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A Taxonomic Study of Cercospora vitis (Lév.) Sacc.,  
the Causal Organism of a Leaf Spot Disease on Grapes.

A Thesis presented in partial fulfilment of  
the requirements for the degree of  
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by

Ian Charles Harvey

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SUMMARY

1. A leaf spot disease of grapes caused by a dematiaceous fungus is described for the first time in New Zealand.
2. Literature on the taxonomy of the causal organism is reviewed and reveals a pleurality of binomials that have been applied to the fungus. The main features in contention are the correct basionym, symptomatology, conidium shape and degree of conidiophore compactness. These were studied in relation to the taxonomy of the causal organism and from results obtained it was finally placed in the genus Cercospora, where the binomial becomes C. vitis; the legitimate specific epithet for the fungus.
3. Cultural studies of the pathogen provided further supporting evidence for placement in the genus Cercospora.
4. An apparatus for photomicrographically recording conidium ontogeny and spore germination patterns of filamentous fungi is described.
5. Classification schemes of the Fungi Imperfecti are reviewed and all were found to have certain shortcomings with respect to the classification of the causal organism. A proposed alternative scheme is outlined.
6. The disease does not become manifest until late in the growing season and hyphal swellings in the stomata (stomatopodia) are found to constitute the form in

which the pathogen persists during a latent infection period. A disease cycle is synthesised from results of glasshouse infection experiments, field observations, and reports from the literature.

PREFACE

There is at present a rapidly increasing area of grape vines being grown in New Zealand. As at the 31st of March, 1969, the Viticultural Advisory Committee of the Department of Agriculture estimated there were 2,200 acres of grape vines in New Zealand, of which 1,750 acres are bearing. It is also estimated that by the end of 1970, the acreage will have increased to 3,000 acres (Thompson, personal communication). There is also a parallel boom in the wine making industry (see table I), with New Zealanders becoming increasingly more conscious of good wines as the number on the market increase and comparisons are made with those produced overseas.\*

TABLE I

Wine production and grape vine acreages in  
New Zealand.<sup>1</sup>

	1961-62	1966-67
Wine put down	1,206,000 gal.	2,298,000 gal.
Vine yard acreage	1,187	1,748

<sup>1</sup>Industrial statistics, 1966-67.

Grape vines grow prolifically out of doors in the northern areas of New Zealand and yields per acre are often very high, especially in the Gisborne and Auckland districts.

\* See Consumer No. 58, 1969 - published by the Consumer Council, N.Z.

However, although the climate is conducive to vine growth, it is also conducive to disease development, and there are several fungal diseases that currently plague the industry. As the acreage increases, disease problems are likely to be of even greater magnitude.

In the Autumn of 1968, the author received a number of severely spotted grape leaves from a Matamata home garden. The disease was tentatively identified as being caused by the fungus Isariopsis clavispora (B. & C.) Sacc., a pathogen not previously recorded in New Zealand (Dingley, personal communication). There are, however, reports of the disease being present in several overseas countries including North America (Berkeley, 1875; Higgins, 1929; Rhoads, 1926; Schwarze, 1917; Tharp, 1917), South Africa (du Plessis, 1942a, 1942b), India (Munjal and Sethi, 1966) and the Philippines (Roldan, 1938). Examination of vineyards in the Gisborne and Auckland areas in February and April 1969 respectively, failed to reveal the presence of the disease.

A review of the literature revealed that the causal fungus has been given several generic and specific names and further, there is considerable confusion as to the correct binomial that should be applied to the pathogen. Accordingly, studies were initiated into all aspects relative to the correct nomenclature of the causal fungus. Associated with this work, the requirements of proof of pathogenicity (Koch's postulates) were fulfilled, and symptoms were recorded as they occurred both in the field and under glasshouse conditions.

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## I INTRODUCTION

There are several organisms capable of causing foliage diseases of grapes, including those inducing powdery mildew, downy mildew, anthracnose and black-rot. Further, there are five fully characterized species of Cercospora reported to attack grape leaves (Chupp 1953), but the organism under study, which has also been placed in the genus Cercospora, is the most prevalent and quite distinct from the others (Appendix I). However, despite its apparent uniqueness, the taxonomy of the causal organism has been the subject of much debate in the literature, as evidenced by the fact that it is currently known under three generic names, namely Cercospora, Isariopsis and Pseudocercospora. Even though several workers have conducted quite detailed taxonomic studies on the causal organism, they have often applied different generic names and specific epithets to the fungus (Higgins, 1929; du Plessis, 1942b; Munjal and Sethi, 1966).

The plurality of names could have arisen from the fact that the fungus was first described in the 19th century when generic limits were broad and vague. As new genera were erected, limits were reduced and defined more precisely as a consequence of which many fungi had to be reclassified into these genera. Placement was often incorrect because many original descriptions were vague and imperfect. The fungus under study illustrates this point in that it was first placed independently into three genera (Cladosporium, Septonema and Graphium) but because

it demonstrates some unusual and variable morphological characteristics, there arose considerable confusion as to its correct nomenclature. Consequently, the taxonomy of the fungus was studied from two aspects:

- (a) The historic development of the nomenclature.
- (b) Morphological and physiological characteristics appertaining to taxonomy.

To determine the correct taxonomic affinity of the fungus, results from the morphological and physiological studies were compared with reported characteristics of the same fungus and closely related fungi.

The confusion in nomenclature arose during the use of the classification system of the Fungi Imperfecti devised by Saccardo in the 19th century. Since modern taxonomy of this group of fungi is based on different criteria, the current confusion was also studied in relation to these present day concepts.

To enable a comparison to be made with other descriptions of the fungus quoted throughout this thesis, a description of the disease in the field and the causal organism as found in New Zealand is now presented.

Lesions first appearing midway through growing season, 2 to 8 mm in diameter, at first as small irregular black areas on upper surface with no necrosis of host tissue, immarginate; later becoming larger and still irregular to angular with red-brown

centres and dark brown-black margins (Fig. 1). Host tissue slightly yellow, becoming more obvious with age of the lesions; lesions often becoming confluent. On lower leaf surface lesions appear light brown through tomentum, sometimes with dark brown centres. Often causing premature leaf yellowing and defoliation.

Fruiting amphigenous; conidiophores long (150 - 450 x 3 - 6  $\mu$ ), arising from a stroma, medium olivaceous brown, but dark brown en mass; unbranched, septate, multigeniculate at tips with no obvious spore scars; proliferating by 'sympodial' growth; in dense fascicles of 10 - 50 units, fused to spreading at base, always spreading at apex, sometimes coremoid (Fig. 16).

Conidia medium olivaceous brown, blastospores, 3 - 14 (8) septa, obclavate, phragmospores, with obconically truncate base and tapering obtuse apex, straight to slightly curved, smooth, often guttulate, caducous, 20 - 100 (63) x 3 - 12 (7)  $\mu$  (Fig. 2).

Habitat: on leaves of Vitis vinifera var. Albany Surprise; from a private garden, Matamata, New Zealand. Herb. P.D.D. 26770 and 26771, Herb. Lev. 4111.

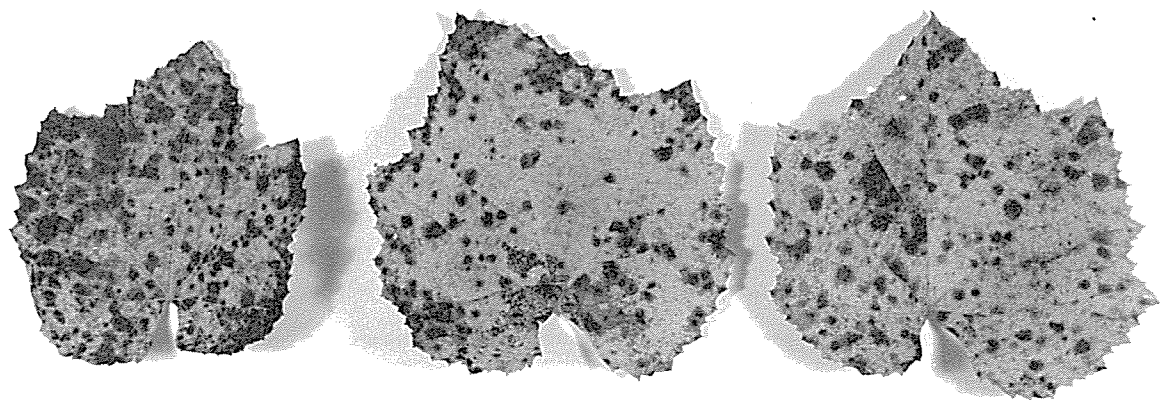
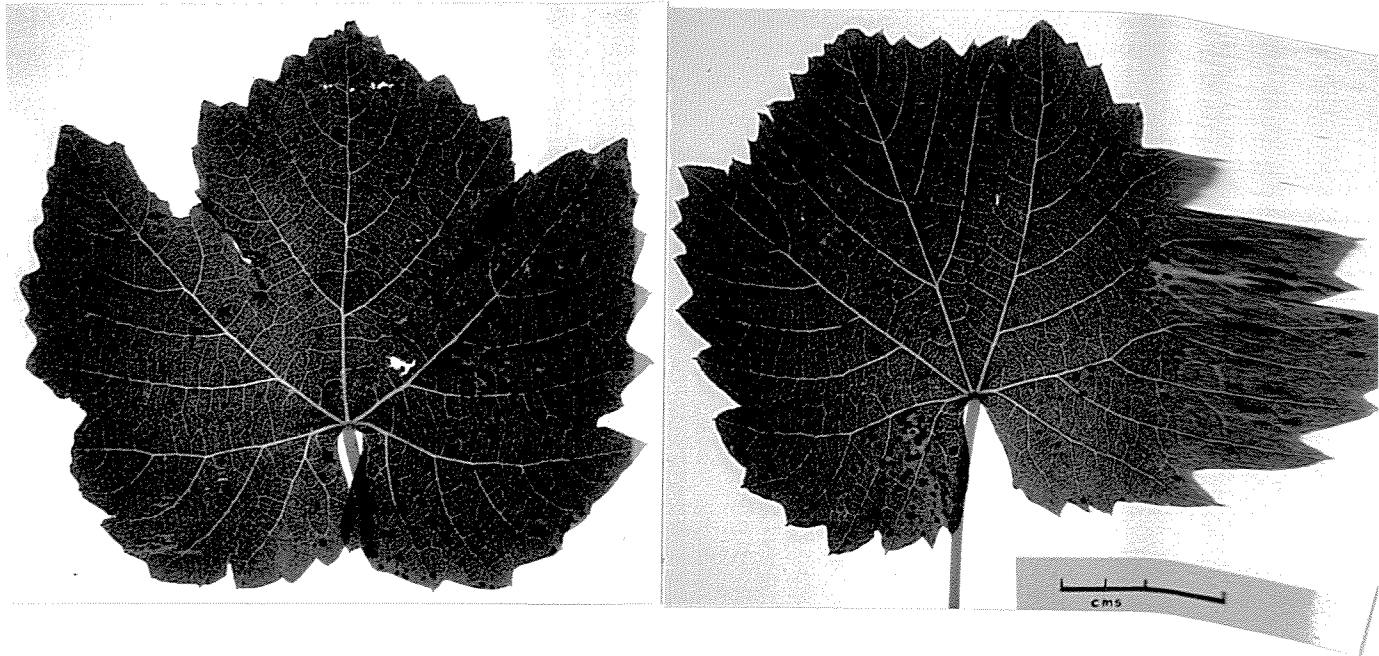


Fig. 1. Field symptoms of the disease on grape leaves (var. Albany Surprise).

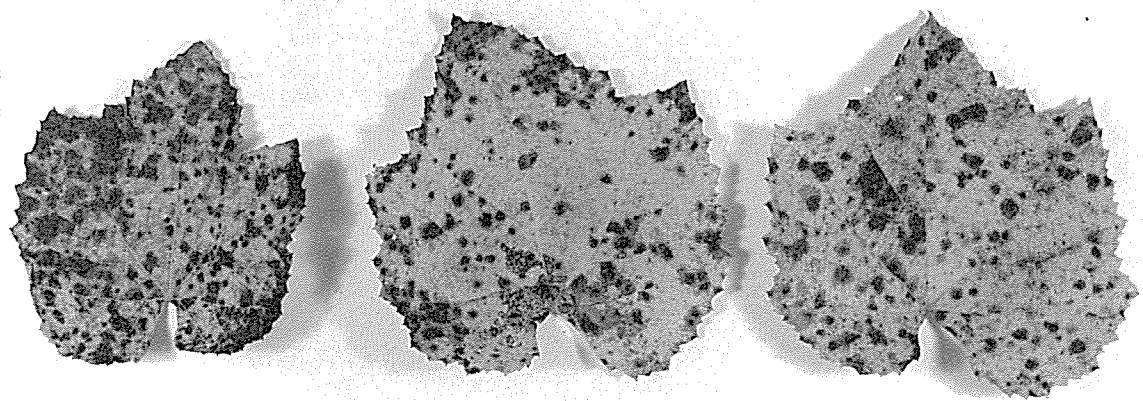
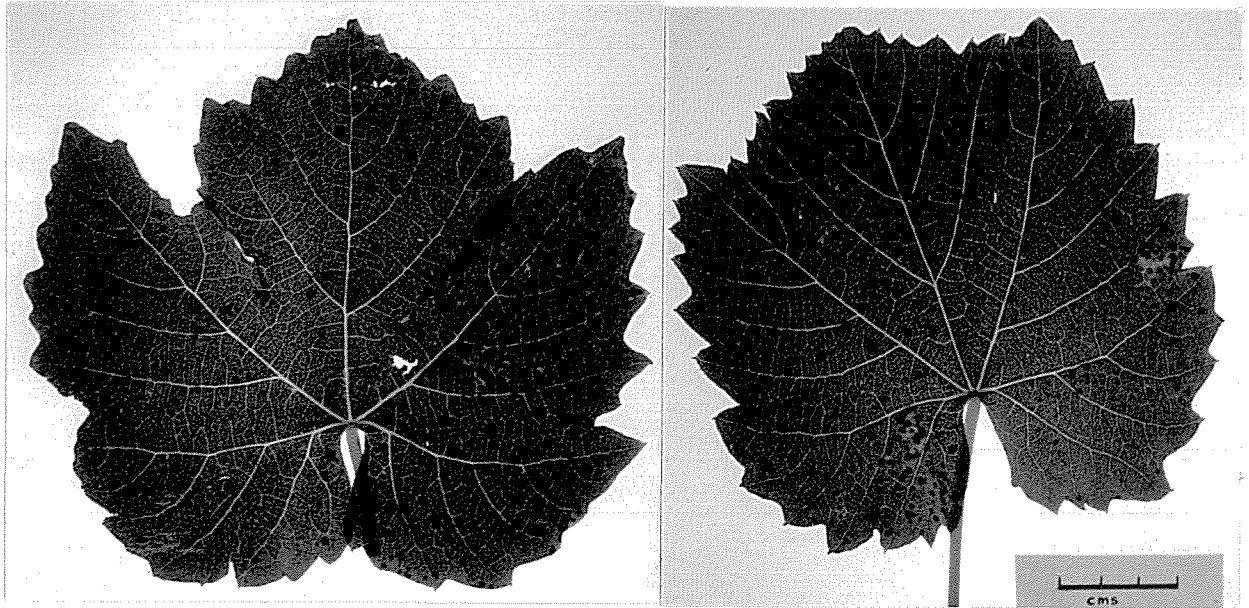


Fig. 1. Field symptoms of the disease on grape leaves (var. Albany Surprise).

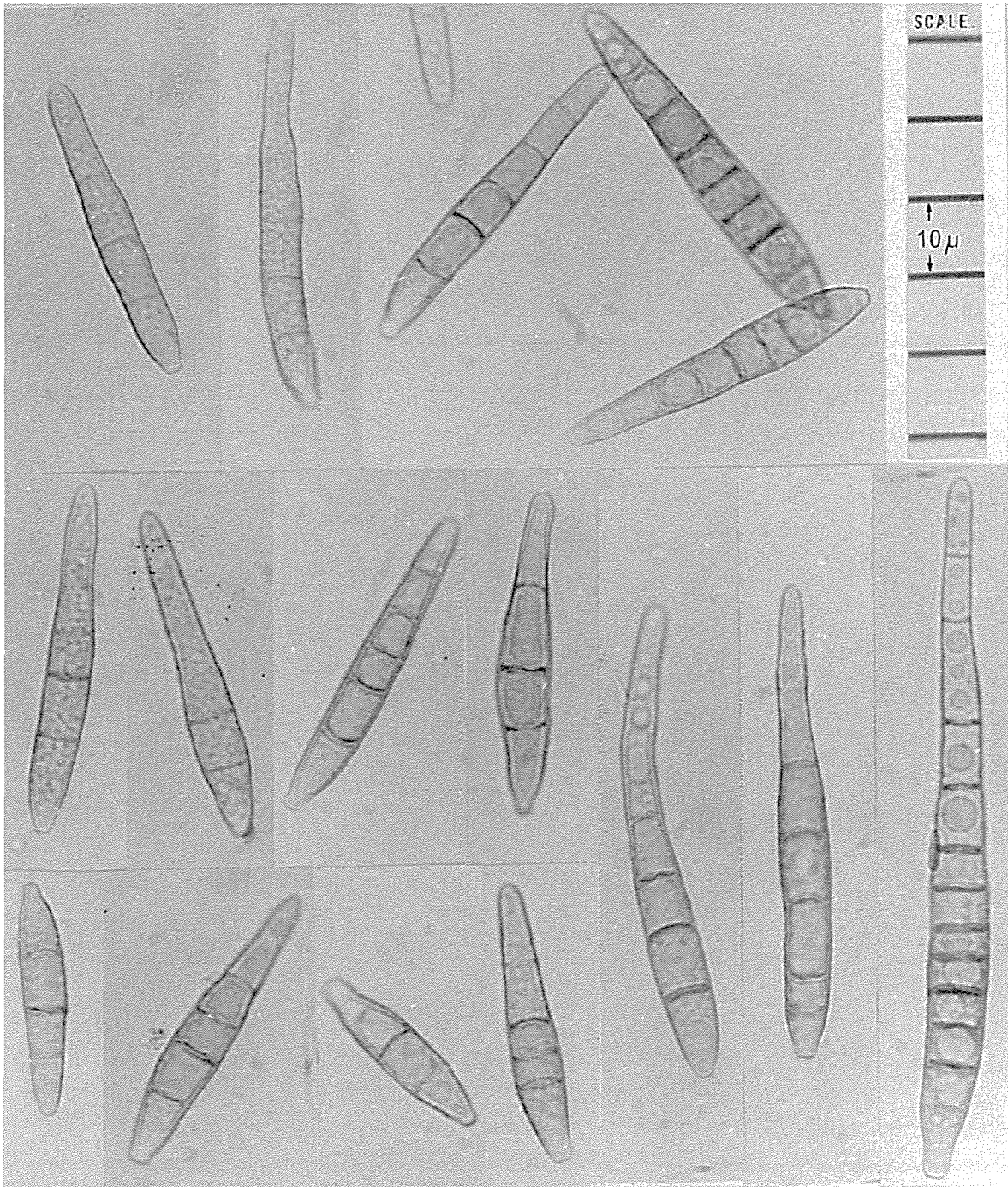


Fig. 2. Conidia of the causal organism from naturally infected field material.

## II HISTORY OF THE TAXONOMY OF THE CAUSAL ORGANISM

The fungus causing this leaf spot disease of grapes has been placed in several genera and has had several specific epithets. In more recent literature, the fungus has been known by the following binomials:

- (i) Cercospora viticola (Ces.) Sacc.
- (ii) Cercospora vitis (Lév.) Sacc.
- (iii) Isariopsis clavispora (B. & C.) Sacc.
- (iv) Pseudocercospora vitis (Lév.) Speg.
- (v) Isariopsis fuckelii (Thüm.) du Plessis.

These four binomials are analysed historically to determine how each of the above binomials came to be applied to the fungus and to help determine the correct genus into which the fungus should be placed.

- (i) Cercospora viticola (Ces.) Sacc.

In 1854, an Italian Cesati named the fungus Cladosporium viticolum, while Passerini in 1872 described the same as Cladosporium ampelinum. Saccardo in 1875, apparently unaware of the earlier records, named it Cladosporium vitis. However, he later considered the fungus to fit in the genus Cercospora erected by Fresenius in 1864 and consequently renamed it C. vitis (Lév.) Sacc. and later C. vitis Sacc. While compiling *Sylloge Fungorum* in 1886, Saccardo discovered the previous reference to this fungus, recognized Cesati's Cladosporium viticolum as the basonym and reduced all other names mentioned in the above paragraph to Cercospora viticola (Ces.) Sacc. His

description was:

"Maculis amphigenis subcircularibus vel irregularibus 2-10 mm diam., arescendo ochraceis, vix marginatis; hyphis saepius hypophyllis hinc inde densiuscule fasciculatis filiformibus, septulatis. 50-200  $\mu$  4-5, rectis, ochraceis, sursum obtuse et obsolete denticulatis; conidiis elongato-obclavatis, sursum attenuatis, 3-4-septatis guttulatis, 50-70  $\mu$  7-8, olivaceo-ochraceis.

Hab. in foliis vivis Vitis viniferae et V. Labruscae cultae et silvaticae in Italia boreali, Austria, Lusitania, Gallia et Germania."

Translation: Spots amphigenous subcircular or irregular 2-10 mm in diam, drying to an ochreous colour, scarcely marginate; hyphae mostly on under surface, here and there forming dense bundles, filiform, septate. 50-200 x 4-5  $\mu$ , straight and ochreous, ends obtuse and weakly denticulate; conidia elongate to obclavate, ends attenuated, 3-4 septate, guttulate, 50-70 x 7-8  $\mu$ , olivaceous to ochreous.

Hab. on living cultivated and wild leaves of vitis vinifera and V. Labruscae from southern Italy, Austria, Lusitania, France and Germany.

Higgins (1929) discovered the sexual phase of the fungus naming it Mycosphaerella personata Higgins and considered the asexual phase to be C. viticola, but observed darker lesions than those described by Saccardo, and further, described a completely different shape for the mature conidia. Munjal and Sethi's (1966) description of the fungus under M. personata and C. viticola also differed

from Saccardo's in that the lesions were described as possessing ash-grey centres and the conidiophores having prominent spore scars.

(ii) Cercospora vitis (Lév.) Sacc.

Deighton (1960) referred to the asexual phase of Mycosphaerella personata as Cercospora vitis (Lév.) Sacc. This combination for the asexual phase was made by Saccardo in 1876 based on Septonema vitis as described by Léviellé in 1848. The following is a description of S. vitis Lév. as given by Saccardo in *Sylloge Fungorum* (1886):

"Hyphis floccosis gregariis hypophyllis fasciculatis longis, cylindricis, continuis, macula exarida insidentibus caespitulosque nigros formantibus; conidiis acrogenis, catenatis, subfusiformibus, 4-6-septatis, deciduis.

Hab. ad folia Vitis viniferae, Bordeaux Galliae. - Diu pro Cercospora viticola habui; nunc vero diversam rem censeo et revera Septonematis speciem."

Translation: Woolly, clustered hyphae on underside of leaf, fasciculate, long cylindric and continuous. Forming small dark tufts on dried out spots; conidia acrogenous, catenulate, subfusiform, deciduous, 4-6 septate.

Hab. on Vitis vinifera leaves in Bordeaux France.

- "For a long time I had thought it to be C. viticola, now I am of the opposite view, it is indeed a Septonema species."

Subsequent to his erection of C. vitis (Lév.) Sacc.,

Saccardo changed his mind and considered S. vitis to be a separate fungus on grapes, due probably to it being described with catenulate, subfusiform conidia. He therefore used Cladosporium viticolum Ces. as the basionym for the fungus. Some mycologists take the original view held by Saccardo, that S. vitis does in fact refer to the same fungus, and that Léviellé probably observed some conidia end to end in his microscope slide preparation and thus described the conidia as catenulate (Deighton, personal communication). If this view is taken, then S. vitis Lév. becomes the legitimate basionym of the fungus. Unfortunately, at the time of publication the type specimen - S. vitis Lév. had not been located or examined by the author, and so the true nature of the conidia remains a matter of conjecture.

When Saccardo rejected S. vitis as the basionym for the fungus in 1876, he reduced the authority from C. vitis (Lév.) Sacc. to C. vitis Sacc. He was not aware at that time of Cladosporium viticolum Ces.

Lindau (1910) presumably considered both C. vitis (Lév.) Sacc. and C. vitis Sacc. to be incorrect: the former because it had been rejected by Saccardo himself and the latter because there were earlier legitimate binomials for the fungus. Hence Lindau named the fungus C. vitis (Lév.) Lindau. Saccardo's previous authority was still valid however, so Lindau's authority is invalid.

Cifferi (1922) reported and illustrated a fungus

on grape leaves that was similar to the one under study, but with smaller (27-32 x 4-5/μ) clavate, one septate conidia. The author believes that Cifferi observed immature conidia which have the features he described (see section IV). Therefore, his construction of a variety - C. vitis (Lév.) Sacc. var. rupestris Cifferi was not warranted.

(iii) Isariopsis clavispora (B. & C.) Sacc.

In 1875, Berkeley described the fungus on grapes from North America and named it Graphium clavisporum B. & C. Saccardo, using this description plus that of von Thümen (1878), reclassified the fungus into the genus Isariopsis which was erected by Fresenius in 1863. Saccardo named the fungus I. clavispora (B. & C.) Sacc., although he also stated this fungus may be "An forma compactior Cercosporae viticolae?" His description was:

"Minuta, olivacea e maculis orbicularibus brunneis oriunda; hyphis sursum relaxatis et flexuosis; conidiis linearibus clavatisve pluri (3-4) septatis, articulis 1-guttatis, 44=4-5 (teste Thüm.).

Hab. in foliis Vitis Labruscae in Carolina inferiore et New Jersey."

Translation: Small, olivaceous, arising from circular brown spots; hyphae loose and wavy at tops; conidia straight, clavate, 3-4 septate, 1-guttulate in nodes, 44 x 4-5/μ (according to von Thümen).

Hab. on foliage of Vitis Labruscae in southern

Carolina and New Jersey.

Tharp (1917) compared the fungus which he found in Texas with Saccardo's descriptions, and considered his I. clavispora to have longer and wider spores (30-56 x 6-8 $\mu$ ) with more septa and no guttulation. Schwarze (1917) described I. clavispora as causing lighter coloured spots than described by Berkeley or Saccardo. He also described the conidia as obclavate in contrast to the clavate conidia described by Saccardo for I. clavispora. Roldan (1938) also described the conidia as obclavate, but the symptoms as chocolate brown spots.

Rhoads (1926) in his semi-popular treatment of the disease in Florida, described and illustrated the conidia of I. clavispora as being clavate, and the symptoms as irregular or angular brown spots with slightly thickened or elevated borders.

(iv) Pseudocercospora vitis (Lév.) Speg.

Spegazzini (1911) considered this pathogen of grape leaves to be atypical of either the genus Septonema or Cercospora and erected a new genus Pseudocercospora to accommodate it. The basionym for the type species P. vitis was Septonema vitis Lév. The author was unable to obtain any of the type specimen to compare with the New Zealand fungus, so was unable to determine if this fungus had catenulate conidia as described by Léviellé for S. vitis.

Chupp (1953) used this binomial (P. vitis) when

describing the fungus in his monograph on the genus Cercospora. Chupp describes Pseudocercospora as "a dematiaceous hyphomycete with large Phragmidium-like conidia." He also states that the conidia have thick walls. Spegazzini's original description of the genus (see section V) described the conidia as Phragmospores - not Phragmidium-like. Chupp then describes conidia of the fungus under study as "rarely with thick walls", which hardly substantiates the fungus being described under his concept of Pseudocercospora.

If S. vitis is taken as the basionym for the fungus, then P. vitis is a legitimate binomial; but whether Spegazzini was warranted in erecting this genus is discussed under section V.

(v) Isariopsis fuckelii (Thüm) du Plessis.

In 1942, du Plessis described the fungus as follows:

".,,Maculis amphigenis, primitus subcircularibus vel angularis, fuscis, demum circularibus, 5-10 mm in diam solitariis, gregariis, vel confluentibus, interdum extensis ad indeterminatis maculis, pallide brunneis; maculis epiphyllis, marginibus distinctis, fuscis, elevatis, mediis punctulatis; maculis hypophyllis subcircularibus, cinereum fuscis, interdum marginatis, marginibus minus elevatis distinctique; conidiophoris basilaribus, septatis, simplicibus, rectis, densiusculi caespitulis, stilbeis, fuscis, exorientibus pseudo-parenchymaticis ac subepidermatis stromatis, interdum sensim dilatis ad apices; apicibus pluri-geniculatis, flexuosis, brunneis, 157.8-278.8 x 3.7-5.4  $\mu$  (217.8 x 4.4  $\mu$ ); conidiis primitus clavatis, continuis, rectis, demum elongato-clavatis vel subfusoides, apicibus attenuatis, rotundatis, basibus obtuso-rotundatis, 2-10 septatis, constrictis ad septis, brunneis granularibus, 34.7-77.8 x 5.4-9.2  $\mu$  (50.0 x 7.1  $\mu$ -).

Hab. in foliis *Vitis viniferae*, Africae australis."

Translation: Spots amphigenous, at first subcircular or angular, fuscus, finally circular, 5-10 mm in diam, solitary, gregarious or confluent, occasionally producing indeterminate spots, pale brown; spots on upper surface with clearly defined dark elevated margins, centres minutely dotted; spots on the lower surface subcircular ash-grey brown, occasionally bordered, the margins less elevated and distinct; conidiophores with a base, septate, simple, upright as dense stalked little tufts arising from a pseudo-parenchymatous subepidermal stroma, sometimes spreading at the apices. Apices pluri-geniculate, flexuous brown 157.5-278.8 x 3.7-5.4/ $\mu$  (217.8 x 4.4/ $\mu$ ); conidia at first clavate, continuous, upright, later elongate - clavate or subfusiform, with attenuated rounded tips, base bluntly rounded, 2-10 septate, constricted at the septa, brown, granular, 34.7-77.8 x 5.4-9.2/ $\mu$  (50.0 x 7.1/ $\mu$ ).

Hab. on leaves of *Vitis vinifera*, South Africa.

Du Plessis produced this new binomial for the fungus because he considered that variation in described symptoms and the shape of the mature conidia did not conform consistently in the literature with the binomials given, namely I. clavispora or C. viticola. His proposed binomial (I. fuckelii) is discussed fully in the next two sections under considerations of symptoms and conidium shape.

Table II presents a summary of the salient mycological features of the fungus as described by the various authors cited in this section.

TABLE II - SUMMARY OF DESCRIPTIONS  
OF THE CAUSAL ORGANISM

Author	Date	Binomial	Conidiophore dim.	Conidiophore characteristics	Conidial dimension	Conidium shape	Septa
Berkeley	1875	<u>Graphium clavispورا</u>	-	compacted of flexuous threads, free at apex and waved	-	linear or clavate, sometimes much attenuated below	pluri-septate
Saccardo	1886	<u>Isariopsis clavispора</u>	-	loose and wavy at tops	44 x 4-5 $\mu$	linear or obclavate	3-4
Saccardo	1886	<u>Cercospora viticola</u>	50-200 x 4-5 $\mu$	dense bundles	50-70 x 7-8 $\mu$	elongate to clavate	3-4
Tharp	1917	<u>I. clavispора</u>	-	-	30-56 x 6-8 $\mu$	-	7-8
Schwarze	1917	<u>I. clavispора</u>	50-200 x 4-5 $\mu$	densely fasciculate	50-70 x 7-8 $\mu$	elongate to obclavate	1-3
Rhoads	1926	<u>I. clavispора</u>	-	closely packed bundle, irregular spreading tips	40-70 $\mu$ (approx)	'clavate'	2-5
Higgins	1929	<u>C. viticola</u>	100-300 x 4-6 $\mu$	dense coremium-like bundle	24-65 x 4.2-7.2 $\mu$	'clavate'	3-9
Roldan	1938	<u>I. clavispора</u>	60-200 x 4-4.5 $\mu$	densely fascicled; tops loose-wavy	30-76 x 6.5-9 $\mu$	obclavate	1-3
du Plessis	1942	<u>Isariopsis fuckelii</u>	157.8-278.8 x 3.7-5.4 $\mu$ (217.8 x 4.4 $\mu$ )	upright tufts, sometimes gradually expanding at apex	50 x 7.1 $\mu$ (34.7-77.8 x 5.4-9.2 $\mu$ )	obclavate	2-10
Chupp	1953	<u>Pseudocercospora vitis</u>	50-400 x 3-4 $\mu$	fascicles usually dense and strikingly coremoid	20-80 x 4-7 $\mu$	obclavate	3-7
Munjal and Sethi	1966	<u>C. viticola</u>	50-400 x 3-6 $\mu$	fascicles dense, also loose but sometimes forming a compact coremium-like mass	20-80 x 4-7 $\mu$	obclavate	multi-septate
Author	1969		150-450 x 3-6 $\mu$	fused to spreading at base, always spreading at apex. Sometimes coremoid	20-100 x 3-12 $\mu$	obclavate	3-14

TABLE II (Cont'd)

Author	Date	Binomial	Conidiophore Dim.	Conidiophore characteristics	Conidial dim.	Conidium shape	Septa
Lindau	1910	<u>Cercospora</u> <u>vitis</u>	50-200 x 4-5/μ	Compact fascicles	50-70 x 7-8/μ	obclavate	3-4
Ciferri	1922	<u>C. vitis</u> v. <u>rupestris</u>	-	-	27-32 x 4-5/μ	'clavate'	0-1

### III INFECTION STUDIES AND SYMPTOMATOLOGY

Since this leaf spot disease of grapes had not previously been recorded in New Zealand it was necessary to prove pathogenicity by fulfilling the requirements of Koch's postulates.

Although the causal organism of this disease is quite distinct and easily identified, the reported symptoms from various world locations are diverse and variable. Saccardo (1886) and Schwarze (1917) described the lesions as dry and ochre-coloured or yellow, and without margins. Du Plessis (1942a, 1942b) and Munjal and Sethi (1966) observed the lesions to have dark brown margins and ash-grey-brown centres. Du Plessis reported elevated margins, as did Rhoads (1926); but Rhoads, Higgins (1929), Roldan (1938) and Berkeley (1875) all described the lesions as dark or chocolate brown.

In view of the reported range of field symptoms characteristic of this disease, four varieties of commercially grown grape vines were artificially inoculated under glasshouse conditions and resulting symptom expressions compared with those of field diseased plants.

#### Materials and Methods

##### (i) Production of host material:

All inoculation experiments were carried out on four varieties of grape vine grown in the glasshouse. The vines were established from cuttings obtained from the Gisborne area, and the varieties involved were two vines

each of Golden Chasselas, and Pinot Chardonnay and three each of Baco 22a and Riesling. They were set out on 2nd September, 1968, each in a one gallon container with two nodes below and three or four nodes above the surface of the potting mix, and then placed outside against the south-eastern wall of a building. When the cuttings had produced three or four leaves from a bud, the containers were returned to the glasshouse bench. At the end of the first growing season the vines were not pruned, but repotted into four or five gallon drums.

(ii) Isolation and Culture of the Pathogen:

Sporulation of diseased field material was obtained by placing portions of leaves exhibiting lesions under high humidity conditions in a petri-dish lined with moistened filter paper, and incubated at 24°C. Isolation of the pathogen to agar was accomplished by picking spores off fascicles, using a sterile needle. All culturing was carried out on PDA and sporulation in culture was induced as described later. (page 45).

(iii) Preparation and application of inoculum:

Spore suspensions were prepared by flooding sporulating plate cultures with sterile distilled water and adding a few drops of 'Tween 80' per plate as a dispersant. Cultures were stroked with a sterile loop to dislodge the spores and the resulting suspension was filtered through muslin to remove large pieces of mycelium. Spore concentrations were determined using a Neubauer counting chamber (Haemocytometer). An atomizer was used to apply inoculum

to both upper and lower leaf surfaces to run-off point. Vines were then placed in large humidity cabinets to facilitate spore germination and establishment of infection. Control vines were sprayed with sterile distilled water and given identical treatment to inoculated vines. Inoculations were carried out on two occasions, as follows:

(1) towards the end of the first growing season, using an inoculum strength of 20,000 spores per ml. After two weeks in the high humidity cabinet the vines were returned to the glasshouse bench.

(2) at the beginning of the second growing season, five weeks after the first leaves had appeared, using an inoculum strength of 30,000 spores per ml. High humidity treatment following inoculation was terminated at ten days on account of the rapid development of Botrytis cinerea. Pers. ex Fr.

In both experiments, care was taken to inoculate leaves representative of all stages of development, since one objective of these experiments was to determine whether leaf age at the time of inoculation had an effect on the degree of symptom expression.

(iv) Recording of Results:

Details of symptom expression were recorded both while plants were in the high humidity cabinet, and later when set out on the glasshouse bench. Humidity in the glasshouse was kept to a minimum to prevent sporulation on developing lesions and the possibility of secondary spread of the disease.

(v) Sporulation on inoculated leaves:

Sporulation on inoculated excised leaves was induced by subjecting them to high humidity within petri dishes (12 cm diameter), as illustrated in Fig. 3.

All experiments and observations recorded in this section were on leaves from these two inoculation runs, unless otherwise stated.

Results

(i) Koch's Postulates:-

- (a) Exposure of diseased field material to high humidity constantly yielded one type of fungal growth associated with all lesions. The fungus had dark, densely fasciculate conidiophores which bore at their apices single pigmented conidia.
- (b) On PDA the fungus produced colonies which grew relatively slowly, were dark olive green beneath and with white-grey aerial mycelium above.
- (c) Within one to two weeks following high humidity treatment, inoculated vines produced numerous dark necrotic areas (Fig. 4) on all lower inoculated leaves. Top growth that was inoculated showed no symptoms, suggesting that only older, more mature leaves were susceptible to the fungus.
- (d) Lesioned leaves given high humidity treatment were observed to sporulate within 24 hours, producing

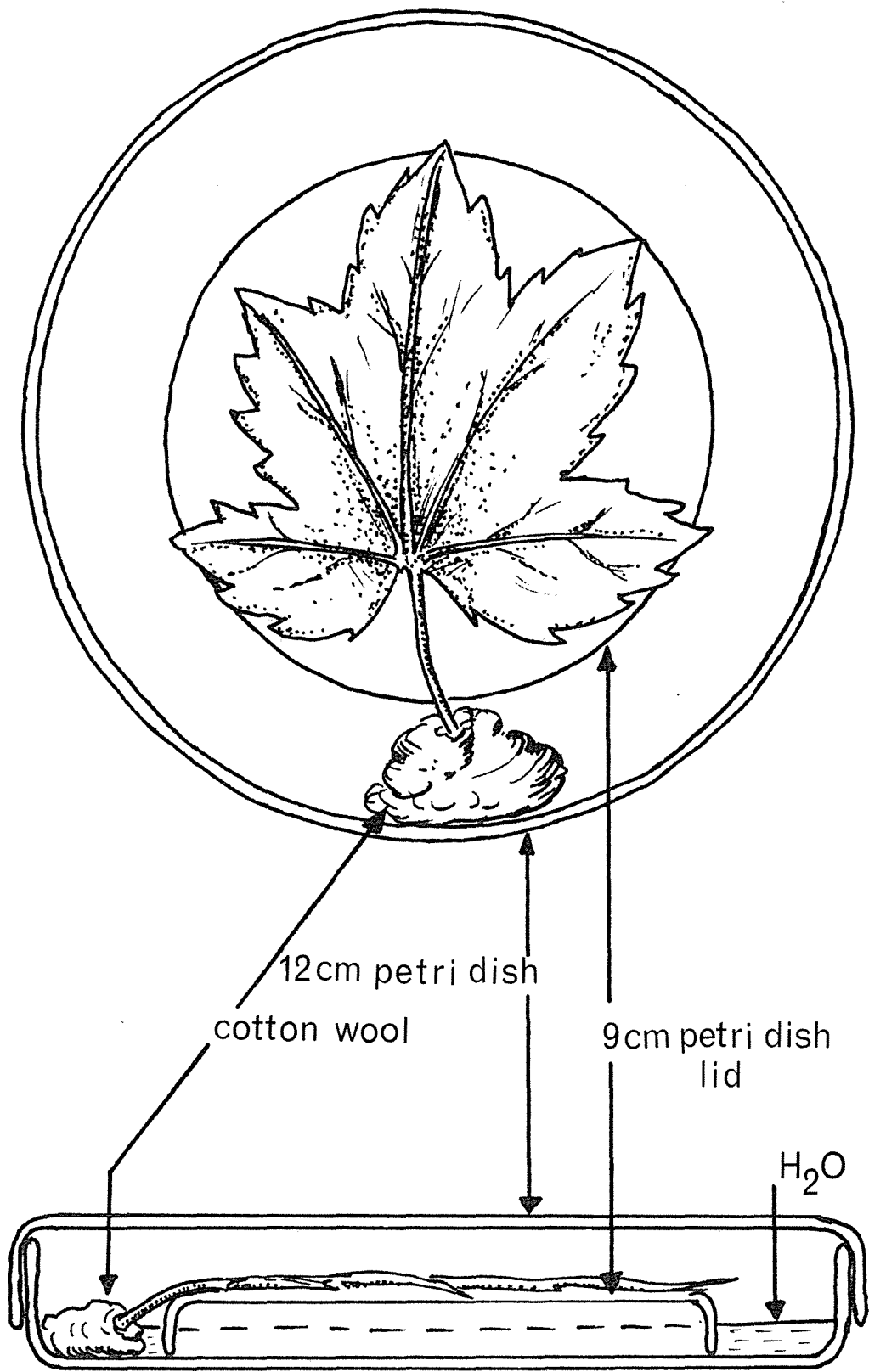


Fig. 3. High humidity apparatus for inducing sporulation on diseased, excised leaves.

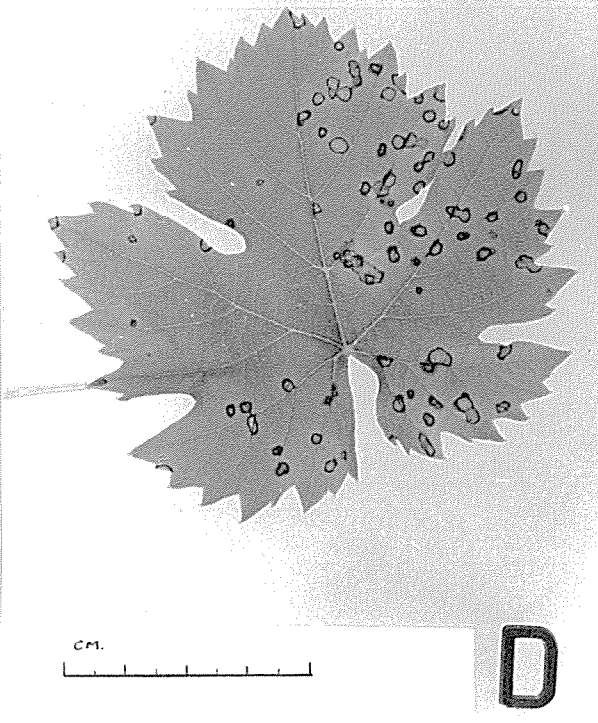
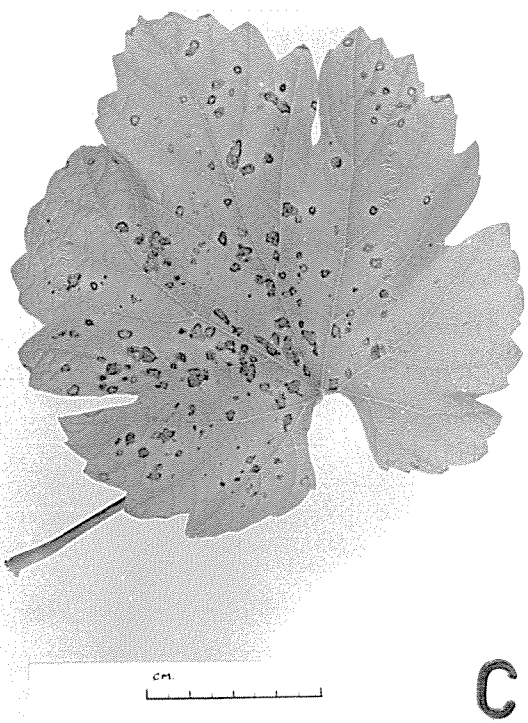
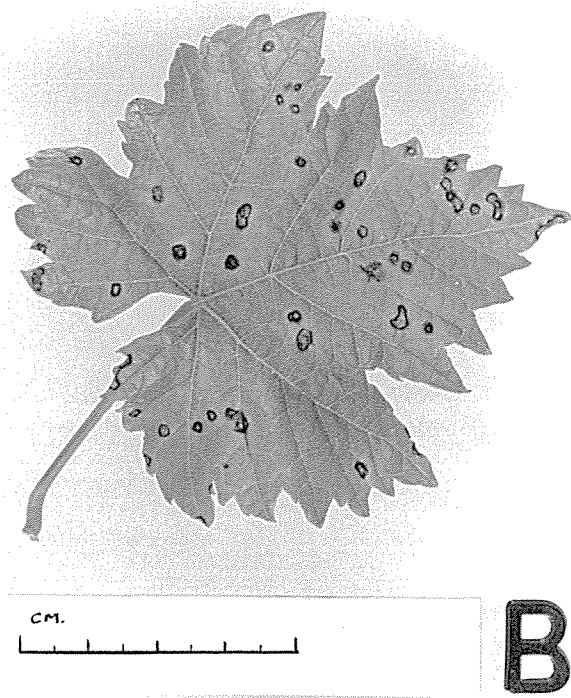
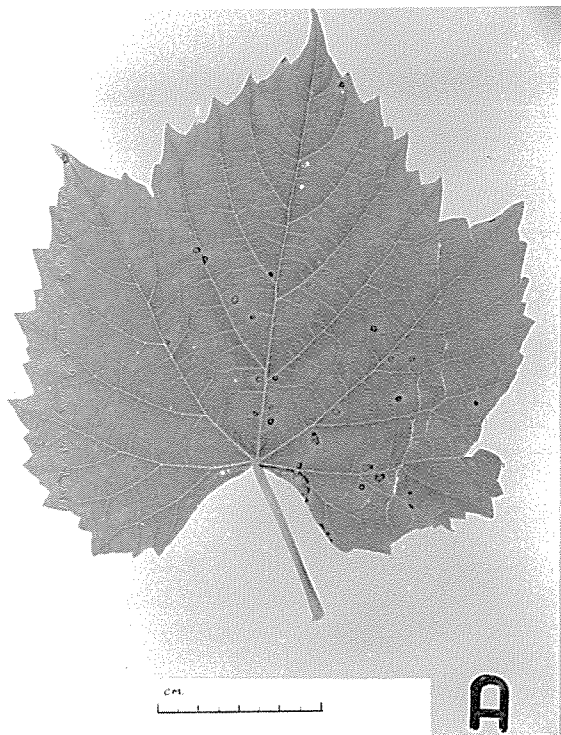


Fig. 4. Symptom expression on artificially inoculated grape leaves. A: var. Baco 22a, B: var. Golden Chasselas, C: var. Pinot Chardonnay, D: var. Riesling.

conidia on dense fascicles that were identical to those produced on field diseased leaf material. Isolation to PDA produced colonies in all respects identical to those described in step (b).

Thus each step of Koch's postulates was satisfied, thereby establishing beyond doubt that the isolated fungus was in fact the pathogen involved.

(ii) Symptoms:

In the two inoculation experiments, the ultimate expression of symptoms was identical, but while in the high humidity cabinets the sequence of symptom development was different. The varieties Pinot Chardonnay and Golden Chasselas developed conspicuous watery-brown lesions on the older leaves within four days of inoculation in the first inoculation experiment, while the varieties Riesling and Baco 22a developed similar symptoms which were much less obvious. All varieties in the second inoculation experiment developed small dark necrotic lesions within ten days in the high humidity cabinet.

Within one to two weeks on the glasshouse bench following removal of the vines from the cabinets, numerous necrotic lesions developed on all older inoculated leaves on the lower portions of all grape varieties (step (c) Koch's postulates). Lesions were from one to eight mm in diameter, circular to irregular, with dark red-brown margins, light tan centres and varying degrees of yellowing of the surrounding host tissue. These symptoms differed from the naturally infected field material which develop initially as small black

spots, which when older, produce red brown instead of light tan centres.

In both inoculations, controls remained disease free.

All apical leaves that were inoculated failed to produce any lesions up to this stage (Figs. 5, 6, 7, 8 and 9). This clearly demonstrated that older, more mature leaves were susceptible to the fungus, whereas actively growing young leaves were not. If leaves must therefore reach a certain age before they become susceptible to the fungus, this would explain the delayed manifestation of the disease observed in the Matamata area, where the disease first became obvious late in December. The literature reports similar findings overseas where in South Africa (du Plessis, 1942) and in Florida (Rhoads, 1926) the disease is a late season condition of the vines.

Four weeks after inoculation, many of the lower inoculated leaves of all varieties became yellow or moribund and began to fall from the vines. These leaves were collected to prevent them sporulating on the moist glasshouse bench and causing secondary spread of the disease.

The upper limit of disease manifestation on the vines was marked with a foil ring two weeks after removal from the high humidity cabinet so that any further manifestation up the vines would not be overlooked. Within a week, the next leaves up the plants showed scattered lesions, and manifestation continued progressively on the leaves towards the apex. Lesion numbers decreased with time, however, until the leaves that were apical at the time of inoculation showed no lesions six to seven weeks after inoculation.

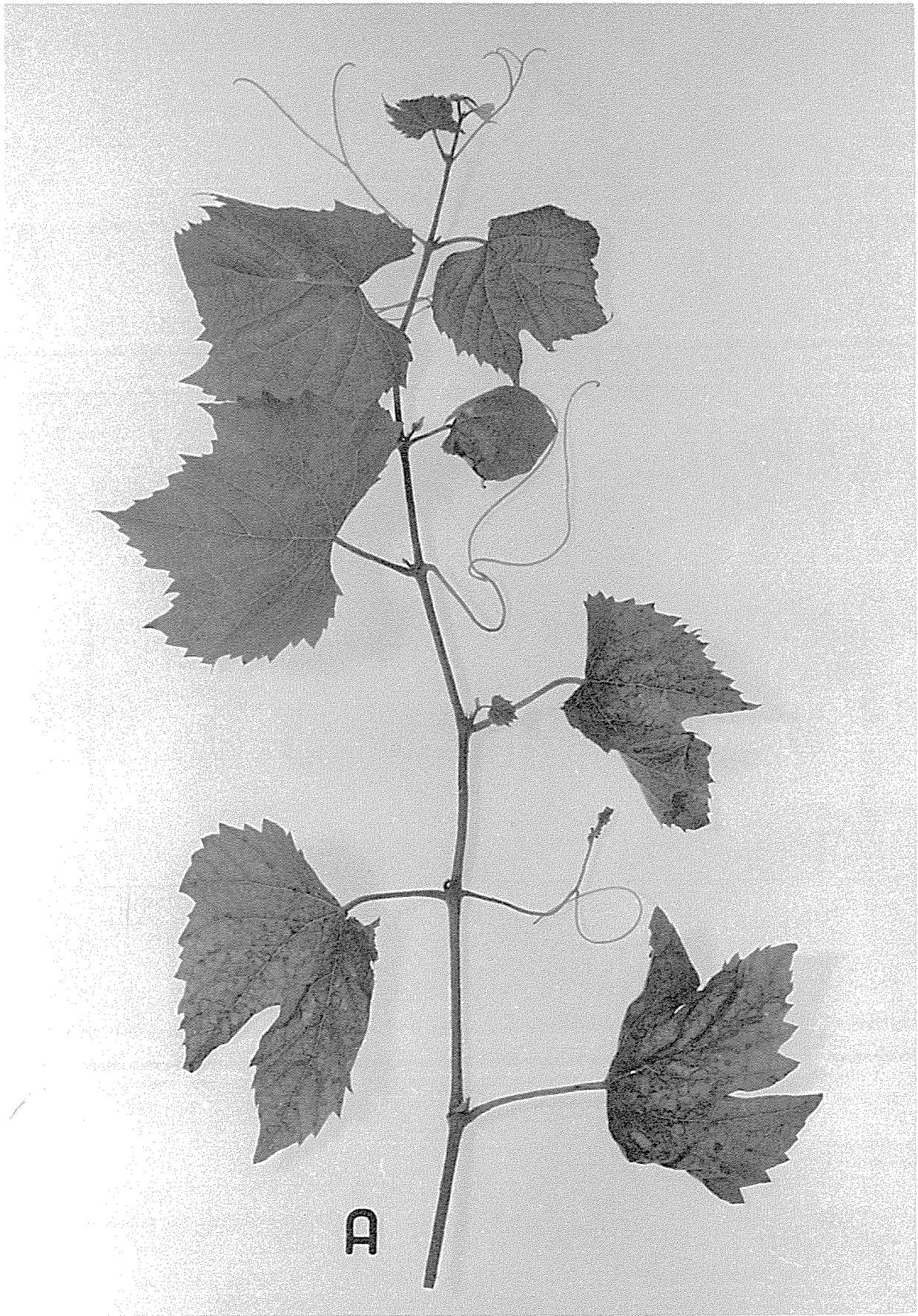


Fig. 5. Artificially inoculated vine (var. Baco 22a) showing the absence of disease on apical leaves.



Fig. 6. Artificially inoculated vine (var. Golden Chasselas) showing the absence of disease on apical leaves.

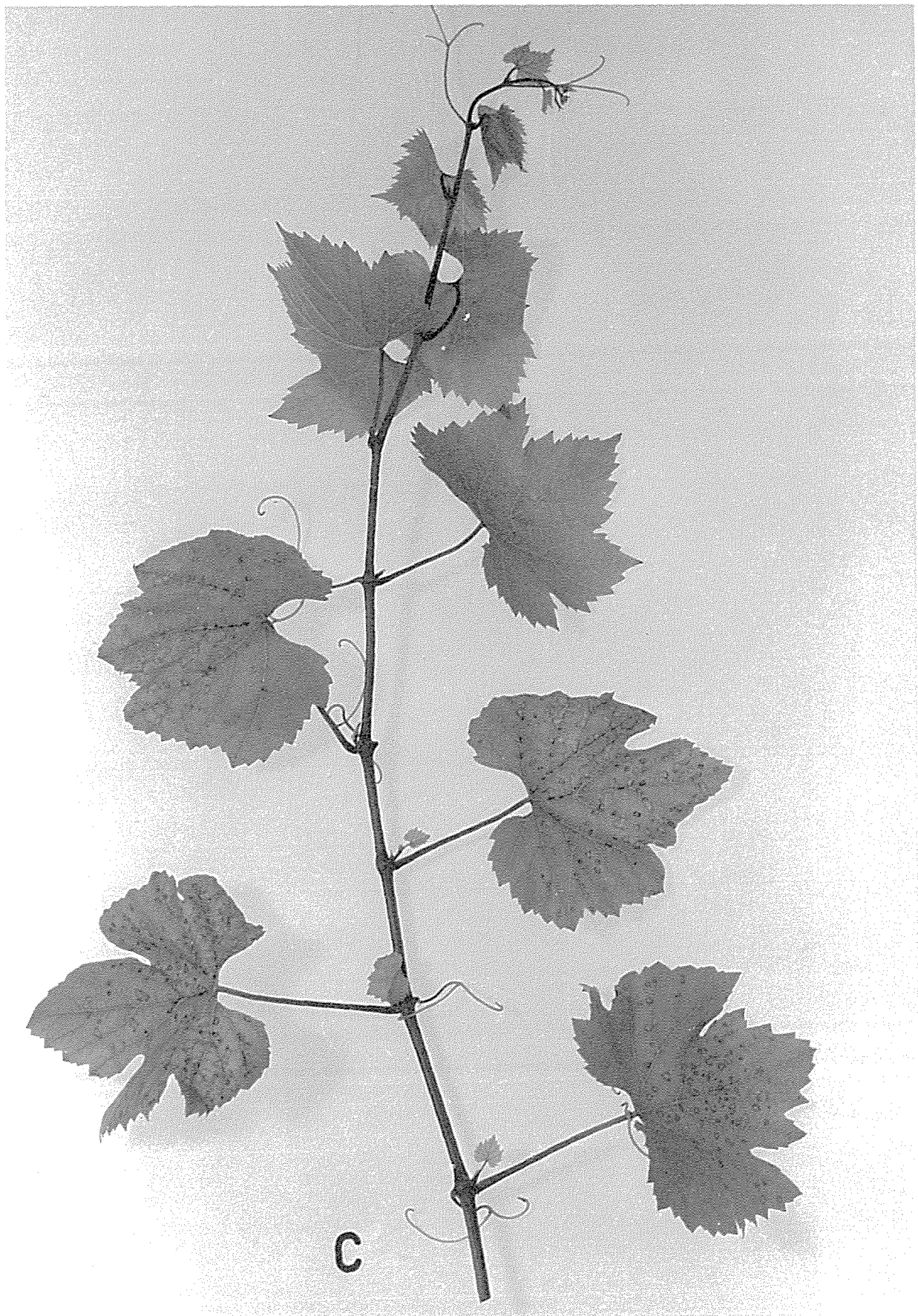


Fig. 7. Artificially inoculated vine (var. Pinot Chardonnay) showing the absence of disease on apical leaves.



Fig. 8. Artificially inoculated vine (var. Riesling) showing the absence of disease on apical leaves.

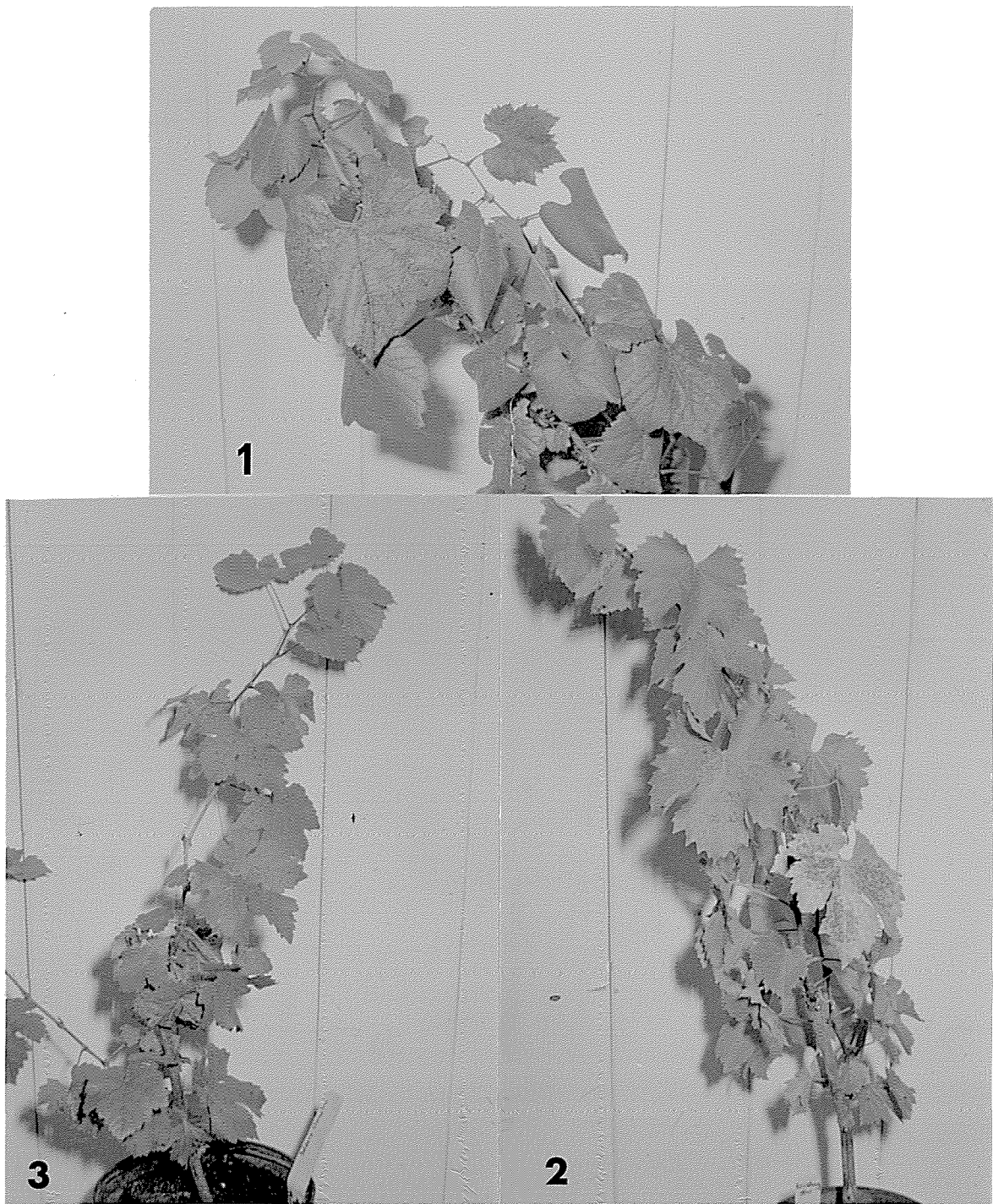


Fig. 9. Artificially inoculated vines (inoculation no. 1) showing symptom expression on all except the apical leaves. 1: var. Pinot Chardonnay; 2: var. Riesling; 3: var. Golden Chasselas.

Discussion:

The organism isolated from diseased field material proved to be pathogenic to the four grape varieties tested. However, the symptoms produced on these varieties were not the same as those described from the field. This demonstrates that the one organism is capable of producing different symptoms on different host varieties and under different environmental conditions. Thus the pleurality of symptoms reported from locations around the world can be explained, not by the disease being caused by more than one organism, but by the disease being observed under different environmental conditions and/or on different grape varieties.

As mentioned previously, du Plessis (1942b) used as part of his argument in proposing the new binomial I. fuckelii for the fungus, the fact that field symptoms in his collection were distinct from those reported for I. clavispora and C. viticola. Such a proposal is hardly tenable in view of the present findings, namely, that depending on prevailing environmental conditions and/or the varieties involved, symptom expression can be extremely variable. In point of fact, early field symptoms in New Zealand conform with those reported by Rhoads (1926) for I. clavispora and glasshouse symptoms resultant on inoculations using field isolates were indistinguishable from those reported by du Plessis for I. fuckelii and Munjal and Sethi (1966) for C. viticola.

It should also be mentioned, however, that symptom expression is a feature of the disease as induced by the

pathogen and not an attribute of the pathogen itself. Accordingly, it is not a criterion to be used in taxonomic studies.

The phenomenon of increased susceptibility of the host with age, as demonstrated in the above inoculations, and the delayed manifestation of symptoms on younger leaves is discussed in detail in Appendix II.

IV CONIDIUM SHAPE OF THE CAUSAL ORGANISM IN RELATION TO ITS TAXONOMY

Quite independent of the generic or specific names that have been applied to the pathogen in the literature, there is an apparent disagreement among workers as to the shape of mature conidia. For example, when describing the fungus under the binomial Isariopsis clavispora, authors have produced conflicting reports. Thus Berkeley (1875) described the conidia of the basionym Graphium clavisporum as clavate (that is, club shaped). Saccardo (1886), when renaming the fungus I. clavispora, likewise described the conidia as clavate, but Schwarze (1917) and Roldan (1938) both described them as elongate to obclavate. Rhoads (1926) described and illustrated the conidia of I. clavispora as elongate and somewhat club shaped, each round at the apex and tapering to a slender stalk-like base (that is, clavate).

A similar series of disagreements is revealed when one reviews the taxonomic history of the fungus under the binomial Cercospora viticola. In the original description by Saccardo the conidia were described as elongate to obclavate, and although he equated I. clavispora with this binomial, Saccardo failed to note the discrepancy in the conidium shape apparent from the two reports. Likewise Higgins (1929), when describing the Cercospora viticola state of Mycosphaerella personata, failed to note a discrepancy between his description of the conidia (which he described as club-shaped varying to fusiform, but when club-shaped attached by the smaller end) and that by Saccardo for C. viticola which he had obviously studied since detailed

references were given to it in his paper. Munjal and Sethi (1966) agreed with the original description by describing the conidia of C. viticola as obclavate, as did Chupp (1953) when he described the fungus under the binomial Pseudocercospora vitis.

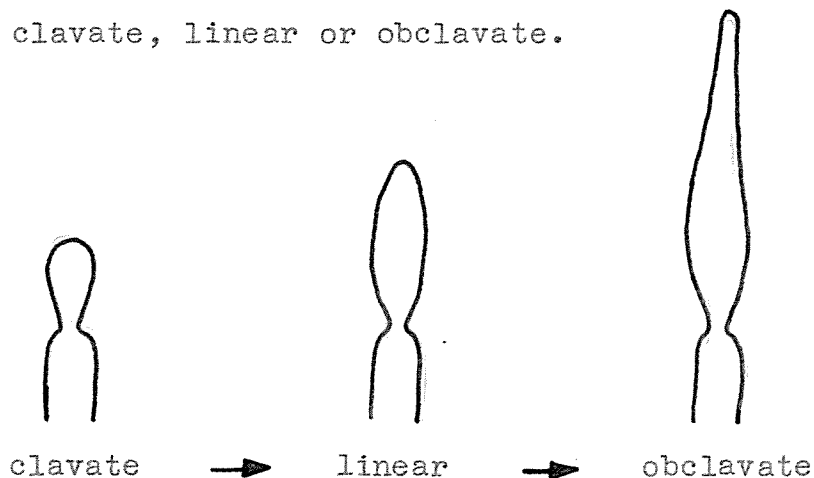
Lindau (1910) described the conidia of Cercospora vitis as obclavate, whereas Cifferi (1922) described those of C. vitis var. rupestris as clavate.

Du Plessis (1942b) considered this fungus on grapes to be a species of Isariopsis, but proposed a new combination I. fuckelii (Thüm.) du Plessis. His stated reason for not accepting I. clavispora was that all reports up until that time described the conidia under this binomial as being clavate, whereas he observed them to be obclavate. However, this is untrue as both Schwarze (1917) and Roldan (1938) described the conidia of I. clavispora as obclavate. It appears that du Plessis conveniently misquoted Schwarze by stating that he (Schwarze) called the fungus I. viticola (B. & C.) Sacc. Other than in du Plessis' paper, this binomial is found nowhere else in the literature. Further, du Plessis listed Roldan's paper in his bibliography, but failed to refer to it in his text.

All workers referred to above were observing conidia of the same organism, yet there was this discrepancy as to the shape of the spores. The following hypothesis is advanced to explain why this occurred.

It has been reported (Munjal and Sethi, 1966) and was observed in this study that the conidia of the fungus are caducous; that is, they fall readily from the conidio-

phores. It is for this reason that in liquid mounts of the fruiting structures prepared for microscopic examination, mature conidia are rarely observed adhering to the conidiophores (see Fig. 16). By contrast, immature conidia formed at the apex of the conidiophores and which have not formed a septum between spore and sporophore, are non-caducous and at this stage are clavate. Therefore, it is postulated that in the process of maturation, the conidia pass through different shapes and depending upon the stage of development of the conidium in contact with the conidiophore at the time of examination, so the spores could justifiably be described as being either clavate, linear or obclavate.



To determine the validity of this hypothesis, it was deemed necessary to follow in detail the sequential development of single conidia so that any changes in shape during their ontogeny could be recorded.

#### Materials and Methods

An apparatus was required which would allow continuous observation of conidium development at high magnifications. Cole and Kendrick (1968) described a thin culture chamber used specifically for such studies, and their subsequent papers (Kendrick, Cole and Bhatt, 1968; Cole and Kendrick, 1968, 1969a,

1969b; Kendrick and Cole, 1968, 1969) indicate effective use of the apparatus in solving taxonomic problems associated with conidium ontogeny. Cole, Nag Raj and Kendrick (1969) have described a further method for observing conidium ontogeny in plate culture. This latter method was not described at the time the present study was initiated, and the former method was found unsuitable in this laboratory. An alternative method using an apparatus of simple construction and found to be equally effective for use in ontogenetic studies of imperfect fungi was developed and is described below.

Basically, the apparatus is a shallow chamber made from clear perspex in which an inoculated agar block was suspended on a coverslip. When growth and sporulation of the fungus was observed to occur at points where the agar and coverslip met, photomicrographs were taken at high magnifications. The design and dimensions of the chamber are given in Figs. 10A and 11A.

The chamber was operated in the following manner. An agar block approximately  $1 \text{ cm}^2$  was cut from a PDA plate and transferred to a sterile coverslip (40 x 22 mm). The block was then inoculated at points where the agar and the coverslip met (Fig. 10B & 11B), using washed, fragmented pieces of mycelium (see sporulation in culture p45). To avoid contamination, inoculation was carried out within a sterile petri dish. Once inoculated, the coverslip preparation was inverted and sealed centrally over the aperture of the chamber so that the agar block was suspended within the cavity of the apparatus. Paraffin wax proved to be the most suitable sealant as it prevented coverslip

Fig. 10. A: The Growth Chamber; external view with dimensions (cm).

B: Inoculation method.

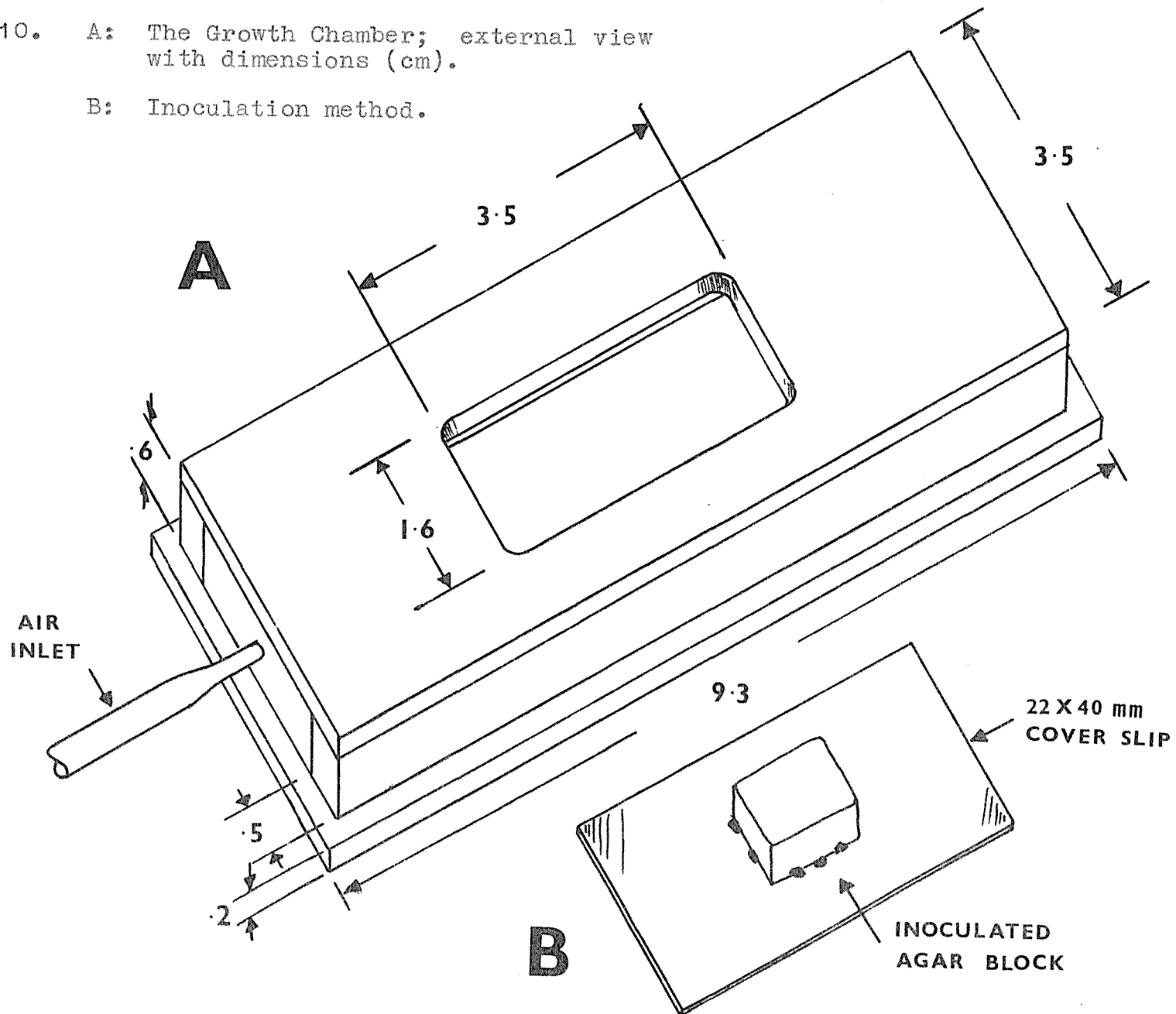
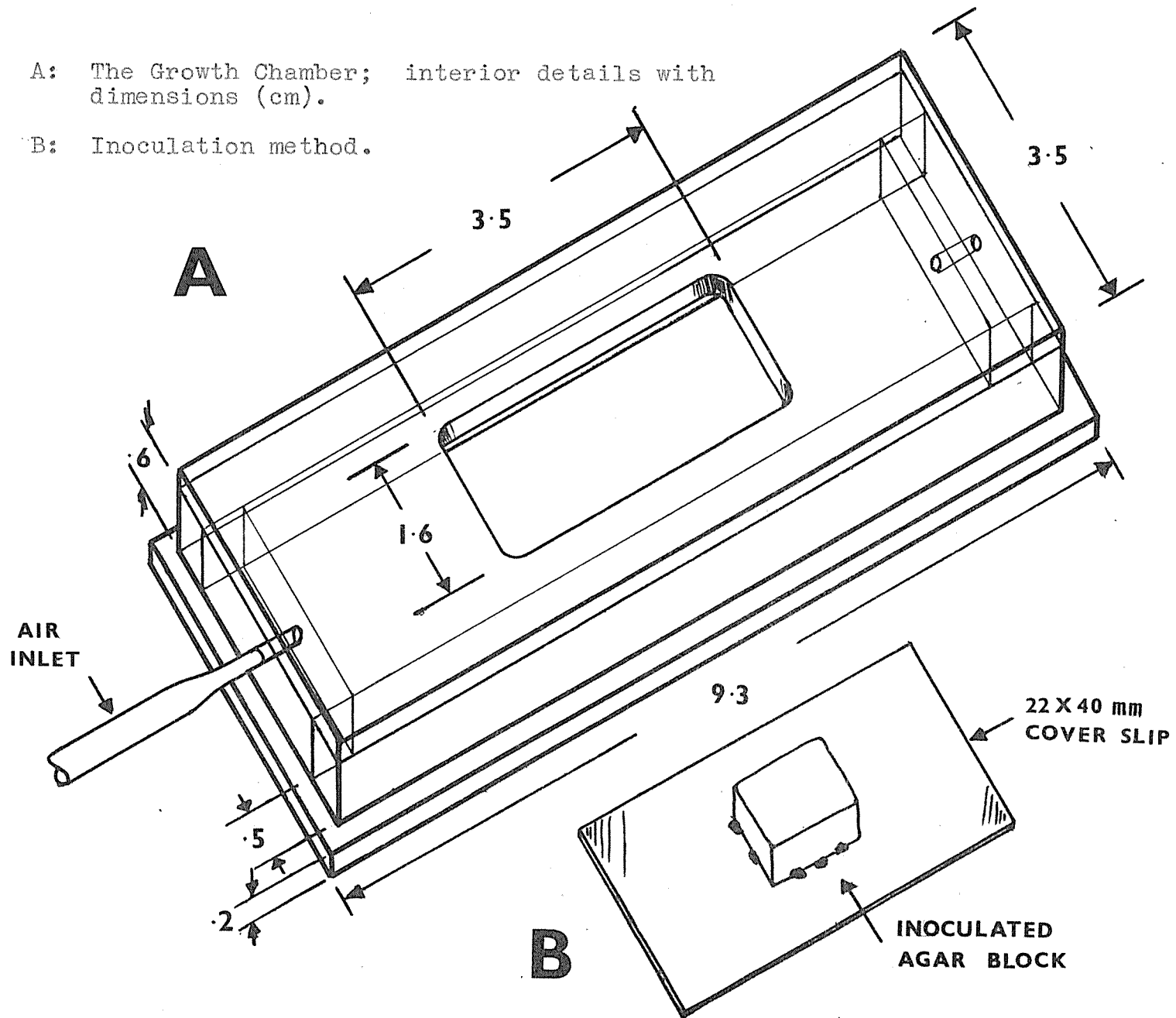


Fig. 11. A: The Growth Chamber; interior details with dimensions (cm).

B: Inoculation method.



movement under oil immersion magnification. High humidity within the chamber was maintained by a moist air flow from a Metcalf "Hyflo" (Model A) air pump. The air was passed through two Drechsel gas wash bottles containing sterile water, and a bleed valve between them allowed adjustment of the air flow (Figs. 12 and 13). Rubber tubing from the second wash bottle directed the air into the chamber through a tapered hard plastic inlet tube plugged with cotton wool. Several chambers were conveniently fed with moist air from the two wash bottles at one time. Besides maintaining high humidity within the chamber, thereby facilitating growth and sporulation of the fungus, the air flow served to prevent excessive build-up of temperature caused by continual illumination.

When sporulation was observed to occur close to the coverslip, a photographic sequence of a suitable conidiophore and conidium was begun. The recording was carried out using a 35 mm camera (Ilford F.P.3 film) fitted to a Leitze Wetzler Ortholux compound microscope (Fig. 13) using x40 or x90 (oil immersion) objectives and a x10 eye piece in the camera.

Cine-photomicrographic recording was also carried out with the apparatus. A Bolex 16 mm camera was mounted on top of the microscope and 400 A.S.A. Ferrina black and white film fed through by a time lapse mechanism at the rate of eight frames per minute, with a quarter of a second exposure. The ontogeny of a conidium was followed for six to eight hours and when the film was projected the stages of growth were condensed into only a few minutes viewing time.

Fig. 12. Diagrammatic illustration of the growth chamber chamber in operation.

- P = Air pump
- W<sub>1</sub> = ) Drechsel bottles containing
- W<sub>2</sub> = ) sterile water
- B = Bleed valve
- M = Microscope
- C = Camera
- G = Growth Chamber

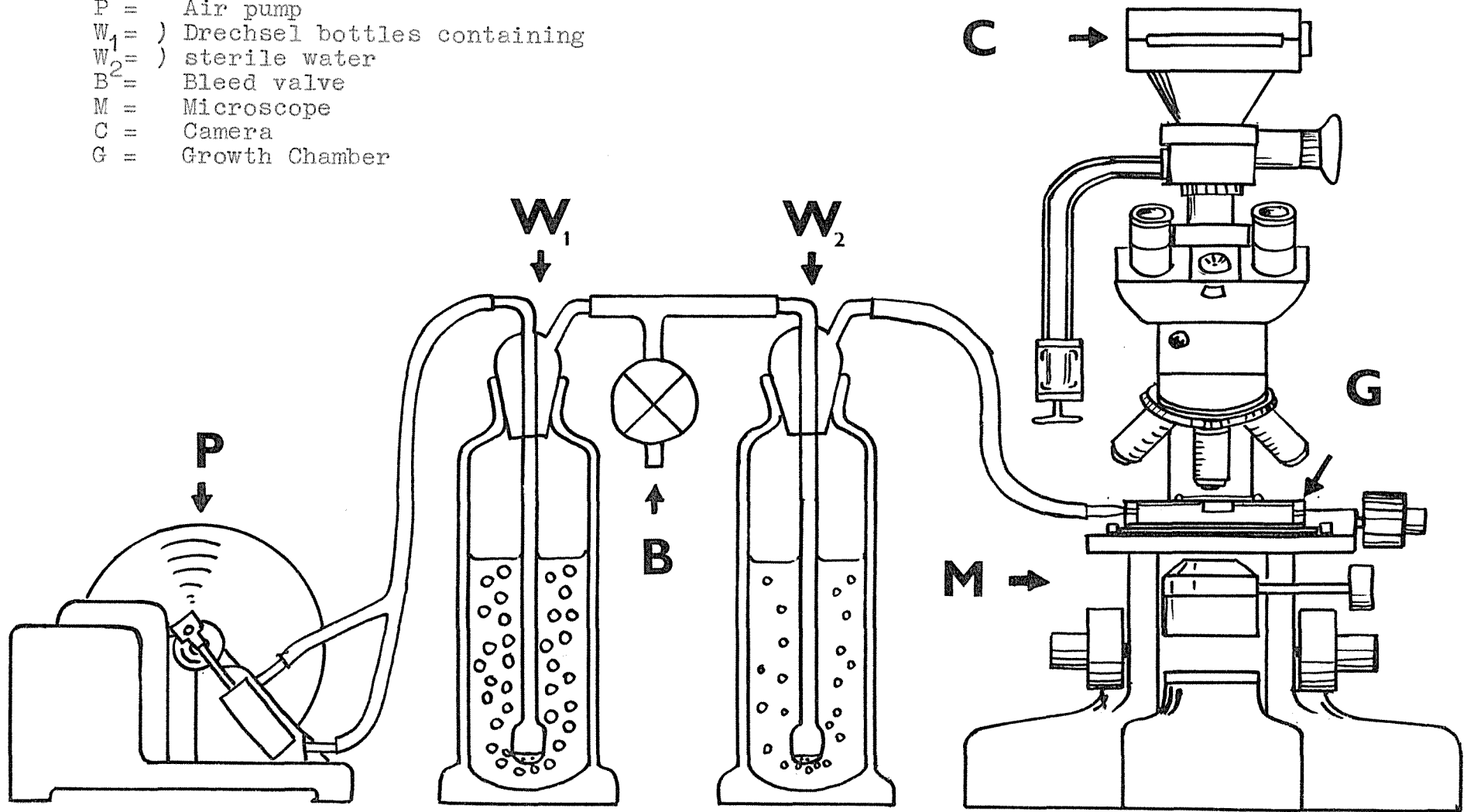




Fig. 13. Further view of the growth chamber in operation.

## Results and Discussion

At 24°C, sporulation occurred on the mycelial fragments within 24 to 48 hours. Photographs were taken at approximately 40 minute intervals in Fig. 14 and 80 minute intervals in Fig. 15. During the course of this study the maturation process was followed many times, and these series of photographs are typical ontogenetic sequences for the fungus.

The photographic series show that the conidia do in fact change shape during their development. In photographs C & D of Fig. 14, the conidium has a rounded apex and a tapering base; that is, it is clavate. In photographs F & G of the same series the conidium has become linear, and in the photographs H to K, the distal portion of the conidium has elongated so that at maturity the conidium is obclavate. The caducous nature of the conidia is illustrated in photograph L. The internal details of the conidia cannot be clearly distinguished because of light refraction in the absence of a mountant.

The contradictions in the literature as to the shape of the conidia can now be attributed to the caducous nature of the conidia and the change of shape during maturation. If a worker observed clavate conidia adhering to the conidiophores (as in photographs C & D of Fig. 14) and noted the club-shaped conidia free from the conidiophores in the mountant, then it is not difficult to comprehend how he would consider that the young conidia retained their clavate shape through to maturity. However, if a mature conidium was observed adhering to the conidiophore, as in photograph K (Fig. 14) then the

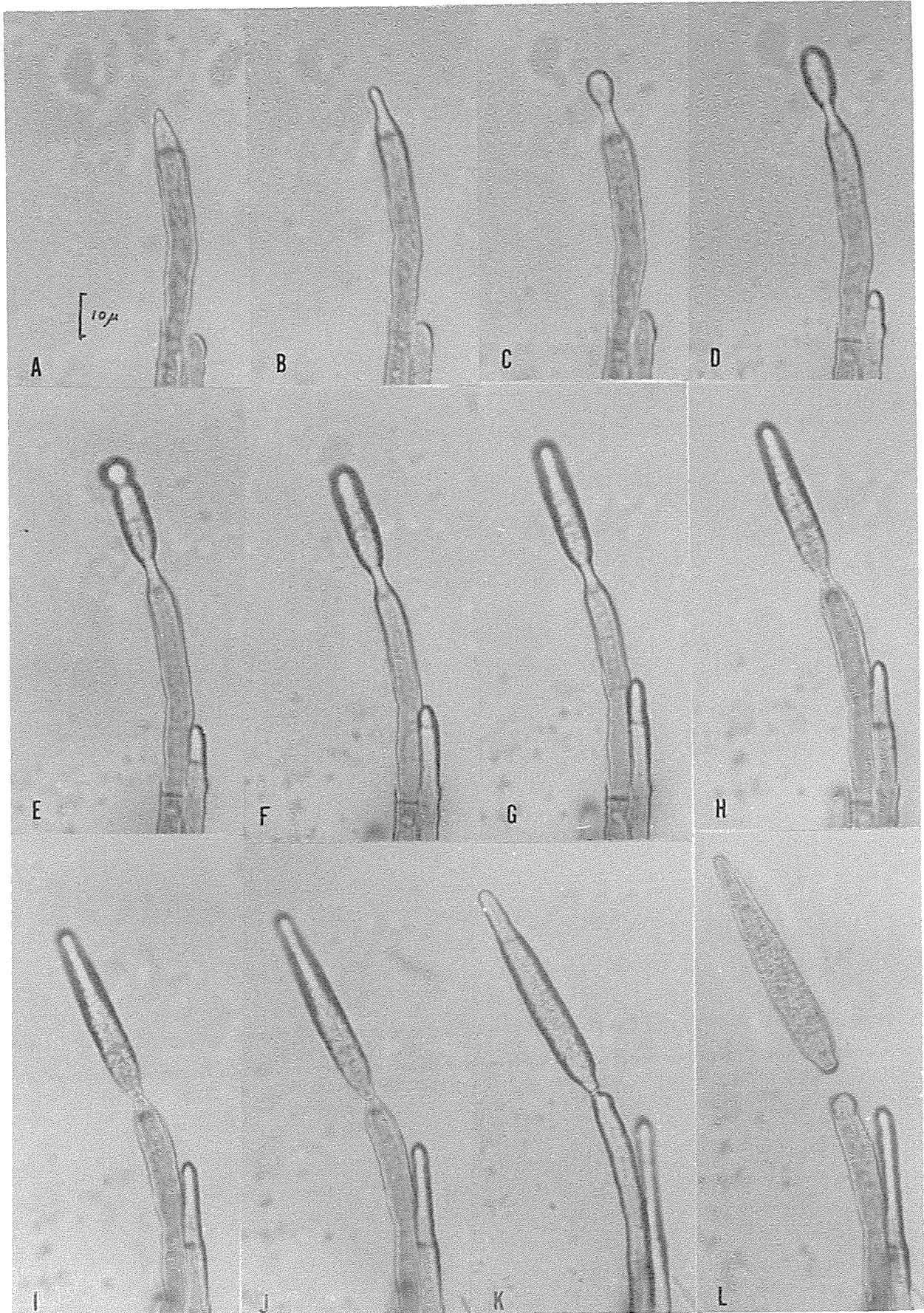


Fig. 14. Conidium ontogeny of the causal organism (x400) at 40 min. intervals.

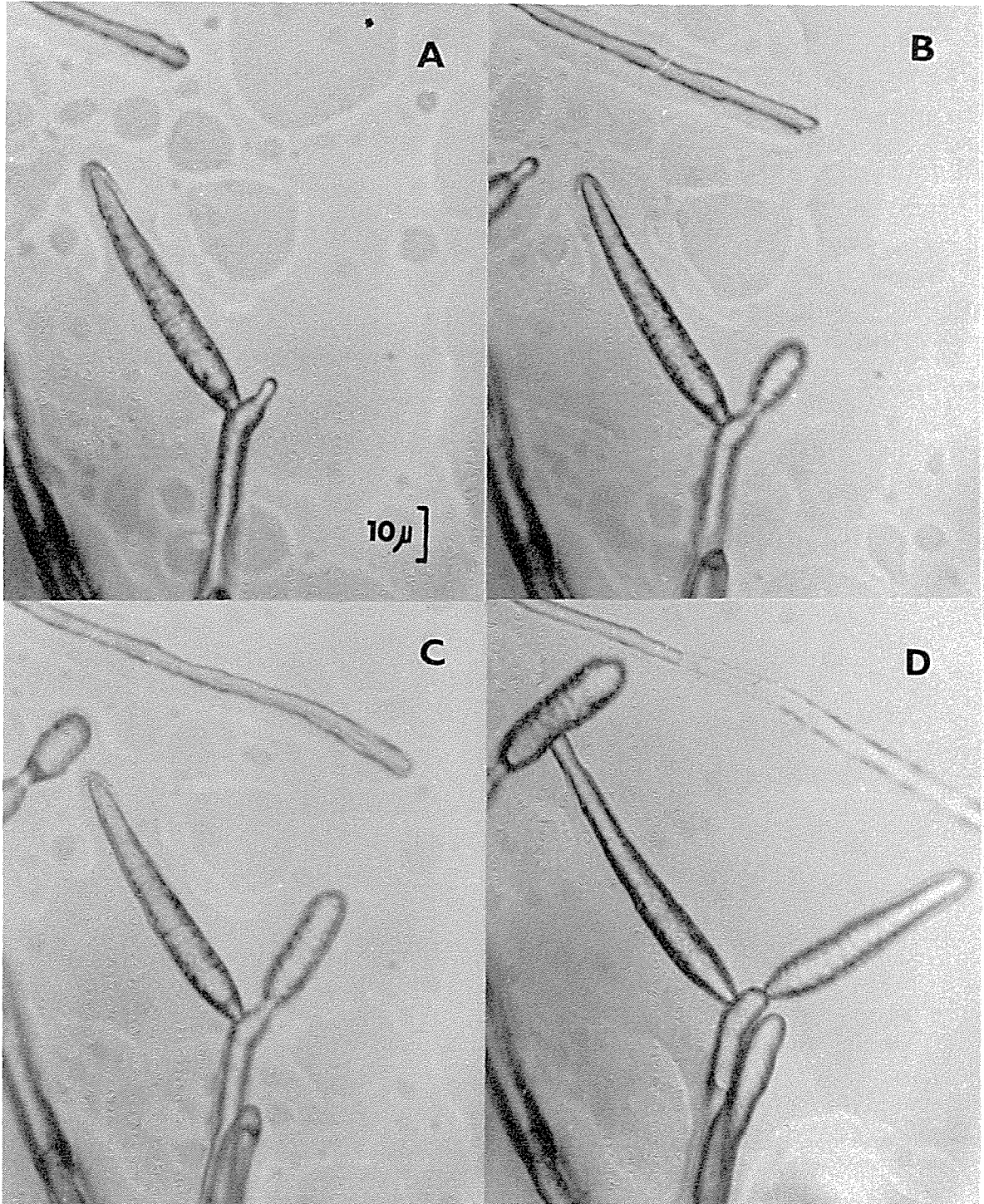


Fig. 15. Conidium ontogeny of the causal organism illustrating development of the second conidium (x400) at 80 min. intervals.

worker would naturally record the mature conidium shape as obclavate.

Du Plessis (1942b) did in fact state that the conidia changed shape during maturation, but never connected this fact with the different reports in the literature of the nature of the mature conidia. For this reason, the author considers invalid the change of specific epithet to I. fuckelii made by du Plessis for this fungus.

The droplet of moisture at the tip of the conidium in photograph E (Fig. 14) is a phenomenon that was invariably observed on the conidium first formed on the conidiophore. It appeared after 100 to 160 minutes of conidium development and lasted for 20 to 30 minutes. Cine-photomicrography revealed that it formed very quickly then reduced in size slowly. After it had disappeared the septum separating the conidium from the conidiophore was laid down. This suggests that it may be associated with a movement of nutrients from the mycelium and conidiophore into the conidium. The droplet was rarely observed on the second or third conidia formed on the one conidiophore.

The blown out end of the conidiophore and the proliferation of the conidiophore, as demonstrated in Figs. 14 and 15 are discussed when considering Deuteromycete classification schemes and the causal organism.

V COMPARISON OF THE CONCEPTS OF THE GENERA CERCOSPORA,  
PSEUDOCERCOSPORA AND ISARIOPSIS

The following is an outline of the original and the modern concepts of the three genera into which the fungus has been placed, namely, Cercospora, Pseudocercospora and Isariopsis.

(i) Cercospora:

This genus was erected in 1864 by Fresenius with C. apii on celery as the type species. Chupp (1953) interpreted the original concept as follows:

"Conidiophores: Biophilous, coloured, geniculate, in fascicles, straight or crooked, with or without septa, bearing one or more spores at one time and bearing spores laterally as well as at the tip.

Conidia: Obclavate, straight or curved, multiseptate, not cylindrical, hyaline."

In his monograph on the genus Cercospora, Chupp (1953) redefines the concept of this genus. He states that conidiophore characteristics can range from widely divergent stalks to fascicles so dense that they resemble a coremium. Conidium colour is stated as mostly hyaline or pale coloured, with only a few having medium dark coloured ones.

A quick perusal of descriptions in the literature of conidium colour in the genus Cercospora reveals a gradation from hyaline, through pale olivaceous to olivaceous brown.

(ii) Pseudocercospora:

Spegazzini erected this genus in 1911 based on the fungus under study. His definition was:

"Genus hyphomycetum dematieum macronemeum phragmosporum a Septonemate vita biophila distinctum."

Translation: A hyphomycete genus with large, dark thread-like phragmospores, distinct from Septonemate parasites.

Spegazzini presumably erected this genus to distinguish dark spored species from hyaline spored Cercosporae. To separate Cercospora - like species into those with dark and those with hyaline conidia would be unwise for the following two reasons:

- (a) A gradation exists in Cercospora species between hyaline and dark coloured conidia, and the splitting of this group into two genera would leave a large number of intermediates that could be placed in either genus.
- (b) Modern taxonomic schemes, based on developmental morphology do not recognise conidium colour as a strong generic criterion, especially where gradations exist.

The author therefore disregards the genus Pseudocercospora in favour of the broader concept of Cercospora which includes a range of conidium pigmentations.

(iii) Isariopsis

In 1863, Fresenius erected this genus based on a single species I. pusilla.<sup>\*</sup> This fungus had previously been called Stysanus albo-rosella by Desmazieres (1853). Thus Saccardo (1882) listed Fresenius as the erector of the genus Isariopsis based on I. albo-rosella (Des.) Sacc. Saccardo (1886) described conidia of the genus in Sylloge Fungorum as:

"Gracilis, fuscescens vel expallens, tere, ex hyphis laxis constans. Conidia in paniculam v. capitulum laxan digesta. - Species quaedam ad Hyalostilbeas nutant et tunc ad Arthrosporium accedunt, quod vero habitu robustiore differt."

Translation: Slender, dark or somewhat paler, smooth, standing up-right from loosely arranged hyphae. Some species tend towards Hyalostilbeas and then some approach Arthrosporium which differs in its more robust appearance.

The genus was included in the Phaeostilbeae; that is, conidiophores fused into synnemata or coremia and conidiophores and/or conidia dark coloured. It is also placed under Phragmosporae; that is, conidia 2 - pluriseptate.

In his monograph on synnematosus Fungi Imperfecti, Morris (1963) describes the genus thus:

"Synnemata dark, composed of loose conidiophores, bearing conidia at or near the tips; conidia dark or pale; 2 or more celled, cylindrical to obclavate, often curved, dry, produced singly."

Ferraris (1909) pointed out the fact that the conidia and conidiophores of I. albo-rosella are hyaline.

\* Plakidas (1960) wrongly states that the genus was erected by Fries.

He therefore proposed a division of the genus into two genera: Isariopsis (for those species with hyaline conidia and conidiophores) and Phaeoisariopsis (for those species with dark conidia and/or conidiophores). The type species for Phaeoisariopsis was given as P. griseola (Sacc.) Ferraris (= I. griseola Sacc.).

Morris's (1963) description of the genus Isariopsis was based on what he thought was I. albo-rosella, but which he later found to be I. griseola (Jung and Morris, 1968). These two workers have followed Ferraris and recognize the two separate genera Isariopsis and Phaeoisariopsis. They describe and illustrate several new species of Phaeoisariopsis. From their illustrations, several of the new species seem to produce conidia porogenously, with one demonstrating percurrent conidiophore proliferation. However, the type species of this genus Phaeoisariopsis (P. griseola) has blastospores with subsequent sympodial conidiophore proliferation (Hughes, 1953). It would appear, therefore, that several of the new species described by Jung and Morris should be placed in a separate genus to either Isariopsis or Phaeoisariopsis. The use of colour as a criterion for separating genera is tenuous, especially in the case of this genus. Savile (1957) has shown that a species of Isariopsis (I. bulbiger (Focke.) Savile) can have dark or light coloured conidiophores depending on the amount of degradation of the host tissues. Therefore, the division of the genus Isariopsis into Isariopsis and Phaeoisariopsis is not considered warranted.

According to the concept of Cercospora, as outlined by Chupp, the genus is broad enough to include species of the genus Isariopsis. That is, species of Cercospora can have

dark coloured conidia as do most of those in Isariopsis, and likewise have coremoid conidiophores. However, these two genera are classified, according to the Saccardoan scheme, into different form - families; Cercospora in the Dematiaceae and Isariopsis in the Stilbaceae (or Stilbellaceae). Since the latter family is characterized by synnemata or coremia, it follows that the major difference between the two genera is the degree of compactness of the conidiophores.

VI CONIDIOPHORE CHARACTERISTICS OF THE CAUSAL ORGANISM  
IN RELATION TO ITS CLASSIFICATION

The previous section has shown that the basic difference between species in the genus Cercospora and those in Isariopsis is that the former have conidiophores grouped into fascicles while the latter have them united into synnemata or coremia. Therefore, the form of the conidiophores en mass on the host tissue will determine the genus into which the fungus should be placed.

In describing conidiophore fasciculation in the genus Cercospora, Chupp (1953) states:

"Occasionally fascicles may be composed of 30 - 50 stalks or more and are then described as being very dense.

Aside from density there are also degrees of compactness. These range from widely divergent stalks to fascicles so compact that they resemble a coremium."

Although species of Isariopsis are synnematous fungi, variation in conidiophore characteristics have been reported for species in this genus. For example, Miles (1917) studied I. griseola Sacc. on beans and described the coremia as:

"Columnar and ... formed of rather dark brownish hyphae, closely aggregated, though seemingly not at all united with each other. The members of the fruiting column tend to separate, especially with age, thus indicating that the

structure should perhaps not be regarded as a typical coremium at all."

According to Higgins (1929) and Munjal and Sethi (1966) the fungus under study, also described under the genus Isariopsis, shows similar variation in conidiophore compactness. Higgins stated:

"If the air is damp these erect branches grow out simultaneously as a dense coremium-like fascicle of slender, simple, olive-green hyphae, which cohere throughout most of their length. ... If only moderately dry, the conidiophores may grow out separately and have all superficial appearances of a typical Cercospora. "

Because conidiophore compactness is of primary importance in the taxonomy of this fungus, the fruiting structures were studied carefully on the host tissue to find if any variation occurred and to determine whether environmental influences changed their basic characteristics.

#### Materials and Methods

Non-sporulating field diseased grape leaves were placed under high humidity conditions at 24°C for 24 to 48 hours. Conidiophore bundles that arose were removed by pricking off stromata and observing in Shear's mounting fluid. It was noted that this mountant had little effect in changing the degree of compactness of the bundles. Consequently, morphological characteristics of the conidiophores that were observed through the microscope

closely allied those on the sporulating host material. Photomicrographic records of conidiophore variation were taken.

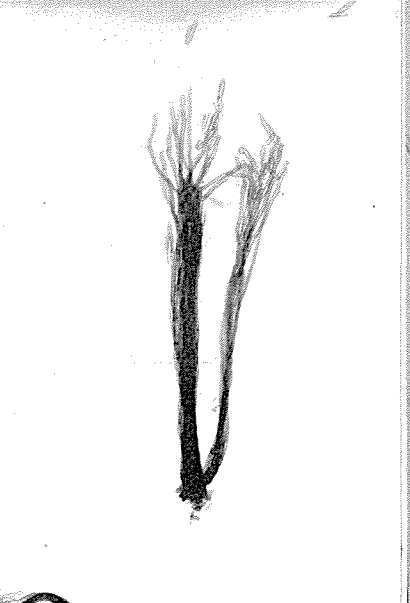
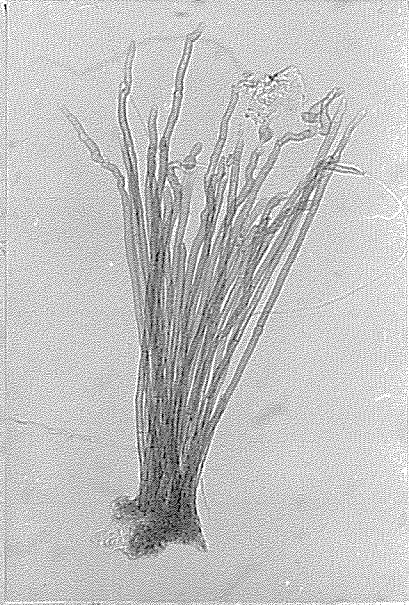
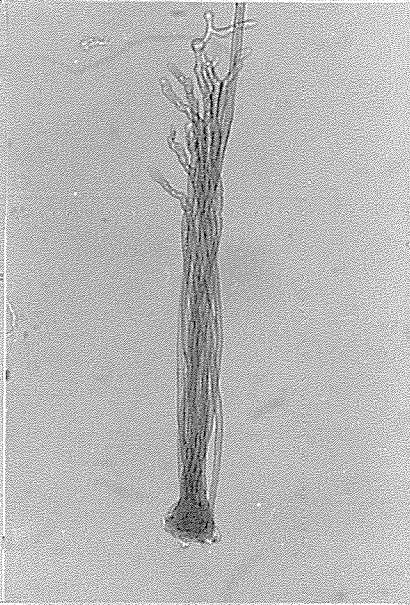
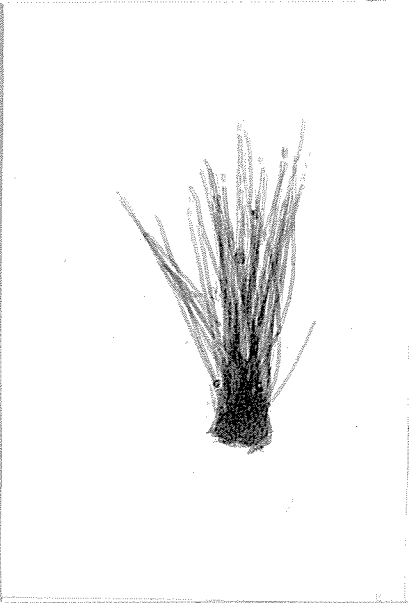
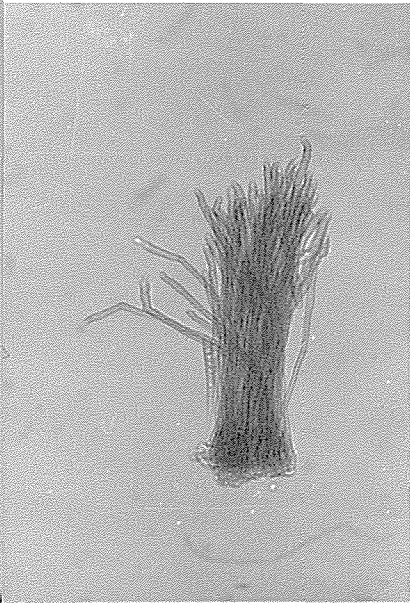
The effect of relative humidity on conidiophore characteristics on both naturally infected and artificially inoculated leaves was tested. Non-sporulating lesions were subjected to a range of humidities, namely, 85, 88, 92.5, 98 and 100%, provided by saturated salt solutions at 25°C (Winston and Bates, 1960).

### Results and Discussion

Variation in conidiophore characteristics from field material is illustrated in Fig. 16. Even though the lesions produced conidiophores and sporulated under constant high humidity conditions a variation in conidiophore compactness is clearly demonstrated. This variation was repeatedly observed both on artificially and naturally infected material when given high humidity treatment. Conidiophores in the left hand column of Fig. 16 could be called coremia, characteristic of the genus Isariopsis, whereas those on the right are dense fascicles, characteristic of the genus Cercospora. Thus the fungus could be placed in either genus, depending upon the number of conidiophores one observed in the different categories of compactness.

Under the range of relative humidities provided, naturally infected leaves from the field produced conidiophores more readily from the stromata at lower humidities than did the artificially infected leaves. At

Fig. 16. Photomicrographic illustration of the variability in conidiophore compactness demonstrated by the pathogen. Conidiophores are all from field diseased material.



100% R.H., a variation in conidiophore characteristics was observed, as in Fig. 16, but the majority of the conidiophores of any one bundle were spreading; that is fasciculate. At lower relative humidity values, diseased field material tended to produce fused conidiophores. These results appear to contradict the observations of Higgins and Munjal and Sethi who considered that increased humidity caused aggregation of the conidiophores.

The above results clearly demonstrate that the degree of compactness of the conidiophores, the prime taxonomic character used for separating the two genera Isariopsis and Cercospora is a variable feature of the fungus, so much so that depending upon the field material studied, so the organism could be placed in either genus. Since in the majority of cases where diseased material was examined following high humidity treatment, the tendency was towards a spreading habit of the conidiophores, the legitimate placement of the fungus would appear to be in the genus Cercospora.

VII CULTURAL STUDIES APPERTAINING TO CLASSIFICATION OF  
THE CAUSAL ORGANISM

In the previous section it was shown, on the basis of conidiophore characters, that the pathogen warrants placement in the genus Cercospora. As supporting evidence, cultural studies were carried out on field isolates to determine whether such features as gross colony characteristics, effect of temperature on radial growth, sporulation in culture and spore germination were typical of species within this genus. Cultural studies per se are not accepted as basic taxonomic criteria, but they do aid in the determination of relationships between fungi.

(i) Colony Characteristics

Growth rate and cultural characteristics of the fungus were studied on seven commercially prepared artificial media. Inoculation was with 4 mm mycelial discs cut from water agar plates with a sterile cork borer and placed centrally in each of the five replicate plates per treatment. All plates were incubated at 25°C for four weeks, with readings on growth rate being taken every 3½ days. In recording diametral colony growth the average of two measurements at right angles was taken for each plate and the average for the five replicates taken for each treatment. Cross hatching on the bottom of the plates ensured that the same diameters were always measured. Total diameters and cultural characters were recorded at the termination of the incubation period.

Table III and Fig. 17 give comparative size, rate

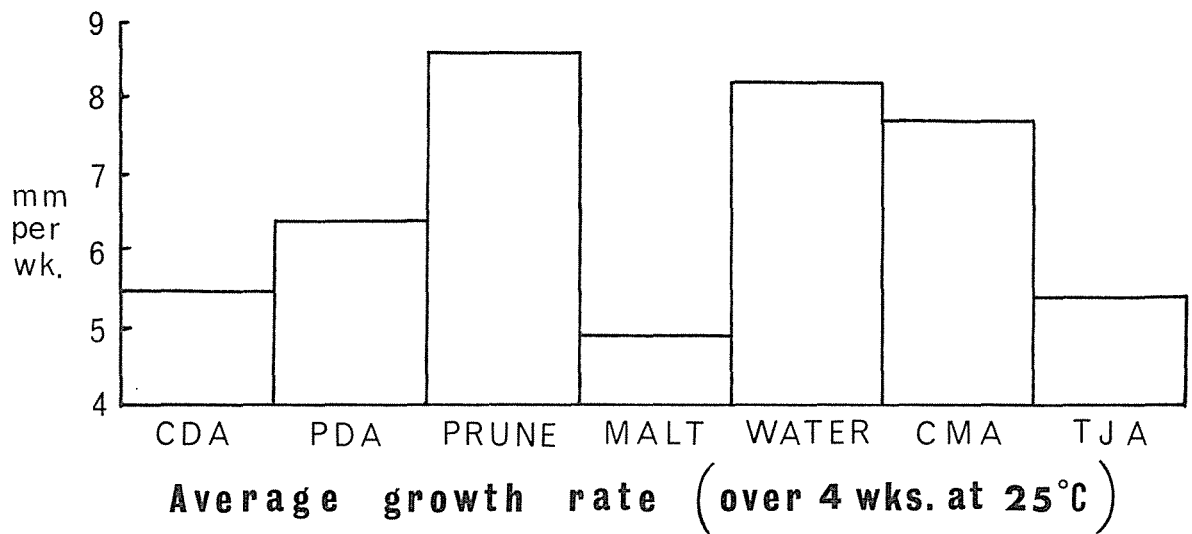
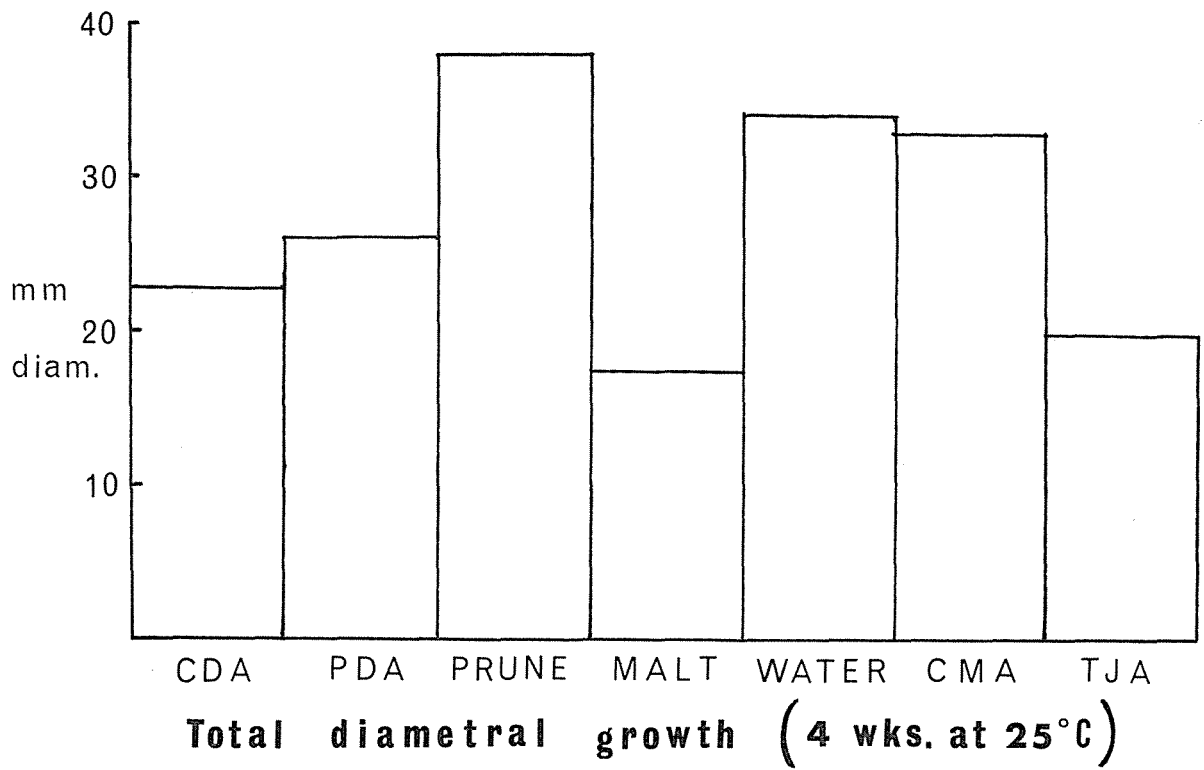


Fig. 17. Total growth and growth rates of the pathogen on various artificial media.

of growth and descriptions of the fungus growing on different media. The rate of growth was relatively slow on all media, with prune and water agars supporting faster, though more effuse radial growth than the other media.

Slow growth in culture has been recorded previously for this fungus (Higgins, 1929; du Plessis, 1942b) and is a common feature of Cercospora species (Latham, 1934; Jenkins, 1938; Wolf, 1940; Plakidas, 1940, 1956; Hansen and Rawlins, 1944; Weimer and Luttrell, 1948; Muntanola, 1954; Anzalone and Plakidas, 1957; Freeman, 1959; Rangaswami and Chandrasekaran, 1962).

Cultural characters were diverse for the one organism on the seven test media, but it showed a fairly uniform slow growth rate throughout, a feature common to species of Cercospora.

TABLE III  
CULTURAL CHARACTERISTICS OF THE CAUSAL ORGANISM

Media	(1) Growth rate	(2) Diameter	Descriptions
Capek Dox Agar (Oxiod) (CDA)	5.5	23	Variable, some white sectoring, raised, smoky grey above, smooth to irregular outline, often with large excreted moisture droplets, dark olive green below, splitting the agar.
Water Agar	8.2	34	Aerial mycelium white with scattered cottony white mycelial balls, submerged growth greeny-grey. Growth very effuse, outline irregular.
Potato Dextrose Agar (Oxoid) (PDA)	6.4	27	Upper surface greeny-grey with a mixture of grey and white effuse aerial mycelium. Compact, regular outline; dark olive green beneath.
Corn Meal Agar (Difco) (CMA)	7.7	33	Flat colony, olive green above but covered with mottled white mycelial patches near the centre. Outline effuse and irregular, dark olive green beneath.
Prune Agar (Difco)	8.6	38	Same as above.
Malt Agar (Difco)	4.9	17.5	Dense raised growth, dark grey periphery with white centre above; outline smooth. Very dark olive green beneath.
Tomato Juice Agar (Oxoid) (TJA)	5.4	20	Dense, low undulant growth. Charcoal grey above, with white sectors. Outline compact but serrated. Black beneath, splitting the agar.

(1) Average growth per week in mm.

(2) Diameter in mm after 4 wks growth at 25°C.

(ii) Effect of Temperature on Growth

The fungus was grown on prune agar for four weeks at a range of temperatures between 5° and 35°C. Prune agar was utilized because it gave the fastest diametral growth (see previous experiment). However, the best medium for radial growth is not necessarily the best for total mycelial growth (Berger and Hanson, 1963) but the faster the radial growth in relation to time, the smaller will be the error of measurement in assessing the effect of a factor on the growth of the fungus (in this case, temperature). Five replicates per treatment were inoculated with 4 mm mycelial plugs from water agar plates and the resultant effects of temperature regimes on the growth of the fungus are illustrated in Figs. 18 and 19.

The temperature range for optimum growth was between 20 and 30°C, with the optimum at approximately 25°C. There was no growth at 35°C, and when, after four weeks at this temperature the plates were returned to the optimum temperature (25°C) the fungus failed to commence growth. Plates held at 5°C grew normally when returned to 25°C. The fungus was therefore inactivated at 35°C. Rand (1914) found a similar situation with Cercospora fusca Rand, which did not grow at 36° - 40°C for two weeks, nor when returned to normal temperatures.

Most plant pathogens have an optimum temperature for growth between 24° and 30°C, with more having an optimum at 25°C than at any other temperature (Cochrane, 1958). The optimum temperature for growth of species of Cercospora

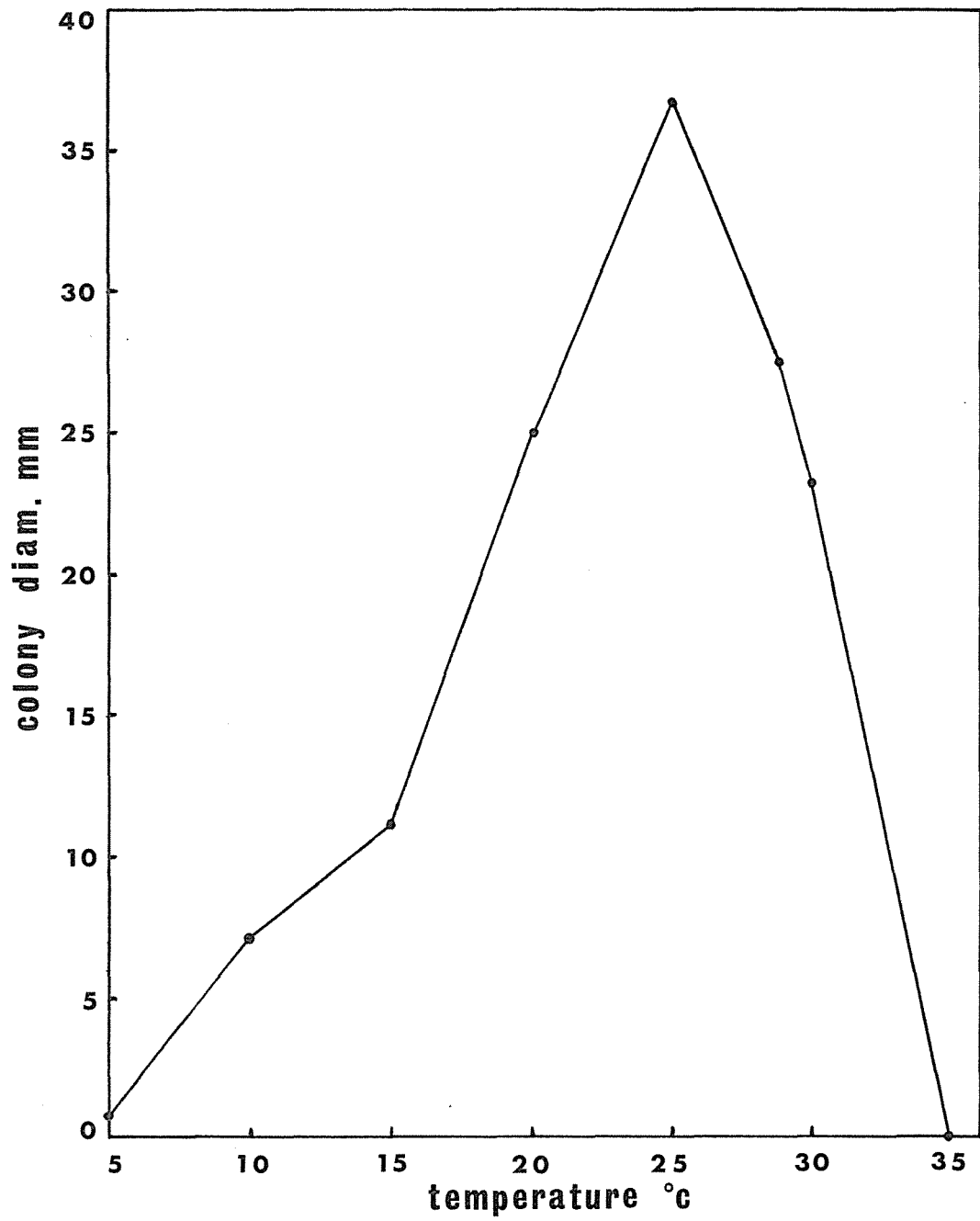


Fig. 18. Temperature/growth curve of the causal organism on prune agar after 4 weeks growth.

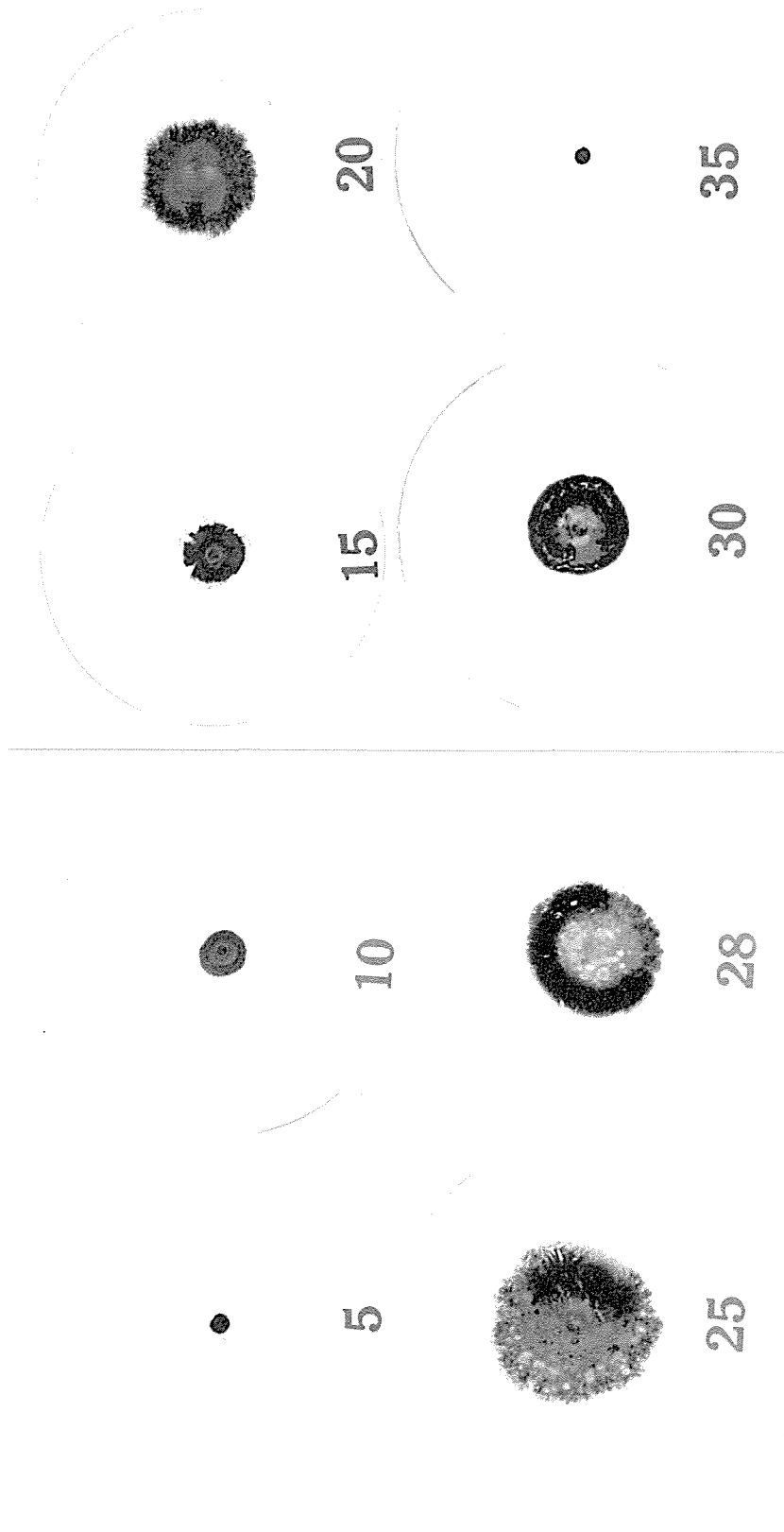


Fig. 19. The effect of temperature ( $^{\circ}$ C) on growth at 4 weeks on prune agar.

that have been studied in culture is reported to be in the vicinity of this temperature (Weimer and Luttrell, 1948; Stavely and Nimmo, 1969).

Although only limited importance can be placed on these comparisons the fungus does, however, have the same approximate temperature optimum for growth in culture as those reported in the literature for other species of Cercospora.

(iii) Sporulation in Culture

The causal organism sporulated sparsely or not at all on all artificial media used in this study. Similar results have been reported for this fungus in culture by Higgins (1929) and du Plessis (1942b). Species of the genus Cercospora have been reported on numerous occasions to sporulate poorly on artificial culture (Rand, 1914; Wormald, 1928; Harrar, 1937; Wolf, 1938; Yu, 1947; Weimer and Luttrell, 1948; Muntanola, 1954; Anzalone and Plakidas, 1957; Chowdury, 1957; Jackson, 1960; Kaiser and Lukezic, 1965).

When working with plant pathogenic fungi, spore production in culture is an important process, as cross inoculations with spore suspensions is an integral part of plant pathological research. Hence, much work has been conducted in discovering ways in which Cercospora species can be induced to sporulate on artificial media. There appears to have been two major approaches towards overcoming this difficulty, namely:

- (a) manipulation of the media and cultural

environment; and

- (b) manipulation of the fungus growing in culture.

Methods used in inducing Cercospora species to sporulate in culture are now reviewed under the above categories.

(a) Manipulation of media and cultural environment

One of the main methods for inducing Cercosporae to sporulate in culture is the use of plant decoction agars (Daichun and Valleau, 1941; Thomas, 1943; Baxter, 1956; Kilpatrick and Johnson, 1956; Murakishi et al., 1960), but other agar types have been used. For example, in their nutritional studies on C. viticola, Sethi and Munjal (1963) obtained spores for inoculation of liquid cultures from colonies growing on Potato dextrose plus yeast extract agar. Berger and Hanson (1962, 1963) obtained sporulation of Cercospora zebrina Pass. on V.8 juice agar but considered environmental factors to be important in influencing both size and amount of conidia produced in culture. Miller (1969) also considered environment, especially aeration, to be important in inducing sporulation of Cercospora gossypina Cke., but found that low levels of glucose increased sporulation. Stavely and Nimmo (1968) reported maximum sporulation of Cercospora nicotianae Ell. & Ev. at 18°C and on an agar medium containing 1.6 g DL - leucine, 5 g sucrose and 3.6 g yeast extract per litre, plus mineral nutrients.

Gased or autoclaved host material has been reported to sustain abundant sporulation with some species of Cercospora

(Lewis, 1940; Baxter, 1956; Kaiser and Lukezic, 1965), and in the present study was found to be effective in producing sporulation. Autoclaved grape leaves inoculated with mycelial plugs and incubated at 25°C on sterile, damp soil in 12 cm petri dishes, sporulated after the formation of dense fascicles within seven days.

(b) Manipulation of the fungus

It has often been observed that fresh conidial isolates of species of Cercospora sporulate within 48 to 72 hours in culture, with a subsequent marked reduction as aerial mycelium increases (Nagal and Dietz, 1932; Nagel, 1934; Latham, 1934; Jenkins, 1938; Wolf, 1943). Latch and Hanson (1962) utilized this principle, and using two to three cycles of conidial subcultures produced sufficient conidia of Cercospora davisii Ell. & Ev. for inoculation purposes. Ryker (1942) found that transferred mycelial tips of Cercospora species performed in the same manner as conidial isolates, with a sterile aerial mycelium eventually overgrowing the original cultures.

Sporulation in culture was obtained with the fungus under study by placing a three to four week old colony from PDA into a McCartney bottle containing approximately one dozen glass beads and 10 ml of sterile distilled water. The bottle was shaken until the mycelium was fragmented into small pieces. The slurry was then poured on the surface of fresh PDA plates and spread evenly over the surface by rotating and tilting. Any excess moisture was poured off and the plates were incubated at 24°C. After 24 to 48 hours, prolific sporulation was observed arising from the mycelial fragments.

Spores were present in numbers sufficient to produce a concentrated spore suspension suitable for spray-inoculation of host plants. Following this initial production of spores and the subsequent growth of aerial mycelium, no further conidia were produced. Sporulation induced by this method appears to parallel the observation that fresh conidial isolates will sporulate in culture, as described above. That is, mycelial fragments act in the same way as fresh conidial isolates in that the fresh growth that is initiated has the same ability to produce abundant conidia.

Similar induction of sporulation by maceration of the mycelium in sterile water has been found to be effective for other species of Cercospora (Plakidas, 1940, 1956; Baxter, 1956). However, Jackson (1960) could not induce sporulation of Cercospora antirrhini Muller & Chupp from Snapdragon by this method.

Forsyth et al. (1963) induced sporulation of Cercospora beticola Sacc. by scraping the aerial mycelium and washing the cultures under running water, as described by Ludwig et al. (1962). This method also was found to induce sporulation of the fungus under study, but was not utilized in the production of inoculum as the fragmenting method described above was more productive. Again, sporulation induction in this case can be attributed to the initiation of fresh growth by the scraping of the aerial mycelium.

Calpouzos (1954) considered the control of sporulation in culture to be more of a factor of genetics than of media used. By removing sporulating sectors from heterogeneous

cultures of Cercospora musae Zimm. from bananas and subculturing these through several generations he obtained cultures that sporulated uniformly. He considered that this was a selection for nuclear types that would give sporulation.

In this study, the causal organism was found to demonstrate the typical low sporulation rate in culture of Cercospora species. The methods used to induce sporulation in this study, namely, using sterile host material, maceration of mycelium in sterile water and scrapping off of aerial mycelium from colonies and washing under running water, are all methods used in inducing sporulation with various Cercospora species, thus further indicating the pathogens close association with this genus.

The genus Isariopsis belongs in the Stilbaceae, a family characterized by the formation of synnemata. Several genera in this family have been studied in culture in an attempt to induce sporulation subsequent to the formation of synnemata (MacLoed, 1956; Taber and Vining, 1959; Taber, 1959; Carlile et al., 1961; Loughheed, 1961, 1963; Wigley, 1968). The fungus under study sporulated without prior formation of synnemata; in fact, it performed more like Cercospora species, which do not produce fascicles in culture, but produce conidia on free conidiophores. Hence, the fungus shows an affiliation more towards Cercospora than to synnematous type fungi.

(iv) Spore germination

Spore germination of the fungus was compared to other reported spore germination patterns of fungi in the genus

Cercospora. The growth chamber utilized in observing conidium ontogeny (page 26) was used for recording patterns of spore germination. A sterile cover-slip (22 x 40 mm) was covered with a very thin layer of agar, allowed to harden, then inoculated with a spore suspension using a platinum wire loop. The agar film was trimmed so as to fit in the aperture of the chamber and sealed in place with paraffin wax. A moist air flow was maintained through the chamber as before. Germination patterns of one or more conidia were continuously observed and photomicrographically recorded, with a three hour interval between each frame.

Figs. 20 and 21 show that the germination pattern of mature and immature conidia is basically the same. That is, germ tubes originate from the end cells first, with some tubes arising from the median cells later. These results are similar to those obtained by Higgins (1929) and du Plessis (1942b) for this fungus.

Germinating Cercospora conidia are reported and illustrated in the literature with germ tubes arising mainly from the terminal cells of the conidia and occasionally and/or later from median cells (Wormald, 1928; Wolf, 1927, 1938, 1940; Woodroof, 1933; Jenkins, 1938, 1945; Thomas, 1943; Kovachick, 1954). The pattern of spore germination illustrated for this fungus shows a close resemblance to the manner in which spores of Cercospora species germinate. Since the pattern of spore germination has been used in the classification of the Helminthosporium group of fungi in the Hyphomycetes (Ito, 1930; Shoemaker, 1959), some weight

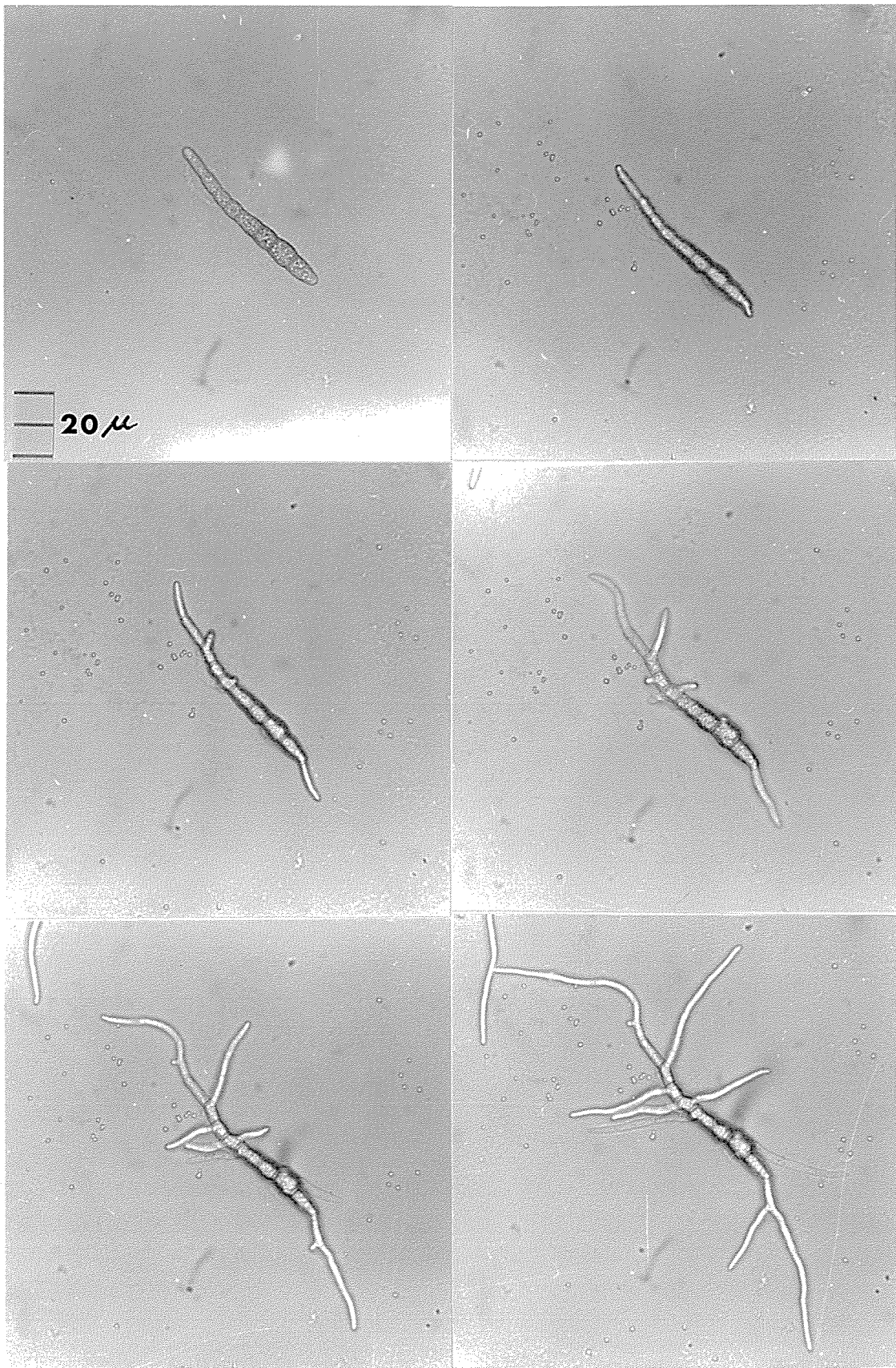


Fig. 20. Germination pattern of a mature conidium at 3 hour intervals.

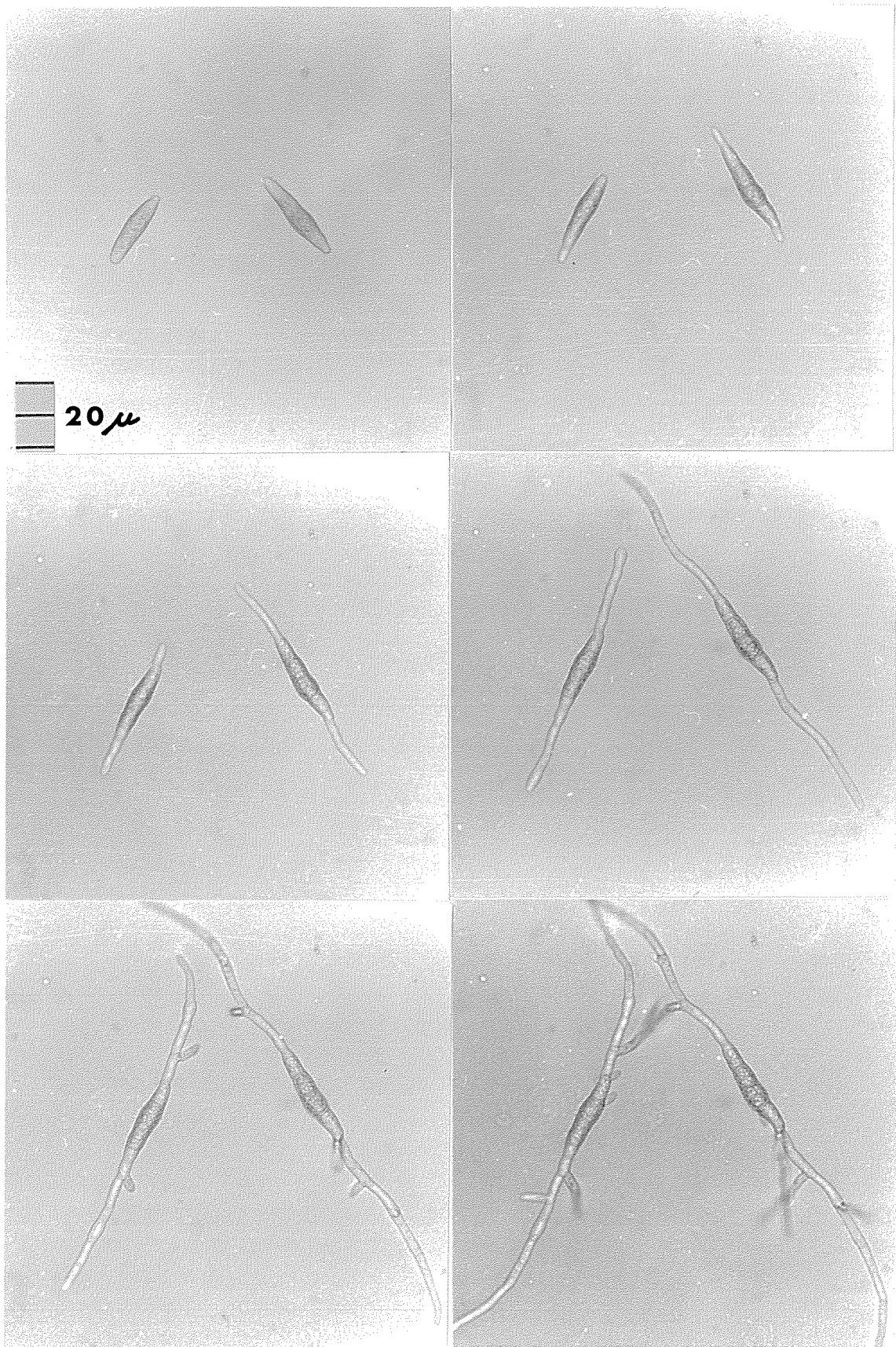


Fig. 21. Germination pattern of 2 immature conidia at 3 hour intervals.

can be assigned to this character in comparing the fungus with other species of Cercospora.

VIII DEUTEROMYCETE CLASSIFICATION SCHEMES AND THE CAUSAL ORGANISM

The Fungi Imperfecti have been termed a 'waste basket assemblage of fungi' (Tubaki, 1963) for here are deposited all fungi which cannot be classified using the morphology of the sexual phase. Consequently, classification schemes evolved solely around the asexual phase have given rise to many contradictions which have caused mycologists to become dissatisfied with one or other of the systems employed. Several classification schemes have been advanced, and a brief outline of these and how they relate to the fungus under study is given below.

(i) Saccardoan classification:

Saccardo, in the 1870's and 1880's devised a master plan of classification for all the fungi. He divided the Fungi Imperfecti into form-orders and form-families on the basis of the type and characteristics of the fruiting structure that produced the conidia. These were further sub-divided into sections depending upon the shape, septation and colour of the conidia. This scheme pigeon-holed all the known imperfect fungi, and even left some holes yet to be filled, but it did not constitute a natural system of classification. However, this was a very convenient system which mycologists the world over have adopted.

The fungus under study is included in the form-order Moniliales, where the conidia are produced free to the atmosphere and not enclosed in a pycnidium or acervulus.

The genus Cercospora is classified in the form-family Dematiaceae and in the section Scolecosporae, characterized by long-filiform or vermicular conidia (Clement and Shears 1930). Pseudocercospora is placed in the same form-family but in the section Phragmosporae. The genus Isariopsis is included in the form-family Stilbaceae (or Stilbellaceae), series Phaeostilbeae (conidia and/or synnemata dark coloured) and section Phragmosporae (Saccardo, 1882). The fungus has been placed in all of these genera, which are closely related and intergrated, yet their classification in the Saccardoan scheme is widely divergent. This illustrates the artificiality of the scheme, which has also been exemplified with many other imperfect fungi (see Hughes, 1953; Goos, 1956; Simmons, 1966).

(ii) Vuillemin's classification (see Langeron and Vanbreuseghem, 1952):

Vuillemin in 1901 proposed a classification of the Hyphomycetes based on spore forms. He recognised two basic types:

- (A) THALLOSPORES - which are essentially non-caducous spores formed at the expense of the thallus by transformation of certain elements.
- (B) CONIDIOSPORES (= Conidia vera) - external spores, terminal or lateral and essentially caducous, which originate upon the thallus as newly formed elements.

The fungus definitely produces conidiospores, but which section within this second category it fits is in doubt.

Unfortunately, in utilizing this scheme Langeron and Vanbreuseghem included in the section Phialidae fungi that produce blastospores and annellospores. There appears to be considerable overlapping of sections which produce conidiospores, making precise classification within this scheme very difficult. Therefore the author is uncertain as to the placement of the genera Cercospora and Isariopsis in Vuillemin's classification scheme.

- (iii) Mason's classification (see Bisby, 1953; Goos, 1956; Langeron and Vanbreuseghem, 1952)

In 1937, Mason proposed two divisions of the Fungi Imperfecti on the basis of type of spore dispersal, namely:

- (a) those dispersed by air or mechanical means - in which case conidia are dry i.e. Xerospores
- (b) those dispersed by water, and conidia produced in slime i.e. Myxospores or Gloiospores.

The fungus is included in the second category, but taxonomically it has little significance as there are inter-grations between the two groups, depending on environmental conditions. Goos (1956) stated in reference to this scheme:

"While graduation between taxonomic categories is to be expected, it does not seem logical to base a major division upon a character that seems so obviously transitional."

- (iv) Hughes' classification (1953)

Hughes divided the Hyphomycetes into eight sections on the basis of the manner of conidium production from the conidiophore and the subsequent growth characteristics of the

conidiophore (if any).

The fungus under study is characteristic of fungi in his Section II, defined as follows:

"Conidia arise as blown out ends of the apex of simple or branched conidiophores, and the ends of successively produced new growing points developing to one side of the previous conidium."

Figs. 14 & 15 illustrate these characteristics and demonstrate the application of the apparatus described in the text for characterising fungi in terms of their conidium ontogeny, - a basic feature used in modern taxonomy. In referring to the genera Cercospora and Isariopsis, Hughes stated:

"In Cercospora spp. the conidiophores are borne on an immersed or semi-immersed stroma and the genus is usually classified amongst the Dematiaceae. Isariopsis griseola Sacc. has almost synnematosous conidiophores with conidia like those of Cercospora and is classified amongst the Stilbaceae. The genera Cercospora and Isariopsis, however, are otherwise similar with respect to conidium origin and development and should appear together in any classification."

On the basis of the findings in the present study, the author would agree fully with the above quotation as far as the causal organism of this leaf spot disease on grapes is concerned.

(v) Tubaki's classification (1958 - 1963)

Tubaki followed Hughes' concept for the classification of the Hyphomycetes, but redefined and provided names for the sections. He placed Cercospora in the Termino-radulaspores of the division Radulasporae. Termino-radulaspores are defined thus:

"Terminates the growth of the conidiophore and is formed side by side upon small denticulate sterigma (spicule projections), usually solitary. Conidiophore increases in length as new ones develop."

Also included in this major division (Radulasporae) is the genus Botrytis. This, however, is included in a completely different section in Hughes' system (Section IB). The denticulate sterigma that is so important in this group appears to the author to be a feature that should not be given such importance, as many fungi that demonstrate this structure are also placed by Tubaki in the division Blastosporae e.g. Gonatobotrys and Cephaliphora. As regards the fungus under study, it appears to produce a denticle early in the formation of the conidium, eventually producing a conidium with an obconically truncate base and conidiophore with a rounded apex. After sympodial growth of the conidiophore (Fig. 15) there is no evidence of a denticle remaining where the previous conidium was formed.

(vi) Barron (1968)

Barron followed Hughes in the division of the Hyphomycetes. However he divided Sections I and III each into two sections, giving them equal rank with all others. To these 10 sections he gave names that as closely as possible described the salient mycological features of the section. The fungus under study is placed in the Sympodulosporae. The term sympodula ("little sympodium") was coined by Kendrick (1962) to describe the characteristic proliferation of the conidiophores in this section. However, there is a paradox here in that fungi in the Porosporae often produce precisely the same proliferation of the conidiophores as that charac-

teristic of the Sympodulosporae. Most fungi in the Sympodulosporae produce conidia blastogenously (as does the fungus under study), but some are also produced murogenously\* (e.g. Pleiocheata setosa; Hughes, personal communication). Therefore, if it was not for the sympodial nature of the conidiophore, fungi in the Sympodulosporae would be distributed amongst the sections Blastosporae and Aleuriosporae. This suggests that this 'natural' classification scheme of Hughes, and Barron may need revision if a more phylogenetic scheme is to be erected.

In the above review the author has emphasised the short-comings in each of the schemes so far devised for the Fungi Imperfecti as they relate specifically to the classification of the fungus under study. In Appendix III an alternative classification scheme for the Fungi Imperfecti is presented where genera can be placed into phylogenetically arranged families and orders. Albeit somewhat tentative, the scheme is proposed in an attempt to overcome some of the difficulties outlined in the review.

\* See Luttrel, 1963.

IX CONCLUSIONS

The basionym of the causal organism of this leaf spot disease of grapes is considered by the author to be Septonema vitis Lév., not Cladosporium viticolum Ces. as taken finally by Saccardo. This means that all combinations of the fungus should be based on S. vitis. Even though there has been much contention as to the correct generic placement of this fungus, the correct binomials for the fungus in the three genera, based on S. vitis are:

Cercospora vitis

Isariopsis vitis

Pseudocercospora vitis

I. vitis is a combination that has never been constructed, even though many workers have considered the fungus to be a member of this genus. In the genus Isariopsis it has been known as I. clavispora and I. fuckelii. The latter binomial proposed by du Plessis cannot be considered a valid binomial for the pathogen. The study clearly shows the spurious nature of the criteria on which he based his conclusion, namely, differential reports in the literature of disease symptom expression and conidium shape of the causal organism. I. clavispora is not a legitimate combination for the fungus as it is not based on the legitimate basionym.

The genus Pseudocercospora is disregarded by the author because of its overlap into the genus Cercospora which is considered broad enough to encompass species with dark conidia.

The fungus is not considered to be a species of Isariopsis on the basis of variation in conidiophore compactness exhibited on host material. Because the majority of conidiophores observed tended to be fascicular in habit, the fungus is precluded from synnematous groups of the Fungi Imperfecti (family Stilbaceae), thus prohibiting its placement in the genus Isariopsis, a Stilbaceous genus. The modern concept of the genus Cercospora has defined limits sufficiently broad so as to include most of the conidiophore variation demonstrated by the fungus. Further, when grown in culture the pathogen was typical of species of the genus Cercospora, especially in respect to slow growth rate, optimum temperature for growth, poor sporulation capacity and spore germination pattern.

On the basis of their identical conidium and conidiophore developmental morphology, modern taxonomic schemes would favour the merging of some Isariopsis species into Cercospora (Hodges and Haasis, 1962). The fungus is therefore considered to be a species of Cercospora, and the basionym is taken as Septonema vitis Lév. The correct binomial for the fungus, therefore, is Cercospora vitis (Lév.) Sacc.

Cercospora vitis (Lév.) Sacc (1876)

= Mycosphaerella personata Higgins (1929)

Synonymy:

Septonema vitis Lév. (1848)

Cladosporium viticolum Ces. (1854)

Cladosporium ampelinum Pass. (1872)

Graphium clavisporum (B. & C.) (1874)

Cladosporium vitis Sacc. (1875)

Cercospora vitis Sacc. (1876)

Cercospora viticola (Ces.) Sacc. (1886)  
Isariopsis clavispora (B. & C.) Sacc. (1886)  
Helminthosporium vitis (Sacc.) Pirota (1889)  
Pseudocercospora vitis (Lév.) Speg. (1910)  
Cercospora vitis (Lév.) Lindau (1910)  
Cercospora vitis (Lév.) Sacc. var rupestris  
Cifferi (1922)  
Isariopsis fuckelii (Thüm.) du Plessis (1942)

The ascigerous state of the pathogen (Mycosphaerella personata Higgins) was never located during the present study, despite constant vigilance. Because of the rarity with which the sexual phase has been reported, the author did not feel justified in following the modern trend in referring to the asexual phase as the Cercospora state of M. personata.

APPENDIX I

Key to the Cercospora spp. on grape leaves (from Chupp, 1953; p.600).

- A. Conidia hyaline, mostly acicular - C. truncata
- AA. Conidia coloured, not acicular.
- B. Conidiophores pale in colour, conidia 2-3.5 to 3-6  $\mu$  wide, fruiting epiphyllous, conidiophores occasionally branched.
- C. Conidia 2-3.5 x 35-70  $\mu$ , conidiophores 2-3.5 x 10-35  $\mu$ . stromata medium, fascicles dense to very dense  
- C. brachypus
- CC. Conidia 3-5.5 x 20-35  $\mu$ , conidiophores 3-5.5 x 20-70  $\mu$ , stromata slight, fascicles not dense  
- C. vulpinae
- BB. Conidiophores medium dark in colour, conidia wide - 3.5-6 to 4-8  $\mu$ .
- C. Fruiting effuse, hypophyllous, fascicles rarely dense, not coremoid. Conidia 4-6.5 x 30-120  $\mu$ , conidiophores 3.5-6 x 15-70  $\mu$ .  
- C. vitis-heterophyllae
- CC. Fruiting not effuse, amphigenous, fascicles dense, coremoid; conidia obclavate, tip sometimes drawn out in beak form, Conidia 4-7 x 20-80  $\mu$ ; Conidiophores not branched, 3-4 x 50-400  $\mu$ .  
- C. vitis  
(Isariopsis clavispora)  
(C. viticola)  
(Pseudocercospora vitis)

APPENDIX IIDELAYED DISEASE MANIFESTATION LATENT INFECTION AND  
THE DISEASE CYCLE

In section III of the text, it was shown that grape leaves became susceptible to C. vitis only after they had reached a certain stage of maturity. This phenomenon of increased susceptibility of the host with age is discussed by Yarwood (1958) under what he calls 'Ontogenetic Predisposition', and he cites several examples. One that parallels the disease under study is Frogeye of tobacco, caused by C. nicotianae, which only becomes manifest on the 'ripe' or mature leaves (Johnson and Valteau, 1949). Brown rot of stone fruits, caused by Monilinia fructicola (Wint.) Honey is a well known example of increased susceptibility of the host with age, but although much research has been conducted on this disease the exact reason for the inability of the fungus to establish an active parasitic relationship in green fruit has yet to be determined (Anon., 1969).

Also in section III, a delayed manifestation of the disease was described on younger inoculated leaves up the vine as they became more mature. Since no secondary spread of the disease took place, this delayed manifestation was postulated to be due to latent infection that had become established after inoculation. This Appendix outlines the studies conducted to determine the nature of this latent infection and to relate it, and the phenomenon of increased susceptibility with age of the host, to the overall disease cycle. All experiments, unless otherwise stated, were conducted on young leaves

from the apical regions of the vines that had shown no evidence of symptoms 20 days after inoculation.

(i) Macroscopic evidence of latent infection

Inoculated, symptomless leaves and similar leaves from the control vine were cleared of chlorophyll by placing in a warm mixture of glacial acetic acid and 95 per cent ethyl alcohol (1:1) for four to five minutes. The leaves were then rinsed in tap water and stained in decolorized basic fuchsins (Preece, 1959), and subsequently mounted on four inch square glass slides and photographed using a green lens filter to increase contrast. The inoculated leaves produced bright red spots where infection presumably had taken place, while the control leaves showed no such reaction (Fig. 22). Preece revealed early infection of Venturia inaequalis (Cke.) Wint. in apple leaves using this periodic acid-Schiff staining method and although these staining results with grape leaves were not as spectacular as his, they nevertheless demonstrated macroscopically that there was possible latent infection in the leaves.

Similar symptomless leaves were placed under high humidity conditions in petri dishes, as in Fig. 3. Within 4 to 8 days the leaves had all produced numerous sporulating lesions. The removal of leaves from the vines was believed to enhance maturity and the results showed that viable fungal structures were still present.

(ii) Microscopic evidence of latent infection

Experimental work up until this stage had demonstrated

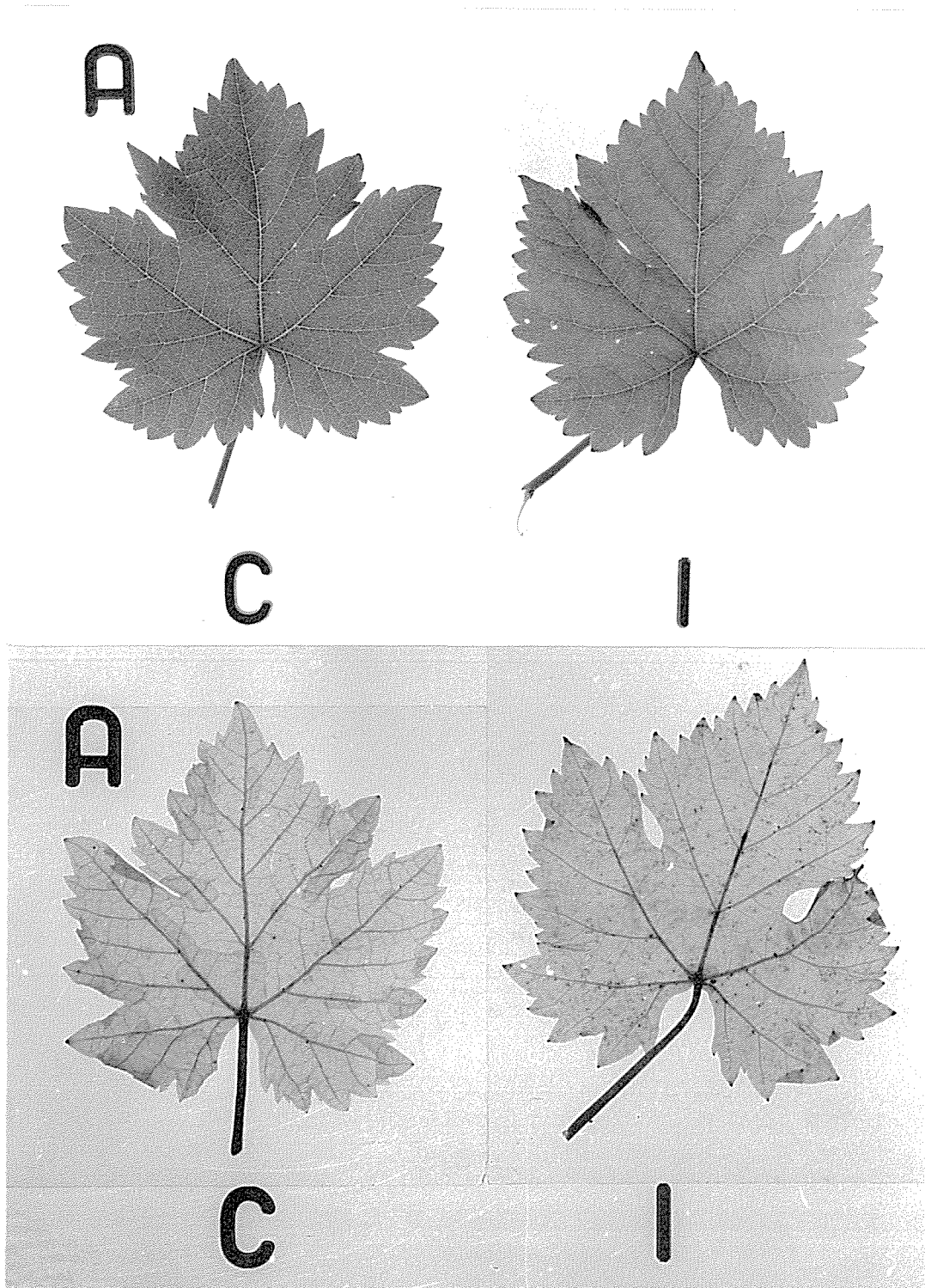


Fig. 22. Latent infection in artificially inoculated grape leaves (var. Golden Chasselas) demonstrated macroscopically by clearing and staining. Top: leaves before clearing and staining. Bottom: after clearing and staining. C = control; I = inoculated leaf. Note that dark speckles on lower right leaf are infection sites of C. vitis.

the existence of latent infection but had not revealed in what form the pathogen had remained viable in host tissues. This was investigated using histological techniques.

Symptomless, inoculated leaves were removed from the vines, cleared as in the previous experiment and placed in various stains. Of the several stains tried, trypan blue (0.2 per cent solution) in lactophenol (Minderman, 1956) was found to produce maximum staining of the fungal structures and minimum staining of host material. After rinsing in tap water the leaves were placed on four inch square glass slides and selected areas covered with a cover-slip, using water as a mountant. Under the compound microscope, conidia of C. vitis were observed to germinate producing long and very thin germ tubes (1-1.5  $\mu$  diam.) which grew extensively over the leaf surface. On the underside of the leaves hyphae were occasionally observed to branch in such a manner as to suggest a tropism or taxis towards stomata, although they would often grow over them. These branches varied in length; if they were long they would swell at the ends to approximately 2 to 3  $\mu$  in diameter as they entered the stomata; if short, the complete branch would swell in the stomata. These structures were recorded by camera lucida drawings (Fig. 23). Photographs were not possible because of the effuse nature of the light transmitted through the cleared grape leaves and the consequent lack of sharp definition and depth of focus of the hyphal structures on the leaf surfaces.

Although the swellings of the hyphae in the stomata were appressorium-like, they should more correctly be called stomatopodia. Similar structures have been reported arising

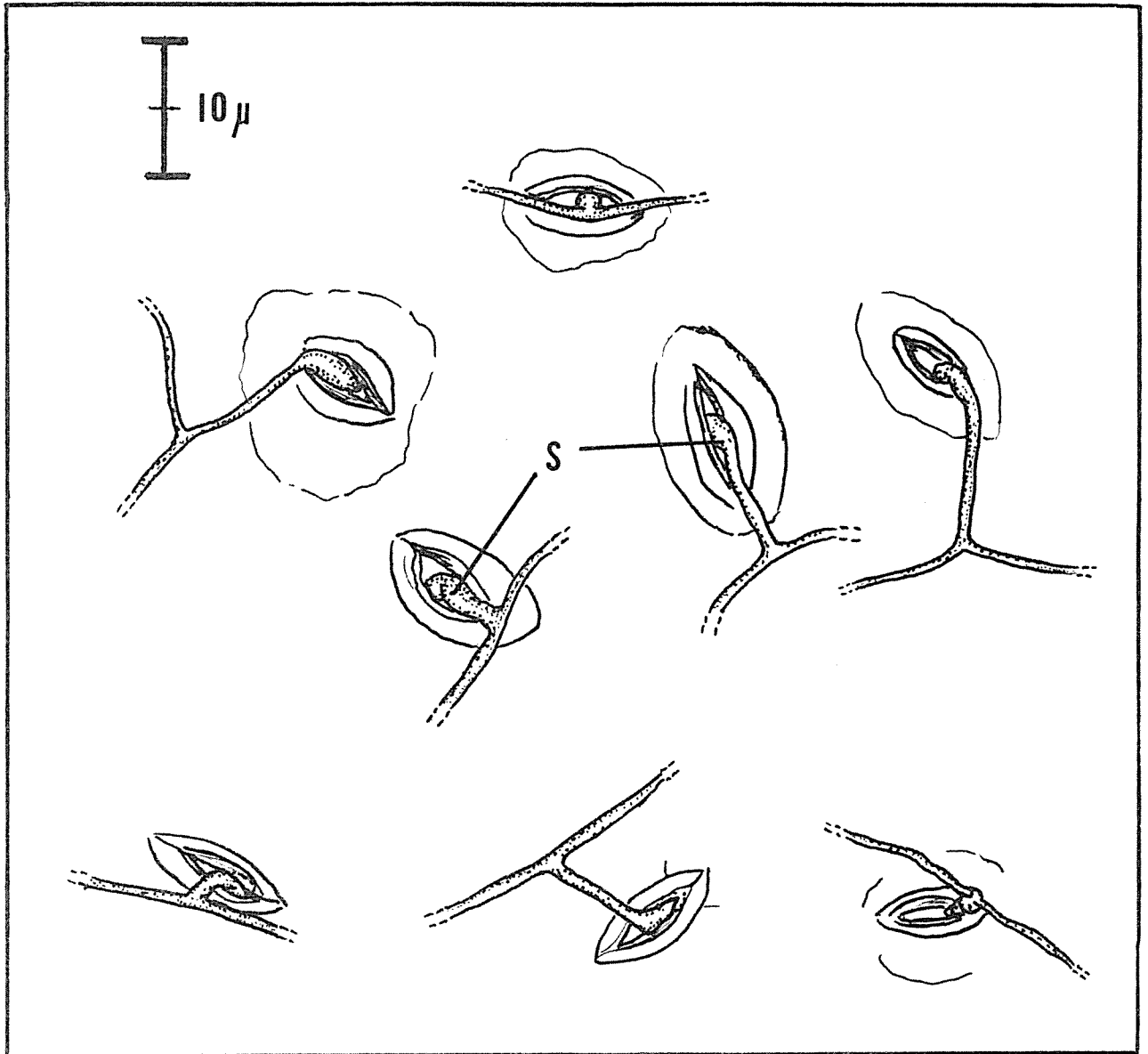


Fig. 23. Camera lucida drawings of host penetration and latent infection. Stomata are from the lower leaf surface of leaves showing no symptoms 20 days after inoculation. S = stomatopodia.

from epiphytic hyphae growing across banana leaf surfaces when infected with Mycosphaerella fijiensis Deighton (Meredith and Lawrence, 1969). However, these workers did not report whether these structures were also produced from germinating conidia or whether they proliferated the disease. Horne and Palmer (1935) described a latent infection process of Botryosphaeria ribis (Tode ex Fr.) in green Avocado fruit which took the form of a hyphal mass in the stomatal cavity. Wade (1956) found with brown rot of apricots that the latent infection in green fruit was in the form of conidia of M. fructicola lying within the stomatal cavities, some of which would germinate and penetrate cells surrounding the cavity. In the present study, however, latent infection by C. vitis was not found to advance beyond the stomata, but in the previous experiment, the decolorized basic fuchsin stained a reaction area about the stoma that had been penetrated.

Latent infection by C. vitis in grape leaves occurs therefore in the form of stomatopodia which can cause establishment of the disease when conditions of the host, and possibly the environment, are conducive to the pathogen. The host condition appears to be associated with age of the leaf because of the differential susceptibility between old and young leaves and the gradual manifestation of the disease up the inoculated vines as the leaves matured. The decrease in the number of lesions with increased time between inoculation and the leaf becoming susceptible, as described in section III of the text, can now be attributed to a loss in virulence of the latent infection present in the form of stomatopodia. The cause of this loss of virulence may be attributed to

either external environmental influences, internal host inactivation or a combination of both.

(iii) The disease cycle

All facts appertaining to the behaviour of the disease in the field are here synthesised into a schematic summary of the disease cycle. The proposed scheme is based on:

- (1) observations of specimens received from the Matamata area at various intervals throughout three growing seasons,
- (2) glasshouse inoculation experiments,
- (3) reports in the literature of the disease overseas.

The cycle is graphically illustrated in Fig. 24 which shows the nature, amount and longevity of each phase of the disease cycle. The trends only are being illustrated although fluctuations in each phase will naturally occur in response to environmental changes. The following notes outline the salient features of each phase of the disease cycle.

(a) Overwintering and Primary inoculum:

It is reported that C. vitis overwinters by way of the conidiophores on diseased fallen leaves (Rhoads, 1926; Higgins, 1929; du Plessis, 1942a). The ascigerous state of the fungus, Mycosphaerella personata has been reported on two occasions to constitute an additional means of overwintering (Higgins, 1929; Munjal and Sethi, 1966), but despite constant vigilance, this state was never located during the present

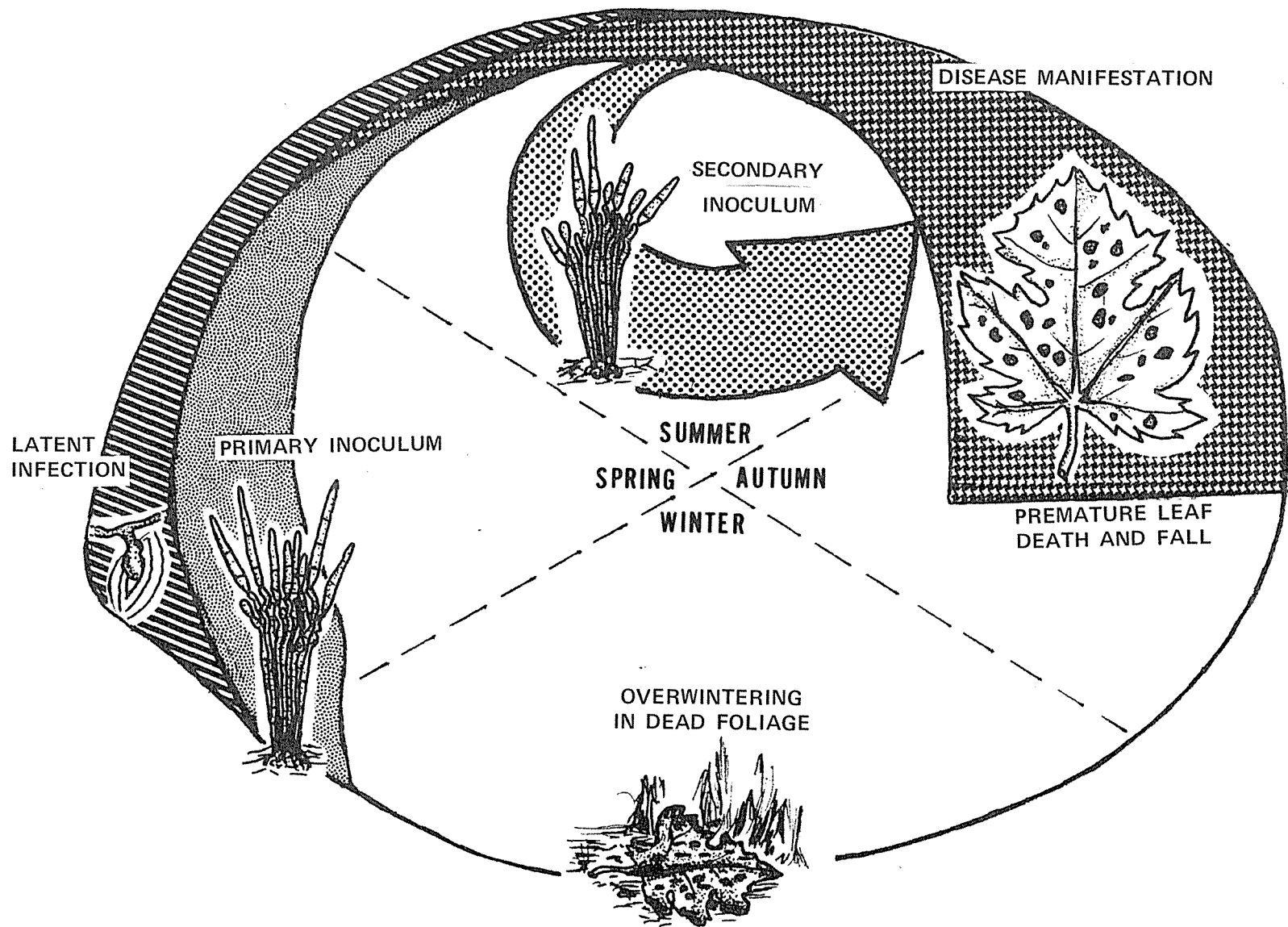


Fig. 24. Schematic summary of the disease cycle of the leaf spot disease of grapes caused by C. vitis.

study. Primary inoculum in the form of conidia, produced on the overwintered conidiophore bundles, arise when conditions in the spring become favourable for sporulation (Higgins, 1929; du Plessis, 1942a, 1942b).

During spring and summer there would follow a steady reduction in the level of primary inoculum as the overwintered host material and the fungus were broken down by weathering and microbial activity. The distant location of the Matamata home garden, the only known site of the disease in New Zealand, precluded the possibility of assessing the inoculum level during this period.

(b) Latent infection:

Hyphal swellings in the stomata (stomatopodia) constitute the form in which the pathogen persists during the latent infection period. The stomatopodia are envisaged as one of the ways the pathogen bridges the gap between the beginning of the growing season and the onset of susceptibility of the host.

Latent infection would be produced in the field by primary inoculum present while the leaves were still resistant to the development of the disease. The number of latent infection sites would not, however, accumulate during this period because:

- (1) the longevity of the stomatopodia is of a transient nature, lasting at the most six to seven weeks,
- (2) the level of primary inoculum would decrease during this period.

Therefore, corresponding to the decrease in the level of the primary inoculum illustrated in Fig. 24, there is a delayed decrease in the level of latent infection.

(c) Manifestation and Secondary Inoculum:

The disease begins to manifest itself from the beginning of summer onwards as the grape leaves mature and become susceptible. This is postulated to be caused by two overlapping factors (see Fig. 24):

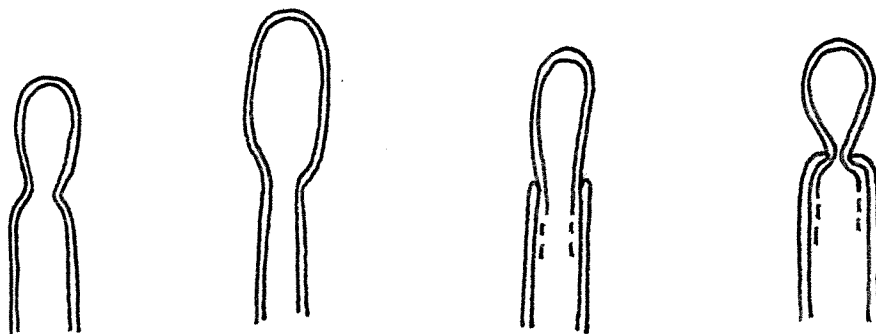
- (1) the end of the primary inoculum phase.
- (2) latent infection (stomatopodia).

Soon after manifestation of the disease, secondary inoculum arises from the lesions leading to a rapid build-up of the disease, as and if conditions become conducive to its development. This would continue until all leaves had fallen from the vines either because of the disease, which can cause severe defoliation, or as a consequence of natural senescence. The pathogen then overwinters associated with these diseased fallen leaves.

APPENDIX IIIA PROPOSED CLASSIFICATION OF THE FUNGI IMPERFECTI  
BASED ON PHYLOGENETIC RELATIONSHIPS

This proposed classification of the Fungi Imperfecti is based on the following fundamental assumptions:

- (i) That there are only four basic types of asexual spores, namely, blastospores, murospores, phialospores and porospores (see Luttrell, 1963). These four types constitute the basis for the four orders of the scheme.



blastospore      murospore      phialospore      porospore

The wall relationships illustrated above indicate the possible phylogenetic trends inherent in the scheme. Families are subdivided within these orders depending primarily on the growth features (if any) of the conidiophores.

- (ii) That a phylogenetic classification of the Fungi Imperfecti is possible based on the manner of asexual spore production and conidiophore proliferation.
- (iii) That asexual and sexual phases of fungi have developed independently on the one organism.

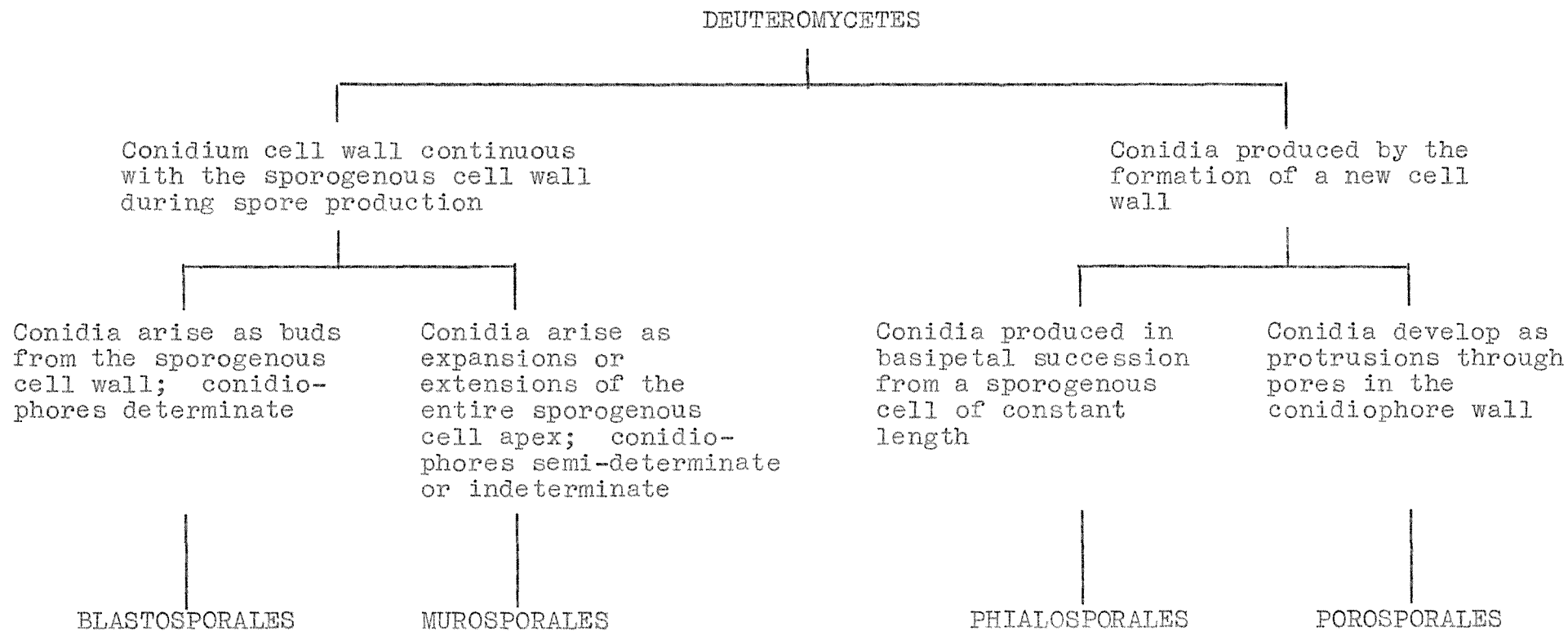
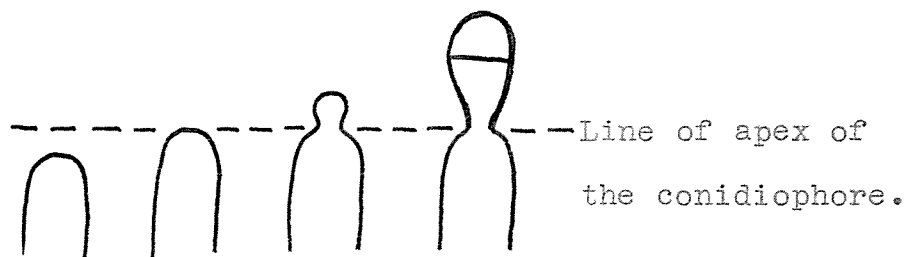


TABLE IV. Proposed orders for the Deuteromycetes.

More often than not the asexual and the sexual phases play quite independent and widely differing roles in the life cycles of fungi and are consequently subjected to differing environmental pressures that will influence their evolution. Therefore the asexual phase per se, be it associated with a sexual phase or not is considered in this classification.

Table IV outlines the basic features of the four orders, which are now individually considered in detail.

1. Blastosporales: Blastogenous conidium production is demonstrated by the yeasts which many mycologists consider to be the most primitive group of Ascomycetes. Hence the Blastosporales is placed as the lowest order of Deuteromycetes despite the fact that this method of conidium production is demonstrated throughout the Ascomycetes. The conidiophores are considered determinate since they stop elongating before producing a bud, with the consequence that the apex of the conidiophore can be determined early in the formation of the conidium.



The order is divided into four families on the basis of conidiophore characteristics:

- (1) Conidia produced singly or in acropetal chains as a bud from the tip of the conidio-

phore or from a previously formed conidium

..... FAMILY BLASTOSPORACEAE

- (2) Conidia produced singly (occasionally in chains) from the conidiophore apex by budding, and from the apex of a sympodial\* proliferation of the conidiophore

..... FAMILY SYMPODULOBLASTOSPORACEAE

- (3) Blastogenous conidia produced simultaneously over the surface of a terminal or intercalary ampulae; sometimes produced in chains

..... FAMILY BOTRYOBLASTOSPORACEAE

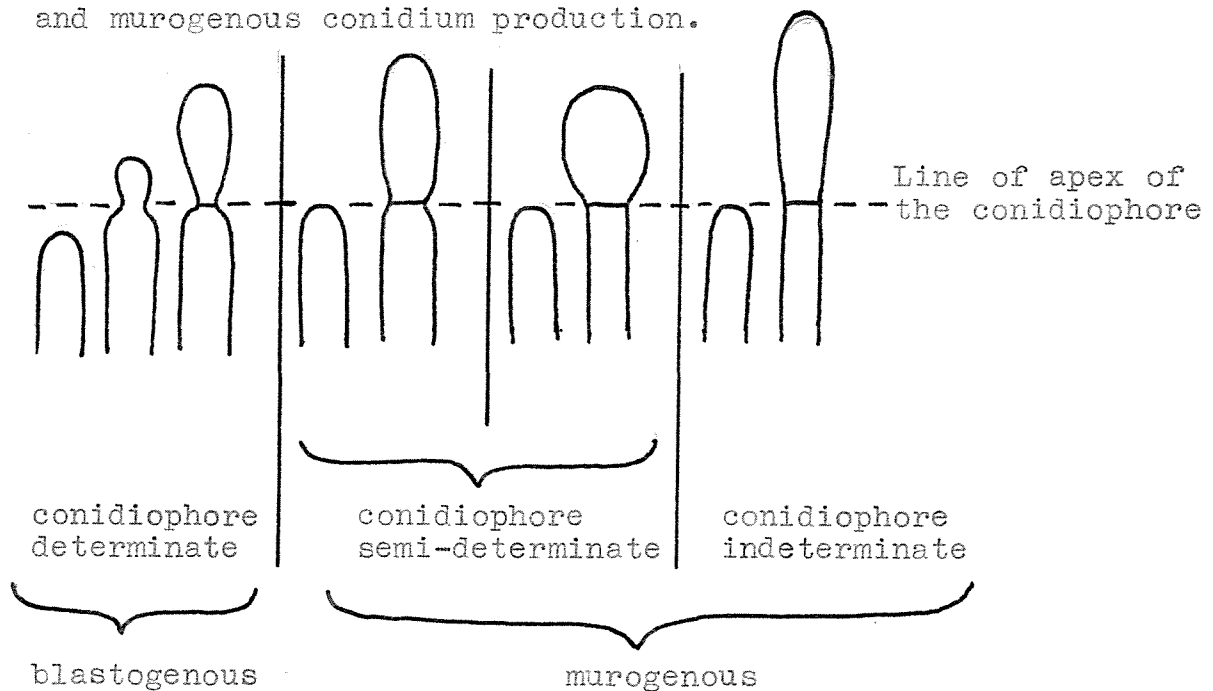
- (4) Conidia produced blastogenously at apex or lateral to the sporogenous cell; conidiophore meristematic from basal region

..... FAMILY MERISTEM BLASTOSPORACEAE

All names for the above families stem from Barron's (1968) nomenclature, but with family suffixes. His Sympodulosporeae is changed to Sympoduloblastosporaceae to give a more precise indication of the type of conidium production and conidiophore proliferation. Such a family title immediately separates it from the identical sympodial type of proliferation found in the Murosporales and the Porosporales.

\* Symodium = a stem whose successive sections are strictly branches, each arising from the preceding branch.  
 Sympodula = a small symodium (see Kendrick, 1962).

2. The Murosporales: The name for this order is derived from the term 'murogenous', coined by Luttrell (1963) to describe conidium production by way of an expansion of the entire cell wall at the apex of the sporogenous cell. Conidia produced in such a manner he termed aleuriospores. However, the term 'murogenous' is extended here to include conidium production by an extension of the sporogenous cell. Conidium production can be considered to be murogenous if more than one half of the width of the conidiophore apex consists of the conidium base. The diagram below illustrates this type of conidiophore apex, which is termed either semi-determinate or indeterminate. The diagram also provides a comparison between blastogenous and murogenous conidium production.



Van der Plank (1963) stated - "Nature seldom draws lines without smudging them;" so with some intermediate cases a decision must be made as to whether the conidia are blastospores or murospores.

The order Murosporales is divided into four families:

- (1) Conidiophores semi- or indeterminate; single conidia murogenously produced at apex with a pleurality often produced on sympodial conidiophore proliferations or branches below the conidiophore apex

..... FAMILY ALEURIOSPORACEAE

- (2) Conidiophores indeterminate; breaking up into many conidia

..... FAMILY ARTHROSPORACEAE

- (3) Conidia formed in a basipetal chain from a meristematic conidiophore zone; the chain may merge imperceptibly with the conidiophore apex

..... FAMILY MERISTEM ARTHROSPORACEAE

- (4) Conidia produced murogenously in basipetal sequence from a conidiophore that increases in length during successive conidium productions to form 'annellations'

..... FAMILY ANNELLOSPORACEAE

Again, these family names are derived from Barron's series names, but by restricting the sense of the term "a blown out end of the conidiophore" as used by Hughes (1953) and Barron, some genera are moved from section II or the Sympodulosporae into the Aleuriosporaceae. Generally an aleuriospore is a thick walled conidium that secodes with difficulty from the conidiophore. The author, however, agrees

with Goos (1956) and Luttrell (1963) in extending the term to include all conidia produced murogenously from the conidiophore, irrespective of subsequent conidiophore proliferation.

Arthrospores, meristem arthrospores and annellospores are all murogenously produced conidia but with various subsequent conidiophore behaviour in the production of successive conidia. Cole and Kendrick (1968) proposed two subdivisions of the group producing meristem arthrospores on the basis of whether there is a cytoplasmic connection throughout the chain or not; that is, whether conidia are completely mature or not when they leave the meristematic zone.

3. The Phialosporales: With the present state of knowledge of this order, it is safest to characterize it by one family.

- (1) Conidia (phialospores) - develop in rapidly maturing basipetal series from the apex of the conidiophore (phialide) which may or may not possess an evident collarette

..... FAMILY PHIALOSPORACEAE

Division of this family was proposed by Tubaki (1958, 1963) on the basis of whether the conidia were delimited inside the phialide or at its apex, but Cole and Kendrick (1969a) considered this division superfluous.

4. The Porosporales: This order can only include one family as divisions based on methods of conidiophore proliferation, as in the Blastosporales, may split some

already existing genera into different families.

- (1) Conidia produced through pores on the conidiophores, singly or in acropetal chains. Conidiophores may proliferate by sympodial or percurrent\* growth

..... FAMILY POROSPORALES

These latter two orders (Phialosporales and Porosporales) are characterized by the conidia being formed from new cell walls extra to the sporogenous cell wall. Electron micrographs by Campbell (1968) show the wall relationships in porogenous spore production while those by Zachariah and Fitz-James (1967) and Buckley et al. (1969) clearly demonstrate the essential features of phialides and phialospores. The former two orders (Blastosporales and Murosporales) produce conidia by the extended growth of the sporogenous cell wall.

Discussion A classification of the Fungi Imperfecti based on developmental morphology and embodying orders and families has not previously been advanced. Although modern schemes have attempted to reflect natural groupings, only one attempt has been made to use the family notation (Subramanian, 1962). The present proposed scheme, therefore, incorporates the ideas of many mycologists and synthesises them into a unified whole which expresses possible phylogenetic relationships between the imperfect fungi. The ten families of the four orders are all based on well defined terms which should

\* Percurrent (sensu Luttrell, 1962) = Proliferation through the tip of the conidiophore, usually through the conidial scar after the conidium is detached.

facilitate the placement of most imperfect fungi in the scheme. As with any classification system, border-line cases are inevitable that will either overlap families or even orders. Nevertheless, it has all the advantages of most of the schemes previously advanced, and further, reduces many of the disadvantages, as outlined in section VIII of the text. A considerable amount of research would have to be carried out in characterizing the numerous genera of the Deuteromycetes into this proposed scheme, especially in the pycnidium and acervulus producing fungi. Some work, however, has already been conducted with the electron microscope on characterizing conidium production in various genera of the Coelomycetes (Brewer and Boerema, 1965; Sutton and Sandhu, 1969).

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