 \times 25.4 \mu$ Rosellinia aquila (8,p.34).
11. Spores pale brown, slightly inequilateral,
 $4.5 - 7.5 \times 10.5 - 16.5 \mu$, av. $5.6 \times 13.3 \mu$
Hypoxylon glomeratum var.1.(10,p.36).
- 11'. Spores chestnut brown, elliptic equilateral,
 $4.5 - 7.5 \times 12.0 - 16.5 \mu$, av. $6.3 \times 14.0 \mu$
Hypoxylon glomeratum var.2.(p.37).
- 12(1) Stromata superficial -----13.
- 12'. Stromata erumpent through the bark and at least partially
embedded in the substrate at maturity -----15.
13. Stromata consisting of small pulvinate glomerules
with the perithecia completely immersed and not evident
in outline
Spores pale brown, oval elliptic, slightly inequilateral
 $4.5-7.5 \times 11.0 - 16.5 \mu$, av. $6.0 \times 14.3 \mu$
Hypoxylon glomeratum var.3.(p.37).
- 13'. Stromata broadly effuse, crustose; papillate ostioles
sometimes indistinct -----14.

14. Perithecia closely crowded in the stroma; spores dark brown, nearly opaque,
3.0 - 7.0 x 7.5 - 13.0 μ , av. 4.9 x 10.1 μ
Hypoxylon glomeratum var.4.(p.37).
- 14'. Perithecia separated in the stroma by wide carbonous bands of tissue; spores dark brown, subhyaline.
3.0 - 6.0 x 7.0 - 13.5 μ , av. 4.6 x 9.7 μ
Hypoxylon glomeratum var.5.(p.38).
- 15(12). Ostioles not raised above the stromal surface -----16.
- 15'. Ostioles clearly evident, papillate -----18.
16. Stroma dark grey or metallic black at maturity; perithecia relatively large and at least 450 μ in long axis -----17.
- 16'. Stroma greenish grey at maturity; perithecia small not exceeding 300 μ in long axis,
Spores elliptic to subglobose,
6.0 - 9.0 x 10.5 - 14.0 μ , av. 6.5 x 12.2 μ
Nummularia uni-apiculata (13,p.39).
17. Spores 5.0 - 9.0 x 13.5 - 19.0 μ , av. 6.7 x 15.5 μ
Nummularia succenturiata (11,p.39).
- 17'. Spores 4.5 - 7.0 x 10.5 - 15.0 μ , av. 5.7 x 12.9 μ
Hypoxylon exutens (12,p.39).
- 18(15). Ostioles truncate, i.e. vertices of the perithecia are without stromal covering. Spores pale brown subhyaline,
1.5 - 4.0 x 6.0 - 9.0 μ , av. 2.3 x 7.1 μ
Hypoxylon sp. 13A (14,p.40).
- 18'. Perithecia entirely immersed in the stroma -----19.
19. Stromata restricted to a definite circular form, normally with a concave surface and raised border
Spores 4.0 - 6.0 x 8.0 - 13.0 μ , av. 5.0 x 10.9 μ
Nummularia kalchbrennera (19,p.42).
- 19'. Stromata variable, usually effuse and never restricted to a circular form; surface sometimes concave but if so, without raised border -----20.

20. Surface of stroma shiny and smooth -----21.
- 20'. Surface of stroma dull, and usually with a coarse surface -----22.
21. Perithecia 100 - 210 x 500-600 μ
 Spores 4.5-8.0 x 8.0 - 17.0 μ , av. 5.5 x 11.8 μ
Hypoxyton merrillii (15,p.41).
- 21'. Perithecia 250 - 400 x 800 - 900 μ
 Spores 4.5 - 7.5 x 9.0 - 17.5 μ , av. 6.2 x 13.3 μ
Hypoxyton nummularium(16,p.41).
22. Spores chocolate brown, narrowly elliptic,
 6.0 - 10.5 x 10.5 - 20.0 μ , av. 7.2 x 15.6 μ
Hypoxyton mediterraneum(17,p.42)
- 22'. Spores dark brown to black, broadly elliptic,
 6.0 - 10.0 x 9.0 - 18.00 μ , av. 7.4 x 14.7 μ
Hypoxyton asarcodes (18,p.42).

In the following descriptions, number of spores measured is given after the average size in each case:

1. Rosellinia protuberans Karst. (Figs. 1 - 3, 6A, 7A).

Stromata anlanate on decorticated wood with the major part immersed in the substrate; ectostroma consisting of a broad carbonous superficial layer surrounding the species of the perithecia; entostroma very scanty and not extending far beyond the perithecial bases; host wood not infected by mycelium to any marked degree. Perithecia 1 - 3 per stroma, globose to coniform, 400 - 700 x 600 - 1000 μ ; walls uncarbonized except at the ostioles; papillate with the perithecial necks reaching the surface of the stroma or extending slightly beyond. Asci clavate, long stipitate, 120-130 x 9 μ , stalk 70-75 x 1.5 μ . Spores broadly oval with rounded ends, light brown, 4.5 - 7.5 x 8.0 - 13.5 μ , av. 5.5 x 10.7 μ (270).
 Hosts. Canthium spinosum and wood unidentifiable.

2. Rosellinia obtusissima (Berk & Curt) Saccardo. (Figs. 4,5, 6B, 7B).

Similar to the above but differing in greater extent of the entostroma which surrounds the perithecia as a well defined layer, and in the shape of the perithecia which is conic and never globose.

Perithecia 1 - 3 per stroma, 200 - 300 x 600 - 700 μ ; walls mainly uncarbonized; ostioles papillate. The surface of the stroma round the ostioles is hardly raised so that the ostioles are often difficult to perceive on superficial examination. Asci clavate, long stipitate, 120-150 x 9 μ , stalk 45-75 x 2 μ . Spores broadly oval to subglobose, dark brown, 6.0 - 10.0 x 10.5 - 16.5 μ , av. 7.4 x 13.3 μ . (90)
Host: Sideroxylon inerme, old decorticated wood.

3. Rosellinia pulveracea (Ehrb) Fuck. (Figs. 10, 14A).

Stromata minute, globose, uniperitheciate but gregarious on the surface of decorticated wood. Perithecia 200-300 x 150 μ , ostioles papillate, indistinct. Asci clavate, short stipitate, 75-90 x 8 μ , stipe 18-21 x 3 μ . Spores oval to subglobose, light brown, 5.0 - 8.0 x 6.0 - 10.5 μ , av. 6.2 x 8.5 μ (60).
Host . Acacia karoo, decorticated wood.

4. Rosellinia moroides (Curt) Saccardo. (Figs. 8, 9, 14B).

Stromata minute, globose, uniperitheciate or with several perithecia under a thin superficial covering that closely follows their outline; superficial on decorticated wood. Ectostroma dark carbonous with uneven surface; entostroma not clearly distinct from the ectostroma but generally lighter in colour and with individual hyphae often evident in longitudinal section. Perithecia globose, 220 - 320 x 300 - 400 μ , with broad carbonous walls. Asci clavate or cylindric, short stipitate, 90 - 110 x 9 μ ; stalk 12 - 15 x 3 μ . Spores oval equilateral with rounded ends, dark grey to black, 6.0 - 9.0 x 10.5 - 15.0 μ ; av. 8.4 x 12.6 μ (160)

Rosellinia ligniaria

Host. Unidentified, decorticated twigs.

5. Rosellinia apiculata, Saccardo. (Figs. 11, 12, 13, 14C).

Stromata small, uniperitheciate globose or with several perithecia under a superficial covering that closely follows their outline, or pulvinate when the perithecia are closely associated. Perithecia 450 - 900 x 650 - 1400 μ ; walls uncarbonized except for the ostioles; ostioles indistinct, papillate. Asci narrowly cylindric, 85-140 x 6 μ , with stalks that vary in length from 20-60 μ . Spores oval with rounded ends, pale brownish grey, subhyaline, 3.0 - 6.0 x 6.0 - 11.0 μ , av. 4.5 x 9.1 μ (140).

Hosts. Passerina falcifolia, bark and decorticated wood

Olea capensis wood, wood unidentified.

6. Rosellinia mammoidea (Cke) Saccardo. (Figs. 15, 16-18, 7C).

Stromata not very variable, uniperitheciate or biperitheciate; globose, immersed at the base, rarely semi-circular due to broadening of the perithecial bases. Ectostroma hard, black and carbonous merging gradually into the entostroma which is brown and uncarbonized. Perithecia globose, 900 - 1400 x 1000 - 1500 μ ; walls uncarbonized except for the ostioles; ostioles papillate and usually distinct. Asci clavate, 120 - 130 x 11 μ , stalk variable but usually fairly short, 35 - 65 x 3 μ . Spores equilateral, oval-elliptic with rounded ends, dark brown, 6.0-10.5 x 10.0-19.0 μ , av. 7.9 x 14.9 μ (220). Hosts. Probably comprise a wide range of species; unfortunately many of which were unidentifiable. The following are known with certainty:-

Olea capensis

Cassine croceum

Gonioma kamassi

Sideroxylon inerme

7. Rosellinia corticalis Allesch. (Figs 19, 20-22, 23, 7C).

Stromata very variable, uniperitheciate, biperitheciate or with several perithecia aggregated under the same stromal covering in ranging degrees of proximity. The similarity of uniperitheciate stromata to those of the previous species makes it hard to separate the two forms with justification. The perithecia are identical in size. Asci 100 - 135 x 8 μ , stalks 15 - 30 μ . Spores oval-elliptic, equilateral, dark brown, 5.0-9.0 x 13.5-18.0 μ , av. 6.7 x 15.7 μ . (60).

Hosts. Wood and bark, unidentifiable.

8. Rosellinia aquila. Ces. and de Notaris. (Figs. 24-28, 29 A, B.).

Stromata uniperitheciate, occasionally biperitheciate and usually closely aggregated. Ectostroma hard, black and carbonous entostroma uncarbonized, dark brown and usually fairly limited in extent. Perithecia globose, relatively large, occupying most of the interior of the stroma at maturity, 1300 - 2100 x 1200 - 1500 μ ; walls uncarbonized except for the ostioles and a short region below; ostioles papillate and conspicuous. Asci cylindrical, short stipitate, 180-200 x 11 μ , stalks 30-45 x 2 μ . Spores elongate elliptic or navicular, inequilateral, dark brown, 6.0 - 10.5 x 17.0 - 39.0 μ , av. 8.0 x 25.9 μ . (210).

Hosts. Royena lucida

Kiggelaria africana

Olea capensis

Gymmosporia buxifolia, and many other species, unidentifiable.

Two points of interest concern this species. The first is the frequent occurrence of a mycelial mat of dark hyphae surrounding the stroma. This is referred to by most authors (Miller 1928, 1942; Arx and Muller 1954) as the subiculum. It consists of numerous stout darkly coloured hyphae that interlock to form a loose mass round individual stromata.

Twigs of Olea capensis were inoculated with R. aquila in the manner described in Chapter II. The twigs that were deposited in the open developed mealy patches of stout dark hyphae, after 3 months, that corresponded both to those observed round the stromata of collected material and to the dark secondary mycelium produced in agar culture (see under microscopic characters for this species, later this chapter). Conidiophores were produced abundantly over the surface of the cultured material, and these corresponded exactly to the ones associated with stromata collected. The failure of stromata to develop on the cultured material and the absence of a dark hyphal layer round many mature stromata collected indicates, however, that the formation of these hyphae takes place independently and that they are probably homologous with the superficial conidial layer developed in many other species. The term "subiculum" is therefore a misleading one.

The second feature of interest is the nature of the entostroma. In the great majority of specimens examined this was found to be dark in colour but in one sample it was white and fleshy. The fleshy type was uni- or biperitheciate, and had the same spore size (4.0-9.0 x 15.0-28.0, av. 6.7 x 22.3 μ). Unfortunately, the ascospores could not be cultured in spite of persistent attempts, so that it could not be found whether the cultural characters agreed with the typical or not. No pronouncement whether the species belonged to R. aquila or to a Penzigia could be made.

9. Rosellinia thelena (Fr.) Raf. (Figs. 23, 30B).

It was impossible to find more than a small sample of this species, so that the following description cannot be taken for a generalized account.

It is clearly similar to R. aquila (normal type) in possession of a subiculum and in general stromal characters. The perithecia are large, globose, 1500 - 2,000 x 1200 - 1700 μ . The difference between the 2 species lies in the spores which are elliptic with acutely pointed ends, inequilateral, usually light brown and measuring 4.5 - 9.0 x 14.0 - 24.0 μ , av. 6.8x18.7 μ (60). The asci are cylindrical with a fairly long stalk, 140-150 x 9 μ ; stalk 30 - 60 x 2 μ .

Host. Schotia latifolia.

10. Hypoxyton glomeratum. Cke.

The actual limits of this species appear to be ill defined, and it seems probable from descriptions in the literature that the present concept may embrace a number of closely related but distinct species. This is supported by the present work on mycelial and conidial characters. Material showing extreme variation in stromal form and spore size was sent to Dr. C. Booth at Kew and to Dr. Miller for confirmation. Since all of this material was referred to H. glomeratum by these authorities, it seems best to give the different forms listed below varietal instead of specific rank for the present:

Variety 1: (Figs. 30-41, 52 A).

Stromata uniperitheciate and semi-circular, or multiperitheciate and pulvinate to slightly effuse, always superficial on bark or decorticated wood. Ectostroma dark, carbonous; entostroma lighter, not carbonized, and small in quantity, closely following the line of the perithecial bases. Perithecia large, broadly globose, occupying most of the stromal interior, and evident in outline, 500-1400 x 450-900 μ ; large and small perithecia occur in the same stroma but usually those developing singly in a stroma are larger (1000 - 1400 x 650 - 900 μ) than those developing severally (500 - 650 x 450 - 730 μ). The perithecial wall is uncarbonized except for the ostioles, and is often very difficult to distinguish from the surrounding tissue. The ostioles are typically papillate or spout shaped. In most cases a layer of ectostroma clearly forms the outer part of the ostiole; in others this layer is very thin and scarcely distinct from the perithecial wall below it and the upper halves of the stromata appear consequently somewhat darker in surface view than the lower. Both uniperitheciate and multiperitheciate stromata were closely associated in the same sample collected and it was difficult to believe that they belonged to different species.

Asci clavate or cylindric, long stipitate, $125-170 \times 6 \mu$, stalk $65-90 \times 2 \mu$. Spores elliptic with rounded ends, slightly inequilateral, pale brownish grey subhyaline, $3.5 - 7.5 \times 10.5 - 16.5 \mu$, av. $5.6 \times 13.3 \mu$. (150).

Hosts: Passerina falcifolia

Olea capensis and wood unidentifiable.

Variety 2: (Figs 42, 43, 49 B, 52 B).

Stromata multiperitheciate (uniperitheciate not found) aplanate, and pulvinate or effuse depending on the extent. Ectostroma comprises a thin superficial carbonous crust; entostroma of the usual type well developed beneath it. Perithecia oval with conical vertices that are evident in outline on the surface of the stroma, $250 - 600 \times 400 - 750 \mu$. Asci cylindric, long stipitate, $110 - 120 \times 6 \mu$, stalk $45 - 55 \mu$. Spores oval, slightly inequilateral, dark brown with a grey tint when seen collectively, $4.5 - 7.5 \times 10.5 - 18.0 \mu$, av. $6.3 \times 14.0 \mu$ (120).

Hosts: Acacia mollissima, bark.

This variety agrees with the material identified by Miller which is now in the Pretoria Herbarium (nos. 27682, 27794) and which was described in his paper (1942, p. 256).

Variety 3: (Figs. 44-46, 52 D).

Stromata very small, pulvinate or globose, uniperitheciate or more commonly, multiperitheciate, and differing from the former types by the complete immersion of the perithecia. Ectostroma a thin carbonous crust; entostroma fairly well developed, light brown. Perithecia ovoid or conic, often flattened by mutual pressure, $170 - 350 \times 350 - 550 \mu$; walls uncarbonized except near the ostioles; ostioles indistinctly papillate. Asci clavate, shortly stipitate, $100 - 110 \times 9 \mu$, stalks $30 - 40 \times 2 \mu$. Spores oval elliptic, slightly inequilateral, $4.5 - 7.5 \times 11.0 - 17.5 \mu$, av. $6.1 \times 14.3 \mu$. (90).

Hosts: wood unidentifiable.

Variety 4: (Figs. 47, 48, 52 C).

Stroma effuse, sometimes with a rather indistinct outline; surface flat or nearly so in contrast to the 2 previous varieties since the perithecia do not appear in outline. Ectostroma and entostroma similar to that in Variety 1.

Perithecia usually densely clustered in the stroma and sometimes flattened laterally by mutual pressure, ovoid to conic, $200 - 400 \times 500 - 600 \mu$; walls usually heavily carbonized from the ostioles almost to the bases; ostioles papillate with conspicuous outer stromal covering. Asci cylindrical or slightly clavate, variable in length depending on the extent of the stalk; $75 - 130 \times 6 \mu$, stalk $20 - 70 \times 3 \mu$. Spores oval equilateral, dark brown, $3.0 - 7.0 \times 7.5 - 13.0 \mu$, av. $4.9 \times 10.1 \mu$. (120).

Hosts: Probably comprise a very wide range. Known hosts are:-

Olea capensis

Cassine croceum

Vepris lanceolata.

Variety 5: (Figs. 50, 51, 73 A, 77 A).

Stroma similar to the foregoing in main character, differing chiefly in the partial immersion within the substrate, and the much greater extent of carbonous ectostroma which extend nearly down to the bases of the perithecia. The entostroma is very small in quantity. Another minor feature is that the perithecia, although growing in numbers under the same stromal covering, are not densely crowded. They are ovoid, or nearly circular in outline, $300 - 350 \times 400 - 450 \mu$; walls heavily carbonized except sometimes at the base; ostioles indistinctly papillate. Asci cylindrical, long stipitate, $130 - 150 \times 5 \mu$, stalk $55 - 100 \times 3 \mu$. Spores oval equilateral, light or dark brown, $3.0 - 5.5 \times 6.5 - 12.0 \mu$, av. $4.6 \times 9.7 \mu$. (90).

Hosts: wood and bark, unidentifiable.

In addition, another type was identified as Hypoxyton glomeratum by Kew which has a clearly defined white entostroma at maturity. This, however, disintegrates with age. The spores are normally somewhat inequilateral, peculiar in shape with one end narrower than the other, $3.5 - 7.0 \times 9.5 - 15.0 \mu$, av. $5.0 \times 11.7 \mu$. This species has been grouped with those of the genus Penzigia, but should obviously be compared with typical members of H. glomeratum. (see pp. 180, 197).

11. Nummularia succenturiata (Tod) Nits. (Figs. 53, 54, 77 B).

Stromata usually indefinitely effused but sometimes aplanate or bicircular, always partly embedded in the substrate though not necessarily erumpent through bark; surface characteristically dull brown to black and coarsely grained and often granulate or warted, never shiny. Ectostroma carbonous, forming bands between the perithecia; entostroma slight in quantity. Perithecia variable in shape depending on their distance apart, ovoid or conic due to mutual pressure, $200 - 400 \times 400 - 600 \mu$; walls carbonous nearly to the bases; ostioles indistinct and not raised above the stromal level. Asci clavate or cylindric, $150 - 180 \times 10 \mu$, stalks $60-85 \times 5 \mu$. Spores broadly oval, equilateral with bluntly pointed ends, in varying shades of brown, $5.0 - 9.0 \times 13.5 - 19.0 \mu$; av. $6.7 \times 15.5 \mu$. (60).
Hosts: Olea capensis and wood unidentified.

This species was not sent for confirmation.

12. Hypoxylon exutans (Cke) Miller. (Figs. 57, 60 B, 78 C).

Stromata restricted and irregular in outline or indefinitely effused, erumpent through bark; surface usually dull brown to black, smooth, sometimes granulate, usually somewhat convex. Ecto- and entostroma as above. Perithecia usually angular or flattened due to mutual pressure, $130 - 420 \times 450 - 500 \mu$; walls carbonous nearly to the bases; ostioles indistinct. Asci cylindric, short stipitate, $80 - 120 \times 6 \mu$, stalks $7 - 30 \times 3.5 \mu$. Spores oval equilateral, dark brown, $4.5 - 7.0 \times 10.5 - 15.0 \mu$, av. $5.7 \times 12.9 \mu$. (60).

Hosts: Twigs, unidentifiable.

The identification of this species was confirmed by Kew.

13. Nummularia uni-apiculata. Penz. and Sacc. (Figs. 55, 56, 78 A).

Stromata aplanate, usually widely effuse but sometimes restricted in extent; surface smooth, dull when young and during early maturity, distinguished by the greenish grey hue. Interior construction similar to that of preceding species but with the important difference that the tissue between the perithecia is uncarbonized and possibly entostromatic in origin. Entostroma fairly extensive, uncarbonous, light brown. Perithecia relatively small, oval, closely packed, $150 - 230 \times 180 - 300 \mu$, walls carbonous only below the ostioles; ostioles apparent in surface view as small dots that are

not raised above the stromal level, and in section comprise narrow necks from the perithecia to the exterior that are lined by perithecial tissue for most or nearly all of the way. Asci cylindric, short stipitate, $95 - 120 \times 8 \mu$, stalk, $17 - 21 \mu$. Spores broadly oval, equilateral, dark brown to nearly black, $6.0 - 9.0 \times 10.5 - 14.5 \mu$, av. $6.5 \times 12.2 \mu$ (90).
Hosts: Kiggelaria africana.

The identification of this species was confirmed by Kew.

14. Hypoxyton 13A. (Figs. 58, 59, 60A, 78D).

Stromata aplanate, orbicular, slightly convex, usually about 3×4 mms. in area. Ectostroma carbonous, rather thin; entostroma conspicuous, dark brown; tissue between the perithecia uncarbonized. Perithecia broadly oval, usually somewhat angular, $400 - 500 \times 500 - 600 \mu$; walls difficult to distinguish from the surrounding stroma except at the apices where they are broader than elsewhere and heavily carbonized. The apices of the perithecia protrude through the surface of the stroma and form distinct ring-like marks on the surface. The ostioles, therefore, are entirely perithecial in nature. Asci $40 - 55 \times 3 \mu$, stalk $9 - 18 \mu$. Spores minute, cylindrical with abrupt rounded ends, very pale brownish grey, subhyaline, $1.5 - 3.0 \times 5.0 - 9.0 \mu$, av. $2.4 \times 7.0 \mu$. (60).

Hosts: Twigs, unidentifiable.

On account of the annulate ostioles, this species could be placed with the next group, dealt with in Chapter IV. However, the sunken stroma, typically flat metallic surface, closely crowded perithecia uncarbonized at the base and the clear differentiation of ecto- and entostroma are all features which, taken collectively, relate the species closer to the typical Nummularium forms. It can thus be regarded as an intermediate between the two groups. The species is close to Nummularia viridis (Theiss.) but it is not certain whether the two are identical. (see Theissen, 1909). Also similar, but differing in stromal area, is N. annulata Rehm (see Saccardo. Syll. Fung. XXIV, 1087, 1928).

15. Hypoxyton merrillii. (Bres.) Mill. (Figs. 61-63, 66, 77C).

Stromata orbicular, linear elliptic or indefinitely effused, aplanate, erumpent through bark at maturity or occasionally superficial on decorticated wood. Surface of stroma characteristically smooth and shiny in contrast to closely allied forms but this feature is not invariable; convex, flat or slightly concave. Ectostroma hard, black and carbonous, extending as bands between the perithecia; entostroma light brown, very slight in quantity. Perithecia closely aggregated and characteristically flattened or distorted by mutual pressure, narrowly elliptic, $100 - 210 \times 500 - 600 \mu$; walls carbonized except near the bases; ostioles papillate, usually conspicuous but sometimes indistinct; often one ostiole serves for 2 or more confluent perithecia. Asci cylindric or clavate, shortly stipitate, $90 - 110 \times 6 \mu$, stalk $7 - 25 \times 3 \mu$. Spores oval, equilateral, dark brown, $4.5 - 8.5 \times 8.5 - 17.5 \mu$, av. $5.7 \times 11.9 \mu$. (270).

Hosts: Probably a wide range. Those known comprise:-

Olea capensis

Trichocladus crinitus

Royena lucida

Gymnosporia buxifolia

Virgilia oroboides

Material sent to Kew and to Dr. Miller was confirmed as this species.

16. Hypoxyton nummularium. Bull ex Fr. (Figs. 64, 65, 66B, 77D).

Similar in general features to the former, but differs in the more robust nature of the stroma and the larger perithecia which are $250 - 400 \times 800 - 900 \mu$. Asci cylindric, $100 - 125 \times 7 \mu$, with stalks of medium length, $20 - 45 \times 3 \mu$. Spores oval equilateral, dark brown, longer than in the previous species, $4.5 - 7.5 \times 9.0 - 17.5 \mu$, av. $6.1 \times 13.5 \mu$ (60).

Host: unidentifiable; dead wood and bark.

This strain was identified as H. nummularium var. merrillii by Dr. Miller. It agrees closely, however, in details of spore size and shiny stromal surface with the description and illustration in his account of the British Xylariaceae (1932), so the full specific name has been retained for the present. The species is clearly distinct from that previously described in the larger spore size.

17. Hypoxyton mediterraneum (de Notaris) Miller. (Figs. 67, 68, 69, 77E).

Stromata indefinitely effused, of varying extent but normally forming large crustose areas on old logs and major branches; similar to H. merrillii in superficial appearance and internal construction but may be distinguished fairly easily by the dull somewhat granulate surface and conspicuous conic papillate ostioles. The perithecia are completely immersed, usually flattened or angular due to mutual pressure; 200 - 400 x 500 - 900 μ .

Asci cylindrical, short stipitate, 120 - 135 x 9 μ , stalks 25 - 35 x 4 μ .

Spores elliptic equilateral, characteristically chestnut brown, 5.0 - 10.5 x 10.5 - 20.5 μ , av. 7.2 x 15.7 μ (240).

Hosts: Gymnosporia buxifolia

Olea capensis

Vepris lanceolata.

The spore size is slightly smaller than that given by Miller (1945) but material sent to Kew was reported to agree closely with typical collections there.

18. Hypoxyton asarcodes. (Th.) Miller. (Figs. 70-72, 73B, 78B).

Stromata orbicular, restricted, or somewhat effuse but not as extensive as H. merrillii and associated species; surface smooth and dull. Interior construction as in H. merrillii. Perithecia completely immersed, 200 - 500 x 300 - 600 μ ; ostioles conic papillate as in H. mediterraneum. Asci cylindrical, short stipitate, 90 - 120 x 10 μ , stalks 15 - 35 x 3.5 μ . Spores broadly oval elliptic with rounded ends, very dark brown to black, 5.0 - 10.5 x 9.0 - 18.0 μ , av. 7.4 x 14.7 μ (150).

Hosts: unidentifiable, wood and bark.

The identification of this species was confirmed by Dr. Miller.

19. Nummularia kalchbrennera. Saccardo. (Figs. 74, 75, 76B, 78E).

Stromata orbicular or almost cylindrical, definitely restricted in extent and never indefinitely effused, usually 4 - 5 mms. diam; surface smooth, often shiny, characteristically concave with a raised margin but varying to nearly flat. Internal construction as in H. merrillii and allied species. Perithecia immersed, large, flask shaped, generally flattened by mutual pressure, 400 - 650 x 750 - 850 μ ; ostioles conspicuously papillate and raised above the stromal level. Asci not available for measurement.

Spores oval elliptic, equilateral with bluntly pointed ends, $3.5 - 7.0 \times 8.0 - 13.0 \mu$, av. $5.0 \times 10.9 \mu$. (60).

Hosts: Gonioma kamassi. Extensive collections have been made of the species on this host and since it has not been found on the wood of other trees, it would appear to be host specific.

This species was compared and found to agree with the type material in the Pretoria Herbarium.

Discussion:

This species group clearly contains a series of forms which represents stages in the development of the stromal covering from a state in which it is restricted or slight in extent to one where it is well developed. Correlated with this development is the increase in number of perithecia from one or a few to several and their progressive immersion within the stromal matrix. These trends are reflected in the gradual change in stromal form; in the supposedly primitive species now in the genus Rosellinia and part of Hypoxyton the stromata are globose or pulvinate and the perithecia evident in outline, while in other species, of Hypoxyton and Nummularia, the stromata are orbicular or effuse with a smooth surface.

The interior of the stroma, however, is remarkably similar in general plan throughout the group. The only noteworthy feature is the greater development of carbonous tissue in the effuse species. (Table IV, Appendix III, shows that the difference in spore size between each species is generally significant.)

The following account of the cultural characters confirm the differences already observed between the perfect stages and may in addition provide extra characters to separate closely related forms that can not be easily distinguished on stromal features:

B. Cultural Characters:

Refer Appendix I § 6 - 12. For descriptions of stain the initial S or W and the following number refer to comparisons made with standard colour charts (§ 12, p.227). Conidial dimensions are based on 30-50 measurements.

I. Growth Character of the mycelium:

The appearance of fungal colonies grown on malt, maize, Leonian's and Czapek agars is usually sufficiently characteristic to distinguish each species from the rest. This can be best appreciated by examination of the following key:

Key:

1. Aerial mycelium scanty or closely appressed to the substrate, subhyaline, white or pale yellow ----- (Group I, p.45) ----- 2.
- 1'. Aerial mycelium felty, floccose, silky, velvety, or fleecy, opaque or rarely subhyaline, usually gleaming white; surface usually smooth but sometimes coarse ----- (Groups II & III, p.55) --- 6.
- 1''. Aerial mycelium coarse, felty, with characteristic granulate or straggling appearance and uneven surface; usually subhyaline at first, later dull opaque white with various coloured tints. -----
----- (Group IV, p.73). -----15.
2. Stain not produced in bottle or plate culture----- 3.
- 2'. Stain produced in bottle culture, sometimes in plate culture as well ----- 5.
3. Aerial mycelium smooth; conidia invariably produced.----- 4.
- 3'. Aerial mycelium moderately coarse; conidia not observed.
Rosellinia apiculata (1, p.46).
4. Colour of mycelium dull white; sometimes pale grey; conidia very profuse and pale grey ----- Hypoxylon glomeratum var. 1. (2, p.47).
- 4'. Colour of mycelium dull white; conidia usually frequent, white or pink ----- Hypoxylon glomeratum var. 2. (3, p.49).
5. Colour of mycelium dull yellow ochre to buff; stain yellow in plate culture; brilliant yellow to saffron orange in bottle culture (all media) ----- Rosellinia moroides. (4, p.51).
- 5'. Colour of mycelium dull white to grey; stain olive green to grey black ----- Hypoxylon asarcodes. (5, p.53).
- 6(1). Colonies characteristically granulate after 8 days; margin divided into small unequal segments ---
----- Rosellinia obtusissima. (6, p.55).
- 6'. Colonies smooth throughout period of growth; margin not as above ----- 7.
7. Colonies downy, appressed, uniform, white opaque, usually densely covered with conidia--- Rosellinia protuberans. (7, p.57).
- 7'. Colonies more luxuriant, felty to lanose, sometimes with a distinct velvet "pile", zonate or uniform, white----- 8.

8. Colonies velvet or felty, at least in the centre ----- 9.
- 8'. Colonies lanose or cottony-felty ----- 14.
9. Colonies velvety at the centre but becoming downy oppressed and
subhyaline towards the margin Rosellinia pulveracea. (8,p.59).
- 9'. Not as above ----- 10.
10. Colonies always strongly zonate; canescent zones alternating
with velvet-felty and sharply distinguished from each other ---
Hypoxyton glomeratum var. 3. (11,p.64).
- 10'. Colonies entirely velvety or fleecy; zonate or uniform ----- 11.
11. Carbonization absent in plate culture ----- 12.
- 11'. Carbonization of substrate visible on reverse side of
plate cultures ----- 13.
12. Colonies velvet felty, usually zonate and with characteristic
plumose margins when old ----- Rosellinia mammoidea. (9,p.61).
- 12'. Colonies velvet felty, uniform and without plumose
margins ----- Hypoxyton glomeratum var. 5. (13,p.68).
13. Colonies fleecy, compact and usually zonate, with coarse surface.
Hypoxyton glomeratum var. 4. (12,p.66).
- 13'. Colonies velvet felty, never zonate, and with smooth
surface ----- Nummularia succenturiata (14,p.70).
- 14(8). Colonies densely cottony lanose, usually with aerial mycelium
several mms. high; texture very light, not depressed
with age ----- Rosellinia corticalis (10,p.63).
- 14'. Colonies at first thin felty, later silky --
Rosellinia aquila and
Rosellinia thelena (15,p.71;16,p.73).
- 15(1"). Aerial mycelium in malt plate culture pure white ----- 16.
- 15'. Aerial mycelium in malt plate culture tinted early with
buff, rose, or olive green ----- 18.
16. Marginal hyphae distinct, forming a velvet ruff; stain
and mycelium orange brown on Leonian and Czapek agars ---
Hypoxyton 13A (17,p.73).
- 16'. Marginal hyphae straggling and not easily discernible;
stain never orange brown ----- 17

17. Stain absent; mycelium with characteristic granulate appearance, especially when young ----- Nummularia uni-apiculata (18,p.75).
- 17'. Stain dark olive green;mycelium typically stragglng -----
----- Hypoxylon exutans. (19,p.77).
- 18(15).Aerial mycelium predominantly dull white; colouration restricted to small scattered areas including the centre ----- 19.
- 18'. Colouration extending to the entire aerial mycelium ----- 20.
19. Surface coarse but never granulate; colouration of mycelium and stain brown pink or olive green; conidia inequilateral, pyriform, av. 2.2 x 5.4 μ . ----- Hypoxylon merrillii. (20,p.78).
- 19'. Surface usually granulate with age; colouration as for H. merrillii; conidia equilateral, oval, av. 3.0 x 5.7 μ . -----
----- Hypoxylon mediterraneum.(21,p.81).
- 20(18).Myceliumlight purple brown on malt and Leonian agars (plate culture) ----- Hypoxylon nummularium. (22,p.83).
- 20'. Mycelium ochre yellow ----- Nummularia kalchbrennera.(23,p.85).

GROUP I. Colonies submersed to appressed, white or pale yellow.

1). Rosellinia apiculata:

Malt. A : Bottle Culture (Fig. 79).

Appearance:

Closely appressed; aerial mycelium scanty and developing from the centre outwards, at first colourless, later white subhyaline with a coarse surface. In old colonies (> 3 months) very small superficial aggregations of mycelium lend the colony a characteristic granulate appearance.

Margin: Submersed, hyaline and colourless, entire, with compact peripheral hyphae.

Conidia: None recorded.

Stain: None.

B : Plate Culture (Figs. 81,82):

Appearance:

Canescent, white subhyaline, with a moderately coarse surface.

Margin: Submersed, in some cases entire, in others lobed or segmented; peripheral hyphae dispersed.

Other details as above; but colonies lack the secondary development of a granulate surface except in rare cases.

Other media: Very similar to malt. We can tabulate the essential features as follows:-

B o t t l e C u l t u r e .		
M a i z e .	L e o n i a n ' s . (F i g . 8 0) .	C z a p e k .
Mainly submersed aerial mycelium very sparse.	Similar to malt except for more scattered development of aerial mycelium as ill defined white areas.	Similar to malt.
P l a t e C u l t u r e .		
M a i z e .	L e o n i a n ' s . (F i g . 8 3) .	C z a p e k .
Almost entirely sub- mersed. Surface slightly gelatinous.	Almost entirely submersed; surface markedly gelatinous.	Distinguished from other media in that the white colour is more opaque and in the dry nature of the surface.

Microscopic characters:

No secondary mycelium. Primary mycelium with no distinguishing character; maximum diam. 2.0μ . Recognition of the species is based on the gross external features just described.

2). Hypoxyton glomeratum var. 1.:

Malt. A : Bottle Culture. (Fig. 84);

Appearance: Mainly submersed, with scant aerial closely appressed mycelium which is usually hyaline and nearly colourless, but white and opaque if occasionally developing in some quantity. Normally conidia are found abundantly soon after inoculation so that the surface becomes characteristically fine granulate. In some cases, however, where the conidia fail to develop the mycelium has a sodden aspect.

Margin: Usually distinct from the interior of the colony by the absence of conidia; submersed, hyaline, colourless and entire; peripheral hyphae compact and closely parallel.

Conidia: Ashy grey (S 233, 234) produced in great numbers over the entire surface of the colony except the margin.

Stain: None.

B : Plate Culture (Fig. 86):

Appearance: Mainly submersed, with canescent subhyaline, colourless aerial mycelium. Growth is normally uniform but sometimes showing ill defined zones 4 - 6 in number and 5 - 6 mm. wide, differing only in concentrations of superficial conidia.

Margin: Other details as above.

: Except for Czapek, cultures on other media are similar.

Other media: B o t t l e C u l t u r e .

M a i z e	L e o n i a n ' s .	C z a p e k . (Fig.85).
Less luxuriant than malt; colony paler and conidia less profuse, at first restricted to the centre and then slowly developing outwards.	Similar to malt.	Growth very slow (1.8 cm. diam. at 25°C after 14 days), and conidia very sparse, though produced over the entire surface.
P l a t e C u l t u r e .		
M a i z e (Fig.87).	L e o n i a n ' s . (Fig.88).	C z a p e k . (Fig.89).
Less luxuriant than malt, with aerial mycelium very scanty, but otherwise similar. Conidia sparse, produced round centre only.	Similar to malt.	Totally submersed with sodden aspect and few conidia. Olivaceous ring 2mm. broad and 2mm. inwards to margin may develop after 4 weeks.

Microscopic characters:

There is no secondary mycelium. The primary mycelium has a maximum diameter of 2.9μ , and an interesting though inconstant feature is the undulating form of the marginal hyphae (see Fig. 161C).

Conidiophores and conidia. (Fig. 98 A, B):

The conidiophores comprise an indefinitely branched system of which the terminal hyphae are fertile. Apart from the production of conidia, the conidiophores can be distinguished from the vegetative mycelium by the predominantly ternate branching. The fertile hyphae are relatively short, ranging from 4-20 μ , and either bear conidia along short spicate terminal portions of the axis or in apical clusters.

The latter is clearly a modification of the former, and represents a shortening of the fertile portion. The conidia are usually borne in 4 rows corresponding to the outward elongation of the tip of the fertile hyphae in 4 different directions, but may be specially arranged, or without apparent order. They are small, equilateral, oval with both ends rounded, hyaline individually but deep cineraceous seen collectively; and measure $1.4 - 2.3 \times 2.6 - 4.6 \mu$, average $1.9 \times 3.7 \mu$.

3). Hypoxylon glomeratum var. 2:

Malt. A : Bottle Culture:

Appearance: Mainly submersed up to 14 days old, later canescent appressed, with very sparse aerial mycelium. This is almost hyaline and colourless at first, then white subhyaline tinged with dark brown, especially near the site of inoculation. Surface completely smooth when young, becoming finely granulate when conidia are produced.

Margin: Submersed, entire, with compact, rather effuse peripheral hyphae.

Conidia: Restricted to the lower parts of bottle cultures and formed rather sparsely over the surface. Colour white, or pale pink to reddish brown when seen collectively but almost colourless otherwise.

The conidia develop 3 - 4 weeks after inoculation.

Stain: Variable in appearance, either absent entirely or appearing after 1 - 2 months; very diffuse, light amber brown. (S 339), darkening with age to dull brown (S. 701).

B : Plate Culture: (Figs. 92,93).

Appearance: Canescent, appressed except for the margin which is velvet silky; white subhyaline, with a fine smooth surface.

Margin: Not clearly distinct from the interior of the colony; entire with peripheral hyphae very closely compact, running parallel to each other.

Conidia: Not produced in culture though obtainable by the Riddell slide technique.

Stain: None.

Other media: In general, similar to malt.

B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s . (Fig.90).	C z a p e k . (Fig.91).
Similar to malt.	Slightly more luxuriant than	Similar to malt and
Stain diffuse through-	malt, forming a white opaque	maize.
out culture, very pale	canescent appressed mat by	
brown.	10 days.	

P l a t e C u l t u r e .

M a i z e . (Fig.96).	L e o n i a n ' s . (Figs.94, 95.)	C z a p e k . (Fig.97).
Similar to malt but	Canescent to fleecy with pecu-	Almost entirely sub-
slightly less luxu-	liar glossy sheen when viewed	mersed and hyaline
riant.	through the reverse of the	except for a canes-
	plate.	cent area surrounding
		centre.

Microscopic Characters. (Fig. 99):

1. Primary mycelium:

No distinguishing character; maximum diameter 1.5μ .

2. Secondary mycelium:

This comprises long stout, frequently septate hyphae $3.5 - 4.0 \mu$ diam. which are only sparingly branched and occur near the centre of the bottle cultures. They are possibly associated with the production of stain. The possession of secondary mycelium sharply distinguishes this species from the others in this group.

Conidiophores and conidia:

The conidiophores form part of an indefinitely branched system, and each is 110-250 μ long, dichotomously branched, and in addition to the closer ramification and production of conidia, are also distinct from the vegetativemycelium in the wider diameter of the hyphae which are 1.7 - 2.9 μ diameter.

The conidia are acrogenous or pleurogenous. The youngest conidium is always in the centre of a fully formed apical cluster. Usually the fertile hypha elongates beyond the site of the first formed conidia and produces a second cluster. This gives rise to a rather characteristic pattern that gives the impression that the conidia arise in fascicles. The conidia are relatively large, oval or oval elliptic, sometimes pyriform, pink when seen collectively, 2.3 - 3.4 x 4.0 - 7.4 μ , average 2.6 x 5.4 μ .

4). Rosellinia moroides.

Malt. A : Bottle Culture:

General appearance:

Mainly completely submersed, canescent, with granulate or smooth surface; no zonation. Aerialmycelium dull yellow to yellow brown with conidiophores scattered throughout the colony and undistinguishable from it, or organized into a thin crust round the centre inoculation point. Centre part of the colony granulate.

Margin: Not distinct from the interior of the colony, entire downy appressed, white to subhyaline.

Conidia: Appear after 8 - 14 days over the entire surface of the colony, dull yellow brown, inconspicuous.

Stain: Dull yellow or orange, (S.226) underneath the entire colony.

B : Plate Culture: (Fig. 102).

Appearance: Canescent with granulate or nearly smooth surface; no zonation. Colour of mycelium variable, sometimes nearly white, or very dark yellow, but always with a saffron tint.

Other characters as above.

Other media: B o t t l e C u l t u r e .

M a i z e . (F i g . 1 0 0) .	L e o n i a n ' s .	C z a p e k . (F i g . 1 0 1) .
Less luxuriant than malt, with very scanty aerial mycelium restricted to the centre part of the colony. No conidia and stain slight.	Similar to malt.	Similar to malt but with sodden aspect and convolution of the medium to form nume- rous irregular radia- ting channels. Mycelium brilliant orange (S.211-246) at first, later (> 3 wks.) becoming duller. No conidia; stain as for malt.

P l a t e C u l t u r e .

M a i z e . (F i g . 1 0 3) .	L e o n i a n ' s .	C z a p e k .
Less luxuriant than malt, submersed to appressed, with gelatinous surface. Stain nil except for zone of faint willow green just behind margin.	Similar to malt but with sodden aspect.	Similar to malt but with slight intensi- fication of colour.

Microscopic Characters:

No secondary mycelium; primary mycelium without distinguishing character, maximum diameter 1.8μ .

Conidiophores and conidia: (Fig. 117, B - D):

The conidiophores are short in comparison with those of other species, only $90 - 140 \mu$ long, and only distinguished from the vegetative mycelium by the slightly greater diameter of the main branches ($1.5 - 3.3 \mu$) and more frequent branching. The main axis of the conidiophore does not branch more than 2 or 3 times and the ultimate fertile branches are usually short.

The conidia are borne from the sides and apices of the fertile branches, and are oval or subglobose, hyaline but tinted light olive yellow, $2.0 - 3.2 \times 2.9 - 5.7 \mu$, av. $2.6 \times 3.7 \mu$; in addition, minute conidia, variable in shape, $1 - 2 \mu$ long, are often produced from the sides of the hyphae when the colony is young.

5). Hypoxyton asarcodes:

Malt. A : Bottle Culture. (Fig. 104):

Appearance: Downy appressed with sparse aerial mycelium; surface fine smooth or slightly cottony, dirty white behind the margin to grey with age. Growth is normally uniform but slight zonation was evident in one out of the 4 cultures examined, 4 zones 2mm. wide appearing behind the margin and distinct from each other in slight differences in colouration.

Margin: Submersed, dirty white subhyaline, entire with compact to slightly effuse peripheral hyphae.

Conidia: None recorded.

Stain: Olive green to grey, eventually turning grey black (S.434) with age; produced after 10 days, up to but not beyond the margin.

B : Plate Culture: (Fig.106).

Appearance: Canescent, finely and densely appressed, dull white subhyaline with a smooth dry surface, sometimes with loose cottony mycelium developing in the centre of the colony up to 4mm. high, with age (>2 weeks).

Margin: Submersed canescent, usually entire, rarely lobed, at 28°C , very closely compact, peripheral hyphae lying parallel and very close together.

Conidia: None.

Stain: Variable in time of appearance, distribution and colour, but normally present; usually some shade of olive grey (W.: willow green 00862/1, 00862/3), but sometimes pale yellow (S. 212.)

Other media:- B o t t l e C u l t u r e .

M a i z e . (F i g . 1 0 7) .	L e o n i a n ' s .	C z a p e k .
Similar to malt at first submersed downy (1-14 days) but later developing white felt mycelium 0.5 mm. high in centre of the colony.	Similar to malt and maize; but also with central mat of scant aerial mycelium with coarse surface, dirty white subhyaline in colour.	Differs from other media in greater luxuriance; aerial mycelium forming a coarse felty mat 0.5 mm. high dull white in colour.
Margin distinct, submersed, 6-14 mm. wide, pale dirty white subhyaline. Stain amber yellow at periphery (S 250) rapidly turning grey brown to olive black (S. 702).	Margin distinct, submersed, 7-8 mm. wide, pale dirty white subhyaline. Stain grey brown (S. 702) throughout colony.	Margin not distinct from centre of the colony, appressed felty. Stain grey brown (S. 702), throughout colony, less conspicuous than on other media.

P l a t e C u l t u r e .

M a i z e . (F i g . 1 0 7) .	L e o n i a n ' s .	C z a p e k .
Completely submersed to faintly appressed with sodden aspect; colour white subhyaline. Stain none.	Almost completely submersed except for slight canescence at centre. Stain pale olive grey.	More luxuriant than on the other media; canescent felty, white, nearly opaque, with coarse surface. Stain indistinct olive brown.

Microscopic Characters:

There is no secondary mycelium. The primary mycelium has no distinguishing character; maximum diameter 3.0μ . (Fig. 224 B).

Conidiophores and conidia: None recorded.

Chlamy dospores: (Fig. 232 C):

These are found from the submersed mycelium one week after inoculation, in bottle culture and sometimes in plate culture as well. They are oval or pyriform with a thick refractive wall, dark amber or dark brown to black, $5.1 - 5.7 \times 6.0 - 8.8 \mu$.

GROUP II. Colonies felty, floccose, velvety, fleecy or lanose, pure white.

6). Rosellinia obtusissima:

Malt. A : Bottle Culture (Fig. 108):

Appearance: At first coarse felty (18 days) with dense white aerial mycelium up to 0.3 mm. high, later floccose with a coarse surface, and developing grey flaring sterile outgrowths up to 1.5 cm. high near the original point of inoculation. With age a distinct rind 1.5 mm. thick, formed partly of the substrate and partly of closely woven mycelia develops underneath the aerial mycelium. The growth of the colony is zonate, 4 bands 3 mm. wide and 5 - 7 mm. apart being formed in 14 days at 25°C and 2 further bands after a fortnight at room temperature (av. 20°C). The colour of the aerial mycelium is white, pearly when young and tinted yellow with age, and shows a transient orange tint in the centre of the colony after 4 weeks.

Margin: Distinct from the centre of the colony as a submersed to downy appressed subhyaline zone 3 mms. broad. The margin is not entire but slightly segmented and the hyphae are compact to slightly effuse.

Conidia: Produced after 6 months, extending from the centre outwards, very finely granulate and pale fawn in colour.

Strain: Appearing after 2 - 3 weeks, faint ochre yellow and diffuse throughout the substrate; also exuded as droplets on the surface of the aerial mycelium.

B : Plate Culture: (Figs. 114, 115).

Appearance: At first felty appressed (1 - 5 days) with a smooth surface, later becoming characteristically coarse in texture (5 - 10 days) and finally floccose with small aggregations of white mycelia 2 mm. diam. scattered over most of the colony or restricted to a wide zone behind the margin. The aerial mycelium is white, sometimes turning very pale cream with age. Growth is uniform instead of zonate as in bottle culture; presumably zonation occurs only when the colony is allowed to attain a certain age on a stable medium.

Margin: Not clearly distinct from the interior of the colony, 2 - 3 mm. submersed, canescent appressed, white subhyaline, and usually uneven and segmented due to the growth of peripheral hyphae in different directions. The peripheral hyphae may present a somewhat plumose aspect due to this manner of growth, though they are compact rather than effuse.

Conidia: None.

Stain: None.

Other media:- B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s . (Fig.109).	C z a p e k . (Fig.110).
Less luxuriant than malt; aerial mycelium much thinner and velvety; not zonate. Stain ochre yellow, appearing late, after 6 weeks.	Similar to malt; zonate. Stain ochre yellow, dense at centre and lightening towards the outside, appearing after 4 weeks.	Canescent at first (10 days) later (4-6 weeks) developing a thin white subhyaline mat growth and flaring growths as in malt; not zonate. Stain diffuse light yellow ochre, appearing after 4 weeks.

P l a t e C u l t u r e .

M a i z e . (Fig.111).	L e o n i a n ' s . (Fig.112).	C z a p e k . (Fig.113).
Less luxuriant than malt and not as dense; otherwise similar with floccose development when old.	Similar to malt, except margin further segmented; each segment 3-6 mm. wide and with the outer part submersed and white subhyaline.	Distinct from all other cultures by the gleaming smooth fleecy nature of the young cultures (1-5 days) Floccose development occurs later as in malt.

Microscopic characters:

No secondary mycelium is formed. The primary mycelium is rather narrow in comparison with other species, with a maximum diameter of 1.7μ . (Fig. 223 B).

Conidiophores and conidia: (Figs. 116, 117 A).

This species has the least specialized type of conidiophore known for the 60 spp. investigated. The conidiophores are scarcely distinct from the vegetative mycelia and are usually extremely long, (at least 500μ). They comprise indefinitely branching systems from which the conidia are budded off almost at random, either singly or in groups of 2 - 3. The conidia are small, oval to subglobose, hyaline, usually tinted pale yellow and $1.7 - 3.2 \times 2.6 - 3.4 \mu$, av. $2.4 \times 3.2 \mu$.

7). Rosellinia protuberans:

Malt. A : Bottle Culture (Fig. 118):

Appearance: Mainly velvety, sometimes velvety felty but with smooth surface, forming a closely appressed mat on the surface of the agar. Growth is zonate, 3 zones and 3 mm. wide and 7 mm. apart being formed in 2 weeks at 25°C and a further 3 after a fortnight at room temperature (in 20°C). After 2 months, scattered floccose dull white to pale grey outgrowths are formed which are similar to those of the previous species and the original zonate pattern is obscured.

Margin: Not distinct from interior of the colony; downy entire, white subhyaline, compact.

Conidia: Appear after 2 weeks over the centre of the colony; very pale fawn.

Stain: None.

B : Plate Culture: (Fig. 121).

Appearance: Canescent to velvety with a very fine smooth surface; white opaque at centre to white subhyaline near the margin.

Margin: Not distinct from interior of colony; entire, compact, with Peripheral hyphae lying closely parallel.

Conidia: Appearing as a thin white or very pale grey covering over the centre of the colony after 7 days.

Stain: Nil.

Other media:- B o t t l e C u l t u r e .

M a i z e.(Fig.120).	L e o n i a n ' s .	C z a p e k.(Fig.119).
Similar to malt, but growth is slower and zonation less marked (2 zones in 10 days at 25°C). Appearance canescent velvety with peculiar dense oval mats similar to those of Czapek culture of <u>Rosellinia obtusissima</u> .	Similar to malt but growth is slower and zonation not at first so marked (2 zones in 10 days at 25°C).	Distinct from other cultures by submersed growth up to 14 days; later canescent with oval areas, white subhyaline to almost colourless. Growth is almost uniform; 2 faint zones being noted after 10 days.

P l a t e C u l t u r e .

M a i z e.(Fig.122).	L e o n i a n ' s .	C z a p e k .
Less luxuriant than malt and Leonian's; canescent with slightly gelatinous surface. No zonation.	Less luxuriant than malt; canescent with slightly gelatinous surface. No zonation.	Almost entirely submersed, aerial mycelium scanty and canescent; surface gelatinous. No zonation.

Microscopic characters:

This species resembles Rosellinia obtusissima in the narrow diameter of the hyphae (max.= 1.7 μ). No secondary mycelium is formed.

Conidiophores and conidia: (Fig. 123).

The conidiophores show distinct resemblance to those of H. glomeratum previously described comprising long, indefinitely branched systems, but can be distinguished by the dichotomous instead of the ternate forking of the fertile branches. The fertile branches are 85-100 x 1.8 μ , and are usually non septate, but may be 1 - 2 septate. The upper 9 - 20 μ of these branches bear the conidia in dense spicate clusters. The conidia are borne on wartlike prominences which arise in the same way as that de-

scribed for H. glomeratum. They are subglobose to oval, rounded at both ends, hyaline individually but collectively pale fawn grey, 1.4 - 2.6 x 2.3 - 4.0 μ , av. 1.8 x 3.0 μ .

8). Rosellinia pulveracea:

Malt. A : Bottle Culture: (Fig 124).

Appearance: Velvety, with granulate surface due to small outgrowths of mycelium; otherwise smooth. No zonation. Aerial mycelium white at first, later dull white to pale grey. With age (6 weeks) dull white or yellow flaring outgrowths develop as in other species of Rosellinia.

Margin: Not distinct from the interior of the colony; downy appressed white, opaque to subhyaline at the periphery.

Conidia: None.

Stain: None.

B : Plate Culture: (Figs. 128, 129).

Appearance: Velvet felty, dense, with very fine smooth surface. Aerial mycelium gleaming white, up to 3.5 mms. high near the centre and thinning gradually towards the margin, and consequently turning from opaque to subhyaline.

Margin: Usually distinct as a zone 3 mm. broad, canescent, white subhyaline, and entire. The marginal hyphae are compact and run closely parallel.

Conidia: None.

Stain: None.

Other media: In contrast to other species, well marked differences are observed when this species is grown on different media.

B o t t l e C u l t u r e .

M a i z e.(Fig.125).	L e o n i a n ' s.(Fig.126).	C z a p e k.(Fig.127).
Less luxuriant than malt; at first canescent, later (6 weeks) velvet felty. No zonation. No stain.	More luxuriant than on malt, felty lanose but characteristically dense as in other cultures. Also distinguished by pock-shaped depressions in the surface, the orange pink tinge to the white aerial mycelium, and the carbonization of the medium that takes place with age. Stain none.	Much less luxuriant than malt at first (10 days) with very sparse growth of aerial mycelium. Later thin closely appressed aerial mycelium develops over the whole surface of the colony, and when 3 weeks old, the surface wrinkles to form folds, and droplets of olive stain are exuded on the surface. Stain olive yellow (S.220) diffuse thro' out substrate and appearing after 4 wks.

P l a t e C u l t u r e .

M a i z e.(Fig.130).	L e o n i a n ' s.	C z a p e k.
Less luxuriant than on malt, and similar to Czapek; comprising a central velvety part and a margin 4 mm. broad, canescent and white subhyaline.	More luxuriant than on malt; comprising a velutine centre and distinct margin 3 mm. broad which is subhyaline white.	The colony comprises a centre which is velvety felty and closely appressed, and with a smooth fine surface, and a broad margin of up to 3 mms, which is subhyaline white, canescent to completely submersed.

Microscopic characters:

Due to the absence of secondary mycelium and of conidiophores, it is impossible to identify the primary mycelium alone. It is very similar to Rosellinia protuberans and R. obtusissima, with a maximum width of 1.4μ . (Fig. 223 A).

9). Rosellinia mammoidea:

Malt. A : Bottle Culture: (Figs. 131, 132).

Appearance: Mainly velvet fleecy, always very dense, and with characteristic rough uneven surface. The degree of luxuriance varies within the same strain, and, moreover, monospore and multispore isolates themselves show the full range. The following malt cultures were examined in detail:-

Strain.	Type of culture	Growth character (after 14 days).
7	Monospore	Velvet fleecy.
	Ascus,	Velvet fleecy.
	Multispore.	Velvety appressed, less luxuriant than the other two.
47.	Two spore.	Both cultures velvet fleecy.
	Ascus.	Velvety, slightly less luxuriant than the monospore.
433.	Multispore.	Velvet-fleecy, very luxuriant.

Growth of the colonies was zonate, 2 - 3 ruffs of mycelium each 2 - 3 mm. wide and about 5 mms. apart being formed at 25°C in 14 days.

Margin: Distinct from the rest of the colony, 2 - 3 mm. broad, submersed, colourless, entire, compact.

Conidia. Produced after 10 months over a small part of the aerial mycelium, usually near the margin; colour pale fawn grey (S. 235).

Stain: None.

B : Plate Culture: (Figs. 137-139).

Appearance: Velvet fleecy, compact, with zonate growth or infrequently uniform; 3 zones each 2 mm. wide and 5 mm. apart are produced in 7 days. With age colonies are distinguished from most other species by the strongly plumose appearance of the mycelia at the margin.

Margin: Not distinct from the interior of the colony, lobed to nearly entire, compact.

Conidia: Only produced in one instance, in a colony 3 weeks old. They are very pale grey and scattered over the entire surface of the colony in small pulvinate masses.

Stain: None.

Other media:- B o t t l e C u l t u r e .

M a i z e . (F i g s . 1 3 3 , 1 3 4) . L e o n i a n ' s . C z a p e k .

<p>Less luxuriant than on malt, dense canescent velvety with an uneven surface. 2-3 zones are produced in 14 days at 25°C. Old colonies (6 weeks) produce flaring outgrowths and mound shaped oval areas as in other <u>Rosellinia</u> spp. Conidia are produced on floccose tufts encircling original point of inoculation, after 8 weeks.</p>	<p>Similar to malt but zonation less evident, shown in one culture as 2 indistinct concentric ruffs of white fleecy mycelium. Flaring outgrowths developed in 1 culture after 5 weeks.</p>	<p>Almost completely submersed, colourless hyaline, with scant downy aerial mycelium confined to 4 small adjacent areas near the centre of the colony.</p>
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 P l a t e C u l t u r e .

M a i z e . (F i g s . 1 4 0 , 1 4 1) . L e o n i a n ' s . (F i g . 1 3 5) . C z a p e k . (F i g . 1 3 6) .

<p>Less luxuriant than on malt, fleecy and closely appressed; otherwise similar.</p>	<p>Similar to malt.</p>	<p>Submersed to finely canescent; white subhyaline; aspect sodden.</p>
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Microscopic characters:

No secondary mycelium. Primary mycelium without distinguishing features; maximum diameter 2.0μ (Fig. 148 A).

Conidiophores and conidia: Fig. 148 B).

The general plan of the conidiophore is the same as that of R. protuberans but the conidia are approximately the same size as those of R. corticalis. The conidiophores are indefinitely branched, with ultimate fertile branches $55-80 \times 1.7 \mu$. The conidia are borne in short spicate apical clusters, and are oval to subglobose, equilateral, $1.4 - 2.3 \times 2.3 - 4.0 \mu$, av. $1.8 \times 3.0 \mu$.

10). Rosellinia corticalis:

Malt. A : Bottle Culture.(Fig. 142):

Appearance: Velvety lanose throughout, changing little with age, with densely packed hyphae up to 15 mms. high and mostly over 5 mms. high. The colour of the mycelium is white, tinted light yellow brown in certain areas with age.

Margin: Not distinct, felty appressed and rising abruptly into the aerial mycelium behind.

Conidia: None.

Stain: None.

B : Plate Culture:(Figs 144, 145).

Appearance: Similar to the above; sometimes partly zonate. Conidia once produced over the surface of a 3 week old colony but otherwise never seen. The conidia are pale fawn grey and give the colony a finely granulate surface.

Other media:- B o t t l e C u l t u r e .

<u>M a i z e.</u> (Fig 142).	<u>Le o n i a n ' s.</u> (Fig 143).	<u>C ě a p e k.</u>
Much less luxuriant than malt; appressed canescent or submersed. Outer part of colony hyaline and centre white subhyaline.	Similar to malt, aerial hyphae up to 9 mm. high.	Submersed entirely, hyaline very restricted and making poor growth.

Plate Culture.

Maize.	Leonian's. (Fig. 144)	Czapek. (Fig. 145).
Similar to but less luxuriant than on malt, velvety, 2 - 3 mm. high.	Slightly more luxuriant than malt, dense velvet lanose up to 6 mm. high and with cushiony appearance.	Appressed, finely canescent with smooth surface; margin submersed completely.

Microscopic characters:

No secondary mycelium. Maximum width of primary mycelium is 1.7μ .

Conidiophores and conidia. (Fig 148 C):-

Similar in general plan to Hypoxylon glomeratum var. 1. with the typical ternate branching. The fertile system is indefinitely branched, each unit ranging from $150 - 250\mu$ in length. The conidia are produced without apparent order in spicate clusters on the ends of the ultimate branches, and are oval to subglobose with rounded ends, pale fawn grey collectively, $1.4 - 2.0 \times 2.0 - 2.9\mu$, av. $1.7 \times 2.3\mu$.

The two species R. mammoidea and R. corticalis are very similar in cultural characters but the greater luxuriance of the latter species and the difference in the conidiophores serve to distinguish them.

11). Hypoxylon glomeratum var. 3:

Malt. A : Bottle Culture: (Fig. 149).

Appearance: At first (up to 7 days) finely canescent with scant subhyaline white aerial mycelium and a silky aspect, especially near the margin; later (3 weeks) becoming denser at the point of inoculation thus differentiating 2 regions, a pale white opaque canescent centre surrounded by a large subhyaline white one. The growth of the colony, however, is never typically zonate.

Margin: Segmented, compact.

Conidia: Produced after one week over the surface of the colony, but very sparingly so that they are indistinguishable from the surrounding mycelium. They are white in colour.

Stain: None.

B : Plate Culture:(Fig. 152).

Appearance: Colonies mainly canescent velvety and strongly zonate; each zone is 2 mm. wide and 5 - 7 mm. apart, and consists of an undulating irregular ridge of velvet white mycelium bearing sparse white conidia. The central zone is the most regular and conspicuous. The mycelium between the zones is almost hyaline and closely appressed. The general surface of the colony is smooth or undulating, and is dry or slightly gelatinous in aspect.

Margin: Distinct from the interior of the colony, 4 - 13 mm. wide, submersed appressed, irregular and characteristically segmented, each segment 5 - 15 mms. wide.

Conidia: White, very sparse, restricted to the zones.

Stain: None.

Other media:- B o t t l e C u l t u r e .

M a i z e.(Fig.150).	L e o n i a n ' s.(Fig.151).	C z a p e k.
Submersed or appressed, canescent with characteristic slate grey tint; otherwise similar to malt.	Similar to malt.	Nearly all submersed with very sparse aerial mycelium that is nearly colourless.

P l a t e C u l t u r e .

M a i z e.(Fig.154).	L e o n i a n ' s.(Fig.153).	C z a p e k.
Less luxuriant than malt but similar in appearance. Surface dry gelatinous.	Similar to malt but with markedly gelatinous surface.	Almost entirely submersed but with characteristic zonate growth. Surface sodden or slightly gelatinous.

Microscopic characters:

There is no secondary mycelium. Primary mycelium with no distinguishing feature, rather narrow, maximum diameter 1.7μ .

Conidiophores and conidia:

The conidiophores are short, easily distinct from the vegetative mycelium by their determinate growth and slightly wider diameter, 21-50 μ long ; each usually once bifurcate producing 2 ultimate fertile branches bearing very shortly spicate or capitate clusters of conidia. Interesting peculiarities are the irregularly swollen axes of the conidiophores, and the broad V-shape of the dichotomous branching. The conidia are relatively small, oval to subglobose, white, 1.1 - 1.7 x 1.7 - 5.2 μ , average 1.5 - 2.1 μ .

12). Hypoxyylon glomeratum. var. 4:

Malt. A : Bottle Culture. (Fig. 156):

Appearance: Colony at first velvety (1 - 5 days) later velvet lanose with the aerial mycelium forming a white mat up to 4 mms. high. Growth is usually zonate but the regularity of this type of growth varies, and may be very indistinct. The zones which vary from 2 to 4 in number after 14 days at 25°C are \approx 2 mm. wide and 1 cm. apart. The surface of the colony is uneven and cushiony in aspect.

Margin: Distinct from the interior of the colony as a canescent or submersed zone 4 - 15 mm. broad; entire, compact.

Conidia: Only 1 out of the 5 multispore cultures examined has produced conidia. These are white or light fawn grey in colour, and cover most of the surface of the colony. They were produced after one month.

Stain: None. Carbonization of the substrate occurs, however, after two months to a varying extent, forming a dark zone 2mm. deep beneath the aerial mycelium.

B : Plate Culture.(Fig. 157, 158):

Appearance: Velvet lanose, similar to bottle cultures but differing in the very pronounced nature of the zonate growth, 4 - 6 zones each 2 mm. wide and 4 - 7 mm. apart being formed in 2 weeks. The zones are clearly formed by the repeated unequal development of the peripheral mycelia, some of which grow faster than the others and increase the diameter of the colony, while the

remainder form a ruff on the surface of the colony. While the new zone is consolidated by ramification of the original pioneer hyphae, fresh hyphae grow out to form the next zone. The velvety appearance of the colony is partly due to the distinct "pile" which results from growth of this nature.

Margin: (= outermost zone). Distinct from the interior of the colony, submersed to canescent, 3 - 4 mm. wide, entire or evenly segmented, compact.

Conidia: None.

Stain: None.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k . (Fig.155) .
Similar to malt but without zonate growth.	Similar to malt. Growth faintly zonate.	Similar to malt, but zonate growth very conspicuous.

P l a t e C u l t u r e .

M a i z e .	L e o n i a n ' s . (Fig.159) .	C z a p e k . (Fig.160) .
Similar to that on malt but fewer zones. (Up to 4 produced in 2 weeks).	Similar to malt; 1 culture produced white conidia after 7 days.	Similar to malt, but slightly less luxuriant.

Microscopic characters:

The primary mycelium is distinguished from all other species in that the septae in the marginal hyphae are accompanied by a conic or globose swelling in the mycelium just below. This feature appears to be constant for the 5 strains studied. The maximum diameter of the hyphae of the primary mycelium is 2.2μ . (Fig. 161 A).

The secondary mycelium consists of long stout hyphae $2.1 - 10.6 \mu$ diam. which are loosely interlocked and anastomosing. They appear when the colony is at least 5 days old and run underneath the surface of the primary mycelium. (Fig.161 B).

Conidiophores and conidia:

The conidiophores are of the general type noted for *H. exutans*. They comprise an indefinitely branched system, whose units are 150 - 200 long. Each unit consists of a main branch which divides dichotomously several times, and the ultimate fertile branches gives rise to conidia which are usually scattered down their length but may be in groups of 2 or 3. The conidia are oval to subglobose, equilateral and rounded at both ends, white or very pale grey, $2.0 - 3.4 \times 2.9 - 4.6 \mu$, av. $2.6 \times 3.5 \mu$. (Fig. 162 A, B).

13). Hypoxyton glomeratum var. 5:

Malt. A : Bottle Culture. (Fig. 163):

Appearance: Velvet felty to lanose, always dense and very luxuriant; the mycelium is white opaque and 1 - 2.5 mm. high, with a fine smooth surface.

Margin: Not clearly distinct, velvety with a pronounced "pile", entire, and with compact peripheral hyphae.

Conidia: Formed after 8 months in 1 out of the 3 cultures on the surface of the aerial mycelium over small areas near the upper edge of the colony. In colour they are light fawn.

Stain: None. Carbonization of the substrate takes place after 2 months, forming irregular isolated dark patches about 2 cms. in diameter.

B : Plate Culture. (Fig. 164):

Appearance: Velvet felty, aerial mycelium up to 2 mm. high and forming a dense opaque white mat with a fine and even surface.

Margin: Not distinct, downy to felty appressed, entire or broadly lobed, and with compact peripheral hyphae.

Conidia: None formed in the culture, though obtainable when the Riddell slide technique is used.

Stain: None.

Other media: B o t t l e C u l t u r e .

M a i z e .

L e o n i a n ' s .

C z a p e k .

Similar to malt; carbonization begins after 1 month, irregular in formation.

More luxuriant than on other media; dense felty lanose, white at first, turning dull yellow with age.

Less luxuriant than on other media; scant felty up to 10 days; later more

Maize.	Leonian's.	Czapek.
	Carbonization also takes place.	luxuriant but always closely appressed. Mycelium becomes furrowed after 6 weeks as in previous species, and stain is also secreted. Stain in substrate appears after 6 weeks, very diffuse light ochre brown.

Plate Culture.

Maize.	Leonian's.(Fig.165).	Czapek.(Fig.166).
Similar to malt but less luxuriant.	Similar to malt.	Similar to malt.

Microscopic characters:

Primary mycelium rather narrow, with maximum diameter of 1.9μ (Fig. 224 A). Secondary mycelium, associated with carbonization with substrate, consists of a closely woven system of branched and anastomosing darkly coloured hyphae that lie below the white primary mycelium. The hyphae are 1.4 - 2.7 μ diam. (Fig.225 A).

Conidiophores and conidia. (Fig. 172 A, B):

As in other species, the conidiophores are not really distinct from the vegetative mycelium, and consists of a loose, dichotomously branched network of hyphae with fertile terminal hyphae. Each branching system may be 200 - 250 μ in total length and the fertile branches are 45 - 80 x 1.7 - 2.5 μ , and normally 1 - 3 septate.

The manner of development of conidia is similar to that seen in Rosellinia obtusissima since there is no recognizable fertile region on the fertile hyphae and the conidia apparently develop at random. The main differences between Rosellinia obtusissima and this species is the much shorter average length of the fertile hyphae, and size of conidia. These are relatively large oval elliptic or subglobose with both ends rounded or sometimes

bluntly pointed, hyaline and colourless individually but collectively fawn coloured, $2.3 - 3.4 \times 4.0 - 6.9 \mu$, average $2.8 \times 4.4 \mu$.

14). Nummularia succenturiata:

Malt. A : Bottle Culture. (Fig. 167):

Appearance: Velvet felty, less luxuriant and more closely appressed than the previous species, uniformly white opaque.

Margin: Canescent to velvety, entire, compact.

Conidia: Pale fawn grey, produced after 8 months.

Stain: None. Carbonization of the medium may take place after 2 months, forming isolated dark patches about 2 cms. diameter.

B : Plate Culture. (Fig. 171):

Appearance: As above, velvet felty, opaque, and with carbonization extending from centre.

Other media: B o t t l e C u l t u r e .

M a i z e . (Fig.169).	L e o n i a n ' s . (Fig.170).	C z a p e k . (Fig.168).
Velvet felty, similar to malt; carbonization begins after 1 month and is irregular in formation.	More luxuriant than on malt or maize but otherwise similar in general features; dense felty lanose with smooth surface. Carbonization after one month; 2mm. deep throughout.	Less luxuriant than malt and downy to felt for the first 10 days, later more luxuriant, but always closely appressed while opaque, with a smooth surface. Stain secreted in droplets from the surface of the colony, and also in the substrate; golden brown.

P l a t e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k .
Similar to malt.	Similar to malt.	Similar to malt.

Microscopic characters:

Very similar to the previous species; primary mycelium with max. diam. of 2.0μ , and identical secondary mycelium $1.4-3.0 \mu$ diam.

Conidiophores and conidia. (Fig. 172):

These are also of the same general type as the previous species but may be distinguished by the tendency of the conidia to arise in groups of 2 or 3 as well as singly, and by their larger average size. They are oval elliptic, equilateral with bluntly pointed or rounded ends, grey or fawn collectively, $1.7 - 3.4 \times 4.0 - 7.4 \mu$, average $2.8 \times 5.2 \mu$.

Group III: Colonies smooth silky to thin felty; never dense.

15). Rosellinia aquila:

Malt. A : Bottle Culture. (Fig. 175):

Appearance: Silky felty, very dense with smooth fine surface. Aerial mycelium 2.5 mm. high, usually gleaming white at first, later dull white, submersed mycelium white subhyaline, up to 1.1 cm. deep.

Margin: Not distinct, downy appressed, rather effuse, entire white subhyaline.

Conidia: None.

Stain: None.

Carbonization usually takes place after 2 months to form dark areas beneath the aerial mycelium of variable extent, together occupying about a third of the total colony surface.

B : Plate Culture (Figs. 177, 178):

Appearance: Silky felty at first, later cobwebby, with loose mycelium up to 12 mm. high; with age the colonies sometimes become depressed in the centre, while the marginal region remains luxuriant.

Margin: Not distinct, felty appressed, effuse, entire with widely dispersed peripheral hyphae.

Conidia: None.

Stain or Carbonization: None.

<u>Other media:</u> B o t t l e C u l t u r e .		
<u>M a i z e .</u>	<u>L e o n i a n ' s .</u>	<u>C z a p e k . (Fig.176) .</u>
Slightly more luxuriant, otherwise similar.	Rather less luxuriant than malt: aerial mycelium forms a marginal ruff up to 5 mm. high, otherwise similar to malt culture.	Less luxuriant than on other cultures, white subhyaline, appressed felty with a rather coarse and stragglng surface. Carbonization begins when about 2 months old, and later becomes very prominent.
<u>P l a t e C u l t u r e .</u>		
<u>M a i z e .</u>	<u>L e o n i a n ' s .</u>	<u>C z a p e k . (Fig.179) .</u>
Not as luxuriant as on malt, velvet felty, cob- webby with age.	Not as luxuriant as on malt, velvet felty and cobwebby with age.	Colony almost all submersed up to 4 days, later develop- ing scant cobwebby white subhyaline mycelium.

Microscopic characters:

The primary mycelium is much broader in comparison with previous species, having a maximum width of 3.1μ . (Fig.180 A).

The secondary mycelium is composed of 2 main types of hyphal systems, one composed of long filaments that branch repeatedly at right angles and interclasp and anastomose to form a felt of indefinite extent, and another lying beneath the first which comprises stouter bulbous hyphae are not so closely associated. The former type of mycelium is $2.3 - 2.9 \mu$ diam, while the bulbous type is $5.4 - 16.6 \mu$. diam. (Fig. 180; B, C).

Conidiophores and conidia:

Twigs infected with the mycelium of Rosellinia aquila were left in the open, and after 3 months, secondary mycelium of the type just described for bottle cultures was found growing in pulvinate masses along the bark.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k . (Fig.176) .
Slightly more luxuriant, otherwise similar.	Rather less luxuriant than malt: aerial mycelium forms a marginal ruff up to 5 mm. high, otherwise similar to malt culture.	Less luxuriant than on other cultures, white subhyaline, appressed felty with a rather coarse and straggling surface. Carbonization begins when about 2 months old, and later becomes very prominent.

P l a t e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k . (Fig.179) .
Not as luxuriant as on malt, velvet felty, cobwebby with age.	Not as luxuriant as on malt, velvet felty and cobwebby with age.	Colony almost all submersed up to 4 days, later developing scant cobwebby white subhyaline mycelium.

Microscopic characters:

The primary mycelium is much broader in comparison with previous species, having a maximum width of 3.1μ . (Fig.180 A).

The secondary mycelium is composed of 2 main types of hyphal systems, one composed of long filaments that branch repeatedly at right angles and interclasp and anastomose to form a felt of indefinite extent, and another lying beneath the first which comprises stouter bulbous hyphae are not so closely associated. The former type of mycelium is $2.3 - 2.9 \mu$ diam, while the bulbous type is $5.4 - 16.6 \mu$. diam. (Fig. 180; B, C).

Conidiophores and conidia:

Twigs infected with the mycelium of Rosellinia aquila were left in the open, and after 3 months, secondary mycelium of the type just described for bottle cultures was found growing in pulvinate masses along the bark.

The surface of this mycelium was covered with conidiophores. Conidiophores have also been found in connection with perfect stages collected in the field. Although they have never been produced in culture it therefore seems almost certain that those developed on inoculated wood and elsewhere belong to R. aquila.

The conidiophores in contrast to the dark mycelium bearing them, are light coloured, and hyaline. (Fig. 181). They are not indefinite in extent as in other species of Rosellinia, and each conidiophore consists of a long stalk $100 - 200 \times 2.3 - 4.5 \mu$, and a terminal multibranched portion giving rise to many fertile branches. These are usually fairly short, $15 - 40 \times 2.3 - 3.1 \mu$, with fertile region $6 - 10 \mu$, and are unseptate. The conidia are acropleurogenous densely clustered and form spicate or almost capitate heads. They are borne on wartlike projections of similar origin and shape to those noted previously, but are apparently produced without definite order; in size relatively large and oval elliptic sometimes pyriform, subhyaline and very pale grey collectively, $2.3 - 3.7 \times 4.5 - 6.9 \mu$, av. $3.1 \times 5.7 \mu$.

16). Rosellinia thelena. (Figs. 173, 174):

This species resembles the former so closely in general features that no separate description seems necessary. Both the bottle and plate cultures and the microscopic characters are identical in form and dimensions except for the following features:-

1. Growth on Czapek is no less luxuriant than on malt;
2. No conidiophores have been developed in culture;
3. The growth rates on malt differ significantly from those of R. aquila. R. aquila will not tolerate a temperature of 28°C while R. thelena will grow well at this temperature.

Group IV: Colonies coarse felty, often with straggling appearance, and usually tinted.

17). Hypoxyton 13A:

Malt. A : Bottle Culture.(Fig. 182):

Appearance: At first canescent, later felty with a coarse surface; aerial mycelium 2 - 3 mm. high, dull white at first subhyaline, and later nearly opaque.

- Margin: Submersed to canescent, centre with effuse peripheral hyphae, but the long exploratory hyphae characteristic of other species to be described were not observed here.
- Conidia: Produced on small floccose tufts of aerial mycelium after 1 - 2 months over the entire surface of the colony. This manner of development and the greenish gray colour distinguish this species from all others.
- Stain: Very light brown (S. 340) produced after one month and gradually deepening with age, to murky nondescript.

B : Plate Culture.(Figs. 186, 187):

- Appearance: Loosely felty with coarse surface but with none of the superficial straggling mycelium found in other species; aerial mycelium up to 2 mm. high, white subhyaline to almost opaque.
- Margin: Canescent to submersed, entire, compact with the peripheral hyphae lying partly // and partly dispersed.
- Conidia: None.
- Stain: None.

Other media:- B o t t l e C u l t u r e .

M a i z e.(Fig184).	L e o n i a n ' s.(Fig.183).	C z a p e k.(Fig.185).
Similar to malt but no conidia.	Similar to malt but no conidia.	Almost completely submersed; aerial mycelium scanty and coarse, colourless, hyaline; surface gelatinous.

P l a t e C u l t u r e .

M a i z e.	L e o n i a n ' s.	C z a p e k.(Fig.188).
Colony divided into: 1. downy to appressed centre. 2. white velvety margin 2.0-2.5 mm.broad and up to 2mm. high, of short filaments; thus rather distinct from	Colony divided into: 1. velvety appressed centre. 2. white velvety margin broad and 2 mm. high. With age, colour of mycelium slowly turns to orange brown.	Colony uniform, canescent to velvety with sodden appearance.

Maize.	Leonian's.	Czapek.(Fig.188).
Stain: None.	Stain: Very faint murky brown.	Stain: Dull diffuse amber brown. (S. 201.)

It can be seen from this table that the more compact type of growth and peculiar behaviour on different culture media separates the species from others.(See descriptions of other spp. further on).

Microscopic characters:(Fig. 224 H):

Primary mycelium:

The marginal hyphae are similar in form to those of H. merrillii and H. asarcodes, and are stout, ranging in diameter from 2.3 - 4.0 μ . There is no distinguishing feature.

Secondary mycelium:

Consists of long sparingly branched darkly stained hyphae 2.3 - 6.3 μ diam.

Conidiophores and conidia: (Fig. 193 B):

The conidiophores are normally distinct from the vegetative mycelium by their compact type of branching, although they are not darkly stained and neither do they have pitted walls. The fertile branches arise either singly or in groups, but apparently not in whorls; and measure 10 - 30 x 1.7 - 3.2 μ . The apices of the fertile branches are conspicuously swollen or grotesque in shape. The conidia are narrow, elongate elliptic, usually inequilateral with one end acute or acuminate, light olive green en masse, 1.1 - 2.3 x 4.3 - 7.7 μ , av. 1.6 x 6.3 μ .

18). Nummularia uni-apiculata:

Malt. A : Bottle Culture (Fig. 190):

Appearance: At first loosely felty, becoming dense with age forming a mat up to 2 mm. high of white subhyaline aerial mycelium with the normal coarse surface. The general features are similar to those of the preceding species but the mycelium is pure white without discolouration by conidia and has a typically straggling surface.

- Margin: Canescent with submersed periphery, entire effuse, and with long pioneer hyphae extending up to 5 mm. in front of the advancing colony.
- Conidia: Produced after 7 - 10 days, white in colour and usually forming a smooth opaque covering over part of the colony.
- Stain: Light buff to ochre brown (S. 695) appearing after 2 weeks and deepening slightly with age.

B : Plate Culture. (Figs. 191, 192):

- Appearance: Loosely felty, at first white subhyaline, later, after 2 days, becoming opaque. The height of the mycelium at that time is about 2 mms. The surface of the colony is coarse granulate.
- Margin: Similar to the above; also with long exploratory hyphae.
- Other details as above.
- Other media: All similar to malt, without significant differences.

Microscopic characters:

Primary mycelium:

Similar to H. mediterraneum and H. merrillii but the marginal hyphae are simpler in form and with branches of nearly the same diameter and length. The exploratory hyphae are very stout ranging from 5.7 - 7.7 μ diameter. (Fig. 224 G).

Secondary mycelium:

In contrast to the other species, the secondary mycelium is light brown in colour to hyaline and colourless, and comprises long branched hyphae 2.7 - 5.1 μ diam. which are loosely associated and do not anastomose frequently.

Conidiophores and conidia: (Fig. 193 A):

The conidiophore is similar to those of H. nummularium and H. merrillii, variable in length but usually long, 100 - 300 x 3 - 4 μ , distinct from the vegetative mycelium due to slightly darker colouration, and fine pitting on the walls. The ultimate fertile branches are borne in single or compound verticils and are 9 - 2 x 2.5 - 3.5 μ . The apices of the fertile branches are swollen or clavate but not as conspicuously stout as in allied species. The conidia are borne in heads from the apices of the branches and are hyaline individually though white en masse; oval equilateral with blunt or rounded ends, 2.3 - 3.4 x 3.4 - 5.7, av. 2.8 x 4.8 μ .

19). Hypoxyton exutans:Malt. A : Bottle Culture. (Fig. 194):

Appearance: Dense felty with coarse surface; aerial mycelium up to 2 mms. high, white subhyaline at first (7 days) later opaque, and tinted faint olive green or ochre brown with age (1 - 2 months). The surface is characteristically coarse with the outermost aerial hyphae straggling towards the margin.

Margin: Canescent, entire with effuse peripheral hyphae, and the usual exploratory hyphae considerably in advance of the rest of the colony.

Conidia: Appearing after 7 - 10 days, white subhyaline, scattered over the surface of the colony but not conspicuous.

Stain: Appearing after 7 days, at first light ochre brown but deepening with age (1 month) to feuille morte (S. 191).

B : Plate Culture. (Figs. 198, 199):

Appearance: Loosely felty, luxuriant; aerial mycelium white subhyaline, up to 5 mm. high with very coarse surface, and straggling superficial hyphae.

Stain: Orange ochre (S.247) or olive green (S.315) usually the latter. Other details as above.

Other media: B o t t l e C u l t u r e .

M a i z e . (Fig . 195) . L e o n i a n ' s . (Fig . 196) . C z a p e k . (Fig . 197) .

Less luxuriant; at first Similar to malt. At first similar to
canescent, and developing malt and Leonian's,
characteristic felty tex- later (3 weeks) cot-
ture after 2 weeks. tony lanose and more

Stain: Plate amber. (S.249).

luxuriant.

P l a t e C u l t u r e .

M a i z e . L e o n i a n ' s . C z a p e k . (Fig . 200) .

Similar to malt. Similar to malt. Similar to malt but
with additional amber
tint to mycelium.

The gross external characters of this species differ little from the preceding except for the deeper hue of the stain and the coarse straggling instead of granulate surface.

Microscopic characters:

Primary mycelium: (Fig. 224 I):

This is similar to that described for the previous species but the marginal hyphae are rather more closely septate. They are: 2.7 - 5.3 μ diam.

Secondary mycelium: (Fig. 225 C):

The odd nature of the secondary dark mycelium distinguishes this species from all others investigated. At first it consists of loosely associated hyphae 3.0 - 4.6 μ ; later the individual cells broaden out and the cells contents round up so that the ultimate appearance of a hypha is that of a string of beads. It is not known whether the individual cells can behave as chlamy dospores.

Conidiophores and conidia: (Fig. 232 A):

The conidiophore is distinct from the vegetative mycelium due to its darker colouration and pitted walls, but varies greatly in manner of branching, from very lax to extremely compact. The latter type resembles that of H. merrillii, H. nummularium, and Nummularia uni-apiculata.

The fertile branches are narrow and elongate (6 - 27 x 1.2-2.0 μ) or short and stout (7 - 12 x 3.0-3.4 μ) with bulbous apices, depending on whether the conidiophore is lax or compact. The conidia are narrowly elliptic to pyriform, with both ends rounded or with one acute or sometimes tailed, colourless and hyaline individually but white en masse, 1.2 - 2.3 x 3.4-5.7 μ , av. 1.7 x 4.5 μ .

20). H. merrillii:

Malt. A : Bottle Culture: (Figs. 201 - 203):

Appearance: At first canescent appressed, with aerial mycelium sparsely developed, later (4 - 7 days) developing a characteristic loose cottony white subhyaline to opaque mat with a coarse straggling appearance. After 5 days the mycelium becomes tinted with various colours, usually buff or dark brown and rose; olive

green near the site of the inoculation. In 4 out of the 8 strains investigated the initial coarse felty mycelium was overlaid after 3 - 8 weeks by a superficial felty velvety opaque layer of mycelium which developed from the centre. The appearance of old cultures may thus differ considerably from the young. In the other 4 cultures the mycelium thickened with age but did not become felty. This difference was reflected also in monospore cultures of the two types as well as in multispore.

- Margin: Entire, with widely effuse submersed hyphae of variable length, 3 - 10 mms. long.
- Conidia: Very variable in colour, white, pale pink or olivaceous; appearing after 10 days over the whole of the colony but not conspicuous.
- Stain: Also variable in colour, but normally in shades of yellow ochre (S. 201) or yellow pink (S. 185; incarnat rosé) but also olive green (S. 426 - 431). With age the stain always turns near black in colour.

B : Plate Culture. (Fig.205):

Appearance: Similar to the above in general features, forming an appressed coarse felty mat which is about 3 mms. high, opaque white in the centre but sometimes white subhyaline towards the margin. The mycelium usually becomes tinted with age. Fawn coloured conidia usually appear after 14 days. Stain light ochre brown (S. 131), diffuse.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k . (Fig.204) .
Similar to malt, some colonies also overlaid by felty mycelium after 14 days.	Similar to malt and maize.	Similar to malt and maize.

P l a t e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k . (Fig.207) .
Similar to malt but slightly less luxuriant; subhyaline. No stain.	Similar to malt, slightly more luxuriant. No stain.	Differs from other cultures by greater, though varying, opacity. Stain very faint amber.

Microscopic characters:Primary mycelium:

Relatively stout, with a maximum diameter of 4.4μ . The marginal hyphae are simple in form compound with some of the species described later. (Fig. 224 D).

Secondary mycelium:

Forms a loosely interclasping and anastomosing felt beneath the primary mycelium but is not so conspicuous as in Hypoxylon glomeratum and H. exutans. The diameter of the mycelium ranges from $2.7 - 6.4 \mu$. (Fig. 225 B).

Conidiophores and conidia: (Figs. 208, 209 A):

In contrast to most of the foregoing species the conidiophores are usually sharply distinguished from the ordinary mycelium by determinate length and dark colouration of the main axis. There is much variation, however, in form of the conidiophore. The simplest type, which occurs towards the outer part and margin of bottle and plate cultures, consists of a main axis which is slightly larger in diameter than the primary mycelium though not darkly stained; and several short fertile hyphae $10 - 40 \mu$ long which branch out singly or in groups of 3 - 4 from the main axis and bear apical clusters of conidia. Intermediates between this and the next type are found where the fertile hyphae become restricted to the terminal part of the conidiophore. The most complex form, and also the most typical is found near the centre of the culture and consists of a long stout axis which is dark olive green in colour and $200 - 300 \times 4 - 7 \mu$, ending in a "head" of closely set fertile branches. These branches arise by dichotomous or ternate forking and may themselves branch again to the first or second degree. They are swollen or clavate, and very short, $6 - 14 \times 3 - 4 \mu$, and bear apical clusters of conidia. Both types of conidiophore are further distinguished by the minute pits that occur extensively along the surface of the walls.

The conidia are oval elliptic, characteristically rather narrow and inequilateral with one end broader than the other, but also oval equilateral. Sometimes the conidia are tailed due to apical prolongation of the conidium into a spicate process. They measure $4.0 - 7.2 \times 1.7 - 2.6 \mu$, av. $2.2 \times 5.4 \mu$ (excluding tails).

21). Hypoxyylon mediterraneum:Malt. A : Bottle Culture.

Appearance: Colony at first (1 - 3 days) appressed felty and subhyaline, later the aerial mycelium thickens to nearly 3 mm. in height and becomes opaque, dull white with olive green colouration round site of inoculation.

Margin: Effuse, entire with widely separated long pioneer hyphae.

Conidia: Produced after 7 days all over the surface of the colony, white subhyaline and not conspicuous.

Stain: Appears after 4 days as a delicate pink and gradually darkens to reddish ochre brown (S.131). It is diffuse through the entire colony but is deeper near the centre.

B : Plate Culture. (Fig. 206):

Appearance: Similar to the above, eventually forming a thin velvet felty mat of aerial mycelium up to 2 mm. high with a coarse surface. With age the cultures often become granulate. Dull olive green colouration develops round site of inoculation.

Margin: Entire, effuse, with widely separated submersed exploratory hyphae.

Conidia: Appear after 7 days, on the surface of the aerial mycelium, but are not very conspicuous.

Stain: Pale ochre brown, variable in hue and sometimes very faint.

Other media: B o t t l e C u l t u r e .

<u>M a i z e .</u>	<u>L e o n i a n ' s . (Fig.210).</u>	<u>C z a p e k . (Fig.211).</u>
Similar to malt but less luxuriant. No stain.	Similar to malt.	Less luxuriant than on other media up to 7 days of age then rapidly developing a dense felt with more intense purple ochre hue than on other media; later grey to dirty ochre yellow. Stain appearing after 10 days, light purple (S.107) deepening to dark ochre and then black.

Plate Culture.

Maize.	Leonian's.(Fig.212,213).	Czapek.(Fig.215).
Similar to malt, sometimes less luxu- riant.	Similar to malt.	Similar to malt.

Cultures of this species and H. merrillii are very similar when both well grown, and can only be distinguished by the following features:-

1. Plate cultures of H. mediterraneum are white subhyaline when 2 days old, while those of H. merrillii are more opaque.
2. Czapek bottle cultures of H. mediterraneum are distinguished by the deep ochre yellow to brown mycelium and initial purple colour of the stain. Similar cultures of H. merrillii are not so strikingly coloured.

Microscopic characters:

The microscopic characters easily distinguish this species from its nearest relatives.

Primary mycelium. (Fig. 224 F):

The primary mycelium is characterized by the presence of large stout hyphae with much narrower short curled branches. The outermost of these stout hyphae often grow to a considerable distance in advance of the colony. They range in diameter from 5.5 - 6.6 μ .

Secondary mycelium. (Fig. 225 D):

This forms a loosely branched network of light brown stout hyphae 3.6 - 9.7 μ diam., underneath the primary mycelium. The hyphae are on the whole larger in size than those of H. merrillii and do not anastomose so frequently.

Conidiophores and conidia: (Fig. 216):

The variation in size, shape and organization of the conidiophores is very great in this species. The conidia may be produced in a group from the side of an ordinary (undifferentiated) hypha, in which case no true "conidiophore" exists, or from the apices of distinct fertile branches 3 - 17 μ

x $2.0 - 3.5 \mu$ that arise singly or in pairs or whorls. In the last case a loosely branched conidiophore $100 - 200 \mu$ can be distinguished from the surrounding mycelium, but it is quite different in form from the conidiophores of H. merrillii since it lacks the long stalk, dark colouration, and pitted walls of that species.

All types of conidiophores can be found in any one plate or bottle culture of H. mediterraneum, whether multi- or monoascospore in origin. Usually, however, the more complex types are to be found in the oldest part of the colony.

Conidia:

These are considerably larger in size than those of allied species. They are oval equilateral with rounded ends, white subhyaline, $1.7 - 3.9 \times 4.0 - 8.8 \mu$, av. $3.0 \times 5.7 \mu$.

22). Hypoxyton nummularium:

Malt. A : Bottle Culture. (Fig. 217):

Appearance: Felty, with very dense aerial mycelium, eventually 1 - 3 mm. high, white at first (10 days), tinted pale yellow at the site of inoculation but later tinged very pale purple throughout. Surface initially coarse, later becoming smooth.

Margin: Canescent to felty, entire with effuse widely separated peripheral hyphae.

Conidia: White subhyaline, tinted purple, produced after 10 - 14 days, but not conspicuous.

Stain: Appears after 7 - 10 days, delicate pink at first, later deepening to ruddy black near the centre of the colony (S.106).

B : Plate Culture. (Figs. 219, 220):

Appearance: Similar to the above, felty cottony with aerial mycelium up to 3 mm. high, loose in texture, white subhyaline with a marked purple tinge.

Margin: As above; peripheral hyphae very effuse, submersed and usually widely separated.

Conidia: Appearing gradually after 7 days, from the centre outwards, over the surface of the aerial mycelium.

Stain: Bright orange pink (S.182; terre cuite) varying to murky dark purple brown, developing after 24 hours.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s . (F i g . 2 1 8) .	C z a p e k .
Similar to malt but stain very reduced in quantity.	Similar to malt.	Similar to malt but colouration of mycelium intensified and purple stain very lurid.

P l a t e C u l t u r e .

M a i z e .	L e o n i a n ' s . (F i g . 2 2 1) .	C z a p e k . (F i g . 2 2 2) .
Similar to malt but purple tint much lighter in hue and stain absent.	Similar to malt but with smoother appearance.	Highly peculiar; velvet felty, appressed with moderately smooth surface; mycelium white on exterior of colony and light yellow ochre at centre. Margin, in contrast to other cultures, not entire, lobed or broadly segmented Stain: lurid purple.

Microscopic characters:

Primary mycelium. (Fig. 224 E):

This species is peculiar in that the long characteristic exploratory hyphae are in this case unusually stout (max. 5.4μ) and bear short and relatively narrow branch hyphae instead of the long branches of equal thickness seen in some other species. The branch hyphae are straight instead of curled as in H. mediterraneum.

Secondary mycelium:

Similar to that in other species, consisting of long anastomosing darkly stained hyphae of max. diam. 4.5μ underneath the primary mycelium.

Conidiophores and conidia:

The conidiophores are of the same plan as those of the specialized type described for H. merrillii. The main axis varies in length from 90 - 140 μ , and is normally wider (3.6 - 5.7 μ), than the vegetative mycelium, and is pink or russet in colour. Branching is dichotomous or ternate, the fertile branches normally being produced in close verticils from the terminal part of the conidiophore. The fertile branches are normally very short and stout, 6 - 16 x 3 - 5 μ , unseptate, and with broad clavate apices. The whole conidiophore is covered with very fine pitting. The conidia are acrogenous and sometimes pleurogenous as well, narrowly elliptic to pyriform with one or both ends acute, purplish brown collectively, 1.4 - 2.6 x 4.3 - 7.6 μ , av. 1.9 x 5.8 μ .

23). Nummularia kalchbrennera:

Malt. A : Bottle Culture. (Fig. 226):

Appearance: Canescent felty, forming a thick, closely appressed mat of aerial mycelium up to 1 mm. high. This is much denser than in H. merrillii and other allied species except for H. nummularium. At first the mycelium is dull white or nearly so, but by a week turns dull yellow, often tinted green. The surface of the colony is smoother than that in other species, and lacks in this type of culture at least, the coarse surface characteristic of other species just described.

Margin: Distinct from the interior of the colony as a submersed subhyaline yellow zone 3 mm. broad with effuse peripheral hyphae.

Conidia: Dull yellow, of similar colouration to the aerial mycelium, and are normally produced after 14 days at the centre of the colony.

Stain: Diffuse orange ochre yellow (S. 246), very conspicuous and produced after 7 days throughout the colony.

B : Plate Culture. (Figs. 228, 229):

Appearance: Velvet felty, dense opaque with a coarsely granulate surface, becoming slightly floccose but never straggling with age. The colour of the mycelium is characteristically yellow buff to pale primrose.

Other details similar to the above but no conidia are produced.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s . (F i g . 2 2 7) .	C z a p e k .
Similar to malt.	Similar to malt.	At first (1-7 days) almost submersed with scantily developed aerial mycelium, later developing a felty layer from the centre so that the colony is ultimately similar to the other cultures.

P l a t e C u l t u r e .

M a i z e . (F i g . 2 3 0) .	L e o n i a n ' s . (F i g . 2 3 1) .	C z a p e k .
Slightly less luxuriant than malt. Stain produced only in part of substrate, pale yellow amber and very diffuse.	Similar to maize and less luxuriant than malt. Surface slightly granulate with age. Stain orange ochre yellow (S.246 & others) deep at centre.	Submersed except for slight canescent aerial colourless mycelium near site of inoculation. Surface smooth, sodden. Stain faint yellow ochre.

On the whole the cultural characters of this species are similar to those of H. nummularium except for the colour of the stain.

Microscopic characters:

Primary mycelium:

Similar to that of H. merrillii; marginal hyphae 2.3 - 3.8 μ diam. (Fig. 224 J).

Secondary mycelium:

Rather slight in quantity, comprising long sparingly branched hyphae 3.6 - 5.2 μ diam. (Fig. 225 E).

Conidiophores and conidia: (Fig. 232 B):

Although of the same basic plan as other members of this group, the conidiophores of this species are sharply distinct on account of the

extremely close type of branching, the swollen and peculiar form of the fertile branches and the basal swelling beneath the point of origin of each verticil of fertile branches. The conidiophores are variable in length, $90 - 150 \times 2.6 - 4.0 \mu$, dichotomously or ternately branched, forming fertile branches in extremely close clusters or in verticils or rarely singly. The fertile branches may be themselves branched to the second or third degree, so that they are often difficult to distinguish apart; and are usually short stout and aseptate, ranging in length from $6 - 40 \mu$, and with elliptic or occasionally clavate apices. The conidia are produced in dense clusters from the apices and upper sides of the fertile branches, are broadly oval or elliptic, equilateral with rounded ends, yellow, tinted green when seen collectively, $1.7 - 3.1 \times 4.0 - 8.0 \mu$, av. $2.5 \times 5.6 \mu$.

Conclusions and Discussion:-

A : The characters of the imperfect stage:

1. Growth characters of the mycelium:

The preceding account of the cultural characters were grouped for convenience according to growth type and the differences pointed out between similar forms in each group. The following general conclusions can be made with regard to the growth characters of the genera on malt:-

<u>G e n u s .</u>	<u>G r o w t h C h a r a c t e r .</u>
<u>Rosellinia</u> and <u>Hypoxylon</u> (Endoxylon)	The range of growth character is identical in both groups; varying from submersed or appressed subhyaline to fleecy or lanose, zonate or uniform; <u>but:</u> (1) the coarse felty type is rare; (2) the mycelium is <u>characteristically</u> pure white (only one exception viz. <u>R. moroides</u>); (3) Stain is rarely produced and, if so, in varying shades of brown or yellow.
<u>Nummularia</u> (including the species withdrawn by Miller as well as those retained in the genus).	Characteristically coarse felty with straggly surface, though other types are also recorded (<u>N. succenturiata</u> , <u>H. asarcodes</u>); <u>and:</u> (1) the mycelium is either pure white or coloured.

G e n u s .

G r o w t h C h a r a c t e r .

- (ii) Stain is produced in all cases except one, in varying shades of ochre brown, olive green or dark purple.

The genera cannot be distinguished absolutely on the basis of growth character, although there is undoubtedly a characteristic growth form. The species within these genera can usually be distinguished clearly by one or more features but there are exceptions. Two pairs of species that are clearly separable on characters of the perfect stage develop respectively a nearly identical type of growth in culture. These are Rosellinia aquila and Rosellinia thelena, and Hypoxyton glomeratum (var.5) and Nummularia succenturiata.

On media other than malt the characteristic features distinguishing each species tend to be less pronounced, often, though not necessarily, due to lesser luxuriance in growth. In general, the growth characters on different media can be compared with those on malt in the following way:-

<u>M a i z e .</u>	<u>L e o n i a n ' s .</u>	<u>C z a p e k .</u>
Normally less luxuriant than malt and with lesser production of stain.	Similar to malt, occasionally more luxuriant.	Normally less luxuriant than malt, similar to maize but often distinguished by intensification of colour of mycelium and stain.

Although it is often possible to tell species apart on these 3 media it is usually difficult to formulate the differences between them in precise terminology. The characters on malt, with some striking exception, should therefore be given more emphasis in description.

2. Germination:

A minor feature observed was the difference in manner of growth of the hyphae from the germinating ascospores on malt agar between the great majority of Rosellinia species on one hand and Hypoxyton and Nummularia on the other. In all species of Rosellinia except R. aquila and R.

thelena the young hyphae radiate symmetrically in all directions, while in the others the advancement of the hyphae takes place in one or two directions and is much less symmetrical. See Fig. 233.

3. Growth Rates:

For details of the results obtained and their illustration and statistical analysis, Appendix III, Table V, should be consulted together with Graphs B - F.

The graphs show that the considerable variation in growth rate between the strains cultured is correlated with specific differences expressed by sporographs. Both sporographs and growth rate curves are shown together for easy comparison.

Two groups of species can be roughly distinguished on rate of growth at the optimum temperature. (See classification at end of Chapter II).

(1). Growth slow to moderate:

Rosellinia species except R. aquila and R. thelena.

Hypoxylon glomeratum (all varieties).

Nummularia succenturiata.

H. asarcodes.

Hypoxylon 13 A.

H. nummularium.

H. mediterraneum, strain 427.

Nummularia kalchbrennera.

(2). Growth fast:

Rosellinia aquila.

R. thelena.

Hypoxylon merrillii.

H. mediterraneum (strains 19 and 31).

H. exutans.

Nummularia uni-apiculata.

In the first group, all except the last 4 species have a growth character that is not coarse felty. In the second group, the coarse felty type is predominant. Therefore there appears to be a partial correlation between the coarse felty type of mycelium and a high growth rate.

Statistical analysis shows a high degree of significance (at the 5% level) between the growth rates of different strains cultured. In the case of H. mediterraneum, however, where 3 strains were investigated, each strain was found to be significantly different from the two others at one or more temperatures, and thus it was difficult to consider the species as a whole. For this reason too much weight must not be placed on these results.

(3). The Structure of the Conidiophore:

The variations in morphology observed are basically due to differences in development. There is obvious specialization in form from a type where the conidia are pleurogenous, arising more or less at random as the fertile hypha develops, to the one where the conidia are restricted to the apical region because they only appear when the fertile hypha has nearly reached the limit of its growth. The development of the conidia has been observed in detail in Rosellinia corticalis, Hypoxylon glomeratum varieties 1 and 4, and H. merrillii. Slide cultures were made and observed at intervals of 6 hours, areas of development being marked on coverslip. The development of the conidia, as so observed, appears to conform to a general plan throughout the group. The apex of the hypha swells out into a conidium. While the conidium is cut off by a septum, that part of the hypha behind it grows out and continues growth, forcing the conidia to a lateral position (See fig. 169 B). In Rosellinia and in some Hypoxylon species (e.g. H. glomeratum, var. 1) the original apex of the fertile hypha persists as a distinct protuberance beneath each conidium and where the conidia are clustered the fertile portion of the hypha has a distinct serrate outline. In other species of Hypoxylon, e.g. H. glomeratum, var. 3., and in the Nummularium group, however, the short and usually bulbous fertile branches are merely roughened by the close development of the conidia. In contrast with the type just described, the conidia sometimes mature after the apex of the fertile branch has elongated past it. It is often difficult, moreover, to be exactly sure of the manner of development of the youngest conidia; often they appear to form after the fertile branch has ceased to grow.

The most unspecialised types of conidiophores are those of Rosellinia obtusissima, H. glomeratum types 4 and 5, and Nummularia succenturiata, where the conidia are produced singly or in groups of 2 - 4 from the sides of the fertile hyphae, and the fertile hyphae cannot be morphologically

distinguished from the vegetative mycelium. (See Figs. 116, 117 A, 140, 169). From these a series can be traced, firstly to a condition where the conidia arise in distinct pleuracrogenous clusters (Figs. 117 B, 99 C) and thence to where they are acrogenous or nearly so (Fig. 208) correlated with this specialization is a contraction in length of the fertile branches so that in the most specialized forms they are typically bulbous or swollen. Furthermore, the branching of the main axis and side branches changes from a dichotomous to a characteristic ternate or whorled condition and the conidiophores as a whole appears distinct from the vegetative mycelium due to the darker colour, the frequent presence of fine external wall pitting and the development of a broad stipe below the fertile branches. Due to the fact that conidia were not found for all the species can be constructed, but the different types can still be grouped as follows:-

Type I:

Conidia pleuracrogenous and often situated at random; conidiophores not distinct from the vegetative mycelium.

Rosellinia obtusissima.
Hypoxylon glomeratum, var. 4; var. 5.
Nummularia succenturiata.

Type II:

Conidia arising in groups from the sides of indifferentiated hyphae.

Hypoxylon mediterraneum. (part).

Type III:

Conidia pleuracrogenous; normally forming terminal or intercalary spicate clusters; conidiophores dichotomously branched, only distinct from the vegetative mycelium in the determinate and shorter nature of the branches.

Rosellinia protuberans.
Rosellinia moroides.
Rosellinia mammoidea.
Rosellinia aquila.

Type IV A:

Conidia as above; branching of the conidiophores characteristically though not invariably ternate.

Rosellinia corticalis.
Hypoxylon glomeratum, var. 1.

Type V:

Conidia acrogenous forming apical clusters, sometimes accompanied by a pleurogenous cluster beneath but never similar to Type III; conidiophores if present dichotomously branched.

A: Conidia developed from short fertile hyphae not organised in a conidiophore.

H. mediterraneum (part)

H. merrillii (part)

B: Conidia developed from short fertile hyphae arising from a distinct conidiophore.

Hypoxylon glomeratum, var. 2 & 3.

Hypoxylon 13 A.

Type VI A:

Conidia mainly acrogenous, sometimes pleuroacrogenous, in apical clusters; conidiophores normally branched terminally, usually terminate but sometimes dichotomous, giving rise to short swollen or clavate fertile branches. Dark colouration and fine wall pitting distinguish the conidiophore from the vegetative mycelium.

(Includes majority of Nummularium group).

Hypoxylon merrillii (part).

Hypoxylon nummularium.

Hypoxylon mediterraneum (part).

Hypoxylon exutans.

Nummularia uni-apiculata.

Nummularia kalchbrennera.

It can be seen that there are often clear cut differences between conidiophore types so that these can be used as valuable taxonomic characters. In a minority of cases, however, the conidiophores of closely related species are so similar that they cannot be used, as in Group VI. Moreover, in one case, H. mediterraneum, the form of the conidiophore and manner of development of the conidiophore varies greatly within the same species.

B : CORRELATION OF DIFFERENCES IN PERFECT STAGE WITH THOSE IN CULTURAL CHARACTERS.

S P E C I E S.	STROMAL CHARACTER.	AVERAGE SPORE SIZE (μ).	GROWTH CHARACTER (MALT PLATE).	CONIDIO- PHORE TYPE.	STAIN.	PRESENCE OF SECONDARY MYCELIUM.	G R O W T H R A T E.	
							OPTIMUM.	CHARACTER. *
Rosellinia protuberans	Globose,1-2 peritheciate, immersed.	5.5 x 10.7	Mainly velvety, white.	-	-	-	25°C	Very slow
R.obtusissima	Globose,1-2 peritheciate, immersed.	7.4 x 13.3	Coarse felty or velvet - felty white.	I	-	-	20°C	Moderate
R.pulveracea	Globose,1- peritheciate, superficial.	6.2 x 8.5	Velvety white	-	-	-	25°C	Slow
R. moroides	Globose,1- peritheciate, superficial.	7.6 x 12.2	Canescent yellow.	III	Orange- Yellow	-	28°C	Moderate
R. apiculata	Globose,1-4 peritheciate, superficial.	4.5 x 9.1	Canescent ap- pressed,sub- hyaline white	-	-	-	25°C	Moderate
R. mammoidea	Globose,1-2 peritheciate, base immersed.	7.6 x 15.0	Velvet-fleecy, zonate white	III	-	-	20°C	Slow
R.corticalis	Globose to pulvinate 1-∞ peritheciate,base immer- sed.	6.7 x 15.7	Velvet to cot- tony-lanose, uniform	IV A	-	-	25°C	Very Slow
R. thelena	Globose,1-2 peritheciate, superficial	6.8 x 18.7	Smooth silky- felty,white	-	-	+	25° (Grows at 28°)	Fast
R. aquila	Globose,1-2 peritheciate, superficial	7.7 x 25.4	Smooth silky- felty,white	III	-	+	25° (does not grow at 28°)	Fast
Hypoxylon glomeratum var. 1.	Globose or pulvinate,1- several peritheciate, perithecia evident in outline. superficial.	5.6 x 13.3	Submersed to downy; conidia profuse; sub- hyaline white	IV A	-	-	20°C	Slow
var. 2.	Pulvinate or effuse,pe- rithecia evident in outline; superficial.	6.3 x 14.0	Submersed to downy; conidia sparse; subhya- line white.	V B	Dull brown	+	25°C	Moderate
var. 3.	Pulvinate,very small, perithecia not evident in outline. superficial.	6.0 x 14.3	Canescent- velvety,zonate white.	V B	-	-	25°C	Slow
var. 4.	Effuse orbicular,perithe- cia not evident in outline superficial.	4.9 x 10.1	Velvet-fleecy, zonate white.	I	-	+	28°C	Moderate

var. 5.	Effuse orbicular, perithecia not evident in outline, base immersed in substrate.	4.6 x 9.7	Velvet-fleecy, uniform white.	I	-	+	25°C	Moderate
Nummularia succenturiata	Ost. indistinct; indefinitely effused or orbicular, dull, immersed or superficial.	6.7 x 15.5	Velvet fleecy, uniform white.	I	-	+	25°C	Slow
Hypoxyton exutans	Ost. indistinct; indefinitely effused or orbicular, dull immersed.	5.7 x 12.9	Coarse, felty, white.	VI A	Dark olive green.	+(peculiar).	25°C	Fast
Nummularia uni-apiculata	Ost. indistinct; indefinitely effused, rarely orbicular, dull immersed.	6.5 x 12.2	Coarse felty, granulate white.	VI A	Very pale brown or absent.	+	25°C	Fast
Hypoxyton 13A	Orbicular with truncate ostioles, immersed, shiny.	2.3 x 7.1	Coarse felty to velvety dull white.	V B	Murky nondescript brown.	+	25°C	Moderate
H. merrillii	Ost. papillate orbicular or indefinitely effused, immersed, shiny.	5.5 x 11.8	Coarse felty white discoloured.	V A & VI A	Dark ochre brown or olive green.	+	25°C	Fast
Hypoxyton nummularium	Ost. papillate; stroma indefinitely effused, shiny; immersed.	6.2 x 13.3	Coarse felty, dull purple.	VI A	Dull purple	+	25°C	Moderate
Hypoxyton mediterraneum	Ost. papillate; stroma indefinitely effused; dull, immersed.	7.2 x 15.6	Coarse felty, white discoloured.	II, V A VI A (Conidia unusually large.)	Dark ochre brown.	+	25°C	Fast or Moderate
Hypoxyton asarcodes	Ost. papillate; stroma orbicular effuse, dull, immersed.	7.4 x 14.7	Canescent to velvety, dull white subhyaline.	- (chlamydospores present)	slate grey.	-	25°C	Slow
Nummularia kalchbrennera	Ost. papillate; stroma orbicular with definite circular outline shiny, immersed.	5.0 x 10.9	Coarse velvet-felty, granulate, dull yellow.	VI A	Dull yellow to ochre	+(peculiar)	25°C	Moderate

* The following arbitrary standards have been made:

Very slow	<	1.5 mm. per day
Slow	1.5	2.5 mm. per day
Moderate	2.5	4.0 mm. per day
Fast	>	4.0 mm. per day.

It can be seen from this table that the great majority of the species are separable on cultural characters alone and, furthermore, that the small differences in stromal form which divide closely related species are supported by differences in the nature of the mycelium, conidiophores and growth rate, and therefore must be taken as valid criteria in classification. Of particular interest are the following species groups:-

1. Rosellinia mammoidea and R. corticalis:

The separation of the perfect stages is based on differences in degree of aggregation of the perithecia and slight differences in spore size. These characters are fully supported by cultural differences.

2. The Hypoxyylon glomeratum complex:

These 5 types are probably sufficiently distinct on both stromal and cultural characters to be given separate specific rank. Obviously the species needs re-definition.

3. The Nummularium group of species:

Although it can be said that there is a characteristic type of growth character and conidiophore for this group, the group cannot be separated from the other 2 on cultural characters alone because these features are not sufficiently constant. The similarity of stroma form to the effuse types of H. glomeratum has already been noted.

The genus Nummularia, considered under its original broad concept, can therefore, be best regarded as a specialized development in one direction from typical Hypoxyylon forms similar to H. glomeratum, and the boundary between the two is still difficult to define. Miller's transfer of many of the original species to Hypoxyylon is therefore justified. His retention later of the term Nummularia for species with concave stromata cannot be challenged with reference to one species alone, but the similarity of N. kalchbrennera in many other respects to various species indicates that the distinction may be unsound.

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CHAPTER IV.

THE ANNULATUM GROUP OF HYPOXYLON.A : The Perfect Stage.

The species in this group have the following characters in common:-

1. Mature stroma variable in form but both ecto- and entostroma carbonous and impossible to distinguish on anatomical grounds. In some species peculiar superficial particles are formed on the stroma (see p.100 and Figs. 246, 247B, 249, 256).
2. Perithecia occupying the periphery rather than the base of the stroma due to greater development of the tissue beneath.
3. The ostioles are annulate, i.e. the apex of each perithecium projects through the stromal surface when mature. The ostiole therefore does not comprise any stromal tissue. A distinct annular depression is evident on the surface of the stroma where each perithecium breaks through.

The group as a whole is distinct from the preceding but there is an intermediate species, Hypoxyton sp. 13A already described, which has annulate and ostioles. The aplanate erumpent type of stroma with uncarbonized tissue surrounding the perithecial bases is more characteristic of the Nummularia concept, however, so that species has been treated with the members of that group.

Key to the Species:

1. Stromata usually erumpent and always partly immersed in the substrate at maturity, globose or pulvinate; perithecia globose.
Spores 4.0 - 7.0 x 8 - 13.0 μ , av. 5.1 x 10.3 μ .
Rosellinia 3A (1,p.99).
- 1'. Stromata usually superficial, sometimes embedded in the substrate but never erumpent through it; variable in form. Perithecia with flattened or truncate apices ----- 2.
2. Stromata pulvinate or aplanate, sometimes orbicular or effuse
- 2'. Stromata globose, though variable in size ----- 3.
Spores 4 - 6 x 17 - 22 μ . Hypoxyton gilletianum *
* Not dealt with due to lack of viable material (See Fig. 258).

3. Ostiolar discs very small, inconspicuous, and sometimes incompletely formed. Spores $4.0 - 7.0 \times 11.0 - 16.0 \mu$, average $5.3 \times 13.1 \mu$. ----- Hypoxylon michelianum. (2, p.100).
- 3'. Ostiolar discs normally conspicuous, spores below 12μ in length, stroma coloured when young. ----- 4.
4. Spores brown, subhyaline (size $3.0 - 7.0 \times 7.0 - 12.0 \mu$, average $4.1 \times 8.7 \mu$; stroma metallic purple or dull brick red when young fading to black with age). ---
--- Hypoxylon microcarpum. (4, p.101).
- 4'. Spores yellow or amber, subhyaline. ----- 5.
5. Spores amber, subhyaline (size $3.0 - 4.5 \times 6.0 - 9.0 \mu$, average $3.6 \times 7.3 \mu$; stroma purple when young, fading to black). ----- Hypoxylon stygium. (3, p.100).
- 5'. Spores yellow, subhyaline, larger----- 6.
6. Stromata uni- or multiperitheciate, aplanate or aplanopulvinate, samples usually showing clearly all degrees of perithecial aggregation from an entirely separate to a loosely coalesced condition.--- (Size of spores $3.0 - 5.5 \times 7.5 \times 12.0 \mu$, average $4.2 \times 9.4 \mu$; stroma white when young).
Rosellinia nitens. (6, p.102).
- 6'. Stromata occasionally uniperitheciate but normally multiperitheciate with coalesced perithecia; stromata variable in form, aplanate, pulvinate or semi-circular, superficial or partly immersed in the substrate. (Size of spores $3.0 - 6.0 \times 6.0 - 11.0 \mu$, average $4.4 \times 8.5 \mu$; stroma tawny or yellow when young). ---
--- Hypoxylon truncatum. (5, p.101).

1. Rosellinia 3 A: (Figs. 234 - 238, 257 A).

Stromata uniperitheciate globose, or multiperitheciate and pulvinate, but the number of perithecia rarely exceeds 6 per stroma. The surfaces of the naked perithecial walls are shiny. The perithecia are globose with thick carbonous walls, $600 - 900 \times 800 - 1100 \mu$. Asci cylindrical with moderately long stipes, $105 - 115 \times 6 \mu$, stalks $25 - 50 \mu$. Spores oval equilateral with rounded ends, pale amber yellow, $4.0 - 7.0 \times 8 - 13.0 \mu$, average $5.1 \times 10.3 \mu$ (180). The details of this species do not conform to

any description in the literature.

Hosts: Canthium spinosum

Cassine croceum

Tarchonanthus camphoratus, wood and bark.

2. Hypoxylon michelianum. Ces and de Not. (Figs, 239-243, 257 C):

Stromata usually multiperitheciate and aplanate effuse, but sometimes pulvinate when containing one or a few perithecia. Surface of the stroma usually shiny and uneven or smooth depending on the degree of immersion of the perithecia within. Ostiolar discs very small and inconspicuous though the ostioles themselves are often tapering and spout-shaped; perithecia relatively large, with carbonous walls, $750 - 1200 \times 700 - 2000 \mu$. Asci cylindrical, long stipitate, $180 - 250 \times 6 \mu$, stalks $100 - 170 \mu$. Spores oval inequilateral with 1 side straight and the other concave; colour dark chocolate brown; size $3.5 - 7.0 \times 11.0 - 16.0 \mu$, average $5.3 \times 13.1 \mu$ (60).

The average length of the spores is 3μ less than that described for the type (cited by Saccardo: Syll. Fungorum I, 385), but material sent to Kew and to Dr. J.H. Miller was classified as this species by both authorities.

Hosts: Olea capensis

and wood unidentifiable.

3. Hypoxylon stygium (Lév) Saccardo. (Figs. 244 - 246, 257 B):

Stromata multiperitheciate or occasionally uniperitheciate, aplanate or aplano-pulvinate, shiny, with truncate perithecial vertices. Two characters that distinguish this species from the foregoing are also shared by H. truncatum, H. microcarpum and Rosellinia nitens to be discussed shortly. These are the initial bright surface colouration of the stroma and the presence of spherical mucilaginous or granular vacuolate "ectostromal particles".

The latter appear during late development of the stroma but the manner of formation is not known. In H. stygium both the colour of these particles and the rest of the young stroma is dull purple-red. Unfortunately the colour fades with age and so cannot in itself be used as a constant character.

The perithecia are globose, with heavily carbonised walls, $500 - 800 \times 750 - 900 \mu$. Asci cylindrical, long stipitate, $130 - 160 \times 4.5 \mu$, stalks $75 - 105 \mu$. Spores oval, equilateral, amber, subhyaline, $3.0 - 4.5 \times 6.0 - 9.0 \mu$, average $3.6 \times 7.3 \mu$. (90).

Hosts: Acacia mollissima

Olea capensis, wood and bark.

4. Hypoxyton microcarpum. Penz. et Sacc. (Figs. 247 - 249, 257 D):

Stromata aplanate, or pulvinate, normally multiperitheciate but also comprising forms where the perithecia are single or loosely aggregated. The colour of the young and early mature stroma varies from brick-red to metallic purple. Perithecia variable in size, 550 - 1000 x 550 - 1300 μ . Asci cylindrical, 120 - 180 x 4.5 μ ., stalks 50 - 90 μ . Spores oval elliptic, light brown subhyaline, 3.0 - 7.0 x 7.0 - 12.0 μ , average 4.1 x 8.9 μ . (180).

Hosts: Olea capensis

and wood unidentifiable.

H. microcarpum was established by Saccardo (1897) for a species with purple stromata and a range of spore size slightly greater than that of H. stygium. Later the two species were merged by Miller (1932, and probably earlier), although no formal emendation was apparently made.

The spore measurements were as follows:-

Strain 511: 3.5 - 7.0 x 6.5 - 12.0 μ , av. 4.3 x 8.9 μ .

557: 3.0 - 5.5 x 7.5 - 12.0 μ , av. 3.9 x 9.0 μ .

Both of these were higher than the measurements recorded for H. stygium (strain 234) above. None of the descriptions consulted in the literature (Saccardo 1882; Miller 1932, 1942) recorded a mean length of more than 7 μ . Furthermore, the spores of the 2 samples considered here were darker than those of H. stygium. Notwithstanding the fact that both strains 234 and 511 were identified as H. stygium by Kew, the two species will be regarded as separate in this work and the name H. microcarpum will be retained. Statistical analysis (see Table VI, Appendix II) shows that the spore lengths of these species are significantly different. The widths are not invariably so, however, strain 557 being intermediate between strain 234 (H. stygium) and strain 511. Clearly all three strains are closely related, and their classification may still be regarded as a matter of interpretation.

5. Hypoxyton truncatum (Schw. ex Fr.) Miller. (Figs. 251, 252, 254-256, 257E).

Stromata similar to the two species just described in essential form and structure but differing mainly in details of colouration and spore size. They are superficial on bark or decorticated wood, occasionally partly embedded in

the substrate, tawny or yellow when young, turning to shining black with maturity. The stromal form varies greatly in shape and size, depending on the number and degree of immersion and the coalescence of the perithecia, from aplanate and aplanopulvinate to pulvinate and nearly globose. Perithecia large, 1200 - 1400 x 1400 - 1700 μ . Asci cylindric, usually long stipitate, 110 - 150 x 4.5 μ , stalks 45 - 105 μ . Spores oval equilateral, occasionally tinted pale brown with age, 3.0 - 6.0 x 6.0 - 11.5 μ , av. 4.4 x 8.5 μ .

Hosts: Probably comprise a very wide range. Those known are:-

Cassine croceum

Royena lucida

Gymnosporia buxifolia

Trichocladus crinitus

Olea capensis

6. Rosellinia nitens. Ces. (Figs. 250, 253).

Stromata similar to the aplanate forms of H. truncatum, forming crusts of indefinite extent and about 2 mm. thick, but with a lesser degree of perithecial coalescence. Initial stage of the stroma white or nearly so. Perithecia 700 - 800 x 1000 - 1200 μ , asci 130 - 170 x 5 μ , stalks 55 - 85 μ . Spores oval equilateral, yellow, 3.0 - 5.5 x 7.5 - 13.0 μ , average 4.2 x 9.4 μ (90).

Hosts: Olea capensis,

Trichocladus crinitus.

This species is merged with H. truncatum by Miller. The material collected and discussed here appears to differ from H. truncatum in several respects but to correspond with the description of Rosellinia nitens given by Saccardo (1882). The name has therefore been retained in this connection. The important points of difference from H. truncatum are:-

1. the lesser degree of perithecial coalescence
2. the white initial stage
3. the difference in spore length (1 μ) which statistical analysis shows to be significant.

Discussion:

The unspecialized members of the group Rosellinia 3A and Hypoxyton michelianum, are similar to H. glomeratum (varieties 1 and 2) in appearance, but the remainder where the ostioles are broadly truncate, form a unique series. The walls of the perithecia in these species are thickened at vertices to form a distinct "shoulder",

the rim of which forms the characteristic ring mark on the stromal surface. The individual species are hard to separate on any one character, since the ranges of spore size overlap and only the averages differ significantly (see Table VI); furthermore the characteristic colour of the group stroma does not usually persist after maturity.

B : Cultural Characteristics:

Key to the species. (Based on malt plate cultures):

- | | | |
|-----|--|--|
| 1. | Stain, if produced, amber yellow to dull brown ----- | 2. |
| 1'. | Stain invariably produced and not as above ----- | 3. |
| 2. | Colonies submersed to canescent, subhyaline --- | <u>Rosellinia 3 A.</u> (1, p.103). |
| 2'. | Colonies velvet-cottony to lanose, opaque ---- | <u>Hypoxylon michelianum.</u>
(2, p.104). |
| 3. | Stain varying from pale olive green to chestnut brown ----- | 5. |
| 3'. | Stain ochre yellow or ochre brown ----- | 4. |
| 4. | Colonies appressed velvety to felty, with a smooth surface throughout development, stain always developing behind the margin of the colony and spreading inwards ----- | <u>Hypoxylon stygium.</u> (3, p.106). |
| 4'. | Colonies velvet-felty with coarse surface, stain sometimes spreading from centre sometimes inwards from the margin- | <u>Hypoxylon microcarpum.</u>
(4, p.109). |
| 5. | Felty; with a coarse surface ----- | <u>Hypoxylon truncatum.</u>
(5, p.111). |
| 5'. | Velvety; surface smooth ----- | <u>Rosellinia nitens.</u>
(6, p.113). |

1. Rosellinia 3 A:

Malt. A : Bottle Culture: (Figs. 259, 260).

Appearance: Mainly submersed; aerial mycelium sparse, closely appressed, not more than 0.2 mm. high, and with a characteristic coarse granulate surface.

Margin: Submersed, almost colourless, entire, peripheral hyphae compact to somewhat effuse.

Conidia: None recorded.

Stain: Variable, sometimes absent altogether but normally apparent after 14 days, at first light orange brown (S. 186: brun garance clair, 174, 193) or amber yellow (S. 250) and deepening to dull brown with age.

B : Plate Culture:

Appearance: As above, almost completely submersed; aerial mycelium very sparse, appressed, white subhyaline, with a smooth fine surface.

Other details as above.

The appearance of both cultures is very similar to that of Rosellinia apiculata. Bottle cultures of Rosellinia 3A can sometimes be distinguished by the production of stain; plate culture by the lack of floc-cose outgrowths developed in the other species.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k .
Less luxuriant than malt, submersed except for scattered silky -plumose filaments near the margin; surface of colony sodden.	Similar to malt.	Similar to maize, aerial mycelium very thin, colourless, hyaline; surface sodden.

P l a t e C u l t u r e .

M a i z e .(Fig. 261).	L e o n i a n ' s .(Fig.262).	C z a p e k .
Submersed except for faint aerial mycelium behind margin; aspect dry.	Similar to malt but surface sodden.	Similar to maize, aspect sodden.

Microscopic characters:

No distinguishing characteristic.

Maximum diameter of primary mycelium = 1.5μ . (Fig. 290 A).

No secondary mycelium.

2. Hypoxylon michelianum:

Malt. A : Bottle Culture (Fig. 263):

Appearance: Velvet lanose with aerial mycelium 2 - 3 mm. high, growth uniform except for a "halo" encircling the inoculum in one culture consisting of a ruff of mycelium 2mm. wide and 2 cm. from the centre. Surface smooth.

Margin: Distinct from the interior of the colony, 3 - 5 mm. broad, submersed to downy, colourless, entire, with compact peripheral hyphae.

Conidia: None recorded.

Stain: Varies with strain, in one (91) light chocolate brown (Séguy 247; orange ochre), in the other (288) light purple (S.4), appearing after 14 - 18 days. The stain does not deepen with age. After 8 months carbonization of the medium begins and a black layer 0.3 mm. deep is formed underneath the colony.

B : Plate Culture (Figs. 266, 267):

Appearance: Velvety lanose, gleaming opaque white; surface smooth but usually rising from the centre to the margin.

Margin: Not distinct from the interior of the colony, usually forming a ruff 3 mm. broad of velvet-silky hyphae, entire or very slightly segmented; peripheral hyphae lying parallel.

Conidia: None recorded.

Stain: None.

Other media: B o t t l e C u l t u r e .

<u>M a i z e .</u>	<u>L e o n i a n ' s . (F i g . 2 6 5) .</u>	<u>C z a p e k . (F i g . 2 6 4) .</u>
Similar to malt. Dense white, velvet lanose.	Similar to malt and maize but with production of conidia in fawn grey crustose areas.	At first (up to 7 days) submersed to downy with sparse aerial mycelium though still white opaque; later felty appressed but never velvety or lanose. With age (> 15 days) the aerial mycelium appears very compact and radia- ting furrows develop from the centre to the margin. Margin distinct, 5 mm. wide downy appressed, white subhyaline. Stain dark chocolate brown produced after 10 days and restricted to the centre of the colony.

Plate Culture.

Maize.	Leonian's.(Fig. 268).	Czapek.(Fig.269).
Less luxuriant than malt, velvety to appressed, white sub-hyaline, surface coarse. Margin not distinct downy to slightly plumose, or submersed. Conidia formed profusely over an area extending from centre to margin, white, granulate.	Similar to malt. Conidia formed over a large part of the centre of the colony.	Similar to maize, less luxuriant than malt; felty velvety to appressed at the margin. No conidia.

Microscopic characters:

Maximum diameter of primary vegetative mycelium = 2.2μ .

Secondary mycelium crustose, composed of very short branched hyphae closely anastomosed, $1.7 - 4.2 \mu$ diameter (Fig.271 C, D).

Conidiophores and conidia: (Figs. 270, 271 A, B):

Conidiophores comprising an indefinitely branched fertile system whose ultimate branches bear small clusters of pleuracrogenous conidia. The main axes, $\approx 2.5 \mu$ diam., are strongly monopodial and usually the fertile hyphae are relatively short laterals. Conidia elongate elliptic or pyriform, usually with one end acute and the other rounded, $2.0 - 3.4 \times 5.1 - 12.5 \mu$, average $2.7 \times 7.4 \mu$.

3. Hypoxyton stygium:

Malt. A : Bottle culture. (Fig. 273):

Appearance: Felty, aerial mycelium up to 2 mm. high, with a moderately coarse to smooth surface. The mycelium is usually tinted cream or green after 2 weeks, and with age finally turns dull grey. In one culture, the colours were restricted to zones of the aerial mycelium so that there was alternation between shades of yellow and yellow-grey.

Margin: Usually distinct from the interior of the colony, depending on the degree of development of the aerial mycelium; 5mm. broad, downy

to submersed, colourless, entire, effuse.

- Conidia: Pale yellow green, formed sparsely or in small pulvinate groups over most of the colony after 7-10 days; rarely absent altogether.
- Stain: Variable, usually dull chestnut brown (unrecorded) but sometimes dark olive green, produced after a variable period of time, usually 6-10 but up to 20 days.

B : Plate Culture:

- Appearance: As above, felty velvety with a moderately coarse to smooth surface, dull white in colour.
- Margin: Entire, effuse.
- Conidia: Rarely produced, if so, in pulvinate pale fawn coloured masses over the entire colony when 14 days old.
- Stain: Chestnut brown, rarely olive green, characteristically first appearing as a broad ring just behind the margin of the colony and spreading inwards to the centre.

Other media: B o t t l e C u l t u r e .

<u>M a i z e .</u>	<u>L e o n i a n ' s .</u>	<u>C z a p e k .</u>
Similar to malt, felty, forming a dense mat up to 2 mm. high with a coarse surface. Interior part of colony light yellow (S.260) margin white subhyaline tinged pink. Margin distinct, up to 5 mms. submersed to canescent, entire. Conidia sparse, produced after 7 days. Stain dark olive brown; also secreted as blood red drops on centre and lower surface of 2 month old colony.	Similar to malt, felty, coarse, pale yellow at first, acquiring a dark olive tint after 10 days and finally turning dull grey brown. Margin distinct, 5-6 mm., submersed to downy, white subhyaline, entire, compact to slightly effuse. Conidia inconspicuous. Stain deep reddish brown, darker towards centre of colony than outside.	More luxuriant than on other media but otherwise similar. Colour of mycelium pale yellow, tinted olive green after 10 days and turning pale grey with age. Margin distinct, 5-6 mm., submersed to downy, white subhyaline with a pink tint and bearing pink or red drops of stain. Conidia inconspicuous. Stain deep reddish

M a i z e .

L e o n i a n ' s .

C z a p e k .

brown darker at centre than outside and extending to 5 mm. beyond margin. Droplets are also produced over entire colony after 14 days.

P l a t e C u l t u r e .

M a i z e .(Fig.274).

L e o n i a n ' s .(Fig.275).

C z a p e k .(Fig.276).

Similar to malt, slightly less luxuriant, appressed felty with coarse surface.

No stain.

Appressed felty, white, mostly subhyaline with coarse granulate surface; slightly depressed towards the centre.

Otherwise very similar to malt.

Felty, white opaque, Colour more intense than on other media and tinted yellow green with age (> 2 weeks) Stain typical, reddish ochre to chestnut (S.153-158).

Microscopic characters:

Primary mycelium similar to that of the preceding, unspecialised, but the marginal hyphae are broader, 1.6 - 2.6 μ diam.(Fig. 290 B).

Secondary mycelium consists of long, frequently branched hyphae loosely anastomosing, maximum diameter 3.1 μ .

Conidiophores and conidia: (Fig. 277):

The conidiophores are clearly distinct from the vegetative mycelium by the greater width of hyphae (max.diam. 4.2 μ), the fine wall pitting, and the deep yellow colour. They are 120 - 160 x 3 - 4 μ long, and are branched to the first or second degree, dichotomous, ternate or quadrately, but usually near the terminal portion of the main axis only. Fertile branches 9 - 30 x 1.4 - 3.0 μ , very rarely septate; apices sometimes globose but not otherwise swollen. Conidia acrogenous, in clusters of 6 - 12 ; oval, equilateral and usually rounded at both ends, yellow green collectively, 2.0 - 2.6 x 3.1 - 5.4 μ , average 2.2 x 3.7 μ .

4. Hypoxylon microcarpum (Fig. 272).Malt. A : Bottle Culture:

Appearance: At first (1 - 4 days) appressed felty, later characteristically dense felty with coarse cottony even surface. Aerial mycelium up to 3 mm. high white subhyaline near the margin but variable in colour towards the centre, normally cream to primrose yellow (S.265) at first, later tinted dull green and after a month finally murky grey.

Margin: Felty appressed, white, sometimes with a broad zone 1.2 cm. wide behind it where the stain shows up conspicuously from beneath; entire, somewhat effuse.

Conidia: Produced after 2 months, forming a dense smooth to finely granulate covering over the surface of the colony, pale purplish brown to mouse grey in colour (S.180 - 705). Two monospore and 2 multispore cultures were grown and both the former were much less luxuriant in conidial production.

Stain: Produced after 4 - 6 days, extending to but not going beyond the margin where it shows up faintly beneath the mycelium. It is roseate brown at first (S. 116 : warm sepia) deepening to chestnut and finally to dull black.

B : Plate Culture: (Fig.278, 279).

Appearance: As above, felty; aerial mycelium up to 2 mm. high, white, tinted dull greenish at the centre, and with the stain showing up conspicuously from beneath. The surface of the colony is smooth at first but later very coarse and uneven. The coarser surface and thicker felt are the main features distinguishing this species from the preceding.

Margin: Usually distinct from the centre of the colony, up to 6 mm. broad, effuse, entire.

Conidia: Produced in some cultures but not in others after 5 days, granulate even, mouse grey in colour.

Stain: Variable, even within mono- and multispore cultures of the same strain. Strain 511 was mainly chestnut brown as in H. stygium but sometimes pale olive green (S.216-218). Strain 557 was light orange brown, tinged green (S.215), or pale olive green (S.218). The time of appearance of the stain varied from when the culture was 5 - 10 days old, and spread from the centre outwards or vice-versa.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k .
Similar to malt but without conidia and stain very much lighter.	Similar to malt, margin not distinct.	Similar to malt but becoming furrowed with age (>14 days), also bearing secreted drops of dark brown stain over the centre. Stain very intense reddish brown, and extending beyond borders of colony.

P l a t e C u l t u r e .

M a i z e .	L e o n i a n ' s . (F i g . 2 8 0) .	C z a p e k . (F i g . 2 8 1) .
Slightly less luxuriant than Leonian's, appressed felty. Stain very dense chestnut brown forming wide zone 13-19 mm. wide on outside of colony; centre colourless.	Similar to malt but margin not distinct. Stain deep chestnut brown, uniform.	Velvet felty with rather slow growth; centre depressed, outer part up to 2mm. high. Colour of mycelium very intense white. Margin indistinct. Stain brilliant rose-ate orange (S.197 on outside to S.181 inside) spreading well beyond margin of colony.

Microscopic characters:

Primary and secondary mycelium very similar to that of the previous species; with maxima of 2.9 and 3.6 μ respectively.

Conidiophores and conidia: (Fig. 282).

Conidiophores essentially similar in most respects to H. stygium but are reddish instead of yellow in colour. They are long, up to $200 \times 1.5 - 2.9 \mu$, dichotomously or ternately branched. Fertile branches $13-40 \mu$ long, normally unseptate but may be up to 2 - septate. Conidia acrogenous, and occasionally also produced either singly or in groups of 2 to 3 from the sides of the fertile and other branches, usually close to a septum. Conidia variable in form, usually oval-elliptic but occasionally elongated and sausage shaped, equilateral or inequilateral and pyriform with one end bulbous and the other acute or acuminate; $1.1 - 2.3 \times 3.7 - 9.7 \mu$, average $1.9 \times 5.3 \mu$.

5. Hypoxylon truncatum:

Malt. A : Bottle Culture: (Fig. 283).

Appearance: Dense felty, forming a mat of aerial mycelium up to 2mm. high, and with characteristic coarse sometimes straggling surface. The colour of the aerial mycelium is white at first (1 - 5 days) then pale yellow (10 days) and finally dull brown to grey with age. The intensity of the colouration varies from gleaming to dull (S.233-245).

Margin: Distinct from the interior of the colony, 5 - 15 mm. broad, submersed to canescent, white subhyaline to colourless, entire with effuse peripheral hyphae.

Conidia: Formed over the whole surface of the colony after 10 - 14 days, pale yellow green but not conspicuous.

Stain: Usually tawny or ochre brown, sometimes slightly reddish but not typically so, (S. 201 - 306; feuille morte) extending 5mm. outside the margin of the colony.

B : Plate Culture:

Appearance: At first (1 - 2 days) appressed subhyaline, later developing characteristic coarse felt of about the same density as H. stygium.

Usually the colony when 8 - 14 days old is differentiated into:

1. a wide centre of felty appressed dull yellow mycelium with darker secondary mycelium and stain conspicuous beneath;
2. Marginal zone, canescent, 7 mm. broad, white with stain paler in colour developing beneath.

- Margin: Distinct (as just described) entire, effuse or compact with the peripheral hyphae lying mainly parallel.
- Conidia: Sparsely produced in the centre of the colony, yellow, inconspicuous.
- Stain: Very deep yellow ochre to tawny (S. 201 - 191, to darker) at the centre, lighter outside.
- Other media: B o t t l e C u l t u r e.

M a i z e.	L e o n i a n ' s.	C z a p e k.
Similar to malt, but mycelium pearly white except for occasional slight yellow tint. Stain more restricted, deep brown to black.	Similar to malt; in l culture zonate with 4 broad zones 6-10 mm. wide of alternating pale yellow and yellow-green. (Colouration fades after 6 weeks).	Similar to malt, but denser aerial mycelium up to 3mm. high. Colour of mycelium varies from white to pale buff or pale pink within the same culture. Stain roseate to reddish ochre (S.122-121) deepening to dull red black.

P l a t e C u l t u r e.

M a i z e.	L e o n i a n ' s. (Fig. 286).	C z a p e k. (Fig. 289).
Less luxuriant than malt, appressed felty; stain reduced.	Similar to malt.	Denser than on other media and with finer surface, colour more intense white to yellow. Stain reddish ochre (S.82-173).

Microscopic characters:

Primary mycelium with no distinguishing character apart from the parallel marginal hyphae, with a max. diam. of 3.4μ .

Secondary mycelium similar to that in the 2 preceding species, max. diam. 4.6μ .

Conidiophores and conidia: (Fig. 291 A).

Of the same type as the 2 preceding species, differing only in details. They are 100 - 200 x 1.5 - 3.0 μ , deep yellow with characteristic pitted walls, dichotomous, ternate or quadrately branched. Fertile branches 10 - 36 μ , very occasionally 1 - septate, with tapering or acute ends. Conidia acrogenous, occasionally pleurogenous, in clusters of 3 - 8, variable in form, usually oval-elliptic but occasionally sausage shaped, equilateral or pyriform with one end bulbous and the other acute or acuminate, yellow green collectively, 1.4 - 2.9 x 3.7 - 9.7 μ , average 1.8 x 5.8 μ .

6. Rosellinia nitens:

Malt. A : Bottle Culture: (Fig. 284).

Appearance: Coarse felty, very similar to H. truncatum except that the colony becomes floccose with age and the mycelium is not as uniform in colour, being mainly white with parts tinted orange yellow or pink.

Margin: Distinct, up to 15 mm. wide, submersed, colourless.

Conidia: Appear only after 8 months, dull fuscous brown forming a thin granulate crust over part of the colony.

Stain: Variable, different in various parts of the colony, mainly amber brown, deeper near the centre than the outside of the colony.

B : Plate Culture: (Fig. 288).

Appearance: Similar to H. truncatum but smooth felty and mycelium pale yellow to white in colour.

Margin: Sometimes entire but usually lobed, not distinct, canescent, with the peripheral hyphae lying parallel.

Conidia: None observed.

Stain: Tawny (S. 246) extending up to 6 mm. beyond the margin.

Other media: B o t t l e C u l t u r e.

<u>M a i z e.</u>	<u>Le o n i a n ' s.</u>	<u>C z a p e k.</u>
Similar to malt but less luxuriant. Stain slight in quantity.	Similar to malt.	Similar to malt but aerial mycelium denser, colour intense white or yellow. Stain roseate to tawny.

Plate Culture.

Maize.	Leonian's.	Czapek.
Similar to malt but less luxuriant.	Similar to malt.	Similar to <u>H. truncatum</u> on Czapek; coarse felty white on outside to pale cream further inside (S.260) turning yellow green (S.273) with age. Stain intense fiery roseate, at first tinted purple, later tawny (S.172).

Microscopic characters: (Fig. 290 C, D).

Maximum diameter of primary mycelium = 6.0μ .

Secondary mycelium formed of loosely anastomosing hyphae, max. diam. 4.6μ , and can be distinguished from H. truncatum and other species by the large dark bodies that develop from it. The function of these bodies is unknown; they first appear as small apical or intercolony swellings of the hyphae, each swelling corresponding to a segment of the hypha. The growth increases to a max. diam. of 19μ , and eventually becomes black and carbonous.

Conidiophores and conidia: (Fig. 291 B).

Conidiophores identical with H. truncatum but paler in colour. Conidia oval or elliptic, equilateral pale yellow to buff collectively, $1.7 - 2.3 \times 3.7 - 5.0\mu$, average $1.9 \times 4.8\mu$.

Discussion and conclusions:A : Cultural Characters:1. Growth Form:

The group can be split into two parts, one comprising Rosellinia 3A and H. michelianum where the growth character is variable, either appressed

or velvety, and the other H. truncatum and its close relative where the growth character conforms to a definite felty type and there is production of stain of a characteristic colour. The latter group of 4 species shares many important stromal characters, the most pronounced being the broad truncate ostioles. The other 2 species are more diverse in stromal character and this is reflected by the broad differences in growth character.

2. Conidiophore:

The types of conidiophores found in the group support the conclusions first made. The conidiophore of H. michelianum is similar to that of H. glomeratum var. 2., and the rest of those species included in Type V B, but differs from most of them in the strongly monopodial branching of the main axes. In H. truncatum and its relatives, the conidiophores are determinate and more specialized with the fertile branches much shorter and developed only from the terminal part of the main axis. The conidiophores resemble those of Type VI discussed in the previous chapter but the apices of the fertile branches are tapering instead of swollen or clavate. For this reason it is best to class them separately as Type VII.

3. Growth Rate: (See Appendix III, Table VII and Graph G.)

The results obtained cannot be used as successfully as those obtained for the species considered in the previous chapter. Although the differences in temperature - growth reaction were found to be significant in the majority of cases, the value of the results was offset by:-

- 1) the variability of H. truncatum. The two strains tested grew at significantly different rates.
- 2) The similarity in growth rate of the slower strain (311) of H. truncatum to R. nitens, and of the faster (44) to H. stygium.

More work requires to be done in order to find out whether each species can be recognised by a characteristic growth rate.

The following table shows clearly how differences in cultural characters supports small differences in stromal characters.

S P E C I E S.	STROMA TYPE.	AVERAGE SPORE SIZE (μ).	GROWTH CHARACTER (ON MALT).	CONIDIO-PHORE TYPE.	PRESENCE OF SECONDARY MYCELIUM.	G R O W T H R A T E.	
						OPTIMUM.	CHARACTER.
Rosellinia 3A	Globose / pulvinate, uni- or multiperitheciate; Ost. globose.	5.7 x 10.3	Submersed to appressed, sub-hyaline.	-	-	20°C	Moderate
H. michelianum	Aplanopulvinate, rarely uniperitheciate globose, ostioles narrowly truncate.	5.3 x 13.1	Mainly velvety to lanose, white. Stain nil.	VB	+	20°C	Slow
H. stygium	Aplanopulvinate, rarely uniperitheciate globose; ostioles broad truncate; initial stage purple.	3.6 x 7.3	Felty with moderately coarse surface. Stain usually chestnut, sometimes olive green.	VII	+	28°C	Fast
H. microcarpum	Aplanopulvinate, rarely uniperitheciate globose; ostioles broad truncate; initial stage purple.	4.1 x 8.9	Felty, surface very coarse. Stain chestnut, sometimes olive green.	VII	+	20°C	Slow
H. truncatum	Aplanopulvinate, rarely uniperitheciate globose; ostioles broad truncate; initial stage yellow or tawny.	4.4 x 8.5	Felty, surface moderately coarse, stain tawny yellow or brown.	VII	+	28°C	Fast or Moderate
Rosellinia nitens	Aplanopulvinate or uniperitheciate globose, very variable; ost. broad truncate; initial stage white.	4.2 x 9.4	Felty velvety, surface smooth fine. Stain tawny yellow or brown.	VII	+	25°C	Moderate

References.Hypoxyton michelianum:

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de Notaris, G. : Schema di classificazione degli
Sferiacei Italici. 1861.

Saccardo, P.A. : Sylloge Fungorum I, 385, 1882.

Hypoxyton microcarpum:

Penzig, O. &
Saccardo, P.A. : Malpighia 11, 492, 1897.

Saccardo, P.A. : Sylloge Fungorum 14, 510, 1899.

Hypoxyton stygium:

Ellis, J.B. & Everhart : North American Pyrenomycetes, 649, 1892.
(as H. platystomum)

Leveille, J.H. : Ann. Sci. Nat. 5, 258, 1846.

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253, 1942.

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Soc. 17, 130 - 1, 1932.

Saccardo, P.A. : Sylloge Fungorum I, 379, 1882.

Hypoxyton truncatum:

Miller, J.H. : Ascomycetes from the State of Minas Geraes.
Mycologia 32, 172-304, 1940.

: South African Xylariaceae. Bothalia IV,
254, 1942.

Saccardo, P.A. : Sylloge Fungorum II, 401, 1883.
(as Sphaeria truncata).

Rosellinia nitens:

Berkeley, M.J. &
Broome, C. F. : The Fungi of Ceylon. Jour. Linn. Soc. 14,
no. 1066, 1875.
(as H. chalyboeum).

Cesati, V. : Note botaniche, Napoli, 1872.

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CHAPTER V.

THE RUBIGINOSUM GROUP OF HYPOXYLON.A. The Perfect Stage:

The members of this group are more closely related to each other than are those of the other groups just discussed, and so are more difficult to distinguish. Theissen (1909), suggested that, on account of the similarities in colouration and stromal morphology, they should be given varietal status within the same species. Miller, however, contested this view, (1933), pointing out that the different geographical distributions of the various types throughout the world provides strong evidence for speciation. The following features are characteristic about the stromata:-

1. Stroma variable in form, always fibrous or leathery in texture, and never carbonous.
2. The interior of the stroma shows all stages from uniformity to clear differentiation into ecto- and entostroma. The ectostroma is usually predominant. In some species it bears a superficial crust, pale or dark in hue, that is usually granulate in the form of spherical or elliptical particles. These particles, readily observed in vertical sections of the stromata, are similar to those found in the Annulatum Group. It is not known how they are formed, but they do not appear to be connected in any way with the conidial layer, of which no trace is left at maturity. No description of these particles appears to have been made before.
3. Broad differences in superficial colouration may be used to separate the species. These colours are:-
 - grey or grey brown
 - brown or reddish brown
 - red-purple
 - orange-yellow-ferruginous.

Occasional variants are olive green, yellow green, or blue grey, and should be treated with those species in the 4 groups which they most resemble.

4. Perithecia immersed or protruding in outline under the stromal surface; occasionally widely separated from each other.
5. Ostioles umbilicate; i.e., not raised above the stromal surface. Often the ostioles cannot be distinguished clearly in surface view.

Identification without recourse to type material was found to be an especially difficult task when dealing with the species considered here. The method finally adopted was to give all strains, even those differing on slight characters, specific rank, and an appropriate name was applied when the material agreed with one of the descriptions cited by Saccardo (Syll. Fung. I, 1882, et seq.). This account of Saccardo was one of the earliest written of the group as a whole, and although many of the species described were probably synonymous and the differences between closely related forms not clearly expressed, it was nevertheless preferred to later accounts by Theissen (1909) and Miller (1933, 1942), where many of the species were found to be merged without adequate reasons given. Much of the material sent to Dr. J.H. Miller and to the Kew Herbarium was also classified as H. rubiginosum in spite of obvious discrepancies in spore size and stromal colouration. These discrepancies were explained as being due to the "enormous variability" of the species, H. rubiginosum according to age and habitat. If this is the true explanation then it is indeed questionable whether species originally separated from that species on relatively slight characters have any real validity. No evidence, however, has hitherto been brought forward to support or contradict this view. The work presented here aims to solve the problem at least partially.

Key to Species:

1. Stroma grey or grey brown ----- 2.
 1'. Stroma brown or lightly coloured ----- 4.
2. Stroma grey ----- 3.
 2'. Stroma grey brown, characteristically orbicular effuse; vertices of perithecia evident and usually conic in outline.
 Spores 4.5 - 8.5 x 11.0 - 16.0 μ , av. 5.7 x 13.4 μ . -----
 ----- Hypoxyton 18 A * (1,p.123).
3. Spores 4.5 - 8.5 x 10.5 - 18.0 μ , av. 6.1 x 13.6 μ . -----
 ----- Hypoxyton 18 B * (2,p.123).
- 3'. Spores 4.5 - 7.5 x 8.0 - 15.0 μ , av. 5.6 x 11.2 μ . -----
 ----- Hypoxyton plumbeum. (3,p.124).

- 4.(1) Interior of ectostroma dark brown to black or purple, or red, when
examined in thin section ----- 5.
- 4'. Interior of ectostroma yellow or orange in section; normally with
characteristic discrete coloured granules -----18.
5. Ectostroma brown or reddish brown ----- 6.
- 5'. Stroma red, purple or olive green but without trace of brown
colouration -----10.
6. Maximum spore length exceeding 14.5μ ----- 7.
- 6'. Maximum spore length not greater than 14.5μ ----- 8.
7. Spores dark opaque brown, $5.0 - 9.0 \times 12.5 - 19.5 \mu$, -----
average $7.2 \times 15.3 \mu$. ----- Hypoxylon 19 *(4,p.124).
- 7'. Spores dark opaque brown, $3.5 - 8.5 \times 9.5 - 18.0 \mu$, -----
average $6.0 \times 13.2 \mu$. Hypoxylon vogesiacum (9,p.126).
(occasional form).
- 7". Spores amber, subhyaline, $5.0 - 9.0 \times 9.0 - 16.5 \mu$, -----
average $6.3 \times 13.4 \mu$, Hypoxylon 18F (5,p.125)*
8. Spores uniformly elliptic, equilateral or inequilateral ----- 9.
- 8'. Spores variable in shape and size, globose to elliptic, the latter
characteristically narrow and tapering one end;
 $3.0 - 7.0 \times 7.5 - 14.5 \mu$, av. $4.4 \times 10.4 \mu$; stromata brown or partly
reddish brown. ----- Hypoxylon 18 E * (7,p.125).
9. Spores $3.0 - 6.0 \times 6.5 - 10.5 \mu$; av. $3.9 \times 8.1 \mu$;
stroma uniformly brown. ----- Hypoxylon 18 D * (6,p.125).
- 9'. Spores $3.0 - 5.5 \times 5.0 - 9.0 \mu$, av. $3.5 \times 6.8 \mu$;
stroma reddish brown to brown. ----- Hypoxylon coryphae. (8,p.126).
- 9". Spores $3.5 - 7.0 \times 7.5 - 13.5 \mu$, av. $4.8 \times 10.5 \mu$;
stroma reddish brown to ochraceous.----- Hypoxylon rubiginosum (11,p.127).
(occasional form).
- 10.(5) Surface of stroma red or purple ----- 12.
- 10'. Surface of stroma differently coloured ----- 11.

11. Surface of stroma olive green; stroma superficial;
Spores $4.5 - 7.0 \times 9.5 - 15.0 \mu$, av. $5.1 \times 11.7 \mu$. -----
----- Hypoxylon 18 G * (16,p.130).
- 11'. Surface of stroma yellow green to ferruginous; stromata erumpent
through bark; Spores $3.5 - 7.0 \times 7.5 - 11.5 \mu$, av. $5.1 \times 9.8 \mu$. -----
----- Hypoxylon luridum var. minus.
(17,p.130).
12. Interior of the stroma dark olive brown or black in thin section -----13.
- 12'. Interior of stroma some shade of red or purple -----14.
13. Spores opaque black, elliptic; $3.5 - 8.5 \times 9.5 - 18.0 \mu$,
av. $6.0 \times 13.2 \mu$; stroma exterior pale purple. -----
----- Hypoxylon vogesiacum.(9,p.126).
- 13'. Spores opaque black, subglobose, $4.5 - 7.5 \times 7.5 - 12.0 \mu$,
av. $5.5 \times 9.5 \mu$; stroma exterior deep vivid purple to maroon. -----
----- Hypoxylon 38 (10,p.126).
14. Ectostroma bearing superficial granular particles visible in thin longi-
tudinal section; surface of stroma pale purple; stroma pulvinate or
effuse with characteristic undulating or uneven shape;
Spores $4.5 - 9.5 \times 11.0 - 19.0 \mu$, av. $6.7 \times 15.2 \mu$. -----
----- Hypoxylon 2 C * (13,p.128).
- 14'. Ectostromal particles lacking; ----- 15.
15. Maximum spore length not exceeding 15μ . ----- 16.
- 15'. Spores $5.0 - 9.0 \times 11.0 - 19.5 \mu$, av. $7.1 \times 15.1 \mu$. -----
----- Hypoxylon haematostroma.
(15,p.129).
16. Spores opaque, dark brown to black, $4.5 - 7.5 \times 9.0 - 13.5 \mu$,
av. $6.2 \times 11.8 \mu$; stroma very thin, aplanate crustose deep violet
purple, with conspicuous white ostiolar mouths, asci nearly
sessile. ----- Hypoxylon tenue.(14,p.129).
- 16'. Spores light brown, subhyaline; stroma more robust, aplano pulvinate,
asci with distinct stalks -----17.

17. Stroma pale purple; ostioles usually distinct; initial stage pale purple to white; spores $3.5 - 7.0 \times 7.5 - 13.5 \mu$, av. $4.8 \times 10.5 \mu$. ----- Hypoxyton rubiginosum. (11, p.127).
- 17'. Stroma deep purple to maroon; ostioles never distinct and very difficult to perceive; initial stage bright saffron yellow; Spores $4.5 - 7.0 \times 9.0 - 15.0 \mu$, av. $5.5 \times 11.5 \mu$. -----
----- Hypoxyton ~~strepurpureum~~. (12, p.128).
- 18.(4) Interior of stroma yellow in thin vertical section -----19.
- 18'. Interior of stroma orange -----20.
19. Surface of stroma yellow to ferruginous; Spores $4.5 - 7.5 \times 8.0 - 15.0 \mu$, av. $5.6 \times 11.6 \mu$. -----
----- Hypoxyton piceum. (18, p.131).
- 19'. Surface of stroma yellow orange to ferruginous; Spores $3.5 - 7.0 \times 7.5 - 13.5 \mu$, av. $5.0 \times 9.4 \mu$. -----
----- Hypoxyton ochraceo-fulvum. (19, p.131).
20. Surface of stroma ferruginous to dull yellow -----21.
- 20'. Surface of stroma orange or orange yellow or purple -----22.
21. Stroma with superficial ectostromatic particles visible in section; Spores $3.5 - 7.0 \times 7.5 - 13.0 \mu$, av. $5.0 \times 10.6 \mu$. -----
----- Hypoxyton ferrugineo-rufum, var 1. (20, p.131-132).
- 21'. Stroma without superficial ectostromatic particles; Spores $3.5 - 7.0 \times 7.5 - 15.0 \mu$, av. $4.9 \times 10.8 \mu$. -----
----- Hypoxyton ferrugineo-rufum, var.2. (p.131-132).
22. Stromata pulvinate or globose, with orange surface; entostroma well developed. Spores $4.5 - 8.5 \times 12.5 - 25.5 \mu$, av. $7.0 \times 15.6 \mu$. -----
----- Hypoxyton vividum. (22, p.133).
- 22'. Stromata aplanate or aplano-pulvinate; surface smooth, deep lateritic orange. Spores $5.0-6.5 \times 8.0 - 11.0 \mu$. Hypoxyton hypomiltum. (21, p.132).
- 22''. Stromata variable, surface deep purple; Spores $5.0-9.0 \times 11.0-19.5 \mu$, av. $7.1 \times 15.1 \mu$. Hypoxyton haematostroma. (15, p.129)

1. Hypoxyylon 18 A: (Figs. 292, 293, 297B, 298C).

Stromata orbicular or irregular in form, thin; perithecia usually closely associated, with the vertices conic and evident in outline, but sometimes widely separated. Colour of the stroma variable, from grey brown at maturity to black with age. Perithecia small, $250 - 350 \times 300 - 450 \mu$. Asci cylindric, long stipitate, $120 - 220 \times 8 - 10 \mu$, stalks $42 - 130 \mu$. Spores elliptic, equilateral or with one side straight or concave, dark brown, $4.5 - 8.5 \times 11.0 - 16.0 \mu$, av. $5.7 \times 13.4 \mu$. (100).

Hosts: Olea capensis

Vepris lanceolata

Cassine croceum, and wood unidentifiable.

Classified as H. rubiginosum by Dr. J.H. Miller. This species, however, has been kept apart due to the difference in surface colouration from the one described in the literature (Saccardo 1882, Miller 1942).

2. Hypoxyylon 18 B: (Figs. 295, 296, 297A & C, 298B).

Stromata aplano-pulvinate, superficial or erumpent through bark, forming crustose areas of irregular shape 5×7 mm. Ectostroma bearing superficial granular dark grey to black particles; colour of the stroma as a whole pale grey. Interior of the stroma pale grey tinted yellow; entostroma (beneath perithecia) dull brown, very scantily developed and not easily recognizable as a definite layer. Perithecia immersed or evident slightly in outline, globose to saccate, $250 - 600 \times 450 - 550 \mu$, with wide ostiolar mouths; ostioles not, however, clearly visible on the surface of the stroma. Asci cylindric with distinct stalks, $120 - 230 \times 7.5 - 10 \mu$, stalks $45 - 130 \mu$. Spores elliptic mostly equilateral but sometimes concave on one side, dark brown, $4.5 - 8.5 \times 10.5 - 18.0 \mu$, av. $6.1 \times 13.6 \mu$. (150).

Hosts: Passerina falcifolia

Populus canadensis

Tarchonanthus camphoratus

Classified as a variety of H. hypomiltum by the Kew Herbarium.

The original descriptions for either species do not agree, however, so this form is probably new.

3. Hypoxyylon plumbeum: (Figs. 294, 297B, 298A).

Stromata aplano-pulvinate, superficial on wood and bark; forming small globose or linear masses. Ectostroma without granular particles, grey superficially; interior of stroma dark grey throughout so that the entostroma is not recognizable. Perithecia usually evident in outline above the stroma surface, globose, $800 - 1,000 \times 700 - 800 \mu$, with wide ostiolar mouths; ostioles not, however, clearly distinct on the surface of the stroma. Asci cylindrical with distinct stipes, $110 - 130 \times 7 - 8 \mu$, stalks $30 - 55 \mu$. Spores elliptic, equilateral dark brown, $4.5 - 7.5 \times 8.0 - 15.0 \mu$, av. $5.6 \times 11.2 \mu$. (120).

Hosts: Passerina falcifolia

Populus canadensis

Classified as H. rubiginosum by Dr. J.H. Miller. There was not sufficient material available to send to Kew. The description cited by Saccardo (Syll. Fung. IX, 550, 1891) for H. plumbeum appears to agree with the observations better.

4. Hypoxyylon 19: (Figs. 299, 300, 305, 307D).

Stroma aplano-pulvinate to very thinly effused, superficial on bark and decorticated wood. Surface of ectostroma dull brown to black; interior of stroma dark brown, without distinct entostroma. All material collected, from 2 regions 250 miles apart, was infected by a parasite Calculosphaeria sp. . This formed small protruding perithecia and a white granulate coating on the surface of the ectostroma that often gave the species a characteristic appearance. The perithecia are large, sometimes polystichous, globose to flask-shaped, $400 - 450 \times 500 - 800 \mu$, reaching the surface by short ostiolar necks; ostioles, however, are invisible on the surface of the stroma. Asci not observed. Spores elliptic, usually strongly inequilateral with one side concave, dark chestnut brown $5.0 - 9.0 \times 12.5 - 19.5 \mu$, av. $7.2 \times 15.3 \mu$.

Host: Unidentifiable, comprising decorticated wood and bark.

Species not identified by either Dr. Miller or by the Kew Herbarium. Since only old material has been available it is not possible to say whether descriptions from fresh material would agree with any in Saccardo's Sylloge Fungorum, but it appears probable that this form is really a new species.

5. Hypoxylon 18 F: (Figs. 301, 306B, 307E).

Stromata crustose, forming thin, of varying extent but usually not covering more than 3 cms. in any 1 direction. Surface of ectostroma brown, turning black with age; interior of stroma dark brown, without differentiation into ecto- and entostroma. Perithecia usually evident in outline, globose, sometimes with conic vertices, 200 - 300 x 500 - 600 μ ; ostioles very indistinct, sometimes appearing papillate when the perithecia are narrowly conic. Asci cylindric or clavate, with distinct stipes, 130 - 170 x 10 μ , stalks 45 - 80 μ . Spores oval elliptic equilateral light amber brown, 5.0 - 9.0 x 9.0-16.5 μ , av. 6.3 x 13.4 μ . (150).

Host: Olea capensis, decorticated wood and bark.

Apparently restricted to this host.

Classified as a variety of H. haematostroma by the Kew Herbarium but clearly different in colouration from published descriptions (Saccardo 1882, Miller 1942).

6. Hypoxylon 18 D: (Figs. 302, 306C, 307A).

Stromata crustose, aplanopulvinate, confluent usually indefinite in extent. Surface of the stroma very uneven and corrugated, dark brown turning black with age, interior dark brown, concolorous. Perithecia immersed or slightly evident in outline, situated rather far apart, 250 - 300 x 400 - 500 μ ; ostioles very indistinct. Asci cylindric with distinct stalks 75 - 130 x 30 μ , stalks 45 - 75 μ . Spores oval-elliptic equilateral pale amber brown, 3.0 - 6.0 x 6.5 - 10.5 μ , av. 3.9 x 8.1 μ . (100).

Host: Wood and bark unidentifiable.

Not sufficient material was available for identification by the authorities. The details do not agree with any published description.

7. Hypoxylon 18 E: (Figs. 304, 306A, 307B).

Stromata aplanate, crustose, sometimes uniperitheciate globose, superficial. Ectostroma reddish brown to brown, often varying in colour in different parts of the same stromal area; interior of ectostroma reddish brown while the entostroma, below the perithecia, is dark brown to black and contrasts sharply. Perithecia evident in outline, globose to oval, 350 - 400 x 600 - 700 μ ; umbilicate, usually distinct. Asci cylindric 100 - 120 x 6 μ , stalks 35 - 60 μ .

Spores very variable in shape, ranging from sub-globose to narrowly elliptic, the latter usually with one end blunt and the other tapering; dark grey, $3.0 - 7.0 \times 7.5 - 14.5 \mu$, av. $4.4 \times 10.4 \mu$.

Hosts: Lycium campanulatum

Virgilia oroboides, decorticated wood.

8. Hypoxyylon coryphae: (Figs. 303, 307C, 320B).

Stroma similar in all essential features to the preceding species, but in addition has superficial ectostromal particles. Perithecia $200 - 300 \times 250 - 400 \mu$; asci cylindric, long stalked, $90 - 120 \times 6 \mu$, stalks $45 - 60 \mu$. Spores oval-elliptic equilateral light brown, $3.0 - 5.5 \times 5.0 - 9.0 \mu$, av. $3.5 \times 6.8 \mu$.

Host: Unidentifiable, wood and bark.

9. Hypoxyylon vogesiacum: (Figs. 308 - 311, 333A).

Stromata aplano-pulvinate, forming small glomerules $3 - 8$ mm. diam., or irregularly effused on bark and decorticated wood. Surface of ectostroma usually light purple red to pink, sometimes purple or reddish brown. Interior of stroma dark brown to ochraceous, concolorous, without differentiation. Perithecia completely immersed, globose, $450 - 500 \times 600 - 700 \mu$; ostioles distinct, and conspicuous on the surface of the stroma due to the profuse growth of the periphyses in the ostiolar mouths. Asci $120 - 170 \times 7.5 \mu$; stalks $45 - 90 \mu$. Spores oval-elliptic, equilateral, dark brown, $3.5 - 8.5 \times 9.5 - 18.0 \mu$, av. $6.0 \times 13.2 \mu$.

Hosts: Olea capensis, wood and bark; other species unidentifiable.

Confirmed as H. vogesiacum by the Kew Herbarium.

10. Hypoxyylon 38: (Figs. 312, 313, 333C).

Stromata effuse, orbicular or irregular in form, sometimes rather thin, superficial on bark or wood. Surface of stroma deep purple to maroon; interior olive green to black, concolorous, undifferentiated. Perithecia immersed entirely globose, rather small, $130 - 250 \times 250 - 350 \mu$. Ostioles not distinguishable. Asci cylindric, $80 - 115 \times 7 \mu$, stalks short, $15 - 15 \mu$. Spores oval to sub-globose, equilateral, dark brown to black, $4.5 - 7.5 \times 7.5 - 12.0 \mu$, av. $5.5 \times 9.5 \mu$.

Host: Passerina falcifolia, bark and wood.

Wood unidentifiable.

Classified by Kew as H. rubiginosum. The equilateral black spores of this material, however, do not correspond with Miller's description (1942) of H. rubiginosum or with those of the herbarium specimens at the Pretoria Herbarium which were identified by him. On these grounds this strain is treated as separate.

11. Hypoxylon rubiginosum: P. ex Fr. (Figs. 314 - 320A, 333B).

Stromata variable in form, pulvinate or effuse and indefinite in extent, but always superficial. Ectostroma normally bright purple superficially, but sometimes reddish brown to ochraceous. Interior of ectostroma always pale red to purple, sometimes scarlet, entostroma, beneath the perithecia, rather slight in quantity and dark red to red brown. Perithecia usually evident in outline above the general level of the stroma, sometimes developing singly and far apart from each other and only rarely completely immersed in the stromal matrix; globose, variable in size, 200 - 400 x 250 - 700 μ ; ostioles usually distinct because of the conspicuous growth of periphyses. Asci cylindrical with a short though distinct stipe, 85 - 130 x 7 μ , stalks 15 - 70 μ . Spores oval, equilateral with one side deeply concave, light brown, subhyaline, 3.5 - 7.0 x 7.5 - 13.5 μ , av. 4.8 x 10.5 μ . (210).

Hosts: Probably comprises a wide range:

Those recognised are:- Acacia mollissima

Olea capensis

Passerina falcifolia

Populus canadensis

Scutia myrtina

Sideroxylon inerme

Material of this type agrees with descriptions in the literature (Saccardo 1882, Miller 1942). It was confirmed by the Kew Herbarium as H. rubiginosum and also found to correspond exactly with material in the Pretoria Herbarium identified by J.H. Miller. The name H. rubiginosum has, therefore, been applied to this material.

12. Hypoxyton purpureum: Nits. (Figs. 321, 322, 332B, 333E).

Stromata aplanate or aplanopulvinate, consisting of a matrix in which the perithecia develop independantly so that at maturity they are closely aggregated or far apart. The globose outline of the mature perithecia, indistinct ostioles, brilliant yellow initial stage and deep purple colour at maturity, distinguish this material from samples of other species. The interior of the stroma is deep purple, usually concolorous and the entostroma is hardly distinct. Perithecia $250 - 350 \times 250 - 400 \mu$; asci $105 - 160 \times 6 \mu$, stalks $35 - 70 \mu$. Spores elliptic equilateral, or sometimes with one side straight, light brown, $4.5 - 7.0 \times 9.0 - 15.0 \mu$, av. $5.5 \times 11.5 \mu$. (150).

Host: Olea capensis, decorticated wood.

This material was not sent away for confirmation. The description cited by Saccardo (1882) mentions the changing colour of the initial stage which appears to be a strong distinguishing character, and since the other features agree closely, this identification was considered appropriate.

13. Hypoxyton 2 C: (Figs. 323, 324, 327B, 341C).

Stromata pulvinate to aplanopulvinate, sometimes effuse, superficial. Surface of ectostroma pale purple, interior deep red; entostroma beneath the perithecia very dark red to black. The ectostroma bears superficial granulate particles easily visible when a thin section of the stroma is cut. Perithecia completely immersed, rather large, $200 - 400 \times 350 - 650 \mu$; ostioles usually faintly visible as white dots on the stroma surface. Asci clavate or cylindrical, short stipitate, $100 - 120 \times 9 - 12 \mu$, stalks $20 - 40 \mu$. Spores elliptic strongly inequilateral, with one side concave, dark brown, $4.5 - 9.5 \times 11.0 - 19.0 \mu$, av. $6.7 \times 15.2 \mu$.

Hosts: Curtisea faginea

Rhus legati, bark.

Apparantly restricted to these 2 hosts.

An interesting feature is the similarity in type of bark of the two trees. This may have some influence on stromal form but whether this is really so remains to be proved.

This species was classified as H. rubiginosum var. tropica by Miller, who apparently introduced the name soon after receipt of the material (September, 1958). The name has not yet been published to my knowledge in any botanical

journal however, since that date.

Whether this species is really a variety of H. rubiginosum is rather doubtful. The differences between the two species is at least as great, and as easily perceived, as between say, H. rubiginosum and H. vogesiacum or H. haematostroma. On these grounds it seems best to recognise this as a new species.

14. Hypoxyylon tenue Starb. (Figs. 325, 326, 327A, 341B).

Stromata crustose, aplanate effuse, rather thin, up to 2.5 x 2 cm. in area, partly embedded in, though not erumpent through, bark. Surface of ectostroma deep purple, interior deep red; entostroma dark brown and so distinct from the tissue above. Perithecia evident in outline as globose protuberances with flattened vertices and are rendered more conspicuous by the tufts of white periphyses in the ostiolar mouths; size of perithecia 200 - 250 x 450 - 550 μ . Asci cylindric, nearly sessile, 90 - 125 x 7.5 μ , stalks 15 - 35 μ . Spores oval-elliptic, equilateral or with one side straight or slightly concave, dark grey to black, 4.5 - 7.5 x 9.0 - 13.5 μ , av. 6.2 x 11.8 μ . (100).

Hosts: Trichocladus crinitus

Trichocladus ellipticus, bark.

Apparently restricted to these two species.

Classified as a variety of H. haematostroma by the Kew Herbarium. The description cited by Saccardo for H. tenue suits the species better, however.

15. Hypoxyylon haematostroma Mont. (Figs. 328; 329; 340C, D; 341E).

Stromata usually pulvinate or glomeruliform, up to 5 mms. diam., but also sometimes aplano-pulvinate and effused, forming crusts 2 x 1 cms. wide; superficial on bark or decorticated wood. Ectostromal surface dark red to purple red; interior scarlet, often showing orange yellow granules when broken. Entostroma scanty, dark brown. In thin section (Δ 15 μ) the stroma appears very pale red or pink subhyaline when examined under the microscope, in marked contrast to the opacity of other species. Perithecia usually evident in outline, rather large, globose, 280 - 450 x 250 - 650 μ ; ostioles clearly visible on the surface of the stroma. Asci clavate or cylindric, with stalks that vary greatly in length even within the same group of stromata, 120 - 220 x 8 - 10.5 μ , stalks 50 - 120 μ . Spores oval, inequilateral with one side concave, dark

brown to nearly black, $5.0 - 9.0 \times 11.0 - 19.5 \mu$, av. $7.1 \times 15.1 \mu$.(180).

Hosts: Leucadendron adscendens

Passerina falcifolia

Populus canadensis

Pterocelastrus tricuspidatus

Virgilia oroboides.

Confirmed as H. haematostroma by the Kew Herbarium. This material obviously conforms to the concept of that species as it is recognised today (see Miller : Bothalia IV 2, 1941 - 48) but does not tally in spore measurement with the description given by Theissen (1909). Saccardo does not give the spore size in the Sylloge Fungorum (I, 1882, p. 376). It is thought best to retain Miller's name for the species until this discrepancy in spore size can be explained satisfactorily.

16. Hypoxylon 18 G: (Figs. 329, 338A, 341D).

Stroma aplanopulvinate, similar in form to H. haematostroma. Surface of ectostroma olive green, interior pale yellow green, concolorous, without differentiation. Perithecia $500 - 700 \times 600 - 800 \mu$; asci cylindrical. Spores $4.5 - 7.0 \times 9.5 - 15.0 \mu$, av. $5.1 \times 11.7 \mu$.(75).

Hosts: Wood and bark, unidentifiable.

This species appears to be new, since it does not agree entirely with any published description.

17. Hypoxylon luridum: Nits. var. minus. Sacc. (Figs. 330, 331, 332A, 333D).

Stromata aplanate, erumpent; surface olive or yellow green to ferruginous. Interior of stroma concolorous, dark olive green to black. Perithecia immersed or evident in outline, $400 - 500 \times 600 - 800 \mu$; ostioles clearly evident on the surface of the stroma. Asci cylindrical with distinct stipes, $120 - 160 \times 7 \mu$, stipes $70 - 100 \mu$. Spores oval, usually equilateral but sometimes with one side straight or concave, light brown, $3.5 - 7.0 \times 7.5 - 11.5 \mu$, av. $5.1 \times 9.8 \mu$.(100).

Host: Olea capensis, bark.

Apparently restricted to this species.

Classified as H. rubiginosum by the Kew Herbarium. Material, however, agrees better with the description for the species cited in Saccardo: Syll. Fung. I, p. 357.

18. Hypoxyylon piceum: Ellis. (Figs. 334, 335, 338A).

Stromata pulvinate or aplanate, superficial. Surface of stroma deep golden yellow to ferruginous; interior yellow, concolorous, without differentiation. The yellow granules embedded in the stroma which are characteristic of the species, can easily be seen on breaking open the stroma and on examination of the fragments in water under the microscope. Perithecia usually immersed, but sometimes evident in outline, $450 - 700 \times 450 - 900 \mu$; ostioles occasionally distinct but usually not visible. Asci not seen.

Spores oval-elliptic, equilateral or with one side straight or concave, dark brown, $4.5 - 7.5 \times 8.0 - 15.0 \mu$, av. $5.6 \times 11.6 \mu$. (180).

Hosts: Cassine croceum

Curtisia faginea

The details of the material agree well with Saccardo's description for H. piceum (1891). Dr. Miller, however, regards H. piceum and H. rubiginosum as the same species (Bothalia IV, p. 258, 1941-48: list of synonyms). Material sent to him was classified as H. rubiginosum, but he did not state whether it conformed to the original H. piceum nor did he explain the discrepancy between this species and H. rubiginosum in spore size and stromal colouration.

19. Hypoxyylon ochraceo-fulvum: Berk. (Figs. 337, 338A, 341A, 343A).

Stromata similar in all essential respects to the preceding species, also with yellow granules. The main external colouration is yellow orange however. Perithecia $400 - 600 \times 450 - 650 \mu$; ostioles indistinct or conspicuous. Asci $90 - 120 \times 6 \mu$, stalks $35 - 50 \mu$. Spores oval-elliptic mainly equilateral, light brown, $3.5 - 7.0 \times 7.5 - 11.5 \mu$, av. $5.0 \times 9.4 \mu$. (100).

Hosts: Passerina falcifolia

Canthium spinosum

Not sent for confirmation. The material agrees well with the description given by Saccardo (1891).

20. Hypoxyylon ferrugineo-rufum: Henn. (Fig. 338B).

Stromata similar in essential respects to that of the preceding 2 species but differs in that the surface is dull ferruginous yellow and the interior bright orange. The interior also contains embedded orange yellow granules.

Another minor characteristic is the much closer aggregation of the perithecia within the stroma and the dark entostroma beneath them which contrasts sharply with the orange tissue above. Perithecia oval to saccate, 250 - 450 x 600 - 700 μ , ostioles not distinct. Asci 80 - 130 x 8 μ , stalks 30 - 50 μ .

There were two varieties found:-

1. With superficial orange ectostromal particles. Asci cylindrical. Spores oval, mainly equilateral, light opaque brown, 3.5 - 7.0 x 7.5 - 13.0 μ , av. 5.0 x 10.6 μ . (100).
2. Without superficial orange ectostromal particles. Asci cylindrical. Spores oval, mainly equilateral, light opaque brown, 3.5 - 7.0 x 7.5 - 15.0 μ , av. 4.9 x 10.8 μ . (100).

Hosts: Wood unidentifiable.

Not sent for confirmation. The material agrees well with the description given by Saccardo (1902).

21. Hypoxylon hypomiltum: Mont. (Fig. 336).

Stromata aplanate, rarely aplanopulvinate, usually covering large areas of host wood and bark. Surface of stroma smooth, deep vivid lateritic orange to brick red; interior with orange granulate particles. Perithecia immersed, not evident in outline; ostioles indistinct. Asci cylindrical 105 - 130 μ long, stalks 50 - 60 μ (Miller's measurements, Bothalia IV, p.257, 1941 - 48). Spores elliptic navicular, 8 - 11 x 5 - 6.5 μ (Miller, ibid).

Host: Olea capensis

The material collected was immature. However, comparison with the mature stromata preserved at the Pretoria Herbarium (nos. 110390, 27532, 28893, 33181, 34937) and the fact that there is no other Hypoxylon species described which has this brilliant orange colouration, indicates that this identification is correct beyond reasonable doubt. In a recent private communication (1958), Miller considers the species H. hypomiltum merely to be a variant of H. rubiginosum.

Miller; also confirmed by Kew).

Hypoxyton 18 A: (Miller).

22. Hypoxyton vividum: (Figs. 339, 340A, B; 341F, 342, 343B).

Stromata pulvinate, glomeruliform, up to 5 mm. broad, on bark and decorticated wood. Surface of stroma dull orange to reddish orange; interior of ectostroma brilliant orange. Entostroma normally well developed, dark chestnut brown. Perithecia usually clearly evident in outline, 350 - 800 x 450 - 1,000 μ ; ostioles clearly distinct on the surface of the stroma. Asci cylindric or clavate, distinct stalks, 135 - 180 x 8 - 10.5 μ , stalks 40 - 75 μ . Spores elliptic, equilateral or inequilateral with one side concave, sometimes broad at one end and narrow at the other, dark brown, 4.5 - 8.5 x 12.5 - 25.5 μ , av. 7.0 x 15.6 μ . (220).

Classified as H. haematostroma Mont. by Dr. J.H. Miller. This appears to be unsatisfactory in view of the differences in stroma colour. The material agrees much better with the descriptions given by Theissen (1909) and Saccardo for H. vividum. Miller obviously regards H. vividum and H. haematostroma as the same species (Bothalia IV, p. 257, 1941 - 48).

Discussion:

The species show a well marked trend from a condition where the mature stroma is dull coloured and the interior undifferentiated to that where the stroma is brightly coloured and the interior is separable into a coloured ectostroma and a dark entostroma. The successive gradations in colouration and internal differentiation are often very slight so that several species may only differ from each other in a single, sometimes minor respect only. This fact may be partly the reason for the confusion of terminology and many conflicting opinions as to the specific limits within the group.

The following list summarizes the main differences of interpretation concerning the classification of the material in this chapter. In the left column is a list of species identified by personal examination and judgement of the strains collected. On the right is a list of the species to which these strains were assigned by the authorities. The name of the authority is indicated in parentheses:

<u>H. hypomiltum</u> , normal lateritic type (corresponding with material at Pretoria Herbarium, identified by Miller.)	} <u>H. hypomiltum</u> .
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Hypoxyton 18 B. (Kew).

Group I: Stain pink to reddish orange.

The three species, H. 18A, H. 18 B and H. plumbeum are broadly similar in many respects and with one exception, do not differ greatly on different media. The similarities and variations can be best appreciated by examination of the following table:-

Bottle Culture:-Hypoxylon 18 A:

Medium.	Growth Characters.
Malt:	Canescent white subhyaline, mainly submersed; aerial mycelium not very dense. Margin entire; compact. Conidia sparse, fawn grey produced after 2 months. Stain dull orange brown to roseate.
Maize:	Similar to malt but margin distinct, 5 - 6 mm. wide, submersed completely; interior thin white granulate. Stain restricted very faint roseate.
Leonian's: (Fig.344).	Similar to malt but margin distinct, 2 - 5 mm. wide, submersed interior thin white granulate. Stain dull orange brown; also secreted on surface of colony as rose coloured drops.
Czapek: (Fig.345).	Thin, compact velvety at first subhyaline (14 days) later opaque; margin 4mm. wide submersed. With age typically furrowed from the centre outwards. Stain brilliant roseate orange (S.181) darkening to black at centre; stain extending 1 cm. beyond margin of colony.

Bottle Culture:-Hypoxylon plumbeum:

Medium.	Growth Characters.
Malt: (Fig.348).	Thin velvety, smooth; aerial mycelium opaque white. Margin variable, when distinct forming a broad colourless zone 15 - 18 mm. wide with stain showing conspicuously beneath; always compact and entire. Conidia produced after 4 - 8 months, fawn coloured, in dense scattered areas. Stain usually orange pink (S.204), but sometimes yellow ochre (S.203-337) to roseate ochre brown; appearing late, (14-17 days).
Maize:	Similar to malt, but no conidia.
Leonian's:	Similar to malt, but no conidia.
Czapek: (Fig.349).	Similar to malt and to <u>H. 18 A</u> on Czapek but not as dense white as the latter; also furrowed but no external secretion of stain. Stain orange pink, not produced beyond borders of colony.

Bottle Culture:-Hypoxyton 18 B:

Medium.	Growth Characters.
Malt: (Fig.352).	Similar to above.
Maize: (Fig.353).	Similar to above.
Leonian's:	Similar to above.
Czapek:	Similar to above.

Plate Culture:-Hypoxyton 18 A:

Medium.	Growth Characters.
Malt: (Fig.346).	Canescent velvety white subhyaline to opaque, usually covered with fawn conidia when old (>12 days). Margin entire compact. Stain, nil.
Maize:	Less luxuriant than on malt white subhyaline. No conidia. No Stain.
Leonian's:	Similar to malt but aerial mycelium almost opaque. Stain faint brandy colour.
Czapek: (Fig.347).	Markedly different from preceding; colony 10 days old divided into:- (a) centre 2.0-2.5 cm. with downy appressed mycelium white opaque to pale orange yellow; (b) Marginal zone 1.3 cm. submersed nearly colourless with scant aerial mycelium and stain showing beneath. Stain brilliant roseate orange (S.153-158) deepening to very dark with age (S.156).

Plate Culture:-Hypoxyton plumbeum:

Medium.	Growth Characters.
Malt:	Velvety appressed, but rather variable either smooth or coarse; usually opaque white. Margin indistinct entire, compact to effuse. Conidia none. Stain faint pink, at centre only.
Maize: (Fig.350).	Less luxuriant than on malt. Velvety, white subhyaline. No stain.
Leonian's.	Similar to malt.

Medium.	Growth Characters.
Czapek: (Fig.356).	Similar to malt but stain slightly more intense, though never approaching that of <u>H. 18A</u> .

Plate Culture:-

Hypoxyton 18 B:

Medium.	Growth Characters.
Malt: (Fig.354).	Canescent velvety, white subhyaline, sometimes slightly gelatinous. Margin entire, compact. Stain light pink at centre only.
Maize:	Less luxuriant than malt, canescent, almost hyaline. Stain, nil.
Leonian's: (Fig. 355).	Similar to malt. Stain pink at centre.
Czapek: (Fig.356).	Canescent, white opaque tinted yellow near centre. Margin entire or slightly segmented; compact. Stain inconspicuous yellow butt (S.193).

From this table it can be seen that the most striking differences lie in the colour and intensity of the stain, with growth character secondary in importance. The 2 latter species are, however, very difficult to distinguish, the main character being the density of the mycelium, which is greater in H. plumbeum.

Microscopic Characters: (Fig. 453 D,E).

There is no secondary mycelium in any of the species. Primary mycelium without distinguishing features:

Max. diam. in H. 18 A ----- 2.3 μ ;

H. plumbeum ----- 2.7 μ ;

H. 18 B ----- 1.7 μ .

Conidiophores and conidia:

Key:

1. Conidia strictly acrogenous ----- Hypoxyton 18 A.
- 1'. Conidia at least partly pleurogenous ----- 2.

2. Conidia acropleurogenous, spicate, on the terminal parts of the fertile hyphae ----- Hypoxylon plumbeum.
- 2'. Conidia pleurogenous in small subterminal clusters, never spicate and rarely apical ----- Hypoxylon 18 B.

Hypoxylon 18 A: (Fig.357 A).

Conidiophores scarcely distinct from the vegetative mycelium, 100 - 200 μ long, dichotomously or rarely ternately or quadrately branched. Conidia forming dense apical clusters, broadly oval to globose, fawn coloured collectively, 1.7 - 2.9 x 2.0 - 3.7 μ , av. 2.2 x 2.8 μ .

Hypoxylon plumbeum: (Fig.357 B).

Conidiophores indeterminate, 100 - 300 μ , dichotomous compound branched, the ultimate fertile hyphae 20 - 45 x 1 - 2 μ , rarely septate with elliptic, not swollen, apices. Conidia acropleurogenous, usually forming spicate clusters, hyaline individually but collectively fawn brown; oval-elliptic with both ends rounded; 1.4 - 2.3 x 2.6 - 4.3 μ , av. 1.9 x 3.4 μ .

Hypoxylon 18 B: (Fig.357 C).

Conidiophores not distinct from the aerial hyphae of the vegetative mycelium; mainly submersed, rarely dichotomous and usually narrow (1 μ diam.) The terminal fertile segments of the mycelium are 24-36 μ long; apices unmodified, acute or bluntly pointed. Conidia oval-elliptic both ends rounded, fawn brown collectively, 1.4 - 2.6 x 2.9 - 5.1 μ , av. 1.9 x 3.9 μ .

Group II:

This comprises 5 species which may produce a brown stain in culture. Though each can be recognised fairly easily the differences between the growth characters of each species are minor ones and are best tabulated:

Bottle Culture:-Hypoxyton 18 F:

Medium.	Growth Characters.
Malt: (Fig.362).	Slow growing, dense velvety with smooth fine surface, intense white; later (14 days) when conidia develop, granulate pink. Margin entire, compact. Conidia as above. Stain nil or very faint amber brown.
Maize: (Figs.363, 364.)	Slow growing but faster than on malt; thin velvety, subhyaline to opaque white. Margin entire, compact. Conidia, none. Stain, nil.
Leonian's:	Similar to malt but no conidia.
Czapek:	Similar to malt but correlated in radial furrows after 14 days and without conidia.

Bottle Culture:-Hypoxyton 18 D:

Medium.	Growth Characters.
Malt: (Fig.367).	Velvety with moderately coarse surface, dull white, with age (> 15 days) discoloured by sparse dark brown conidia. Margin entire, compact. Stain, none.
Maize:	Similar to malt but less luxuriant.
Leonian's:	Similar to malt.
Czapek:	Similar to malt but less luxuriant.

Bottle Culture:-Hypoxyton 18 E:

Medium.	Growth Characters.
Malt: (Fig.373).	Canescent to thin velvety appressed, surface smooth. Aerial mycelium dull white subhyaline; after 4 days becoming densely covered with pink conidia. Margin, entire compact to slightly effuse. Stain, dull olive brown becoming almost black.
Maize:	Similar to malt but conidia less profuse and stain lighter.
Leonian's:	Similar to malt.
Czapek:	Similar to malt but less luxuriant.

Plate Culture:-Hypoxyton 18 F:

Medium.	Growth Characters.
Malt:	Slow growing, very dense opaque white, velvety, with smooth surface. Conidia pink finely granulate, appearing after 8 days. Stain, none.
Maize:	Similar to malt but without conidia and with faster rate of growth.
Leonian's:	Similar to malt, slow growing, but without conidia.
Czapek:	Less luxuriant than malt, canescent, dull white subhyaline.

Plate Culture:-Hypoxyton 18 D:

Medium.	Growth Characters.
Malt: (Figs.368, 369).	Velvet felty with moderately coarse surface dull opaque white. Conidia sparse, at first (8 days) pink, but later dull brown. No stain.
Maize:	Almost entirely submersed, sodden, smooth white, nearly hyaline. No stain.
Leonian's: (Fig.370).	Similar to malt but slightly less luxuriant. Stain developing after 3 weeks, conspicuous tawny brown.
Czapek:	Thin velvety to canescent surface smooth; opaque white. Stain none.

Plate Culture:-Hypoxyton 18 E:

Medium.	Growth Characters.
Malt: (Figs.379, 380).	Canescent velvety with smooth surface; white subhyaline. After 4 days conidia develop over the entire colony: these are deep salmon pink (S.188-197). Stain, pale willow green, diffuse.
Maize:	Similar to malt, but stain fainter.
Leonian's:	Similar to malt.
Czapek:	Less luxuriant than malt, sometimes nearly submersed, otherwise similar.

Microscopic Characters: (Fig.453 A).

There is no secondary mycelium. Primary mycelium without distinguishing character:

Max. diam. H. 18 F. ----- 2.3 μ ;
H. 18 D. ----- 2.3 μ ;
H. 18 E. ----- 2.3 μ .

Conidiophores and conidia:Key:

1. Conidiophores dichotomously branched -----2.
- 1'. Conidiophores ternately or quadrately branched, sometimes verticillate -----3.
2. Conidia elongate elliptic, usually inequilateral 1.1 - 2.6 x 4.6-9.7 μ , av. 2.0 x 5.8 μ ; apices of fertile branches unspecialized ---
 --- Hypoxylon 18 F.
- 2'. Conidia oval to globose, equilateral, 1.4 - 2.3 x 2.0 - 3.0 μ ; av. 1.9 x 2.7 μ ; apices of fertile branches globose, sometimes with knoblike projections ----- Hypoxylon 18 D.
3. Conidia elongate elliptic, usually equilateral, 1.5 - 2.1 x 4.2-6.0 μ ; av. 1.8 x 4.5 μ . ----- Hypoxylon 18 E.

Hypoxylon 18 F: (Fig.371 B,C).

Conidiophores not distinct from the vegetative mycelium, forming branching systems 130 - 180 μ long, dichotomous, or rarely ternate. The fertile branches vary in length from 20 - 130 μ . The conidia are elliptic to pyriform, typically inequilateral with one end broad and the other tapering to a point, and are borne in clusters, either apically or from the swollen side of the fertile hyphae. They are pale pink collectively. Other details as in Key.

Hypoxyton 18 D: (Fig. 371 A).

Conidiophores not distinct from the vegetative mycelium, but usually with a more compact type of branching than that just previously described; forming dichotomous branching systems 100 - 200 μ long. The fertile branches vary in length from 20 - 95 μ , and the apices are characteristically swollen; sometimes partite to form distinct knobs. The conidia are borne in apical, rarely sub-apical clusters, and are elliptic to globose, pale pink to dark brown collectively. Other details as above.

Hypoxyton 18 E: (Fig. 381 A, B).

Conidiophores usually distinct from the vegetative mycelium by the whorled or verticillate nature of the ultimate branches, sometimes also rather highly refractive when viewed dry under a coverslip; but usually not exceeding 150 μ . The fertile branches are relatively short, 10 - 20 μ long, with elliptic or bluntly pointed, rarely swollen, apices. Conidia in apical clusters of 6 - 10, elliptic equilateral with bluntly pointed ends, ranging in colour from salmon pink to brick orange collectively. Other details as above.

The remaining species in the group are difficult to distinguish. The following table gives a broad impression of the main visual similarities and differences:

SPECIES.	MALT BOTTLE SLANT			MALT PLATE.		
	<u>Mycelium type.</u>	<u>Margin.</u>	<u>Stain.</u>	<u>Mycelium type.</u>	<u>Margin.</u>	<u>Stain.</u>
<u>Hypoxylon 19</u>	Dense velvety	Distinct, submersed 4mm.	Deep golden brown	Thin velvety	Distinct submersed 1 - 2 mm.	Deep golden brown.
<u>H. vogesiacum</u>	Dense velvety	Not distinct	Dark yellow ochre	Velvety/appressed.	Distinct submersed 1 - 4 mm.	Red ochre.
<u>H. coryphae</u>	Moderate velvety	Not distinct	Red brown	Coarse velvety-felty.	Not distinct.	Dark olive red.
<u>Hypoxylon 38</u>	Dense velvety	Distinct, submersed 3 - 5 mm.	Dull murky red brown.	Dense velvety	Distinct, submersed 2-3 mm.	Nil.
<u>H. rubiginosum</u>	Velvety, moderate or dense.	Distinct, submersed 5 - 13 mm.	Dull red	Velvety, moderate or dense.	Distinct, submersed 1 - 3 mms.	Pale red.
<u>H. purpureum</u>	Velvety, moderate.	Distinct, submersed 3-6 mm.	Dull red	Thin velvet-felty.	Distinct, submersed 2-3 mm.	Pale red.
<u>Hypoxylon 2C</u>	Felty-velvety; surface moderate coarse.	Not distinct	Dull chestnut to red.	Coarse felty	Not distinct	Very pale red, to absent.
<u>Hypoxylon tenue</u>	Dense velvety	Distinct, submersed 2 mm.	Chestnut red, peculiar distribution.	Dense velvety	Distinct, submersed 3-12 mm.	Chestnut red, + peculiar distribution.
<u>H. haematostroma</u>	Velvet, moderately dense.	Distinct, submersed 5-10 mm.	Deep red.	Moderately dense velvety.	Distinct, submersed 3-5 mm.	Deep red.
<u>Hypoxylon 18C</u>	Velvet, coarse to smooth.	Distinct, submersed 7-9 mm.	Deep olive green.	Coarse felty.	Distinct, submersed 2-3 mm.	Pale olive green.
<u>Hypoxylon 18G</u>	Moderate velvet	Distinct, submersed 12-13 mm.	Dull red.	Dense velvety	Distinct, submersed 12 mm.	Deep red to nearly black.
<u>H. piceum</u>	Velvet, moderate to dense.	Distinct, submersed 10-15 mm.	Dull red.	Velvety, moderately dense.	Distinct, submersed 3-4 mm.	Sherry or absent.
<u>H. ochraceo-fulvum</u>	Dense velvet to fleecy.	Not distinct.	Dull red-brown to murky, very restricted.	Velvety-fleecy, coarse.	Not distinct.	Nil.
<u>H. ferrugineo-rufum</u> <u>var.1.</u>	Thin velvet to appressed.	Distinct, submersed 5-10 mm.	Dull orange	Velvet smooth	Not distinct	Sherry colour.
<u>var.2.</u>	Thin velvet	Not distinct	Red black	Coarse felty	Not distinct	Orange
<u>H. hypomiltum</u>	Thin felty to velvety, dense.	Distinct, submersed, 4 mm. wide.	Violet to purple	Dense velvety	Not distinct, canescent appressed.	Nil.
<u>H. vividum</u>	Moderately dense, velvety.	Not distinct.	Red.	Moderately dense, velvety.	Not distinct	Light red.

Detailed descriptions:Hypoxyton 19.

Malt. A : Bottle Culture: (Fig.358).

Appearance: Dense velvety, smooth opaque; colour of aerial mycelium variable, basically white but usually tinged yellow or buff. Usually the centre of the colony is raised above the general level.

Conidia: Sparse, inconspicuous, dull brown; produced after 2 months.

Stain: At first roseate orange (S.158-186), deepening to red brown and finally black; very conspicuous through the colony and extending 1.5 cm. beyond the margin; stain also secreted as roseate / amber drops on the surface of the colony.

B : Plate Culture: (Figs. 360, 361).

Appearance: Thin velvety, subhyaline to opaque, with smooth surface. Aerial mycelium dull white to orange brown.

Conidia: None.

Stain: Duffuse throughout, initially light brandy colour, darkening with age (S.246 - 196 - 193 - 191).

Other media: B o t t l e C u l t u r e .

<u>M a i z e .</u> (Fig.359).	<u>L e o n i a n ' s .</u>	<u>C z a p e k .</u>
Similar to malt, velvety though with rather coarser surface, and with broad marginal zone 4 mms. wide, submersed-appressed white subhyaline. Stain roseate, (S.190) extending 7 mm. beyond margin of colony.	Similar to malt, but with marginal zone up to 1.1 cm. wide, appressed subhyaline. Stain deep ochre red (S.193-196).	At first appressed, pure white, later (>10 days) becoming similar to malt.

Plate Culture.

Maize. (Figs.360,361).	Leonian's.	Czapek.
Canescent to thin velvety, smooth, my- celium pure white. Stain brandy colour (S.246-196).	Similar to malt, but less luxuriant.	Canescent with gela- tinous appearance, especially near the margin; smooth except for centre. Colour of mycelium dull white tinted dull brown. Stain faint roseate (S.134,135).

Microscopic Characters:

Maximum diameter of primary mycelium = 1.7μ . Secondary mycelium consists of long sparingly branched loosely associated stout hyphae $3.5 - 5.0 \mu$. diam.

Conidiophores and conidia: (Fig. 402).

Conidiophores distinct from the vegetative mycelium by the shorter length of the branches, $110 - 130 \mu$ long, usually dichotomously but sometimes ternately branched, fertile branches $13 - 50 \mu$, usually with broad recurved apices. Conidia borne in apical clusters, oval to oval-elliptic, equilateral, dull brown collectively, $1.4 - 3.1 \times 3.1 - 5.4 \mu$, av. $1.9 \times 4.0 \mu$.

Hypoxyton vogesiacum:

Malt. A : Bottle Culture (Fig. 383).

Appearance: At first (1-14 days) closely appressed with scanty subhyaline dull yellow mycelium, later thin velvety and nearly opaque, smooth. The colour of the mycelium becomes deep yellow ochre with age.

Conidia: Dull red with a greenish tint, not conspicuous on the surface of the colony; produced after 14 days.

Stain: At first orange (S.196) to orange yellow, very diffuse, later later deepening to dark ochre yellow or coffee brown.

B : Plate Culture:

Appearance: Submersed-appressed or canescent - thin velvety depending on strain, but always with a coarse surface. Colour of mycelium dull white subhyaline.

Conidia: None.

Stain: Orange yellow, but variable according to stain.

The following colours were recorded:-

Strain	2	7
	S. 214	S. 215
	196-181	225
	246	212
	214	211
		246

Other media: B o t t l e C u l t u r e .

M a i z e.(Fig.382)	L e o n i a n ' s.(Fig.383)	C z a p e k.
At first closely appressed, later (10 days) canescent velvety with small floccose outgrowths, otherwise similar to malt. Stain roseate orange turning coffee colour with age.	Similar to malt. Stain deep tawny.	Similar to other cultures but eventually more luxuriant. Stain yellow orange. (S.213).

P l a t e C u l t u r e .

M a i z e.(Figs.384, 385,386).	L e o n i a n ' s.(Fig.387)	C z a p e k.(Fig.388)
Almost submersed to very finely canescent. Surface somewhat gelatinous. Stain warm yellow orange (S.215) or brandy colour.(S.246).	Slightly more luxuriant than malt, with moderately coarse surface. Stain warm yellow orange (S.212,211,213).	Canescent to submersed with gelatinous surface. Stain deep orange yellow (S.246) or lighter (S.211-212) usually intense.

Microscopic characters: (Fig. 453 I).

Max. diam. of the primary mycelium = 2.3μ . Secondary mycelium consists of long lax hyphae loosely associated, $3.4 - 5.1 \mu$ diam.

Conidiophores and conidia: (Fig. 402B)

Conidiophores distinct from the vegetative mycelium by the shorter branches, $100 - 200 \mu$ long, usually dichotomously branched but sometimes ternate. Fertile branches $12 - 30 \mu$ long, mallet or skittle shaped, with swollen, rounded or truncate apices. Conidia borne in small apical clusters of $5 - 8 \mu$; oval to globose, rarely somewhat pyriform; hyaline when examined singly with a faint reddish tint but collectively faint orange; $1.4 - 3.1 \times 3.1 - 4.6 \mu$, average $2.3 \times 3.7 \mu$.

Hypoxyton coryphae:

Malt. A : Bottle Culture: (Fig. 372)

Appearance: At first (10 days) appressed felty with a coarse surface, later becoming denser and velvety. The aerial mycelium is dull white to olive brown.

Conidia: White to very pale grey, in granulate masses covering part of the lower half of the bottle slant; appearing after 5 months.

Stain: At first orange red (S.246) later turning to dull red - brown.

B : Plate Culture: (Fig.376).

Appearance: Canescent velvety, with a coarse surface, dull white subhyaline.

Conidia: None.

Stain: Variable, usually roseate at first (S.249) but sometimes orange yellow (S.215,227) or greenish yellow (S.250) but always turning dull red brown after 10 days.

Other media: B o t t l e C u l t u r e .

<u>M a i z e.</u> (Fig.373)	<u>L e o n i a n ' s .</u>	<u>C z a p e k.</u> (Fig.374)
Similar to malt but less luxuriant.	Similar to malt. Stain roseate at first (10 days:S.249)	Similar to malt but mycelium is grey.
No stain.	later deep chestnut black.	Stain at first faint pink (S.221-246) later murky dull brown.

Plate Culture.

Maize.(Fig.377)	Leonian's.	Czapek.
Almost entirely submerged aerial mycelium fine canescent, white stain greenish brown (S.337) restricted to centre region.	Similar to malt. Stain rose-ate to dull reddish olive.	Thin velvety, white subhyaline. Stain deep ochre to tawny.

Microscopic characters:(Fig. 453 B).

Max. diam. of the primary mycelium = 2.7μ . Secondary mycelium consisting of long sparingly branched, loosely associated hyphae $1.4 - 3.8 \mu$ diam.

Conidiophore and conidia: (Figs. 381 C, D).

Conidiophores scarcely distinct from the vegetative mycelium by the shorter ultimate branches; $50 - 130 \mu$ long, usually dichotomously but rarely ternately branched. Fertile branches $20-35 \mu$ long, straight or somewhat curved, normally with unmodified apices; conidia borne in apical clusters of 3 - 8, oval-elliptic equilateral, white collectively, $1.4 - 2.3 \times 2.9 - 4.6 \mu$, average $1.6 \times 3.5 \mu$.

Hypoxyton 38:Bottle Slant and Plate: (Figs. 382, 390).

Essentially similar on all media, though slightly less luxuriant on maize and Czapek. Colonies on malt white velvety opaque, with a coarse surface, gleaming white in colour.

Margin: Not distinct, entire.

Stain: None.

Microscopic characters:

Max. diam. of primary mycelium = 2.3μ , of secondary mycelium 2.8μ . Secondary mycelium closely associated, forming a network beneath the primary mycelium in bottle slant culture.

Conidiophores and conidia:

No conidiophores or conidia recorded.

Hypoxyton rubiginosum:

This species is similar in cultural characters to the former, except for the production of stain and the colour of the mycelium.

Malt. A : Bottle Culture: (Figs.391,392,393).

Appearance: At first appressed velvety up to 3 - 5 days, later velvet felty with a smooth surface. Generally the level of the colony is higher at the centre than the periphery. The colour of the mycelium varies from pink to saffron yellow, and does not appear to be constant for any one strain. Monospore and multispore cultures of each strain showed the full range of colouration. Normally the yellow (S.229) or pink (S.129) colour appears directly or soon after inoculation, forming a large central blaze or a halo round the centre. The colour reaches its maximum intensity after 14 days and then gradually fades.

Conidia: Produced after 4 - 8 weeks, and in varying degrees of profusion, usually sparse and inconspicuous, orange brown (S.178) in colour.

Stain: Variable in colouration and occurrence. Typically it is roseate orange (S.153) but may be murky olive brown. Usually the stain diffuses up to but not beyond the margin, but in some cases it is restricted to the central portion of the colony. It appears after 7 - 21 days.

B : Plate Culture: (Figs. 396, 397, 436).

Appearance: Variable, thin velvety to dense velvet felty, but usually the latter. Surface of colony smooth. Colouration variable according to strain, either gleaming white or discoloured, dull yellow or olivaceous red.

Conidia: None.

Stain: Variable, absent entirely or produced after 3 days, light roseate orange (S.153) to olive green or dull olive brown.

Other media: B o t t l e C u l t u r e .

M a i z e .(Fig.394)	L e o n i a n ' s .	C z a p e k .(Fig.395)
Similar to malt but sometimes slightly less luxuriant, and colouration always fainter. Stain roseate orange (S.249, turning to S.246-247) orange pink (S.190) or dark brown.	Similar to malt. Stain roseate brown. (S.203).	Similar to malt but differs by the tendency for radiating furrows to develop from the centre. Stain variable in time of appearance 14 - 28 days after inoculation; roseate (S. 204) mainly developed in the centre.

P l a t e C u l t u r e .

M a i z e .(Figs.398, 399).	L e o n i a n ' s .	C z a p e k .(Figs.400, 401).
Similar to malt but slightly less luxuriant and without stain. Stain none.	Similar to malt. Aerial mycelium tinted orange green (S.210) or orange yellow (S.215).	Similar to malt but colour of mycelium more intense. Stain deep yellow buff (S.246-337) or warm orange yellow (S.215) to roseate.

Microscopic characters: (Fig. 453 H).

Max. diam. of the primary mycelium = 2.0μ . Secondary mycelium forming a mat of closely associated frequently branched hyphae, $1.9 - 3.6 \mu$ diam.

Conidiophores and conidia: (Fig. 402C).

Conidiophores only distinct from the vegetative mycelium by the shorter ultimate branches, about 100μ long, usually dichotomously branched but sometimes ternate; fertile branches $12 - 30 \mu$ long, with unmodified apices. Conidia produced in small apical clusters of 4 - 6, equilateral or pyriform, sometimes with one end tapering to a point; $2.0 - 3.1 \times 4.3 - 5.4 \mu$, average $2.7 \times 4.8 \mu$.

Hypoxyton purpureum:

Malt. A : Bottle Culture: (Fig. 403)

Appearance: At first (1-7 days) submersed and hyaline, later (10 days) developing sparse appressed white subhyaline aerial mycelium, and finally (12-20 days) forming a thin aerial mat which gradually becomes dense velvety with age. The surface of the colony is fine and smooth, becoming granulate when conidia develop. Colour of mycelium dull opaque white in latter stages of development.

Conidia: Appressed after 3 months in one culture, and after 2 months in the other; pale grey to mouse colour (S.232) covering most of the colony to a depth of about $\frac{1}{2}$ mm.

Stain: At first roseate brandy colour (S.186), later deepening in time (S.81-126) after 14 days and finally, turning a dull grey black by 3 months.

B : Plate Culture: (Fig.405).

Appearance: Downy appressed to thin velvety; surface normally moderately coarse to fairly smooth.

Conidia: None.

Stain: Variable in occurrence, sometimes absent or very slight but usually conspicuously developed from the centre outwards, roseate (S.174, 193, 194-247) or very faint yellow, or reddish brown (S.248 deepening to S.173).

Other media: B o t t l e C u l t u r e .

M a i z e.(Fig.404)	L e o n i a n ' s .	C z a p e k .
Similar to malt but without conidia, and strain paler in colour.	Similar to malt. Mycelium white, tinted yellow (S.265) at the centre. Stain reddish orange (S.211-196).	Slightly less luxuriant than on malt, appressed. Stain dull tawny.

Plate Culture.

Maize.	Leonian's.(Fig.406)	Czapek.
Canescent appressed; aerial mycelium scanty white subhyaline. Stain light tawny (S.131).	Similar to malt but with slightly coarser surface Stain various shades of pale green, (S.405) to olive (S.217,218) ap- pearing late (72 weeks).	Restricted in growth, appressed velvety; mycelium white tinted pale red. Stain very deep red (S.81) spreading slightly beyond the border of the colony. General appearance si- milar to <u>Hypoxyton 18A</u> on Czapek.

Microscopic characters: (Fig. 437 D).

Max. diam. of the primary mycelium = 2.6μ . Secondary mycelium similar to that of Hypoxyton rubiginosum, $1.9 - 2.9 \mu$ diam. Conidiophores not apparent; conidia produced from the apices or sides of unmodified hyphae or from hyphal branches that are much narrower ($0.6 - 1.0 \mu$) than the vegetative mycelium ($1.5 - 2.6 \mu$). The conidia are linear elliptic with rounded or bluntly pointed ends; grey collectively, $1.7 - 2.6 \times 6.6 - 17.1 \mu$, average $2.0 \times 8.7 \mu$.

This species can be clearly distinguished from Hypoxyton rubiginosum on account of the deep red stain in bottle culture and the characters of the conidia.

Hypoxyton 2C:

Malt. A : Bottle Culture: (Fig. 412).

Appearance: Velvet felty, with surface initially coarse, later somewhat smoother, at first (1-15 days) white, later tinted fawn to brown, especially near the centre.

Conidia: Produced after 15 - 20 days over the greater part of the colony as a thin fawn to dull orange brown crust.

Stain: Roseate orange to deep red, produced up to the margin and deepening in intensity towards the centre.

B : Plate Culture (Figs. 413,414).

Appearance: Felty with characteristically coarse though not straggling, surface; dull white in colour and usually opaque.

Conidia: None.

Stain: Roseate orange (S.247) very diffuse, spreading from the centre towards the margin.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k .
Similar to malt.	Similar to malt, but with	Similar to malt.
Stain diffuse orange yellow (S.211).	wider range of mycelial colouration, from buff to tawny brown and green.	
	Stain reddish (S.196).	

P l a t e C u l t u r e .

M a i z e .(Fig.415)	L e o n i a n ' s .	C z a p e k .(Fig.416)
All basically similar to malt culture.		
Stain diffuse roseate orange (S.247).	Stain diffuse pink (S.174-158).	Less luxuriant than malt, appressed.
		Stain conspicuous deep orange.

Microscopic characters:

Max. diam. of the primary mycelium = 2.7μ . Secondary mycelium similar to that of Hypoxyton rubiginosum.

Conidiophores and conidia: (Fig. 437C).

Conidiophores only distinct from the vegetative mycelium by the shorter branches; about 90μ long, dichotomously branched. Fertile branches $20 - 45 \mu$ long, with characteristic globose swollen apices. Conidia borne in dense apical clusters, oval-elliptic, equilateral or pyriform with one end acuminate, reddish brown collectively, $1.1 - 2.3 \times 3.4 - 4.8 \mu$, average $1.7 \times 4.1 \mu$.

Hypoxyton tenue:

Malt. A : Bottle Culture: (Fig. 407).

Appearance: Velvet felty, aerial mycelium smooth, opaque, dull white.

Conidia: None recorded.

Stain: Dark red brown to chestnut, produced up to the margin and appearing after 7 days.

B : Plate Culture: (Figs.409,410).

Appearance: Dense opaque velvet felty, rarely subhyaline, dull white, sometimes tinted pale red. Saltants of much less luxuriant type frequently occur.

Conidia: None recorded.

Stain: Characteristically irregular in formation, either in large semi-circular areas concentrically arranged formed the centre of the colony or in small flecks, but very rarely diffuse and evenly spread. The colour varies but is always some shade of dark red or reddish black, (S.201, 211, 131, 111-116).

Other media: B o t t l e C u l t u r e .

M a i z e.(Fig.408)	L e o n i a n ' s .	C z a p e k .
Canescent velvety, white subhyaline. Stain none.	Similar to malt.	Canescent velvety, dull red, with surface thin on maize, but otherwise similar. Stain diffuse reddish orange (S.247).

P l a t e C u l t u r e .

M a i z e.(Fig.411)	L e o n i a n ' s .	C z a p e k .
Appressed to submersed, gelatinous, white subhyaline. Stain chocolate brown, only produced at site of inoculation.	Similar to malt.	Thin velvety, opaque smooth, dull white tinted saffron in parts. No stain.

Microscopic characters:

Max. diam. of primary mycelium = 2.7 μ . Secondary mycelium similar to

that of Hypoxyton rubiginosum.

Hypoxyton haematostroma:

Malt. A : Bottle Culture:(Fig. 417).

Appearance: At first appressed subhyaline up to 4 days old, but later becoming dense velvet felty, white subhyaline tinted pale yellow to dull red. Colonies show the following appearance after 10 - 20 days:-

1. Marginal zone 5 - 10 mms. broad, colourless to white subhyaline.
2. Large pale reddish grey area of variable extent.
3. Centre, pale yellow to dull red, raised above the level of the rest of the colony.

After 20 days the centre part of the colony invariably turns dull red and bears secreted drops of deep red stain.

Conidia: Pale red, not conspicuous on the surface of the colony, and produced after one month.

Stain: Dull red, very conspicuous and penetrating the whole of the medium (S.112).

B : Plate Culture: (Fig.420).

Appearance: Similar to the above in main features, velvet felty, pale red or rarely white, usually opaque; surface smooth to rather coarse. No secreted drops of stain.

Conidia: None.

Stain: Dark red, not extending beyond the margin (S.112).

Other media: B o t t l e C u l t u r e .

<u>M a i z e</u> .(Fig.418)	<u>L e o n i a n ' s</u> .	<u>C z a p e k</u> .(Fig.419)
Similar to malt but mycelium opaque white, scarcely tinted by any colour, and stain dull red to murky or roseate orange (S.196), but not deep red.	Similar to malt but colouration not quite as vivid.	Less luxuriant than malt, canescent to sub-felty with finely granulate coarse surface. Stain faint roseate orange (S.190), diffuse throughout colony.

Plate Culture.

Maize.	Leonian's.(Fig.421)	Czapek.
Similar to malt but less luxuriant and without as profuse production of stain.	Similar to malt.	Downy to thin velvety, opaque or subhyaline dull white tinted yellow. Stain dull red.

Microscopic characters:

Max. diam. of the primary mycelium = 1.7 μ . Secondary mycelium similar to that of Hypoxyton rubiginosum.

Conidiophores and conidia: (Figs. 454 A, B, C).

Conidiophores distinct from the vegetative mycelium by the shorter branches; consisting of a compact dichotomous ramifying system 150 - 220 μ long. Fertile branches 12 - 70 μ long, with unspecialized apices. Conidia borne in apical clusters, oval equilateral, usually with bluntly pointed ends, dull red collectively, 1.4 - 2.3 x 3.1 - 5.1 μ , average 1.8 x 3.9 μ .

Hypoxyton piceum:

Malt. A : Bottle Culture:(Fig. 434).

Appearance: Velvety, forming a thin mat of gradually decreasing thickness towards the margin. Colour of mycelium white at first, but gradually turning brilliant orange in the centre by one month.

Conidia: Sparse, rusty red in colour and inconspicuous; produced after one month.

Stain: Dull reddish brown, not reaching the margin of the colony, and darkening abruptly at the centre.

B : Plate Culture: (Fig. 435).

Appearance: Velvety, opaque white with smooth fine surface.

Conidia: None.

Stain: Variable, either deep sherry brown or faint reddish brown or absent. The same degree of variation was to be found in all strains examined.

Other media: Essentially similar, though less luxuriant on maize. (Fig. 445).

Czapek bottle culture with typical radiate furrowing after 20 days growth.

Microscopic characters: (Fig. 453 C).

Maximum diameter of the primary mycelium = 2.6μ . Secondary mycelium $2.0 - 3.5 \mu$ diameter.

Conidiophores and conidia: (Fig. 437 B).

Essentially similar to those of Hypoxylon rubiginosum and Hypoxylon 2C. Fertile branches $15 - 25 \mu$ long with globose or swollen apices. Conidia oval to elliptic, sometimes pyriform, dull rusty brown collectively, $2.1 - 2.4 \times 4.2 - 6.9 \mu$, average $2.3 \times 5.4 \mu$.

Hypoxylon ochraceo-fulvum:

Bottle Cultures (All media) (Fig. 441):

Similar to the preceding species on malt in general appearance, but fleecy rather than velvety, dense opaque and with a coarse surface. Colour white, often tinted orange or dull brown in parts but never showing the orange centre colouration that is characteristic of Hypoxylon piceum. Conidia sparse, rusty brown. Stain scanty, dull murky red-brown.

Plate Cultures (All media) (Fig. 442):

Velvet fleecy, dense opaque white, with coarse surface. Conidia nil. Stain nil.

Microscopic characters:

Maximum diameter of the primary mycelium = 2.6μ . Secondary mycelium similar to that of Hypoxylon rubiginosum, $2.1 - 3.4 \mu$ diam.

Conidiophores and conidia:

Similar to that of Hypoxylon piceum; conidia measure $2.1 - 2.6 \times 4.0 - 6.7 \mu$, average $2.2 \times 5.6 \mu$.

Hypoxylon ferrugineo rufum:Var. 1. Bottle Cultures (All media) (Fig.438):

Appearance: Thin velvety, smooth, colour of mycelium opaque white tinted primrose throughout.

Conidia: Dull rusty brown, inconspicuous and sparse, produced after 14 days. Czapek culture cast into radiating folds after 14 days, as with other species.

Stain: Roseate brown, spreading beyond the margin of the colony.

Plate Culture (Malt, maize and Leonian's) (Fig.440):

Appearance: Thin velvety to appressed, subhyaline, surface smooth; colour of mycelium dull brandy colour.

Conidia: None.

Stain: Deep brandy to sherry brown in colour. Slow growing.

Czapek Plate Culture:

Almost completely submersed, otherwise identical
Conidia produced sparsely behind the margin.

Microscopic characters:

Maximum diameter of the primary mycelium = 2.0 μ Secondary mycelium similar to that of Hypoxylon vogesiacum, 2.6 - 4.0 μ diam.

Conidiophores and conidia:

Conidiophores of the Hypoxylon rubiginosum type. Conidia oval equilateral, dull rusty brown collectively, 1.9 - 3.0 x 3.9 - 5.7 μ , average 2.4 x 4.3 μ .

Var. 2.Bottle Cultures (All media) (Fig. 439):

Appearance: Thin velvety, smooth, colour of mycelium dull reddish brown, subhyaline. Colonies on malt eventually (14-21 days) becoming densely covered with granulate dull reddish brown conidia.

Stain: Brilliant sherry colour, not darkening with age.

Plate Culture (Malt, maize and Leonian's) (Fig.440):

Velvet felty, with a coarse surface. Colour of aerial mycelium orange. No conidia. Stain brilliant orange to sherry colour. Colony fast growing. Czapek canescent velvety, with smooth fine surface. Colour of mycelium and stain cinnabar orange.

Microscopic characters:

Maximum diameter of primary mycelium = 3.0μ . Secondary mycelium $2.6 - 4.0 \mu$ diameter.

Conidiophores and conidia:

As above for Var. 1.

Hypoxyylon hypomiltum:Bottle Culture (Malt and Leonian's) (Fig. 433):

Appearance: At first (1-14 days) mainly downy appressed, divided into a large marginal zone with scant white subhyaline appressed aerial mycelium and a centre with more developed mycelium, thin velvety, smooth, white opaque tinted pale cream.

Conidia: Sparse, rusty brown, produced after 1 month.

Stain: Variable in time of appearance; in one malt culture it was evident by 14 days, in the other by 28 days; colour at first deep mauve pink (S.16-51) turning with age to a rich orange red (S.172-173) and finally, deepening to black.

Maize: As above but no stain.

Czapek: At first (1-17 days) almost completely submersed, with very sparse appressed aerial hyaline mycelium; later sparsely canescent, white subhyaline throughout.

Stain: None.

Plate Culture (Malt, maize and Leonian's) (Figs. 443,446):

Velvety, opaque white with smooth fine surface; similar to Hypoxyylon piceum but slower in rate of growth. Maize colonies slightly less luxuriant than on other two media. Czapek submersed except for the centre of the colony which is canescent (Fig. 444). Colour hyaline colourless to white subhyaline. No stain.

Microscopic character.

Maximum diameter of the primary mycelium = 3.0μ . Secondary mycelium absent.

Conidiophores and conidia:

Similar to *Hypoxylon piceum*; conidia $2.1 - 2.6 \times 4.0 - 7.0 \mu$, average $2.3 \times 5.7 \mu$.

Hypoxylon 18C:

Bottle Cultures (Malt, Maize, Leonian's) (Fig. 422):

Appearance: Thin velvety to felty, surface coarse; mycelium dull opaque white, tinted pale olive green in parts.

Conidia: Produced in pulvinate masses over most of the surface of the colony after 2 months and are pale green to pale brown in colour.

Stain: Olive green (S.426-431), produced by 14 days and deepening to black with age; lighter towards outside than centre of colony.

Czapek: (Fig. 423):

Appearance: Less luxuriant than above, canescent felty, white opaque with a moderately smooth surface.

Conidia: None.

Stain: Very faint green near centre of the colony.

Plate Culture:

Malt and Leonian's (Figs. 424, 426):

Velvet felty, with a coarse surface, dull white, opaque. No conidia. Stain pale green (S.224) to dull brown (S.250) deepening to dark brown (S.201), usually forming a band 5 - 9 mm. broad, 8 mms. from the periphery of the colony.

Czapek: (Fig. 427):

Appearance: Similar to the above but canescent velvety with a smooth surface.

Stain: None.

Maize: (Fig. 425):

Appearance: Submersed except for ruff of sparse white subhyaline mycelium behind the margin; surface otherwise smooth and sodden.

Stain: None.

Microscopic characters:

Maximum diameter of the primary mycelium = 2.2μ . Secondary mycelium similar to that of Hypoxylon rubiginosum.

Conidiophores and conidia: (Fig. 437 A):

Conidiophores similar to those of Hypoxylon haematostroma, 100 - 200 μ long, compactly multibranched; fertile branches 15 - 75 μ long, with unmodified apices; conidia relatively small, globose to oval, equilateral, olive green or olive brown collectively, 1.4 - 2.3 x 2.0 - 4.0 μ , average 1.9 x 2.7 μ .

Hypoxylon 18 G:

Malt. A : Bottle Culture: (Figs. 428, 429).

Appearance: Velvet felty, forming a thin mat with rather a coarse surface, colour of mycelium white to very pale grey, and tinted pale reddish brown (S.162) near the centre of the colony after 7 days, changing to reddish orange with age (> 3 weeks).

Conidia: Formed in fawn coloured irregularly pulvinate groups after 2 months, scattered over the whole colony but aggregated near the shallow end of the slant culture.

Stain: Roseate brown (S.131 - 191)(bruin havane or deep bistre) appearing by 7 days and becoming very conspicuous beneath the aerial mycelium, usually extending 5 mm. beyond the border of the colony.

B : Plate Culture: (Fig. 430).

Appearance: Velvety smooth; colony usually clearly divisible into a large opaque white centre and a subhyaline canescent margin with the stain showing conspicuously beneath.

Conidia: None.

Stain: Deep chestnut, uniform throughout and sometimes spreading beyond the margin.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k .
Canescent, subhyaline white. Conidia none. Stain produced in the centre only, very faint amber orange (S.250).	Similar to malt.	Shows the typical reaction of most spp; thinner but more opaque, and typically cast into radiating furrows with age (> 3 weeks). Aerial mycelium white tinted pink. No conidia. Stain orange yellow (S.186) in the centre of the colony, lightening to yellow (S.193) outside; produced after 1 week.

P l a t e C u l t u r e .

M a i z e .(Fig.431)	L e o n i a n ' s .	C z a p e k . (Fig.432).
Canescent velvety, usually opaque with smooth surface, white.	Similar to malt.	Submersed, almost colourless, hyaline.

Microscopic characters:

Maximum diameter of the primary mycelium = 3.3μ . Secondary mycelium similar to that of Hypoxylon vogesiacin, $1.6 - 3.4\mu$.

Conidiophores and conidia: (Fig. 454 E).

The conidiophores differ considerably from the normal in several interesting features. They are broader than those of other species, measuring $2.3-3.9\mu$ diam., sometimes amber coloured instead of hyaline, and the fertile branches narrow terminally to form peculiar hooked apices from which the conidia are borne. The conidiophores are $150 - 200\mu$ long, dichotomously branched; fertile branches $10 - 60\mu$ long; conidia larger, oval-elliptic, reddish brown collectively, $2.3 - 3.7 \times 4.0 - 7.4\mu$, average $2.8 \times 5.3\mu$.

Hypoxyylon vividum:

Malt. A : Bottle Culture (Figs. 447, 448):

Appearance: Velvety, forming a very dense smooth mat up to 2mm. high. With age (> 21 days) the centre of the colony becomes raised above the outer part. The colour of the aerial mycelium is at first white (1-10 days) later turning yellow buff at the centre of the colony and reddish (S.172,168) towards the outside. After 21 days drops of reddish brown stain appear over the centre of the colony.

Conidia: Very infrequent, only appearing in 1 out of 4 cultures examined.

Stain: Light roseate (S.153-173) deepening after 20 days to reddish brown.

B : Plate Culture (Fig. 450):

Appearance: Velvety, smooth; aerial mycelium white, tinted pale yellow to saffron at the centre.

Conidia: None.

Stain: Deep red, (S.171) uniformly diffused.

Other media: B o t t l e C u l t u r e .

M a i z e .	L e o n i a n ' s .	C z a p e k . (Fig.449).
Similar to malt but less luxuriant. No conidia. Stain roseate but only developed at the centre and not extending to the margin.	Similar to malt. No conidia.	Similar to malt but with radiating furrows in the mycelium developing after 3 weeks.

P l a t e C u l t u r e .

M a i z e .(Fig.451)	L e o n i a n ' s .	C z a p e k .(Fig.452).
Similar to malt but less luxuriant appressed velvet felty with coarse surface. Stain dull red.	Similar to malt.	Submersed to appressed, stain dull red, limited in quantity.

Microscopic characters: (Fig. 453 G):

Maximum diameter of the primary mycelium = 1.4μ . Secondary mycelium similar to that of Hypoxylon vogesiacum, $1.7 - 5.2 \mu$ diam.

Conidiophores and conidia: (Fig. 454 D):

Conidiophores unique, $100 - 200 \mu$ long, sympodially branched with the main axes terminating in small apical clusters of conidia and the side branches continuing the fertile system. Conidia elliptic equilateral with bluntly pointed ends, $1.1 - 2.0 \times 3.1 - 4.6 \mu$, average $1.4 \times 3.7 \mu$.

Discussion and Conclusions:

In comparison with the species of preceding groups, it can easily be seen that differences in cultural characters between members of this group are not nearly as marked. The main differences lie in the colour of the stain and in the conidial and conidiophore characters. The variations in growth character of the majority of the species have been tabled on p. 144. The following points are important:-

1. Only those species with grey stromata (H. plumbeum, H. 18A, H. 18B), show absolute correlation between colour of the stroma and colour of the stain (pink or to red orange). Although the majority of the species with brown or reddish-brown stromata also produce brown or olive-brown stains, a minority, comprising Hypoxylon 19 and Hypoxylon coryphae, do not. These, with the remainder of the species that are characterized by purple, green, golden, or yellow stromata, produce stains varying from olive green or tawny ochre to red or dull brown.
2. The main differences in growth character between the species lies in the texture (whether smooth or coarse) and colouration of the mycelium. The density and degree of luxuriance and the colour of the stain, are of secondary importance, although of undoubted interest in some cases.
3. There are 9 types of conidiophore, 4 of which correspond to those described for other species groups (see p. 91). These are:-

Type II : H. purpureum (part)
 III : H. plumbeum

- Type V A : H. rubiginosum
Hypoxyton 18B
- V B : Hypoxyton 18F
Hypoxyton 18D
H. vogesiacum
Hypoxyton 18C
H. haematostroma
Hypoxyton 2C
H. piceum
H. ochraceo-fulvum
H. ferrugineo-rufum
H. hypomiltum

- Type IV A : Hypoxyton 19

The two types which are apparently peculiar to the group are:-

- Type IV B : Fertile branches of the conidiophores whorled,
sometimes verticillate
Hypoxyton 18E
H. coryphae
- Type VIII : Conidiophores dichotomously branched, distinct
from the vegetative mycelium by the shorter
ultimate branches; terminal part of the fertile
branches distinctly narrower than the remainder.
H. purpureum (part)
Hypoxyton 18G
- Type IX : Conidiophores sympodially branched, the main axes
ending in short apical conidial clusters
Hypoxyton vividum.

4. Growth Rate. (Table IX, Graphs H-J):

In general, the differences between strains were found to be significant at one or more temperatures. The variability of some of the species,

which had been noted prior to testing, was confirmed, however, by statistical analysis. The strains of each pair tested for H. vogesiacum and H. 18E differed significantly between themselves. No difference existed, however, between the respective perfect stages.

In contrast to these inconclusive results, a close measure of agreement existed between the values obtained for different strains of H. rubiginosum. These strains were drawn from perfect stages which conformed only to the narrow definitions of the species quoted by Saccardo (1882, p.375) and given earlier in this chapter (p.127). Conversely, strains drawn from perfect stages that differed slightly from this type also showed differences in growth temperature reaction that were significant. Table IX shows that these included the strains that were originally placed by modern authorities in H. rubiginosum (refer p.133). Thus the work on growth rates indicates that there is variability in behaviour among the forms at present grouped under H. rubiginosum but that there is constancy among individuals of a certain section of that species.

Similar conclusions can be drawn for H. haematostroma. There is no agreement between the results obtained for Hypoxylon 18F, H. haematostroma (as defined in the text) and Hypoxylon vividum, all of which were placed in the same species by the authorities.

5. Correlation between stromal and cultural characters.

This is shown by the table on the following page.

SPECIES.	STROMAL CHARACTER (COLOUR AND PRE- SENCE OF ECTOSTRO- MAL PARTICLES).	AVERAGE SPORE SIZE (μ).	GROWTH CHARACTER (MALT) PLATE.	CONIDIO- PHORE TYPE.	STAIN (MALT EXCEPT WHERE STATED).	PRESENCE OF SECONDARY MYCELIUM.	GROWTH RATE	
							OPTIMUM.	CHARACTER
ypoxylon 18A	Grey-brown.	5.7 x 13.4	Canescent, dull white subhyaline.	VB	Roseate or orange brown; brilliant ro- seate orange on Czapek.	-	25°	Very slow
. plumbeum	Grey.	5.6 x 11.2	Velvety, white sub- hyaline to opaque.	III	Pink.	-	20°	Very slow.
ypoxylon 18B	Grey + ectostromal particles.	6.1 x 13.6	Canescent velvety, characteristically very smooth and fine.	VA	Pink.	-	25°	Very slow.
ypoxylon 19	Dark grey brown to black.	7.2 x 15.3	Dense velvety.	IVA	Deep golden brown.	+	25°	Slow.
ypoxylon 18F	Brown	6.3 x 13.4	Dense velvety.	VB	Nil	+	25°	Very slow.
ypoxylon 18D	Dark brown	3.9 x 8.1	Velvety opaque	VB	Nil (orange brown on Lecnian's).	-	28°	Moderate.
ypoxylon 18E	Red-brown	4.4 x 10.4	Canescent to thin velvety.	IVB	Dull olive brown.	-) 418 25°) 556 28°	Slow. Fast.
. coryphae	Red-brown + ectostromal parti- cles.	3.5 x 6.8	Thin velvet-felty.	IVB	Orange red to dull red brown.	+	25°	Moderate.
. vogesiacum	Light purple, rarely red-brown.	6.0 x 13.2	Thin velvety, sub- hyaline or opaque.	VB	Dull yellow ochre to ochre brown.	+) 2 25°) 312 25°	Slow. Very slow.
ypoxylon 38	Deep maroon purple to purple red.	5.5 x 9.5	Dense velvety opaque.	-	Dull murky red-brown.	+	28°	Moderate.

<i>H. rubiginosum</i>	Light purple red, rarely red-brown.	4.8 x 10.5	Velvety, moderate or dense, mainly opaque.	IVA	Dull red.	+	25°	Slow.
<i>H. purpureum</i>	Deep maroon purple to purple red.	5.5 x 11.5	Thin velvet felty, smooth opaque.	II, VIII	Dull red to roseate orange.	+	25°	}482 Fast }470 Moderate
Hypoxylon 2C	Pale purple red + ectostromal parti- cles.	6.7 x 15.2	Coarse felty.	VB	Very pale red to absent.	+	25°	Moderate
Hypoxylon tenue	Deep maroon purple to dull purple.	6.2 x 11.8	Dense velvety opaque.	-	Chestnut red, peculiar in distribution.	+	25°	Very slow
<i>H. haematostroma</i>	Dark purple	7.1 x 15.1	Velvet, moderately dense.	VB	Deep red.	+	25°	Slow
<i>H. luridum</i> var. minus	Yellow green to olive.	5.1 x 9.8	Coarse felty.	VB	Pale olive green.	+	25°	Moderate
Hypoxylon 18G	Dull olive green	5.1 x 11.7	Velvet, moderately dense.	VIII	Deep red to nearly black.	+	28°C	Moderate.
Hypoxylon piceum	Yellow to ferrugi- neous.	5.6 x 11.6	Velvet moderately dense.	VB	Sherry or absent.	+	25°	Slow.
ochraceo-fulvum	Orange-yellow to ferruginous.	5.0 x 9.4	Dense velvet fleecy, coarse.	VB	Dull red-murky brown.	+	28°	Fast.
ferrugineo-rufum var. 1.	Rusty; interior orange; + ectostromal particles.	5.0 x 10.6	Velvet smooth	VB	Sherry colour.	+	25°	Slow.
var. 2.	Without ectostromal particles.	4.9 x 10.8	Felty coarse	VB	Orange.	+	25°	Fast.
hypomiltum	Lateritic orange	5-6.5 x 8-11 (Miller's measurements)	Mainly thin, velvety smooth.	VB	Violet to purple	-	20°	Moderate.
Hypoxylon vividum	Dull orange.	7.0 x 15.6	Moderately dense, velvety.	IX	Light red.	+	original 28° cultured 25°	Moderate to Fast.

The problem of specific limits:

Without exception, the cultural work shows differences between the species that can be described precisely. This supports the use of minor characters such as differences in colouration and differentiation of the stromata, besides confirming the significance of small differences in spore size. It is thought that the classification of the material collected is basically correct. The following conclusions about individual species can also be drawn:-

(1) Hypoxyton rubiginosum:

This species, as at present recognized, appears to contain several closely allied forms that can be separated on stroma colour, spore size and cultural characters. These forms have already been given separate specific status (see list at end of the description of the perfect stages). The name, H. rubiginosum, should probably be restricted in future to forms with a purple or purple-brown stroma and ascospores measuring $4 - 6 \times 9 - 12 \mu$, av. $5 \times 10.5 \mu$. The loss of colouration with age of this and many other species might make this separation impracticable, however.

(2) Hypoxyton hypomiltum:

This species should comprise only those forms with the characteristic orange stromal colouration, and should exclude others which are yellow, ferruginous, red-purple or grey. The species, Hypoxyton 18B, identified by Kew as Hypoxyton hypomiltum, does not agree with the description of Hypoxyton hypomiltum cited by Saccardo (1882), nor does it agree with material identified by Miller. This and the marked difference in cultural characters, should separate the 2 species. Hypoxyton 18B is probably undescribed.

(3) Hypoxyton haematostroma:

Three forms, classified by the authorities as this species, can be separated on stromal colouration. Clear differences in growth character, growth rate, conidiophores and strain support these differences. On this evidence the following conclusions may be drawn:-

- i. Hypoxyton 18F is probably a new species,
- ii. H. haematostroma should be restricted to forms with purple stromata and spores measuring 5 - 9 x 11 - 20 μ , average 7x15 μ .
- iii. If the identification of strain 268 as H. vividum is correct, then that species should be retained as separate from H. haematostroma. If the identification is incorrect, then a new specific name for the strain should be devised.

When material of strain 268 was sent to Dr. Miller, it was classified as a yellow variety of H. haematostroma. He did not state specifically whether it was also the same as H. vividum, which he clearly regarded (1942, list of synonyms for H. haematostroma) as a synonym.

In addition to the three species just discussed there is a fourth, Hypoxyton 2C which is very similar to H. haematostroma in the perfect stage. The chief points of distinction are the darker interior colouration of the stroma and the presence of superficial ectostromal particles, visible in longitudinal section. The material of this species was classified as H. rubiginosum var. tropica by Dr. Miller. It is presumed that this term was created after receipt of the material since no description appears to have been published. In any case, this form can be distinguished both from H. rubiginosum and H. haematostroma although closer related to the latter, in stromal and cultural characters. Again, it would seem appropriate to give this species a new name.

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Hypoxylon luridum:

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Hypoxylon ochraceo-fulvum:

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Hypoxyylon piceum:

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CHAPTER VI.

THE GENERA DALDINIA, PENZIGIA AND XYLARIA.

The remaining species studied can be separated from Hypoxylon chiefly in the presence of a fleshy entostroma which persists at least up to maturity. They are placed in 3 genera according to the classifications of Miller (1942) and of Clements and Shear (1931):-

- Daldinia Ces. & de Notaris : interior of stroma darkly coloured,
zonate.
- Penzigia Saccardo : interior of stroma white; fertile part
of stroma flat or convex.
- Xylaria Hill ex Fr. : interior of stroma white; fertile part
of stroma cylindrical.

Daldinia Ces. & de Notaris :

Two species are at present recorded by Miller (1942) from South Africa, both of which have been collected and compared with identified material in the Pretoria Herbarium:

A : Perfect Stage. (Refer to Table X, Appendix III, for statistical analysis of spore size).

Daldinia concentrica. (Bolt ex Fr.) Ces. & de Not. (Figs. 455, 457, 458, 460A):

Stromata globose, varying greatly in size from 5 - 50 mms. diam., usually sessile but frequently with a narrow basal portion that is superficial or immersed entirely in the substrate. Ectostroma thin, granular, deep purple, sometimes with an orange tint in section; entostroma dark brown, finally black, with a variable number (5-15) of narrow concentric rings of paler hue. Perithecia 400 - 700 x 1000 - 1200 μ , globose; walls broad basally and membranous except for the partly carbonized vertices; ostioles papillate or punctate, apparently arising by irregular rupture of the ectostroma. Asci clavate, long stipitate, 160 - 220 x 6 μ ; stalks 90-130 μ . Spores oval equilateral with blunt or rounded ends, dark brown, 4.5 - 6.8 x 9.0 - 13.5 μ , average 5.7 x 11.2 μ (90).

Hosts: Passerina falcifolia

Olea capensis; dead wood and bark.

Daldiniaescholzii(Ehr. ex Fr.) Rehm. (Figs. 456, 459, 460):

Stromata globose to aplano pulvinate, varying greatly in size from 5 - 60 mm. across, always sessile on the substrate. Ectostroma smooth, bright orange superficially, saffron yellow in thin section; entostroma dark brown to jet black with 1 - 2 narrow concentric zones. Perithecia 200 - 300 x 600 - 800 μ , globose except for the carbonous vertices; ostioles punctate, very indistinct, apparently arising by rupture of the ectostroma. Asci not seen. Spores oval, strongly inequilateral, with blunt or rounded ends, pale brown, 3.0 - 4.5 x 7.5 - 9.0 μ , average 3.9 x 8.7 μ .

Host: Wood unidentifiable.

B : Cultural characters:

The 2 species bear no similarity to each other. The following table shows how they compare:-

Bottle Culture:Daldinia concentrica:

M e d i u m.	G r o w t h C h a r a c t e r s.
<u>Malt</u> : (Fig.461)	Very coarse felty, luxuriant and spreading very fast; colour dirty white, with grey patches, texture uniform throughout. Margin indistinct, effuse. Conidia inconspicuous dull grey (with red tint when examined closely). Stain dull olive grey turning black with age. Carbonization of the medium occurs after 20 days, forming a thin black layer beneath the surface mycelium.
<u>Maize</u> :	Coarse felty similar to malt. Stain late in appearance (> 16 days) black.
<u>Leonian's</u> : (Fig.462)	Similar to malt but divided after 14 days into:- i. marginal zone 2 - 3 cm. wide appressed but with cottony scattered outgrowths up to 3 mm. high. Margin indistinct, effuse. ii. Central zone 2 - 4 cm. wide, coarse felty dull white to grey. Stain yellow at first (S.227) later dull grey black.
<u>Czapek</u> :	Similar to Leonian's divided into:- a. marginal zone up to 2.5 cm. wide, mainly appressed but with scattered cottony mycelium up to 3 mm. high. Margin indistinct, effuse. b. Central zone 3.7 cm. diam, coarse felty, dull white to grey. Stain light yellow (S.229) at first (14 days) later dull grey black.

Bottle Culture:Daldinia eschscholzii:

Similar on all media; (Malt, maize, Leonian's and Czapek). Mycelium closely appressed, canescent subhyaline to grey opaque. (Fig. 466).
Margin indistinct, compact. Conidia inconspicuous, scattered over the whole surface of the colony.
Stain red ochre, fading to deep black shortly after 14 days.

Plate Culture:Daldinia concentrica:

M e d i u m.	G r o w t h C h a r a c t e r.
<u>Malt:</u> (Figs. 463,464)	<p>Mycelium loose, felty coarse, with characteristic uneven surface, spreading very fast; dull white with small granules of dark colour due to aggregations of closely knit secondary mycelium. <u>Margin:</u> indistinct submersed, entire, effuse. <u>Conidia:</u> dirty white to pale grey, formed after 7 days over the edge of the colony. <u>Stain:</u> poorly developed at 15-20°C, forming a band up to 10 mm. wide 11 mms. from the centre; dull yellow (S.340). This is often preceded by a smaller band encircling the centre of the colony that is brown (S.702-706). At 28°C, stain dark brown, spreading under entire colony.</p>
<u>Maize:</u> (Fig.465).	<p>Canescent appressed thin, white subhyaline. <u>Margin:</u> indistinct submersed, entire, compact to effuse. <u>Conidia:</u> none. <u>Stain:</u> at first amber yellow later deepening to brown, forming an interrupted halo 1.3 - 2.2 cm. broad and 6 - 14 mm. away from centre of colony.</p>
<u>Leonian's:</u>	<p>Appressed felty, divided into:- i. marginal zone 4 - 6 mm. wide, almost submersed, white subhyaline, entire, compact at first, later plumose effuse. ii. centre appressed with coarse felty texture. <u>Conidia:</u> none. <u>Stain:</u> yellow green tinted brown (S.338) spreading outwards beyond boundaries of the colony.</p>
<u>Czapek:</u>	<p>Dissimilar from other colonies in greater luxuriance, divided into:- a. marginal zone 10 - 15 mm. wide, canescent appressed, pale yellow subhyaline entire, compact to effuse. b. centre lanose up to 5 mm. high white to grey; with age becoming depressed. Surface very coarse and uneven. <u>Stain:</u> restricted to centre region, rich yellow brown darkening suddenly to black (S.211).</p>

Plate Culture:Daldinia eschscholzii:

<u>Medium.</u>	<u>Growth Character.</u>
<u>Malt:</u> (Fig.467)	Sparsely canescent, dull subhyaline white. <u>Margin</u> : indistinct, submersed, entire to slightly lobed compact with peripheral hyphae lying closely parallel. <u>Conidia</u> : pink, scattered uniformly, appearing after 7 days. <u>Stain</u> : greenish behind margin (S.263) to dull red (S.177) at centre.
<u>Maize:</u>	Canescent appressed to sparse velvety with advanced age (4 weeks). <u>Margin</u> : submersed, entire, compact. <u>Conidia</u> : none. <u>Stain</u> : formed in outer part of colony only, inconspicuous, orange (S.246) to dull orange brown.
<u>Leonian's:</u> (Fig.468)	Similar to malt and maize but more luxuriant; canescent velvety with smooth fine surface; white subhyaline. <u>Margin</u> : indistinct downy submersed, lobed compact. <u>Conidia</u> : none. <u>Stain</u> : as for malt.
<u>Czapek:</u>	Almost entirely submersed; aerial mycelium faint with granulate appearance, colourless, hyaline. <u>Margin</u> : indistinct, compact. <u>Conidia</u> : none. <u>Stain</u> : fiery red orange at margin deepening abruptly to black.

Microscopic characters:Primary mycelium

Max. diam:	<u>Daldinia concentrica</u>	3.0 μ (Fig.469 A).
	<u>Daldinia eschscholzii</u>	2.2 μ (Fig.469 B).

Secondary mycelium:

Here the 2 species show interesting differences.

The secondary mycelium of Daldinia concentrica is similar to that of Hypoxyton rubiginosum and other species in that group, and forms a closely knit anastomosing system of dark hyphae 1.1 - 4.4 μ (Fig.469 C). The secondary mycelium of Daldinia eschscholzii however, consists of long rope-like strands consisting of several hyphae 1.8 - 3.7 μ diam., united along part of their length from which many branches ramify outwards (Fig.469 D).

Conidiophores and conidia:

- Key:- Conidiophores verticillately branched;
with clavate fertile branches.....Daldinia concentrica.
- Conidiophores dichotomously multibranched;
fertile branches tereteDaldinia eschscholzii

Daldinia concentrica: (Fig. 470 C, D).

Conidiophores distinct from the vegetative mycelium on account of the dull red colour, wall pitting and verticillate branching; each comprising a compound dichotomous and verticillate branched system 150 - 200 μ long. The ultimate, fertile, branches are 8 - 40 μ long, (usually about 15 μ) x 2.5 - 4 μ , short, stout, normally unseptate, with very conspicuous clavate apices 4 - 10 μ long. Conidia borne in clusters of 8 - 30 from the apices and sides of the fertile branches; oval equilateral, with both ends rounded, hyaline, colourless individually but white to pink grey collectively; 1.7 - 3.7 x 3.4 - 5.7 μ , average 2.2 x 4.3 μ .

The conidiophore is structurally similar to that characteristic of the Nummularium group discussed in Chapter III, but differs in the verticillate arrangement of the fertile hyphae. For this reason it is best to denote it as a separate type, Type VI B.

Daldinia eschscholzii (Fig. 470 A, B).

Conidiophores only distinct from the vegetative mycelium on account of the closely dichotomous branching and shorter ultimate branches. They each comprise a compound dichotomously branched system 100 - 300 μ long. The fertile branches are 10 - 25 x 1 - 2 μ long, terete, unseptate, usually with small globose apices. Conidia acrogenous, in clusters of 3-8 μ , oval-elliptic with rounded ends, hyaline, colourless individually but dark grey to black en masse, 1.7 - 2.6 x 2.9 - 4.3 μ , average 2.4 x 3.6 μ .

The conidiophore is structurally similar to those of Hypoxylon 18C and Hypoxylon haematostroma discussed in the previous chapter. This type is labelled as Type V B.

The two species are clearly dissimilar in growth characters and also in growth rate. (See Graph K).

Penzigia:

A : The Perfect Stage: (See Table XI, Appendix III, for statistical analysis of spore sizes).

Key to species:-

1. Perithecia clearly evident in outline through stromal covering, aggregated but not coalesced, and seated in a fleshy crustose matrix. Spores $5.0 - 8.5 \times 14.5 - 19.0 \mu$, av. $6.4 \times 16.4 \mu$.
----- Penzigia 1 (nov.sp.)
- 1'. Perithecia not evident in outline or very faintly so, closely associated with each other in the stroma. -----2.
2. Interior of stroma yellow or golden -----3.
- 2'. Interior of stroma white -----4.
3. Stromata erumpent, spores $3.0 - 5.5 \times 10.5 - 14.0 \mu$, average $3.9 \times 12.2 \mu$. ----- Penzigia 3 (nov.sp.)
- 3'. Stromata superficial, spores $4.5 - 8.5 \times 9.0 - 13.5 \mu$, average $6.6 \times 11.2 \mu$. ----- Penzigia discolor
4. Stromata sessile -----5.
- 4'. Stromata with distinct basal stalks below the fertile heads -----8.
5. Stromata small, globose, containing 2 - 5 perithecia; Spores $8.0 - 14.5 \times 16.5 - 29.5 \mu$, av. $9.1 \times 19.1 \mu$.
----- Penzigia 2A (nov.sp.)
- 5'. Stromata aplanate, aplanopulvinate or pulvinate globose, containing at least 8 perithecia -----6.
6. Surface of young stroma smooth, dark black; carbonous ectostroma predominant. Spores $3.5 - 7.0 \times 9.5 - 14.5 \mu$, av. $5.0 \times 11.7 \mu$.
----- Penzigia 4 (nov.sp.)
- 6'. Surface of young stroma scaly or rough; fleshy entostroma forming major part of stroma. -----7.

7. Stroma aplanate pulvinate or aplanate, Spores $6.0 - 12.0 \times 27.5 - 37.5 \mu$, average $9.2 \times 37.9 \mu$. ----- Hypoxylon deustum.
- 7'. Stroma pulvinate, often irregularly lobed, Spores $3.5 - 8.5 \times 8.0 - 14.5 \mu$, average $5.6 \times 11.3 \mu$.
----- Penzigia berteri.
8. Stromata very small, divided into a flat or convex fertile portion and a basal stalk, Spores $5.0 - 7.5 \times 10.5 - 13.5 \mu$, average $6.4 \times 12.2 \mu$. ----- Penzigia compuncta.

Penzigia 1: (Figs. 471, 483).

Stromata aplanate crustose, consisting of a thin fleshy matrix 1.5 mm. deep with a carbonous surface; perithecia embedded in this matrix, aggregated but not coalesced, and clearly evident in outline on the surface of the stroma. Ectostroma grey superficially, jet-black and carbonous in section, scaling away with age so that parts of the entostroma are revealed beneath; entostroma white and fleshy. The stroma, although closely appressed to the host bark, is actually in connection with it at only a few places where dark mycelium below the entostroma invades the tissue for a short distance. Perithecia globose, $400 - 650 \times 400 - 500 \mu$, sometimes broader than deep; walls carbonized at the vertices, otherwise membranous; ostioles indistinctly papillate. Asci not observed. Spores elliptic-navicular with rounded or bluntly pointed ends, light brown, $5.0 - 8.5 \times 14.0 - 19.0 \mu$, av. $6.4 \times 16.4 \mu$ (60).

Host: Cassine croceum, bark.

This species is unknown at Kew and does not agree with any description given in the literature. Its structure would appear to be unique in the Xylariaceae on account of the morphological similarity to Hypoxylon (effuse types) on one hand and the structural similarity to typical species of Penzigia (e.g. Penzigia berteri) on the other. On this basis it probably deserves separate specific rank.

Penzigia 2A: (Figs. 472, 473, 480, 483).

Stromata pulvinate globose, 1 - 3 mm. wide and 4 mm. high, formed superficially on bark only. Surface of stroma smooth grey-brown to black., usually dull but sometimes shiny with extreme age. Ectostroma predominant, thick, carbonous; entostroma fleshy white, not greatly developed and disintegrating rapidly with age so that old specimens are easily mistaken for Hypoxylon. Perithecia 2 - 5 per stroma and occupying most of it, 530 - 600 x 700 - 800 μ , walls carbonized except near the base of each perithecium; ostioles indistinctly papillate. Asci cylindric or clavate, with stalks of moderate length, 150 - 170 x 15 - 17 μ , stalks 25 - 70 μ . Spores large, oval with rounded ends, dark brown, 8.0 - 14.5 x 16.5 - 29.5 μ , average 9.1 x 19.1 μ . (100).

Hosts: Olea capensis; bark.

The fungus is apparently restricted to this species, since extensive collecting has failed to demonstrate its occurrence on other hosts.

Material of this species was sent to Kew and to Dr. J.H. Miller and both authorities state that it is unknown to them. The material collected closely agrees with the description given by Miller (1933), for H. regale Morgan var. macrosporum Miller, but differs in possession of a basal fleshy entostroma.

Penzigia 4: (Figs. 474,475,481,483).

Stromata aplanopulvinate globose, 5 - 20 mm. wide; superficial on bark or wood, but usually the former. Surface of stroma smooth, dull black. Ectostroma predominant, carbonous; entostroma fleshy, white, variable in quantity but always apparent in thin section of a mature stroma, so that this species, like the previous one, can be easily mis-classified. Perithecia globose, 300 - 400 x 500 - 600 μ ; walls carbonized near vertices, membranous elsewhere; ostioles papillate and usually clearly visible on the surface of the stroma. Asci cylindric, usually long stipitate, 90 - 150 x 6 μ , stalks 35 - 75 μ . Spores oval, majority inequilateral with one end slightly conical, pale brown 3.5 - 7.0 x 9.5 - 14.5 μ , average 5.0 - 11.7 μ (120).

Hosts: Olea capensis and material unidentifiable; wood and bark.

The white entostroma of mature material easily separates this species from Hypoxylon glomeratum (p.36). The rather peculiar subconical spores comprise another feature of diagnostic value. Mature material was identified, however, as Hypoxylon glomeratum both by Dr. Miller and by Kew. Nevertheless, it is treated separately here and will probably have to be given a new specific name since it does not correspond with any published description.

Penzigia 3: (Figs. 476, 477, 483).

Stromata erumpent through decorticated wood, aplanopulvinate, up to 5 x 3 mms. in area. Surface of stroma often depressed by shallow concavities. Ectostroma thin dark and carbonous, entostroma predominant, brilliant yellow orange. Perithecia large, 500 - 600 x 700 - 1000 μ , occupying major part of the stroma; walls carbonous only near the vertices; ostioles punctate, indistinct. Asci cylindrical, long stipitate, 170 - 210 x 5 μ , stalks 80 - 130 long. Spores cylindrical elliptic, equilateral, ends broadly rounded, pale brown, 3.0 - 5.5 x 10.5 - 14.0 μ , average 3.9 x 12.2 μ . (60).

Host: Curtisea faginea; decorticated wood.

This species, which does not agree with any description given in the literature, is unknown at Kew. It is therefore probably new.

Penzigia discolor: (Berk. & Br.) Miller. (Figs. 478, 479, 482, 483).

Stromata pulvinate, small, up to 6 mms. long, sessile or attached to the substrate at a central point only, superficial on wood or decorticated bark. Ectostroma at first scabrous, dirty white, later grey and then black and carbonous; entostroma predominant, sulphur^u yellow, fleshy. Perithecia relatively large, occupying most of the stromal interior, 400 - 600 x 400 - 500 μ ; walls carbonized only at the vertices of the perithecia; ostioles distinctly papillate. Asci not seen: Miller's measurements (1942), are 105 - 140 μ long with stalks 40 - 50 μ . Spores broadly oval, dark brown, 4.5 - 8.5 x 9.0 - 13.5 μ , average 6.6 x 11.2 μ (90).

Hosts: unidentified; wood and bark, usually not greatly decayed.

Material from the Pretoria Herbarium (31062, 32149) has also been examined.

Hypoxylon deustum : (Hoffm. ex Fr.) Grev. (Figs. 484 - 490 A, 491).

Stromata divided into a flat or convex fertile portion and a sterile base of varying extent. From collections made over a period of 2 years from one region, Garden of Eden, Knysna, it has been definitely established that the morphology of the fungus varies according to the nature of the host substrate.

Type 1: On hard wood or bark, not greatly decayed. (Figs. 484, 485 part, 488).

Fertile part of stroma irregularly effused, crustose aplanate, up to 2 cm. diam. and 2 - 3 mm. thick. Sterile base very short or practically undeveloped. The stroma is attached only at its midpoint to the substrate and sectioning of the material fails to reveal any invasion of the tissue apart from this. The general structure is closely similar to that of Penzigia 1, except for the deeper immersion and greater coalescence of the perithecia.

Type 2: (Fig. 485, part). On decorticated soft wood, greatly decayed.

Main features as above, but the attachment portion is greatly expanded into a distinct stipe extending up to 1.5 cms. into the substrate.

Type 3: On bark; wood greatly decayed beneath. (Fig. 489).

Here the fertile part of the stroma varies greatly in form from a small pulvinate or conic type to the flat aplanate just described. The attachment portion is most peculiar, for it ramifies indefinitely underneath the bark and sometimes comes to the surface again at more than one point thus giving rise to more than one fertile head. That part of the fungus underneath the bark apparently serves as a penetrating organ for in several instances new fertile heads were found during the growing season spreading over the remains of the old from the year before.

The morphology of this type approaches that of a Kretschmaria. Specimens of K. cetrarioides from the Pretoria Herbarium were examined to see whether the 2 forms were identical, since the spore size is also the same. Owing to the poor condition of the herbarium material, however, no positive conclusions could be drawn.

Other details:

Ectostroma at first white, bearing greenish grey conidia over the surface except for a sterile marginal zone up to 5 mm. wide; later pale grey brown and scabrous, finally dark grey to black forming a thin carbonous layer over the perithecia. Entostroma white, fleshy, of variable extent according to the variations mentioned above; becoming carbonous at or soon after maturity and later disintegrating. Perithecia large, globose 1200 - 1400 x 1000 - 1200 μ ; walls uncarbonized except at the vertices; ostioles distinctly papillate. Asci cylindric or clavate usually long stalked, 170 - 330 x 11 - 14 μ , stalks 60 - 170 μ long. Spores elliptic navicular with pointed or rounded ends (both occurring within the same stroma), dark brown to almost black, 6.0 - 12.0 x 27.5 - 37.5 μ , average 9.2 x 37.9 μ .(50).

Hosts: Olea capensis, decorticated wood and bark.

Ocotea bullata, bark.

This species appears to be restricted to these 2 hosts and is much more common on the former than the latter.

Hypoxylon deustum has never been placed in the genus Penzigia but if the nature of the entostroma is recognised as a valid criterion to separate Penzigia from Hypoxylon, then it should surely be classified there. Tulasne realised that it was distinct from most species of the Hypoxylon concept when he named it first as Ustulina vulgaris (1863). Later Miller merged the genus Ustulina with Hypoxylon (1928). However, as has already been shown, the great variability of the species itself really defies artificial boundaries.

Penzigia berteri:(Mont.) Mill. (Figs. 490B, 492, 493).

Stromata aplano pulvinate, often divided into 2 - 3 lobes, normally erumpent through bark from the wood beneath and sessile or slightly stipitate depending on whether the latter is hard or soft, but occasionally superficial on exposed areas of wood from which the bark has cracked away formerly. Ectostroma fleshy when young, later scabrous and eventually hard dark and carbonous. Entostroma white, fleshy, sometimes with large cavities; decaying shortly after

maturity to leave a large hollow space beneath the perithecia. Perithecia situated at the periphery of the stroma, broadly globose with thick walls, $1000 - 1200 \times 600 - 900 \mu$; walls uncarbonized except near the vertices; ostioles distinctly papillate. Asci long cylindric, $140 - 160 \times 9 \mu$, $90 - 170 \times 7 - 11 \mu$, stalks $40 - 110 \mu$. Spores broadly oval, with rounded ends, dark brown to nearly black, $3.5 - 8.5 \times 8.0 - 14.5 \mu$, av. $5.6 \times 11.3 \mu$ (100).

Hosts: Olea capensis Curtisia faginea
Gymnosporia buxifolia Rhus legati

and many other species unidentifiable; wood and bark. The material was checked by Dr. Miller and stated to be this species.

Penzigia compuncta: Saccardo. (Figs. 490C, 494 - 496).

Stromata small, clearly divided into a flat or convex fertile portion $3 - 4$ mms. diam. and narrow terete glabrous stipe about 3 mms. long; superficial on decorticated wood. Ectostroma hard carbonous at maturity; entostroma white fleshy, persistent, in marked contrast to the previous species described. Perithecia globose, $500 - 650 \times 600 - 700 \mu$; walls uncarbonized except near the vertices; ostioles indistinct papillate. Asci clavate or cylindric, long stipitate, $115 - 160 \times 10 - 12 \mu$, stalks $50 - 85 \mu$. Spores oval, equilateral with rounded ends, black, $5.0 - 7.5 \times 10.5 - 13.5 \mu$, average $6.4 \times 12.2 \mu$ (100).

Host: Lycium campanulatum, old and very decayed wood.

B : Cultural characters:

Key based on malt plate cultures:-

- | | | |
|-----|--|----------------------------------|
| 1. | Mycelium developing small spherical carbonous bodies. | |
| | | ----- <u>Penzigia</u> <u>1</u> . |
| 1'. | Not so ----- | 2. |
| 2. | Aerial mycelium submersed or canescent, hyaline ----- | 3. |
| 2'. | Aerial mycelium velvet felty, opaque at least at the
centre ----- | 4. |

Microscopic characters: (Fig. 521 A).

Maximum diameter of the primary mycelium = 1.7μ . Secondary mycelium absent. The feature which distinguishes this species from all others studied except Rosellinia nitens is the production of coal black carbonous bodies (Figs. 498, 499) over most of the surface of the mycelium. The manner of formation of these bodies was studied by removal and examination of small pieces of mycelium from the margin to the centre of an actively growing colony. Examination of mycelium just behind the margin shows small aggregations of hyphae anastomosing and ramifying outwards each from a central focus (Fig. 498 A, B). Later these develop into white pseudoparenchymatous spheres about 30μ diam. About 24 - 36 hours after initiation, these spheres increase greatly in size, first losing all evidence of cellular structure and then many of the hyphal contacts with the surrounding mycelium. The remaining hyphal contacts swell up to 6.3μ in width, become slightly stained and may develop wall pitting (Fig. 498 F). Shortly before the sphere reaches its maximum size the cells of the structure break down and the disintegrating walls can be clearly seen (Fig. 498 E). The colour of the sphere now changes from white to pale brown and then, as carbonization takes place, to jet black. The spheres, which are fully formed after 2 - $2\frac{1}{2}$ days have a hard exterior and a centre that may be hollow or filled with loose cells. They range from $100 - 130 \mu$ diameter.

Conidiophores and conidia: (Figs. 527 A, B).

The conidiophores cannot be distinguished from the vegetative mycelium except for the production of spores. They form an indefinite dichotomously branched system $200 - 300 \mu$ long; ultimate fertile branches $130 - 230 \mu$, sparingly septate, and bearing conidia in small fascicles of 5 - 8 along their length and singly on wart-like prominences near their ends. Conidia oval, fawn grey collectively, $1.7 - 2.9 \times 3.1 - 4.6 \mu$, average $1.9 \times 3.7 \mu$.

Bottle Culture:Penzigia 2A:

M e d i u m.	G r o w t h C h a r a c t e r s.
<u>Malt:</u>	Submersed up to 28 days later very finely canescent; colourless. <u>Margin</u> : entire, compact. <u>Conidia</u> : produced after 32 days, forming thin greenish grey superficial mat. <u>Stain</u> : Dark purple, diffuse, appearing after 28 days.
<u>Maize:</u>	Submersed throughout. <u>Conidia</u> : produced after 14 days, sparse, not uniformly distributed. <u>Stain</u> : None.
<u>Leonian's:</u>	Submersed throughout; subhyaline white surface gelatinous; coremioid outgrowths appear after 11 days but no conidia <u>Stain</u> : none.
<u>Czapek:</u>	Submersed, colourless, otherwise similar to Leonian's.

Plate Culture:

M e d i u m.	G r o w t h C h a r a c t e r s.
<u>Malt</u> :(Fig.502).	Submersed to canescent appressed; almost hyaline, colourless. <u>Margin</u> : compact entire. <u>Conidia</u> : none. <u>Stain</u> : none.
<u>Maize:</u>	Similar to malt.
<u>Leonian's</u> :(Fig.503).	Similar to malt, aerial mycelium hyaline to opaque white, buff at centre.
<u>Czapek:</u>	Almost completely submersed, white subhyaline; surface gelatinous.

Microscopic characters:

Maximum diameter of primary mycelium = 2.0μ . No secondary mycelium.

Conidiophores and conidia: (Fig. 511 C).

Conidiophores scarcely distinct from the vegetative mycelium, dichotomously compound branched, $100 - 150 \mu$ long; fertile branches relatively short, $26 - 40 \mu$, normally unseptate but sometimes with one or two septae.

Conidia acropleurogenous, each seated on a wart-like protuberance, apparently spiral in 2 rows; subglobose to spherical, fawn-grey collectively, 2.0 - 2.9 x 2.6 - 3.7 μ , average 2.5 x 3.1 μ .

Penzigia 4:

A : Bottle Culture: (All media) (Figs.504, 506).

Appearance: Aerial mycelium apart from conidiophores submersed for at least the first 10 days of growth, then sparingly canescent, white subhyaline.

Margin: Submersed, entire compact.

Conidia: Produced very abundantly after the colony is 3 - 7 days old, greenish grey. Sometimes (on malt) the conidiophores cluster to form spherical aggregations but this is not typical.

Stain: None.

The Czapek culture of 1 out of 5 strains investigated showed remarkable zonate deposition of conidia. (Fig. 505).

B : Plate Culture:

<u>Medium.</u>	<u>Growth Character.</u>
<u>Malt:</u> (Figs. 507, 508)	Closely canescent appressed, divided when 6-10 days old into:- 1. Marginal zone 2 - 3 mm. broad, dirty white subhyaline, gelatinous, compact. 2. Central zone, closely appressed covered with pale grey conidia. (S.233)
<u>Maize:</u> (Fig.509)	Entirely submersed, colourless, dry to slightly gelatinous. Conidia distributed unevenly.
<u>Leonian's:</u> (Fig.510)	Slightly more luxuriant than malt, downy appressed, white subhyaline, coarse. Conidia almost up to margin in some places.
<u>Czapek:</u>	Less luxuriant than malt, hyaline, with uniform coating of grey conidia.

Microscopic Characters:

Maximum diameter of the primary mycelium = 2.1μ (Fig. 521 B). No secondary mycelium.

Conidiophores and conidia: (Fig. 512 A).

Conidiophores distinct from the vegetative mycelium in the characteristic ternate branching giving rise to the ultimate branches; $100 - 150 \mu$ long and wide. The fertile branches are long, unseptate, $40 - 65 \times 1.5 - 2.1 \mu$; fertile part rather indefinite in extent, occasionally divided by an intercolony sterile portion, but usually occupying $1/5 - 3/5$ of the fertile branch and ranging from $10 - 40 \mu$ in length. Conidia mainly pleurogenous, borne singly on wartlike protuberances arranged spirally in 4 rows. They are hyaline, colourless individually but collectively greenish grey, oval with both ends rounded, $1.4 - 2.3 \times 2.6 - 4.6 \mu$, average $1.9 \times 3.3 \mu$.

Penzigia 3:

A : Bottle Culture:(All media). (Figs. 512, 513).

Appearance: Thin felty with smooth surface, white to pale grey, opaque, divided during early stages of growth into a submersed marginal zone up to 5 mm. wide and a centre with distinct aerial growth.

Margin: Entire, compact.

Conidia: Produced after 2 months in small pulvinate masses over the centre part of the colony; dark nondescript brown.

Stain: None.

B : Plate Culture:

Medium.Growth Characters.

Malt: Appressed canescent, zonate with zones 2 mm. wide and about 5 mm. apart, white subhyaline sometimes with a brownish tinge;
(Figs. 514, 515) Margin: 2 - 3.5 mm. wide, canescent, entire compact.
Conidia: None.
Stain: None.

Maize: Similar to malt but not as strongly zonate and with a gelatinous surface.
Conidia: Appear with extreme age (> 4 weeks) on outer part.

M e d i u m.

G r o w t h C h a r a c t e r s.

<u>Leonian's</u> :	Similar to malt but with gelatinous surface. <u>Margin</u> : lobed. <u>Conidia</u> : appear with extreme age.
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<u>Czapek</u> : (Fig. 516)	Mainly submersed, gelatinous, subhyaline white; zonate with 5 zones corresponding to thickening of the mycelium beneath the agar surface. <u>Conidia</u> : none.
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Microscopic characters:

Maximum diameter of the primary mycelium = 2.1μ (Fig. 521 C). Secondary mycelium very sparse in quantity, developing in small areas under the white primary mycelium, and characteristically indefinitely dichotomously branched, with few anastomoses; $1.3 - 3.8 \mu$ diam. (Fig. 521, D).

Conidiophores and conidia: (Figs. 522 C,D).

Corresponds exactly with that described for Penzigia l., but in addition, they are usually coloured pale amber instead of being colourless. The conidia are oval to subglobose, pale ochre brown collectively, $1.7 - 2.9 \times 1.7 - 3.7 \mu$, average $2.2 \times 3.0 \mu$.

Penzigia discolor: (Figs. 517, 518).

A : Bottle Culture: (All media).

Appearance: Velvet fleecy, very dense, forming a closely knit mat with coarse surface up to 2 mm. high. A characteristic ruff of mycelium is formed encircling the centre of the colony and 5 cm. away from it. The colour of the areial mycelium is gleaming white.

Margin: Canescent, entire, peripheral hyphae compact.

Conidia: Appear late, 9 months after inoculation; very pale fawn grey over small areas of the mycelium.

Stain: None.

B : Plate Culture:Medium.Growth Character.

Malt: Velvet fleecy, white opaque to subhyaline; growth uniform or with 1 - 3 zones irregularly surrounding the centre. (Fig. 519).
Margin: up to 4 mm. wide submersed, entire, compact.
Conidia: None.

Maize: Less luxuriant than malt; canescent appressed white subhyaline, zonate with 3 zones.

Leonian's: Similar to malt also zonate with 2 zones.

Czapek: Canescent, much less luxuriant than other cultures, white opaque at centre to white subhyaline at outside, faintly zonate. (Fig. 520).

Microscopic characters:

Maximum diameter of primary mycelium = 2.9μ . Secondary mycelium consisting of long, sparingly branched and loosely anastomosing filaments $2.7 - 4.3\mu$ diameter, uniformly dispersed.

Conidiophores and conidia: (Figs. 511 B, 523).

Conidiophores of the same general type as described for Penzigia 1 and Penzigia 3, but much longer, being $300 - 500\mu$ in length. A further minor point of distinction is that the conidia arise off the flat surface of the hyphae and have not been observed to be borne on wart-like protuberances. The fertile branches are $75 - 340\mu$ long, multiseptate; conidia acro-pleurogenous, borne singly or in groups of 4 - 9 along their entire length, are oval to subglobose, pale fawn grey collectively, $2.3 - 3.7 \times 2.9 - 4.8\mu$, average $2.8 \times 3.7\mu$.

Hypoxyton deustum:

A : Bottle Culture (All media) (Figs. 524, 525).

Appearance: Velvet fleecy, white opaque, smooth.

Margin: Canescent, entire, compact.

Conidia: None observed.

Stain: Nil. Carbonization of the medium takes place, however, after 21 days to form a jet black layer beneath the mycelium of variable extent.

The Czapek culture became furrowed and distorted after 3 weeks until finally the agar surface broke.

B : Plate Culture:

Medium.

Growth Characters.

<u>Malt:</u> (Fig.526)	Velvet felty compact and dense, rather coarse, opaque white. <u>Margin</u> : 7 - 15 mm. wide, sometimes with characteristic plumose fronds, otherwise canescent, segmented or lobed, compact. Carbonization of mycelium in centre of the colony takes place after 10 days.
<u>Maize:</u> (Fig.527)	Similar to malt but not as compact; cottony lanose, with coarse surface.
<u>Leonian's:</u> (Fig.528)	Silky lanose; mycelium 3 - 4 mm. high; otherwise similar to malt.
<u>Czapek:</u> (Fig.529)	Velvet felty, smooth; <u>Margin</u> : canescent not plumose.

Microscopic characters:

Maximum diameter of primary mycelium = 2.3μ . The secondary mycelium is very peculiar, consisting of wide platelike expanses of large cells $3.0 - 16.0 \times 10 - 18 \mu$ in size which are carbonized and dark in colour. They arise by indefinite branching and anastomosis of very short hyphae (Fig. 541 C).

Conidiophores and conidia: (Figs. 491 A, B; 560 B).

These have never been observed in culture whether on agar or wood. Young stromata during the early stages of growth invariably appear to develop the imperfect stage, however. The following account is based on sections of young stromata and on dissections of fresh material:

The conidiophores give rise to a palisade $85 - 100 \mu$ deep on the surface of the developing ectostroma, and so close are they compacted that it is often very difficult to distinguish each separately from its neighbours. Each consists of a lateral indefinitely branched system producing erect fertile hyphae at intervals. The latter sometimes bear short side branches also giving rise to conidia. The conidia are pleuracrogenous, elliptic to oval, very pale grey collectively, $2.0 - 2.9 \times 4.6 - 7.7 \mu$, av. $2.4 \times 6.0 \mu$.

N.B.: No difference in cultural characters was observed among cultures taken from stromata of widely different form.

Penzigia berteri:

A : Bottle Culture (All media) (Fig. 530).

Appearance: Velvet fleecy, white, opaque smooth.

Margin: Canescent, entire, compact.

Conidia: Formed on the apices of coremioid outgrowths which develop 2 - 4 months after inoculation. These are described in detail below.

Stain: None. Carbonization of the medium begins after 20 days in all cultures excluding malt.

B : Plate Culture:

Malt, Leonian's, Czapek: (Fig. 531).

Appearance: All basically similar; velvety felty at centre to canescent velvety towards the margin, white opaque with coarse uneven surface.

Margin: Canescent, entire, or lobed, compact.

Conidia: Occasionally produced. (See below).

Stain: None. Carbonization occurs after 6 - 9 days and spreads out from the centre of the colony.

Coremioid outgrowths were produced, in all the cultures examined, 3 - 4 weeks after inoculation. A minority of these developed pale grey conidia: (Figs. 533, 534).

Maize: (Fig. 532).

Appearance: Similar to the above but much less luxuriant, canescent velvety throughout, white subhyaline to opaque with a smooth surface.

Margin: submersed, broadly lobed. No coremioid outgrowths or carbonization.

Microscopic characters:

Maximum diameter of primary mycelium = 2.7μ . Secondary mycelium consisting of long branched hyphae $2.1 - 3.4\mu$ diam., which anastomose indefinitely but do not form the plate-like expanses of large cells as in the former species. (Fig. 541 D).

Conidiophores and conidia: (Fig. 560 A).

The conidia are borne on the apices of coremiform growths mentioned previously. The latter vary greatly in size according to the environment. In bottle culture they are large up to 2 cm. long and 1 cm. wide, irregular in shape but usually cylindric or coniform, and are branched once or twice. In plate culture they are much smaller, 5 - 7 mm. long and 3 mm. wide, cylindric, and unbranched. The base of the coremium is dark due to a surface layer of dark hyphae, while the upper part is white or pink and lacks such a covering. The interior is white, fleshy, and made up of tightly packed pseudo-parenchymatous tissue. (See Fig. 560 A). The structure in section is exactly similar except that the dark hyphae are not as regularly arranged).

The conidiophores are tightly packed, giving rise to a palisade tissue similar to, but not as regularly arranged as, that of H. deustum. The conidiophores do not continue indefinitely as in H. deustum, and range from 40 - 60 μ long. They are dichotomous or ternately branched, and the fertile hyphae are 5 - 20 μ long, terete, or are commonly bulbous or irregular in form. The conidia are pleuracrogenous, oval or elliptic, pale grey collectively, 1.4 - 2.3 x 3.7 - 7.6 μ , average 1.7 x 4.5 μ .

Penzigia compuncta:

A : Bottle Culture: Malt and Leonian's: (Fig. 535).

Appearance: Velvety, dense and closely appressed, zonate with 3 zones produced per 14 days; surface rather coarse; mycelium white opaque, tinted pink.

Margin: Canescent, entire, compact.

Conidia: None.

Stain: None. No carbonization of the medium.

Maize:

Appearance: Growth uniform, silky-felty with short aerial tufts of mycelium 8 mm. high at centre and 3 mm. high near the margin.

Czapek: (Fig. 536).

Appearance: Growth uniform, velvet-silky, smooth; mycelium white subhyaline. Texture much less compact than on other colonies.

B : Plate Culture:

Malt and Leonian's.
(Figs. 537, 538).

Maize and Czapek.

Appearance:

Canescent velvety, strongly
zonate dull white, tinted pale
pink.

Appearance:

Submersed except for sparse aerial hyphae
uniform, white subhyaline, gelatinous.

Margin:

Distinct, 1 - 2 mm. wide,
at first submersed to canes-
cent, but usually becoming
plumose with age.

Margin:

Indistinct, irregular segmented, compact.

Microscopic characters: (Figs. 521 E, 541 E).

Maximum diameter of the primary mycelium = 2.6μ . Secondary mycelium
consists of long sparingly branched and infrequently anastomosing hyphae $1.8 -$
 5.4μ diam.

Conidiophores and conidia:

No conidiophores or conidia observed.

The fungus when inoculated on wood, develops long plumose tassels of
mycelium up to 15 mm. long and 2mm. wide. These consist of numerous hyphae
growing parallel and which are very tightly compacted. (Figs. 539, 540).

SUMMARY OF GROWTH CHARACTERS FOR PENZIGIA AND THEIR CORRELATION WITH

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STROMAL VARIATION.

S P E C I E S.	STROMAL CHARACTER.	AVERAGE SPORE SIZE (μ).	GROWTH CHARACTER (MALT PLATE).	CONIDIO- PHORE TYPE.*	STAIN.	PRESENCE OF SECONDARY MYCELIUM.	G R O W T H R A T E.	
							OPTIMUM. ($^{\circ}$ C)	CHARACTER. (at Optimum).
<u>Penzigia 1</u>	Aplanate; perithecia clearly evident in outline; entostroma white.	6.4 x 16.4	Velvet-silky.	I	-	None, but carbonized bodies formed.	20 $^{\circ}$	Very slow.
<u>Penzigia 2A</u>	Globose; perithecia not evident; entostroma white.	9.1 x 19.1	Submersed to canescent.	III	Dark purple.	-	20 $^{\circ}$	Moderate.
<u>Penzigia 4.</u>	Globose to aplanopulvinate; perithecia not evident, entostroma white.	5.0 x 11.7	Canescent appressed; granulate due to conidia.	IVA	-	-	15 $^{\circ}$	Slow.
<u>Penzigia 3.</u>	Aplano-pulvinate, erumpent. Perithecia not evident; entostroma brilliant saffron.	3.9 x 12.2	Appressed canescent, zonate.	I	-	+	20 $^{\circ}$	Very slow.
<u>Penzigia discolor</u>	Globose or pulvinate, perithecia usually evident in outline; entostroma pale yellow.	6.6 x 11.2	Velvet fleecy, sometimes zonate.	I	-	+	25 $^{\circ}$	Slow.
<u>Hypoxyton deustum</u>	Form highly variable; perithecia not evident; entostroma white.	9.2 x 37.9	Velvet felty, smooth	X	-	+	25 $^{\circ}$	Moderate.
<u>Penzigia berteri</u>	Aplano-pulvinate; perithecia not evident; entostroma white.	5.6 x 11.3	Smooth velvet felty to canescent velvety	X	-	+	20 $^{\circ}$	Moderate.
<u>Penzigia ^{Cam}atrepuncta</u>	Aplano-pulvinate, clearly stalked perithecia not evident; entostroma white.	6.4 x 12.2	Canescent velvety, zonate.	-	-	+	28 $^{\circ}$	Slow.

* See discussion at end of chapter.

The following points are clearly of interest:

1. Growth rate: (Graph L).

Table XII, Appendix III, shows that there is a high degree of significant difference between the growth curves obtained for the species. This confirms the differences shown on previous page. One possibly fortuitous feature is the prevalence of low temperature (15 and 20°) optima among the species studied. Most of the Hypoxylon species grew most rapidly at 25° or 28°.

2. The Problem of Specific Limits:

(a) Penzigia 4:

The problems of inclusion of the strain described here as a Penzigia with Hypoxylon glomeratum have been discussed already on pp. 36 & 38. Cultural work shows that there is a close similarity in general characters between Penzigia 4 and H. glomeratum var. 1, and that they both develop an identical conidiophore type. Only the growth rates are markedly different. If the similarities are accepted as an indication of affinity, then the relationship would logically appear to be a close one. Clearly, however, the inclusion of this strain in H. glomeratum would involve modification of the basic principle separating Hypoxylon from Penzigia.

(b) Hypoxylon deustum:

The growth character of this species and/or the conidiophore type clearly agree with those of Penzigia species (P. berteri and P. compuncta) that are also similar in stromal form. Hence the inclusion of this species in Penzigia instead of Hypoxylon is supported by cultural work.

Xylaria:

Since only a few species of this genus have been examined in comparison with relatively large numbers of some preceding genera, it is not proposed to make too many generalizations concerning the structure of the stroma and the cultural characters. The following remarks, are based on examination of 5 species and on review of the work of Saccardo (1882 et seq.), Miller (1942), and of Dennis (1956, 1957):-

1. The external morphology and form of the stroma vary more than the internal anatomy, and have therefore been more extensively employed in classification. Most of the species have a clearly defined sterile base or "stipe", but in Xylaria fioriana there is complete transition, often within the same group of stromata, from a Penzigia type of stroma where the fertile portion is sessile to the typical form.
2. The anatomy of the mature stroma comprises:
 - (a) an outer carbonous layer contiguous with the perithecial apices;
 - (b) an inner white fleshy centre in which the bases of the perithecia are situated.

In addition, in all the species examined, there has been found

- (c) a superficial layer of dark compact anastomosing mycelium, which may be continuous or restricted to the folds of the carbonous layer beneath. This external layer does not appear to have been well described in the literature. Varitchak (1931) and Brown (1913), in their studies of stromal development, make only passing reference to it. Saccardo referred a straggly (hordellus) outer layer in many of his descriptions of Xylaria species throughout the Sylloge Fungorum but does not state whether or not this is universal. Miller (1942) in his classification of South African Xylariaceae refers to a "pellicle" or crust surrounding the outer carbonous layer but does not describe it more specifically. If the pellicle is synonymous with the mycelial layer described here, then it would appear that it is restricted at maturity to certain species only. The homology of the layer in comparison with Hypoxylon is difficult to interpret but it would appear that it is actually the inner persistent part of the conidial layer that forms before perithecial initiation. In Hypoxy-
lon and Penzigia this conidial layer disappears before maturity.

Immature material of X. arbuscula and X. leprosa have been examined and it was found in each case that the conidia were borne on a palisade layer of cells, the cells beneath, which resembled closely those of the outer crust of the mature stroma, though they were not as darkly coloured. This type of conidial organization is recognized by many authors notably Brown(1913), and was first described by Tulasne (1863).

The perithecia of Xylaria are globose, and the walls are membranous except for the vertices which are stout and heavily carbonized. The membranous walls are usually pale brown, but sometimes not clearly distinct from the fleshy tissue beneath.

The Perfect Stage.

1. Xylaria fioriana: Saccardo. (Figs. 542, 548 B, 549 E).

Stromata gregarious, rarely solitary, typically unbranched but sometimes bifurcate, with conic or cylindrical axes 0.5 - 2 cm. long and up to 5 mm. broad; apices blunt; sterile base short to moderate in length but never clearly distinct from the fertile part and always of the same width or slightly broader. The species thus intergrades with the genus Penzigia. Outer mycelial covering very dense; perithecia globose, 300 - 400 x 500 - 600 μ ; ostioles papillate. Asci 100 - 130 x 8 μ ; spores oval dark brown, 4.5 - 7.5 x 8.0 - 12.0 μ , av. 5.7 x 10.3 μ .(60).

On dead wood and bark of Aloe pluridens.

2. Xylaria apiculata : Cooke. (Figs. 548 A, 549 A).

Stromata usually gregarious, 7 - 12 mm. long, each one consisting of a globose elliptic to coniform fertile head, up to 5 mm. wide, with a distinct acuminate apex, mounted on a narrow cylindrical stipe of varying length. Outer mycelial covering feebly persistent. Perithecia few per stroma, globose, prominent. Asci short stalked, 120 - 140 x 11 μ . Spores oval, broad, 6.0 - 10.0 x 15.5 - 22.0 μ , av. 7.7 x 18.2 μ . (60).

3. Xylaria arbuscula : Saccardo. (Figs. 543, 549 D).

Stromata usually gregarious, 1 - 2.5 cm. long, with abruptly pointed sterile apices, narrow, filiform, each divided clearly into an elongate cylindrical stipe and a fertile elliptic or linear club from which the perithecia clearly project. Outer mycelial covering not conspicuous at maturity, being restricted to furrows on the carbonous outer surface. Perithecia few per stroma 400 - 450 x 450 - 550 μ ; asci 105 - 120 x 6 μ ; spores oval, strongly inequilateral, dark brown, 4.5 - 7.0 x 10.5 - 16.0 μ , av. 5.4 x 12.9 μ .(150).

4. Xylaria castorea: Berk. (sensu Miller) (Figs. 544, 549 B).

Stromata stout, 3 - 4 cm. long and 5 - 7 mm. diameter, with a short sterile often pannose, base and a club- or mallet- shaped smooth fertile portion 3 - 4 cm. long and 5 - 7 mm. diameter. Outer mycelial crust not evident at maturity. Perithecia globose, 350 - 400 x 550 - 600 μ ; asci 80 - 130 x 6 μ ; spores oval dark brown, 4.5 - 6.0 x 7.5 - 10.5 μ , av. 5.0 x 8.9 μ . (80).

5. Xylaria leprosa: Speg. (Figs. 545 - 547, 549 C).

Stromata stout, 3.7 cm. long, usually elongate and tapering; basal part narrow stipitate. Outer mycelial crust persistent at maturity. Perithecia globose, 300 - 400 x 500 - 600 μ ; asci 135 - 170 x 10 μ ; spores oval-elliptic, inequilateral, 5.0 - 7.5 x 13.5 - 22.5 μ , av. 5.8 x 17.3 μ . (120).

Cultural characters:

The cultural characters of the 5 species investigated are on the whole, closely similar. In all the species the mycelium is dense white, velvety, with a smooth surface and, except for Xylaria fioriana, carbonization of the medium takes place in all bottle and in malt and Leonian plate cultures after 7 - 10 days growth at 25°C. Secretion of carbonous material also takes place in the aerial mycelium so that the ageing colony normally has a jet black centre surrounded by a white peripheral zone. In all cultures of Xylaria fioriana and in maize and Czapek plate cultures of the other species, carbonization is either delayed or not apparent.

This species are characterized and best distinguished by formation of a long coremiform or aristate outgrowths in all bottle cultures and in malt and Leonian plate culture. The form of these outgrowths appears to be characteristic of each species.

Xylaria fioriana: (Figs. 550 - 553, 558, 559 B, 560 C).

Outgrowths relatively short and stout, 1.0 - 2.5 cm. long and 3 - 5 mm. diam; lower part covered by long setose hairs, upper part pale grey and bearing many conidiophores in palisade formation.

Xylaria apiculata: (Figs. 556, 557).

Outgrowths narrow, 1.0 - 2.5 cm. long, 1 - 2 mm. diam., velvet-black throughout except for the pale grey or white tips; infertile.

Xylaria arbuscula: (Fig. 558 C).

As for Xylaria apiculata but 2.5 - 3 cm. in length.

Xylaria castorea: (Figs. 554, 555).

Outgrowths robust, 5 - 7 cm. long and 3 - 4 mm. diam., smooth, velvet black except for the last 3 - 5 mm. which are paler; infertile.

Xylaria leprosa:

As for Xylaria castorea but usually crooked and somewhat twisted and densely shaggy.

The structures just described appear to be peculiar to Xylaria though not recorded before. No corresponding structure is produced in Hypoxylon, Penzigia or Daldinia.

Conidiophores and conidia:

Young material of the stromata of Xylaria arbuscula, Xylaria leprosa and Xylaria castorea, and the coremium bodies of Xylaria fioriana have been studied. The structure of the young stromata before perithecial formation is closely similar to that of the coremium. Both consist of

- a. a sterile base consisting of
 - 1) a dense outer dark hyphal covering
 - 2) an ill defined carbonous layer
 - 3) a centre part of white fleshy tissue and,
- b. a clavate part consisting of
 - 1) a dense palisade layer of light or darkly stained conidiophores
 - 2) a centre part of white fleshy tissue

The conidiophores are closely intertwined and difficult to separate; each one consists of a short hypha that is branched di- or trichotomously to produce a number of elongate fertile hyphae, 40 - 60 x 2 - 3 μ , from the

apices of which the conidia are budded off. The conidia are not retained after formation as in Hypoxylon, to form a cluster, and examination of the apices of the fertile hyphae suggest that they are indeed formed singly from more or less the same region. The conidia are oval, hyaline, pale grey to white collectively, and may differ in size from one species to another. The following measurements are recorded:-

Xylaria fioriana : 1.1 - 1.7 x 2.3 - 4.6 μ , av. 1.6 x 3.5 μ .

Xylaria leprosa : 3.0 - 4.5 x 4.5 - 8.3 μ , av. 3.5 x 6.6 μ .

The perithecia develop at a later stage under the conidial layer of the young stromata. Full details have not been observed. The coremiform bodies of X. fioriana, however, merely shrivel or disintegrate with age. The growth of these coremia was studied by means of the following experiment.

Three sterilized branch segments 5 - 6" long, of each of the following trees, Olea capensis, Aloe pluridens, and Cassine croceum, were inoculated with mycelium and placed in a sterile ball jar in the manner described on p.10, § 2. The contents of two jars were later placed in the open, near a waterfall in forest; the first jar after one week and the second after two weeks. Coremiform outgrowths had developed in the second jar only at the time of emptying. The third jar was unopened and kept as a control.

In no case did perithecia develop beneath the conidial layer before the coremia finally shrank and died. It is interesting to record, however, that the wood from the first jar developed coremia after the inoculation period in the jar. This would indicate that formation of coremia under natural conditions does take place, even though such structures have not yet been found in field collections. Unfortunately no young material of Xylaria fioriana has yet been collected for comparison.

Secondary mycelium:

This chiefly differs from that of Hypoxylon on account of the straight, clearly visible, and frequently anastomosing nature of the hyphae (see Fig. 559). The general structure is similar to the dark mycelium of Rosellinia aquila. Moreover, the secondary mycelium of each species is closely similar to the mycelial outer crust observed on the exterior of the stroma.

Specific Details:-

- Xylaria fioriana: Mycelium stout, 2.6 - 3.4 μ diam, frequently anastomosing and producing numerous club-shaped branches.
- Xylaria apiculata: Mycelium variable in diameter, 1.4 - 3.2 μ ; distinguished by production of small club-shaped and ramified side branches, some of which swell and become carbonous. This is reminiscent of Hypoxyton truncatum.
- Xylaria arbuscula: Similar to Xylaria apiculata; diameter 1.7 - 2.9 μ .
- Xylaria castorea: Hyphae straight, multibranched and anastomosing but not forming club-shaped side branches; 1.7 - 2.3 μ diam.
- Xylaria leprosa: Hyphae rather stout, multibranched, sinuous, closely anastomosing but not forming club-shaped side branches; 1.3 - 2.5 μ diam.

Discussion and conclusions:Stroma:

The three genera Daldinia, Penzigia and Xylaria have one feature in common, a well-developed entostroma that is non-carbonous at maturity. The main differences occur in the colouration of the entostroma, the texture at maturity whether fleshy or corky and zonate, and the differentiation of the stroma as a whole into a stipe and fertile portion. The two stromal layers, ecto- and entostroma, are covered by a third in Daldinia and Xylaria which is derived from the remnants of the imperfect stage. This type of organization is further advanced than in Hypoxyton and Rosellinia where the outermost mycelial layer disappears early. It has been shown also that there is little or no tissue differentiation in the Annulatum and Rubiginosum groups of Hypoxyton.

Members of this series therefore appear to be clearly separable from Hypoxyton on theoretical grounds. Certain species of Penzigia, however, greatly resemble H. glomeratum superficially. These are Penzigia 4, Penzigia 2A, Penzigia 3 and Penzigia discolor. When old material of such species is collected, the entostroma is usually found to have disintegrated and disappeared and it is easy to confuse them with Hypoxyton if their prior development is not

understood. The position is further complicated by the discovery of Penzigioid stromata that bear strong resemblance to Rosellinia aquila (see p. 35). Clearly, therefore, separation of Hypoxyton from Penzigia is open to objection on strong practical grounds, even though extreme forms of both genera are clearly recognizable.

The actual limits between Xylaria and Penzigia are likewise ill-defined. It is very difficult to exclude stipitate Penzigias (e.g. Penzigia ^{com}atrepuncta) from Xylaria on the one hand and non-stipitate Xylarias (e.g. Xylaria fioriana) from Penzigia on the other. In the latter species, pulvinate and elongate stromata with normal sterile bases have been found growing in close proximity (see Fig. 542 .)

Cultural Characters:

1. The Imperfect Stage: (Refer back to p.91).

The following types are known for the group:-

Type V B	:	<u>Daldinia eschschoizii</u>
VI B	:	<u>Daldinia concentrica</u> (see p.177).
I	:	<u>Penzigia 1</u> ; <u>Penzigia 3</u> ; <u>Penzigia discolor</u> .
III	:	<u>Penzigia 2A</u> .
IV A	:	<u>Penzigia 4</u> .

For other species, Hypoxyton deustum, Penzigia berteri, and the genus Xylaria, a separate conidiophore type must be recognised where the conidia arise from a coremium and the branches of the conidiophores are arranged in a palisade. This will be known as Type X.

2. The use of cultural characters in relation to generic limits:

The species of Daldinia examined resemble many Hypoxyton species in culture which probably indicates that the relationship between the 2 genera is a strong one. Differences in stromal characters, however, are on the other hand sufficient to invalidate arguments for merging them.

Greater difficulty is, however, experienced in dealing with members of the fleshy series. The species Penzigia with advanced stromal construction and the species of Xylaria studied are distinct from most Hypoxyton species in

possession of a dense smooth white velvety type of mycelial growth accompanied by carbonization of the medium, and a different type of conidiophore. Xylaria is, apparently, further distinguished by production of coremiform outgrowths. Again, however, those species of Penzigia that resembled Hypoxylon in the perfect stage also correspond to a certain extent in the cultural characters. Penzigia 2A and Penzigia 4 are similar in growth character and conidial type to Hypoxylon glomeratum vars. 1 and 2; Penzigia 3 and Penzigia discolor to Hypoxylon glomeratum vars. 3 and 4. Clearly, therefore, there is a certain degree of parallelism between stromal features and growth characters in culture, which supports the view of Miller (1942) and of Arx and Muller (1954) that Penzigia is an intermediate, of imprecise definition, between Hypoxylon and Xylaria.

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(as Xylaria compuncta).

Penzigia discolor:

- Berkeley, M.J. &
Broome, C.E. : The Fungi of Ceylon. J. Linn. Soc. 14,
123, 1875.
- Miller, J.H. : South African Xylariaceae. Bothalia IV,
261, 1942.

Hypoxylon deustum:

- Greville, R. : Scottish Cryptogamic Flor, 6.
Edinburgh, 1828.
- Miller, J.H. : British Xylariaceae II.
Trans. Brit. Mycol. Soc 15,
143, 1930.
(as H. ustulatum).
- : South African Xylariaceae
Bothalia IV, 255, 1942.
- Petrak. Beitrage zur Pilzflora von Mahren und Osterr - Schlesien.
Ann. Myc. 19, 279, 1921.
(as Ustulina deusta).
- Tulasne, L & C. : Selecta Fungorum Carpologia II, 23,
tab III, 1863.
(as U. vulgaris).

Xylaria apiculata:

- Cooke, M.C. : New Zealand Fungi. Grevillea VIII, 66, 1881
- Saccardo, P.A. : Sylloge Fungorum I, 334, 1882.

Xylaria arbuscula:

- Saccardo, P.A. : Mycologia Venetae Specimen, Patavii, 1873.
- : Sylloge Fungorum I, 337, 1882.

Xylaria castorea:

- Berkeley, M.J. : Flora Nov. Zelandica I, 204, 1881.
Saccardo, P.A. : Sylloge Fungorum I, 329, 1882.

Xylaria fioriana:

- Saccardo, P.A. : Annales Mycologici VIII, 337, 1910.
: Sylloge Fungorum, 22', 350, 1913.

Xylaria leprosa:

- Spegazzini, C. : F. Puigg. 268, cited in reference below.
Saccardo, P.A. : Sylloge Fungorum IX, 533, 1891.

CHAPTER VII.

CONCLUSION.

An investigation into the stromal variation and cultural characters in 6 genera at present recognized, of the Xylariaceae, Rosellinia, Hypoxyton, Nummularia, Daldinia, Penzigia and Xylaria, has produced important new information which should be taken into consideration in classification of the group. The main review and conclusions will be summarized under the following headings:-

1. Stroma:

An investigation into the anatomy and morphology of the stroma in different species of the genera studied indicated that there were basically 6 main types of variation according to the number and texture of the stromal layers and the persistence of the first-formed conidial layer. The latter was regarded as a separate entity from the rest of the stroma since it does not often persist to maturity. The 6 types defined (p.21), did not always conform to generic concepts, and some of the main characters at present used in generic separation by Tulasne (1863), Saccardo (1882 et seq.) and Miller (1928), were in fact dispensed with. Various objections were raised against the adequacy of these characters (pp. 7-8, 11-13, 20 - 21.), particularly as regards the number of perithecia per stroma and the level of the stroma surface, whether convex or concave. It is felt that the characters listed at the beginning of the paragraph should be given greater weight in classification. Part of the problem has been to determine whether cultural characters support the new classification adopted.

2. Specialization of cultural characters:

While it was realised that cultural characters are to some extent artificial, it was considered likely that specialization in these could occur just as much as in the respective perfect stages. In most taxonomic systems, it is usually assumed that the most specialised features are the most advanced phylogenetically and that the least specialised are also the most primitive. For example, presence of secondary mycelium would be regarded as more advanced than its absence and a type V or VI conidiophore as more advanced than a type I (see p.91). The Table opposite which is drawn up from species of all

groups except Xylaria, shows that different categories of growth characters are accompanied by considerable differences in conidial type, presence or absence and colour of stain, and other features. This appears to indicate that the felty coarse type of mycelial growth is on the whole more specialised than the canescent or velvety, and that it is usually distinguished by a faster growth rate.

3. Growth Character; Stain and Carbonization of the media:

The most interesting information was gained when malt agar was used as the culture medium. Growth characters on other media were usually similar or less luxuriant and were used for confirmation or as diagnostic characters where their appearance was markedly different. The table drawn up on p. 88 for the first group of species investigated is in general true for the whole.

No cultural type was found exclusively for any of the species groups defined above. Submersed, canescent, or velvety types, usually not producing stain, occurred throughout. Yet the following types are considered to be characteristic for each species group:

T y p e.	Found in. (Number of species in parentheses).
Mycelium white, opaque or subhyaline; velvety to fleecy, usually smooth; no stain; carbonization rare.	<u>Rosellinia</u> + <u>Endoxylon</u> group of <u>Hypoxyton</u> . (10/14)
Mycelium dull white, tinted with various colours, predominantly by olive green, rose and buff; felty, coarse and usually straggling; stain dark olive or ochre-brown; carbonization rare.	<u>Nummularium</u> group (6/9)
Mycelium dull yellow, rarely white; felty, moderately coarse; stain chestnut tawny; carbonization rare.	<u>Annulatum</u> group (4/6)
Mycelium thin velvety, dull white, coarse or smooth, stain some shade of red brown; carbonization absent.	<u>Rubiginosum</u> group (20/23)

associated with a coarse felty type of growth character. The differences in germinating patterns of many species of Rosellinia from the typical Hypoxyton - Penzigia type noted on p. 88 is also interesting. The most sig-

T y p e.	Found In. (Number of Species in parentheses).
Mycelium white, velvety to fleecy, usually smooth; stain none; carbonization very marked and usually conspicuous beneath the surface.	<u>Xylaria</u> (5/5)

4. Microscopic Characters:

(a) Mycelium:

The variations in diameter of primary and secondary mycelium between species are not great enough to be used as a reliable diagnostic character. It is perhaps significant that a wide diameter of mycelium is associated with a coarse felty type of growth character. The differences in germinating patterns of many species of Rosellinia from the typical Hypoxyton - Penzigia type noted on p. 88 is also interesting. The most significant feature, however, is the correlation between the presence of dark secondary mycelium in culture with advancement of stromal type in any species group. This can be seen by examination of the tables at the end of Chapters III - V, and in Chap. VI (pp. 93, 116, 168, 196).

(b) Conidiophore type:

The present study of 55 conidiophores support the following conclusions:-

- A. There is a gross similarity in type of conidiophore and its organization among the members of Hypoxyton and its allies on one hand, and Xylaria on the other. Those of Penzigia fall into both categories. The conidiophores of the former group are hyphomycetous, arising from the upper part of the aerial mycelium, but not organized into a coremium or any type of solid structure. They are similar to certain form genera of the Macronemeae of the Moniliaceae as defined by Clements and Shear (1931). The manner of their organization is clearly different from that seen in Xylaria where spicate or aristate coremia appear to be produced. These are similar in form to the imperfect stage borne on the young stromata preceding perithecial formation that have been figured and described by Tulasne (1863) and referred to by many other workers since. This distinction between Hypoxyton and Xylaria does not

appear to have been stressed by them, however.

The conidia are in all cases similar in general appearance, being oval, elliptic or pyriform, one celled, hyaline or subhyaline, and colourless, or tinted by varying shades of grey, fawn or yellow green, rarely red. Usually they are very small; conidia over 10μ long have not been found and the majority are no more than 6μ . According to the manner of production, they can be termed blasto-spores since they originate by growth and septation of a swelling beneath part of the wall of a hypha. The manner of development, described in detail on p. 90, appears to be universal but great variation occurs concerning the distance between one conidia and the next; a whole range is to be found from elongate spicate to globose fructifications. The order of development of the conidia on the branches appears to be acropetal with the youngest spore at the apex and the oldest below. Sometimes conidia are spirally arranged; in other cases they are clustered without apparent order.

- B. There appears to be a characteristic type of conidiophore developed in each of the 5 groups of Hypoxylon and in Xylaria. Daldinia would appear to be similar to Hypoxylon in this respect, while Penzigia contains types that are characteristic both of Hypoxylon and Xylaria. The distinction between the groups of Hypoxylon, Rosellinia and Daldinia does not appear to be absolute, since a basically similar type of conidiophore, possibly primitive, is very widespread. This has been called Type I, and is distinguished by conidia which are mainly pleurogenous in position. They are scattered or associated in groups of 2 - 5. The conidiophores as a whole is little modified and is scarcely distinct from the normal vegetative branches of the mycelium. In spite of its universality, this type is definitely connected with the genus Rosellinia (see p.91). From Type I may have arisen other more specialised in the groups now to be discussed.

i. Hypoxyton glomeratum complex:

Conidiophores are similar to those of the form genus Haplaria (Clements and Shear, 1931), forming an indeterminately branching system, either dichotomous or ternate, the latter being characteristic. The conidia are acropleurogenous and borne on distinct wart-like protrusions over an area which varies greatly in length, even in the same species. There appears to be a series from a long, rather ill-defined fertile area with many pleurogenous conidia to a spicate type with clustered conidia and finally to a capitate type normally termed a "head" where the conidia are relatively few in number and almost all acrogenous. (see Types III - V, p. 92).

ii. Annulatum and Nummularium groups:

The line of development appears to be continued in these groups where the conidiophores bear relatively short, fertile branches, usually in whorls of 3 but often of 2 or 4, or occasionally singly. The conidia form terminal globose heads. The groups may be distinguished as follows:-

Annulatum: Fertile branches narrow, apices elliptic or acuminate (Type VII, p. 115). Similar to Verticillium. (Clements and Shear, 1931, p. 203 and plate 54).

Nummularium: Fertile branches swollen, apices clavate or rounded (Type VI A). Similar to Verticillium (Clements and Shear, 1931, p. 203).

In both groups less elaborate conidiophore types also occur. These are Type V A, Type V B (see p. 92 and p. 115), and Type I, and are clearly similar to those of (i) preceding.

iii. Rubiginosum group :

This group is characterized by ramose or unbranched conidiophores normally bearing apical clusters of conidia although the fructification may be spicate with acropleurogenous conidia. Frequently also the conidia are borne in groups from the sides of the mycelium. Those conidiophores in which the fertile branches are whorled are clearly the most elaborate. (see pp. 143, 149).

The simpler conidiophores of the group are difficult to assign to any form genus. Those of Type IV B are often similar to Verticillium but vary in number of whorls of fertile branches per single conidiophore from several to one only, and so do not conform strictly to the form genus as defined by the constant compound branching. There is also a clear resemblance towards the Haplaria type of the H. glomeratum complex (Types IV A, V B, p.166) but the absence of visible protuberances subtending the conidia, and, in general, the capitate development of the conidia, separate the members of this section.

iv. Daldinia:

Since only 2 species have been examined it is difficult to make a definite conclusion, but it would appear that more than one type of conidiophore is to be found. That of D. concentrica clearly belongs to the Nummularium type, corresponding rather more closely to the Verticilliosis form genus since it is distinguished by several successive whorls of branches on the same conidiophore axis. D. eschscholzii, however, resembles the typical Rubiginosum form (Type V B).

v. Penzigia and Xylaria:

The variation in form of conidiophore in Penzigia from that of a typical Hypoxylon (Types I, III, IV A) to the complex coremiform one (Type X) which is characteristic of Xylaria, has been already described on pp.204,205. Type X is unknown for any Hypoxylon species studied except H. deustum, but for reasons discussed on pp.182-183, it is thought that the latter species has been misclassified and that its true affinities lie with Penzigia on stromal anatomy alone.

In general we can conclude that there is a most interesting parallelism between conidiophore type and degree of stromal variation. The presence also of an unspecialised form of conidiophore in many different species groups probably indicates a common derivation from a fundamentally similar type.

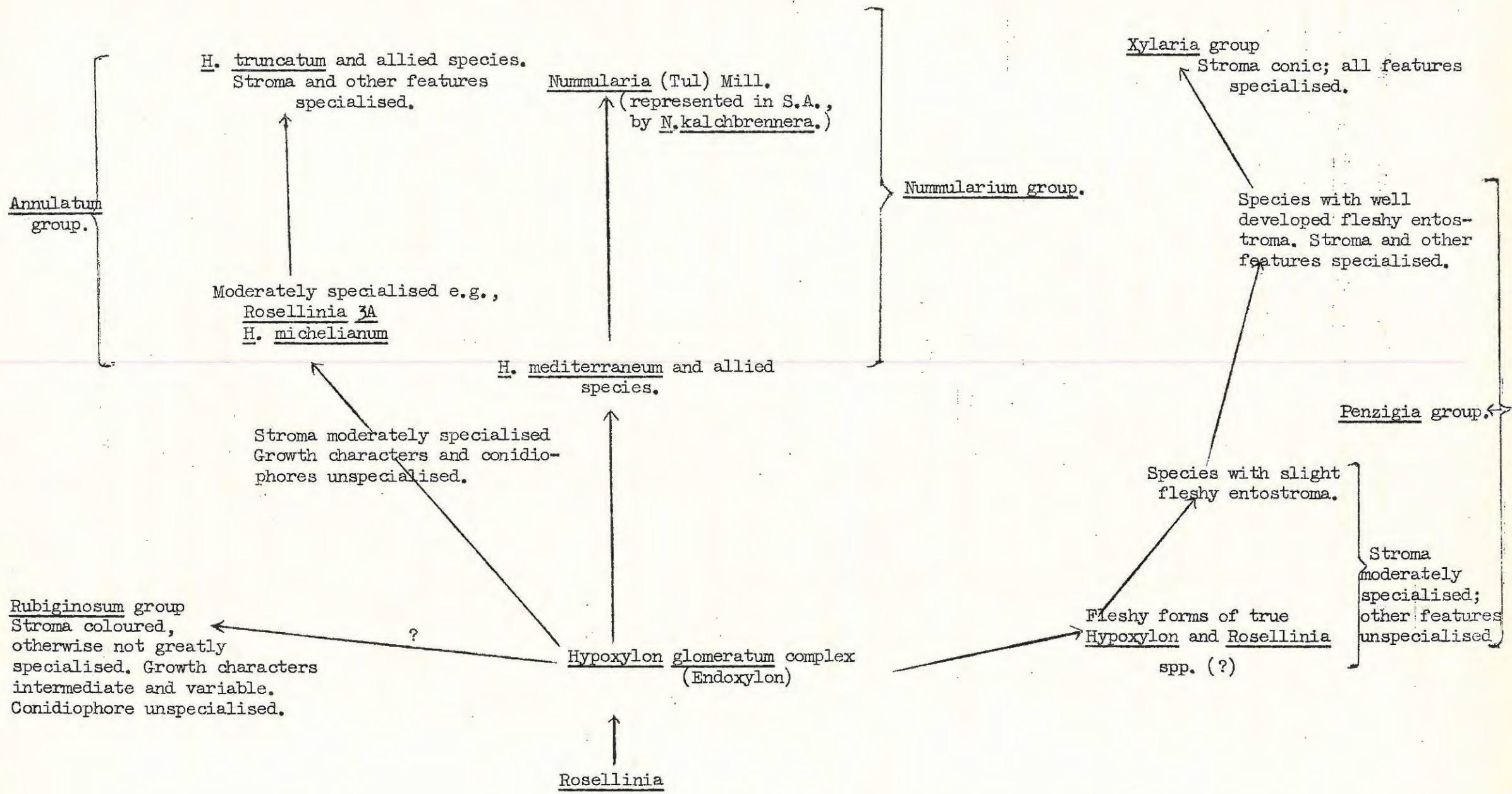
4. Growth Rate:

The range in growth rate from slow to fast appears to be much the same for all the species groups except Penzigia and Xylaria where the rate is consistently slow. Thus growth rate cannot be used as a criterion at generic level. Furthermore, the great variability of some of the species tested would appear to limit its value as a diagnostic character. Much more quantitative information concerning the growth rates of further strains of these species is required before one could assign a strain of unknown origin to its correct taxon. The significantly different growth-temperature reactions among the many strains studied here, however, indicate that the latter proposition is practicable. The results obtained for the Hypoxyton glomeratum and Hypoxyton rubiginosum species groups (Tables V and IX) are considered to be especially significant.

5. Spore Size:

Throughout this work, species definition has rested to a large extent on minor differences in spore size, which were shown to be significant by statistical methods. An important question is: how reliable are these spore measurements in establishing specific identity? Wherever available, more than one and usually several strains having identical ascospores size were cultured. In no case did one set of strains differ amongst themselves in any major respect in culture, but precise differences could be formulated between the cultural characteristics of groups of strains drawn from ascospores of different size. This surely indicates that spore size is a reliable criterion in classification, provided that the average lengths and breadths are carefully determined.

Cultural characters have, therefore, been of great value in supplementing the characters of the perfect stage used in classification and they also support the system of classification proposed on p.21, since there is a broad degree of correlation between the specialisation of general growth characters and conidiophore type with that of the stroma.



It is always difficult to make generalizations concerning the phylogeny of any group. Nevertheless, it is felt that the evidence gained supports the hypothesis that the genus Rosellinia and the Endoxylon group of Hypoxylon are the least specialised and form a plexus from which the other species groups may have been derived. The proposed phylogenetic scheme is illustrated on the opposite table. Each species group has its own trend of stromal specialization which is accompanied by peculiarities in the mycelial and conidial characters.

References:-

- | | |
|---------------------------------|---|
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Shear, C.L. | : The Genera of Fungi. Wilson 1931. |
| Saccardo, P.A. | : Sylloge Fungorum I, 1882.
Reprinted by Edwards Bros; Michigan, 1945. |
| Tulasne, L. & C. | : Selecta Fungorum Carpologia II. Paris, 1863. |

A P P E N D I X I.General Techniques and Information.A : Treatment of Material.§1. Collection:

Perfect stages of Hypoxylon and its allies were collected in the forest and bush areas in the following regions of the Cape Province:-

Nature's Valley (Groot Rivier)	District: Knysna, Western Cape.
Wilderness	George, Western Cape.
Hogsback	Victoria East, Eastern Cape.
Grahamstown	Albany, Eastern Cape.

Fungal material was stored for herbarium purposes in white cardboard boxes 5 x 8" in size, and given a herbarium number.

§2. Identification:

Great difficulty was found in identifying the species with certainty due to their great variability in form and to the incomplete or ambiguous descriptions given in the literature. In all cases a preliminary identification was made with reference to the work of Saccardo (1882 et seq.) and Miller (1928 et seq.), and samples of the majority of the species were then sent to Kew and to Dr. Miller at Athens, Georgia (U.S.A.) for confirmation. Some species, however, could not be tested because it was not possible to find them in sufficient quantity.

Full agreement between the authorities was not always reached and their identification, moreover, was sometimes rejected due to differences of interpretation. These differences are fully discussed in the text. The following table shows how the most important strains were classified together with other relevant information:

IDENTIFICATION TABLE.

217A

Species as finally termed; in order as referred to in text.	Number of Strains studied.	Chief strains sent for confirmation and subsequently referred to in text (Herb. Nos.)	Material personally compared with at Pretoria Herbarium (Herb. Nos.)	Species to which material was referred by the authorities below:		
				Dennis.	Booth.	Miller.
<u>GROUP I.</u>						
(Chapter III, p. 29)						
<u>Rosellinia protuberans</u> *	5	149,237	-	Not determined.	-	-
<u>Rosellinia obtusissima</u> *	2	257	-	"	-	-
<u>Rosellinia pulveracea</u> *	1	269	-	"	-	<u>R. pulveracea</u>
<u>Rosellinia moroides</u> *	1	412	-	"	-	-
<u>Rosellinia apiculata</u> *	5	76,256,430	-	"	-	-
<u>Rosellinia mammoidea</u> *	14	7, 47,433	(present but unidentified).	"	-	-
<u>Rosellinia corticalis</u> *	1	531	(present but unidentified).	"	-	-
<u>Rosellinia aquila</u>	6	22, 28,548	41131,31065	-	-	-
<u>Rosellinia thelena</u> *	1	508	5887	-	-	-
<u>Hypoxyton glomeratum</u>						
<u>Var.1.</u> *	2	119,524	-	-	<u>H. glomeratum</u>	<u>H. glomeratum</u>
<u>Var.2.</u>	1	212	27682,27794 34039 (id. Miller)	-	<u>H. glomeratum</u>	-

<u>Var.3.</u> *	1	396	-	-	<u>H. glomeratum</u>	-
<u>Var.4.</u> *	4	17,336	-	-	<u>H. glomeratum</u>	<u>H. glomeratum</u>
<u>Var.5.</u> *	1	397	-	-	<u>H. glomeratum</u>	
<u>Hypoxyton exutans</u>	1	382	-	-	<u>H. exutans</u>	-
<u>Nummularia succenturiata</u> *	6	218	-	-	-	-
<u>Nummularia uni-apiculata</u> *	1	372	-	-	<u>N. uni-apiculata</u>	
<u>Hypoxyton 13A</u> * (unidentified)	3	127	-	-	Not determined.	
<u>Hypoxyton merrillii</u> *	24	226	-	-	<u>H. merrillii</u>	<u>H. merrillii</u>
<u>Hypoxyton nummularium</u> *	1	275	-	-	<u>H. merrillii</u>	<u>H. nummularium</u> var. <u>merrillii</u>
<u>Hypoxyton mediterraneum</u>	4	19, 31,427	33946	-	<u>H. mediterraneum</u>	
<u>Hypoxyton asarcodes</u> *	1	262	-	-	-	<u>H. asarcodes.</u>
<u>Nummularia kalchbrennera</u>	4	35,402	20818 (type material.)	-	-	-
<u>GROUP II</u>						
(Chapter IV, p.98)						
<u>Rosellinia 3A</u> (unidentified)*	4	434	-	Not determined.	-	-
<u>Hypoxyton michelianum</u> *	6	91,288	-	-	<u>H. michelianum</u>	<u>H. michelianum</u>
<u>Hypoxyton stygium</u>	1	234	27760	-	<u>H. stygium</u>	<u>H. stygium</u>
<u>Hypoxyton microcarpum</u>	2	511,557	-	-	<u>H. stygium</u>	-
<u>Hypoxyton truncatum</u>	19	44,146,311	32148	-	<u>H. truncatum</u>	<u>H. truncatum</u>
<u>Rosellinia nitens</u> *	2	370	-	-	<u>H. truncatum</u>	-

Species, as finally termed; in order as referred to in text.	Number of Strain studied.	Chief strains sent for confirmation and subsequently referred to in text (Herb. Nos.)	Material personally compared with at Pretoria Herbarium (Herb. Nos.)	Species to which material was referred by the authorities below:		
				Dennis.	Booth.	Miller.
217B						
<u>GROUP III.</u>						
(Chapter V, p.118)						
<u>Hypoxyton 18A</u> *	4	220	-	-	-	<u>H. rubiginosum</u>
<u>Hypoxyton plumbeum</u> *	3	193	-	-	-	<u>H. rubiginosum</u>
<u>Hypoxyton 18B</u> *	1	376	-	-	<u>H. hyponiltum</u>	-
<u>Hypoxyton 18F</u> *	1	379	-	-	<u>H. haematostroma</u>	-
<u>Hypoxyton 18D</u> *	1	389	-	-	Not determined	-
<u>Hypoxyton 19</u> *	1	221	-	-	Not determined	Not determined
<u>Hypoxyton 18E</u> *	2	418,561	-	-	Not determined	-
<u>Hypoxyton coryphae</u> *	1	534	-	-	-	-
<u>Hypoxyton vogesiacum</u> *	9	2,312	-	-	<u>H. vogesiacum</u>	-
<u>Hypoxyton 38</u> *	3	555	-	-	<u>H. rubiginosum</u>	<u>H. rubiginosum</u>
<u>Hypoxyton rubiginosum</u>	24	219,538	31068,35870	-	<u>H. rubiginosum</u>	<u>H. rubiginosum</u>
<u>Hypoxyton purpureum</u> *	2	470,481	-	-	<u>H. rubiginosum</u>	-
<u>Hypoxyton tenue</u> *	3	374	-	-	-	-
<u>Hypoxyton 2C</u> *	4	378	-	-	Not determined	<u>H. rubiginosum</u> <u>var. tropica</u>
<u>Hypoxyton haematostroma</u>	2	238	9519,30748 31058,40705 (id. Miller)	-	-	-
<u>Hypoxyton luridum</u> *	2	504	-	-	<u>H. haematostroma</u>	<u>H. haematostroma</u>
<u>Hypoxyton 18G</u> *	1	487	-	-	-	-
<u>Hypoxyton piceum</u> *	3	326,330	-	-	<u>H. rubiginosum</u>	<u>H. rubiginosum</u>
<u>Hypoxyton ochraceo-fulvum</u> *	2	482	-	-	-	<u>H. rubiginosum</u>
<u>Hypoxyton ferrugineo-rufum</u> *						

<u>Var.1.</u>	1	287	-	-	-	<u>H. rubiginosum</u>
<u>Var.2.</u>	1	222	-	-	-	-
<u>Hypoxylon hypomiltum</u>	1	490	10390,27532 28893, etc.	-	-	-
<u>Hypoxylon vividum</u> *	1	268	-	-	-	<u>H. haematostroma</u>
<u>GROUP IV.</u> (Chapter VI, p.173).						
<u>Daldinia concentrica</u>	2	419	-	-	-	-
<u>Daldinia eschscholzii</u>	2	235	-	-	-	-
<u>Penzigia 1.</u> *	1	362	-	-	-	-
<u>Penzigia 2A</u> *	3	122	-	-	Not determined	Not determined but confirmed as <u>Penzigia</u> .
<u>Penzigia 4.</u> *	5	302,513	-	-	<u>H. glomeratum</u>	<u>H. glomeratum</u>
<u>Penzigia 3</u> *	1	390	-	-	Not determined	-
<u>Penzigia discolor</u>	3	365	31062	-	-	-
<u>Hypoxylon deustum</u>	5	552	34941	-	-	-
<u>Penzigia berteri</u> *	8	366	-	-	-	<u>P. berteri</u>
<u>Penzigia compuncta</u> *	1	410	-	-	-	-
<u>Xylaria fioriana</u> *	2	510	-	-	<u>X. fioriana</u>	-
<u>Xylaria apiculata</u>	1	295	-	-	<u>X. apiculata</u>	-
<u>Xylaria arbuscula</u>	3	57	-	-	-	-
<u>Xylaria castorea</u>	3	456	-	-	<u>X. castorea</u>	-
<u>Xylaria leprosa</u> *	4	133,507	-	-	<u>X. leprosa</u>	-
	241					

* Indicates new record for South Africa.

§ 3. Sectioning of stromata and other material:

Sections were cut by means of the freezing and Cambridge **Rocker** microtomes:

A. By freezing microtome:

Fresh material was immersed for 24 hours in gum arabic and then frozen and cut by a CO_2 - freezing microtome. Sections were mounted in 1% acid fuchsin in lactophenol.

B. By embedding in paraffin:

The method adopted was essentially similar to that outlined by Johansen (1940 p.126). Fresh material was fixed for 24 hours in formalo-acetic alcohol, then dehydrated by immersion in successive strengths of alcohol. After changing the immersion fluid gradually to chloroform, the material was impregnated with wax and serial sections were cut by means of a Cambridge Rocker microtome. These were mounted in Canada balsam.

Sectioning of carbonous stromata was facilitated by macerating the material prior to dehydration in boiling Schweizer's reagent (50 cc. HNO_3 + 1 gm. KClO_3) for a period of 5 - 15 minutes depending on the hardness of the stroma. This treatment made it possible for the wax to penetrate into the interior of the stroma but great care had to be taken not to allow the disintegrating action of the fluid to proceed too far. This was done by careful washing of the material in water after maceration. Other macerating fluids were found to be unsatisfactory as complete disintegration was difficult to prevent.

§ 4. Isolation of spores:

The main instrument used in isolation was a micropipette, and this was prepared in the following way: One end of a short length of glass tubing of 5 mms. bore was drawn out to a fine capillary over a Bunsen flame. The length of the tube was now cut to 4 - 6 cms. and a rubber teat was attached to the broad end. Complete sterilization of the tube could not be effected of course but frequent washing and intake of sterile distilled water before and after use reduced subsequent contamination to a minimum.

When isolation was undertaken, part of the stroma of the species in question was crushed in sterile water and examined under the low power of

the compound microscope. Spores were removed by the micropipette under constant supervision through the microscope and expelled in small droplets on to the surface of a plate of clear malt agar. The area covered by the water droplet was noted by marking the reverse of the plate directly underneath. When the concentration of spores was found to be unduly high or when there was a chance greater than normal of contamination from decaying stromata, dilutions were made before final transference by pipetting spores into droplets of water on further sterile slides and examining as before.

During the isolation process, great care was taken to ensure maximum sterile conditions by spraying the atmosphere and the bench with alcohol and menthol. The true secret of success lay, however, in dexterity of manipulation.

The inoculated plates were stored in incubators at a temperature of 25°C and were examined critically each day in order to watch the progress of the germinating ascospores and to exclude contaminants. Both multiple and single spore cultures were eventually obtained by transference of marginal hyphae from the initial colonies to fresh agar plates.

§ 5. Preservation of stock cultures:

During the early stages of the project, stock cultures were made by inoculating test tube agar slopes. This, however, resulted in two disadvantages: the inconvenience caused by replication at 3 - monthly intervals, and the deterioration of the strains themselves, which lost distinguishing characters after the third or fourth subculturing.

Stock cultures were, therefore, left on duplicate agar slopes in 4- or 8 oz. medicine bottles. These cultures remained viable for over a year, during which time a large number of subcultures could be made from them. If contamination occurred at any stage, the stock was immediately replaced.

B : Cultural Work.§ 6. Synthesis of media:

4 media were selected for culture work: malt, maize, Leonian's and Czapek. These were prepared according to the formulae cited by McLean and Cook (1941) and Wehmeyer (1924).

(a) Malt agar:

Formula :	Water	1000 cc.
	Agar	20 g.
	Malt extract	20 g.

The malt extract is a viscous solution with a density of $\underline{\Lambda}$ 1.4 gm. dry wt./ml.

The solution contains:-

- > 50 % maltose
- small quantities of dextrin, dextrose and other carbohydrates
- $\underline{\Lambda}$ 4 % protein.

Procedure: (up to pouring).

The measured quantity of agar was heated with 950 mls. of water in a 2,000 ml. flask in a steamer or water-bath. When the agar was dissolved, the malt extract was added with enough water to make 50 mls, mixed thoroughly and heated with the agar for 15 minutes.

(b) Leonian's and Czapek agars:

<u>Formulae:-</u>	<u>Leonian's.</u>	<u>Czapek.</u>
Water	800 cc.	1000 cc.
Agar	16.00 g.	20.00 g.
Malt extract	0.50 g.	-
Maltose	0.50 g.	-
Peptone	0.05 g.	-
Sucrose	-	30.00 g.
KCl	-	0.50 g.
K ₂ HPO ₄	-	1.00 g.
KH ₂ PO ₄	0.10 g.	
MgSO ₄	0.05 g.	0.50 g.
FeSO ₄	-	0.01 g.
NaNO ₃	-	2.00 g.

Procedure:

As for malt agar: the substances listed were dissolved in water beforehand and then added to the molten agar. A slightly lesser total quantity of water was used in preparation of Leonian's agar.

(c) Maize agar:

Formula: Water 1000 cc.
 Agar 20 g.
 Maize powder 50 gms.

Procedure:

50 gms. of powder ground from maize fruits were boiled for one hour in 2000 cc. water. After being left to settle for a further hour, 1 litre of the clear supernatant fluid was drawn off. 20 gms. of agar were then dissolved in this by further heating.

Pouring of media:

As soon as the constituents were dissolved and shaken to ensure even distribution, the various media were poured, either into test-tubes in 12-15 cc. portions or into 8 oz. medicine bottles in larger quantities of about 80 cc. Both tubes and bottles were corked tightly and autoclaved for 15 minutes at 15 lbs. pressure at a temperature of about 110°C. They were then ready for use; the contents of the tubes normally being poured with sterile plates as soon as possible. Bottles were tilted at a slight angle to the horizontal after removal from the autoclave, so that the agar inside should set in a slope on cooling.

§ 7. Plate Cultures:

Plates were sterilized by heating in a hot air oven at 140°C for one hour or twice at 100°C for 1 hour on successive days. After cooling, a tube of sterile liquid agar was poured into each plate. When the medium had solidified, the plates were inoculated. A metal rod was heated to red heat, cooled suddenly in alcohol and then used to transfer a small piece of marginal or other mycelium from the original culture, obtained directly from the ascospores, on to a fresh plate.

Malt agar was used as the chief medium throughout the project since the fungi grew on it with greater luxuriance than on other media and showed differences from each other that could be expressed in precise terms. At least

15 and usually 20 - 25 plate cultures of each strain were examined in order to make a description of the external characters. These cultures were incubated at 5 temperatures, 15°, 20°, 25°, 28° and 31° C.

Duplicate and often triplicate plate cultures were also prepared, using the other three media. All observations were made 3 - 10 days after inoculation. Due to the varying rates of growth of the different strains it was impossible to describe them all at the same age. Fortunately, however, the appearance of the majority of the strains remained approximately the same throughout their period of growth. Except where specifically mentioned, all descriptions are based on cultures 5 - 8 days old.

§ 8. Bottle Cultures:

Each strain was cultured in a bottle of maize, Leonian's and Czapek agars. Malt cultures were prepared in duplicate and often in triplicate or quadruplicate. One of the malt cultures was kept as a stock culture.

The bottle cultures were incubated at 25° C for 14 days, after which they were removed and kept out of the direct sunlight in a room where the temperature fluctuated between 18 - 23° C. Observations were made after 7 days, at short intervals up to 21 days and fortnightly thereafter. Except where specifically mentioned, all descriptions of the bottle cultures were based on their appearance when 14 days old.

§ 9. Terms used to express the growth characters of the mycelium:

I. Growth Character:

It is very difficult to describe a fungal colony unambiguously, especially when a large number of types are considered that only differ in small details. For this reason as many photographs as possible were taken of the fungi on different culture media to supplement the descriptions given. The following terminology was adapted from Long and Harsch (1918) and new terms have been added to express those growth characters not already described:

1. Submersed: Colony growing entirely below the surface of the agar; aerial mycelium none.

2. Canescent/Downy: Aerial mycelium consisting of short fine hyphae, loosely scattered over the surface of the agar. In most of these colonies the majority of the growing hyphae are still below the agar surface, however.
3. Felty: Aerial mycelium matted, with intertwined hyphae resembling a thin felt; surface coarse or smooth.
4. Cobwebby: Aerial mycelium intermediate between felty and canescent; usually characterized by long weak intertwined hyphae lying in all directions.
5. Silky: Similar to the above, but the hyphae lie parallel, giving the appearance of combed silk.
6. Cottony: Aerial mycelium comprising a loose mass of erect rather long (3-5 mm.) hyphae.
7. Velvety: Aerial mycelium dense and usually rather compact in contrast to the above types; surface smooth resembling the "pile" of velvet due to many short, straight closely growing hyphae. The velvety appearance is usually enhanced by zonate growth.
8. Fleecy: Aerial mycelium very dense though not necessarily very thick, opaque and with a characteristic uneven surface similar to that of a fleece.
9. Lanose/woolly: Aerial mycelium forming a dense opaque mass, usually with a smooth surface; normally very thick (3-5 mms.) and consisting of long tortuous hyphae.
10. Floccose: The aerial mycelium may be initially appressed velvety or fleecy, but is distinct in that scattered pulvinate or irregular patches of short mycelium arise all over the surface of the colony as it grows older.
11. Zonate: The aerial mycelium differs in character within the same colony, forming concentric zones of different degrees of luxuriance. Typical of velvety colonies.

II. Aspect:

1. Gelatinous: The surface of the colony is smooth and to some extent shiny, resembling gelatine. A gelatinous surface is characteristic of some but not all submersed or canescent colonies.
2. Sodden: The mycelium has a water-soaked appearance. The colony varies from appressed to velvety but is never fleecy or lanose.
3. Dry: The opposite of that just described. It is characteristic of the great majority of the species investigated.
4. Appressed: The aerial mycelium is very compact and tends to grow more or less parallel with the agar surface. This aspect is typical of canescent and velvety colonies.
5. Plumose: Dry, with tufts of mycelium, particularly near the margin, that have a central core from which short hyphae radiate.

III. Margin:

i. Distinctness:

It is characteristic of Hypoxylon and its allies that the aerial mycelium, when present, follows closely behind the outermost submersed marginal hyphae, and in some cases the two develop almost simultaneously. Nevertheless, variations in the period required for the aerial mycelium to appear lead to differences in appearance of the colony.

In the majority of species investigated, the colony is uniform in appearance, i.e., there is no great difference in nature between the centre and margin of the colony because where the aerial mycelium is delayed a distinct submersed subhyaline marginal zone is apparent on the outside of the colony. For the purposes of this work the marginal zone in this case has been termed distinct. Throughout the descriptions, however, it is assumed that the margin is not distinct from the centre of the colony unless specifically stated.

ii. Entire:

Margin even and circular in outline, or almost so.

iii. Segmented:

Margin of colony broken into small segments, each independent in growth.

iv. Effuse:

Peripheral hyphae widely dispersed.

v. Compact:

Peripheral hyphae lying together, usually parallel.

§ 10. The microscopic characters of the mycelium:

Two types of mycelium are found in the Xylariaceae, the primary mycelium which develops first and is usually white or yellow, and the secondary mycelium which is dark brown to black and develops much later. Since the secondary mycelium is frequently not developed, its very presence can be used as a distinguishing character.

(a) Primary Mycelium:

This comprises the marginal hyphae and the derivatives from them which make up the bulk of the colony. The primary mycelia, inner to the margin of all the species investigated, branches indefinitely and the ultimate branches are well below 1μ diameter. Consequently one species often appears extremely similar to another when this character is considered only. Large differences in the maximum diameter of the marginal hyphae are much easier to appreciate, however, since the range of diameter is much closer. This and the mode of branching of the marginal hyphae have been used to separate the species. To a lesser extent the pattern of the young germinating ascospores is also important (see Chapter III; discussion).

(b) Secondary Mycelium:

The useful characters of the secondary mycelium, which develops beneath the primary in certain species, are:-

- the depth of colouration,
- the mode of branching, and
- range of diameter.

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