PLANT PATHOGENIC SPECIES OF

STEMPHYLIUM WALLR. IN NEW ZEALAND

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy at
Massey University

by

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June 1977

ACKNOWLEDGEMENTS

I am sincerely grateful to Dr. H.T. Wenham and Dr. K.S. Milne for their advice and encouragement throughout the course of this study and in the preparation of this thesis.

I would also wish to thank:

Mr. D.H. Hopcroft of the Electron Microscope Laboratory, D.S.I.R. for his assistance with the transmission and scanning electron micrographs.

Mr. A.K. Hardacre of the Plant Physiology Division, D.S.I.R. for use of climate rooms.

Mr. H.F. Neilson for technical assistance and valuable discussions.

The staff of the Central Photographic Unit, Massey University, for photographic processing.

Mrs. J. Parry for typing the thesis.

Dr. Ranjit Singh and Dr. Lalit Mehta for their constant encouragement and financial support during this study.

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ABSTRACT

A taxonomic study was conducted of 48 isolates of plant pathogenic Stemphylium Wallroth species, all of which produced a Pleospora Rabenhorst perfect state in culture. Specific identification was attempted using gross colony characters and morphological features of the imperfect and perfect states. Only conidial and ascospore features proved to be of taxonomic value. The diagnostic conidial features were shape, dimensions, length/width ratio, number of longitudinal and lateral septa, number of lateral septal constrictions, ornamentations and pigmentation. Ascospore features of taxonomic significance were dimensions, and shape of both juvenile and mature spores.

On the basis of these criteria it was concluded that three Stemphylium species were present, namely Stemphylium botryosum Wallroth, Stemphylium globuliferum (Vestergen) Simmons, and Stemphylium vesicarium (Wallroth) Simmons, each with a corresponding Pleospora state. This cross-pairing had value since identification of form-species allowed reliable prediction of the Pleospora species, and vice-versa.

The cultural conditions providing the best expression of conidial features (and thus facilitating separation of Stemphylium species) were 5% V-8 agar, pH 7.5, 20C and an 8h photoperiod. Synchronous production of conidia was induced with an injury technique and ascostromata were matured most rapidly at either 12C or a diurnal temperature regime of 8h at 16C/16h at 8C.

A taxonomic survey of 166 Stemphylium isolates from 12 host species revealed the three previously mentioned species and a further undescribed species. The latter was isolated from annual phlox and was characterized by exceptionally large ascospores. S. vesicarium was by far the most common species, occurring on asparagus, chrysanthemum, blue lupin, Russell lupin, tree lupin, onion, pepper and tomato. The legumes were hosts of more than one species; lucerne for instance was a host for S. botryosum, S. globuliferum and S. vesicarium, while Russell lupin and tree lupin were only infected by S. botryosum and S. vesicarium. By contrast, lettuce and carnation were only infected by S. botryosum.

Cross-pathogenicity tests indicated that host specialization was relatively uncommon. S. botryosum from lettuce and the Stemphylium sp. from annual phlox were the only isolates exhibiting host specificity.

The results of ultrastructural studies of conidiogenesis, the

phenomenon of vegetative reversion of conidiophores and juvenile conidia, and the mechanism of ascospore release in Pleospora are discussed.

A previously undescribed, saprophytic, chain-forming species of Stemphylium is also described.

INTRODUCTION

The genus *Stemphylium* was erected by Wallroth in 1833 with *Stemphylium botryosum* as type species. According to Wiltshire (1938), the salient diagnostic characters implied in Wallroth's definition of the genus are:

(i) conidiophores swollen at the apex and bearing a single terminal spore;

(ii) growth of conidiophores may be continued through the terminal scar, with the successive swellings recognisable in an old conidiophore marking the places where conidia have been borne;

(iii) spore shape oval or subangular, muriform, frequently constricted markedly at the median lateral septum.

In 1871, Harz (quoted from Wiltshire, 1938) assigned a fungus found on honeycomb to the genus, naming it *Stemphylium lanuginosum*. This identification was incorrect by reason of the conidiophore features being quite foreign to the original conception of the genus. However, largely because of his excellent description and illustrations of *S. lanuginosum* this modified concept of *Stemphylium* came to be followed by most systematists. Abetting the error was the identification of an isolate by Oudemans as *S. botryosum*, and which Bolle (1924) regarded as agreeing with Wallroth's description, but later demonstrated by Wiltshire (1938) to be either closely allied to, or even synonymous with *S. lanuginosum*. That is, the characters of this latter species came to be widely accepted as typical of the genus, namely:

(i) conidiophores without apical or nodular swellings;

(ii) growth of the conidiophore may be continued by a lateral outgrowth just below the apex, giving a cymose arrangement to an old conidiophore;

(iii) conidia ovate, borne singly and lacking any obvious constriction at the median lateral septum.

Wiltshire (1938) critically considered the whole question of *Stemphylium* taxonomy and nomenclature and concluded that Wallroth's characters for *Stemphylium* must be retained. To avoid the necessity of transferring species of *Stemphylium* sensu Harz to another genus, he proposed that both concepts be retained in the one genus, the essential characters of which would be the dark-coloured, muriform, ovate or

subangular spore. To differentiate the two types he proposed two subgenera:

subgen. Eustemphylium - those species with the original characters of the genus as proposed by Wallroth;

subgen. Pseudostemphylium - those species allied to Stemphylium lanuginosum Harz.

While Wiltshire's sectional treatment of Stemphylium became widely accepted in principle (Groves & Skolko, 1944; Neergard, 1945), it was not put into practice nomenclaturally because the subgeneric interpretation of subsequently described species in either group was never indicated. For example, Stemphylium loti Graham (1953), a Eustemphylium species; and Stemphylium dichroum Petrak (1950), and Stemphylium tritici Deshpande & Deshpande (1965) correspond to Pseudostemphylium spp.

Subramanian (1961) in part rectified this inadequacy by elevating Pseudostemphylium to generic rank, with Pseudostemphylium lanuginosum as the type species. Some acceptance of this proposal was evident when Hoes, Bruehl & Shaw (1965) described a new species Pseudostemphylium chlamydosporum on winter wheat.

A proposal for overcoming the problem of differentiating pseudostemphyloid fungi from true stemphylia was put forward by Simmons (1967), and has been widely accepted (Barnett & Hunter, 1972; Barron, 1972; Ellis, 1971). From a comparative study of several hundred collections and cultures in addition to type species, Simmons concluded that fungi of the S. lanuginosum group had the greatest overall resemblance to the type species of Ulocladium botrytis Preuss. Accordingly, he proposed that S. lanuginosum be transferred to Ulocladium, and this imposed the taxonomic synonymy of Stemphylium subgen. Pseudostemphylium Wiltsh. and Pseudostemphylium (Wiltsh.) Subr., since both are typified by S. lanuginosum. This in turn meant that Wallroth's original concept of Stemphylium was restored.

The question of Stemphylium taxonomy however cannot be resolved in isolation but must be considered in conjunction with perfect states, where these exist. The association of Pleospora as the perfect state of Stemphylium was first established by the brothers Tulasne (quoted from Wiltshire, 1938), and has since been confirmed by several investigators (Groves & Skolko, 1944; Rotem, Cohen & Wahl, 1965; Simmons, 1969; Smith, 1940; Webster, 1969; Wehmeyer, 1962). This Ascomycete genus is

characterised by the production of muriform, yellowish-brown to golden-brown ascospores in bitunicate asci. The fruiting body is an ascostroma consisting of a central locular cavity bearing asci interspersed amongst pseudoparaphyses, and bound on the outside by stromatic tissue. Although Pleospora is the perfect state for several Deuteromycete genera, it is the only one which has been associated with Stemphylium.

Although Wiltshire (1938) was essentially concerned with mycologically defining the genus Stemphylium it is apparent that he considered only two species should be recognised within the original generic definition of Wallroth, namely:

Stemphylium botryosum Wallroth - for species producing a perfect state, and having echinulate - verrucose conidia ; and

Stemphylium sarcinaeforme (Cav.) Wiltsh. - for species not producing a perfect state, and having smooth conidia.

Wiltshire's opinion was widely accepted in that it became common practice to regard all isolates producing an ascigerous stage as Pleospora herbarum (Pers. ex. Fr.) Rabenh., with S. botryosum as its imperfect state (Chilton, 1942; Dingley, 1969; Rotem, Cohen & Wahl, 1965; Smith, 1940; Tate, 1970). However, several stemphylia additional to S. sarcinaeforme have since become recognised; these do not produce a perfect state and have been differentiated primarily on conidial features, but with some regard to host association and presence or absence of stromatic bodies. Such species include Stemphylium astragali (Yoshii) Yamamoto, Stemphylium bolicki Sober & Seymour, Stemphylium callistephi Baker & Davis, Stemphylium chicha (Nisikado & Hiura) Yamamoto, Stemphylium floridanum Hannon & Weber, Stemphylium lancipes (Ellis & Everhart) Simmons, Stemphylium loti Graham, Stemphylium lycopersici (Enjoyi) Yamamoto, Stemphylium melanopus (Schiv.) Hughes, Stemphylium nabarii Sarwar, and Stemphylium trifolii Graham.

Simmons (1969) was the first to question the justification for regarding just one ascocarpic state being genetically connected with Stemphylium, namely P. herbarum with S. botryosum. Following study of stemphylia collected world wide, many of which produced ascocarps on culture media, Simmons concluded that four additional Stemphylium - Pleospora connections could be distinguished on the basis of conidium and

ascospore characteristics, namely:

Stemphylium globuliferum (Vestergen) Simmons - Pleospora sp.
Stemphylium majusculum Simmons - Pleospora sp.
Stemphylium triglochinicola Sutton & Pirozynski - Pleospora sp.
Stemphylium vesicarium (Wallroth) Simmons - Pleospora sp.

Regarding the specific epithet for the Pleospora states of S. globuliferum, S. vesicarium and S. triglochinicola, in each instance Simmons made tentative suggestions only, viz. Pleospora armeriae (Cda.) Ces. & de Not., Pleospora allii (Dub.) Ces. & de Not., and Pleospora maritima Rehm. A perfect state binomial for S. majusculum was not considered. The choice of P. maritima as the perfect state of S. triglochinicola was questioned by Webster (1969) because it is a later homonym of Pleospora maritima Bonn., Rouss. & Sacc., which is a quite different fungus with five septate ascospores.

In New Zealand Stemphylium occurs as a saprophyte, as a weak parasite or as a primary pathogen of a diverse range of plants. Although a number of species are recognised overseas (Baverman, 1968; Graham, 1953, 1957; Graham & Zeiders, 1960; Hannon & Weber, 1963; Weber, 1930; Wells et al., 1956), it has been customary in this country to consider all plant pathogenic isolates as S. botryosum, the imperfect state of P. herbarum (Dingley, 1969; Findlay, 1970; Tate, 1970; Whitwell, 1974). However, in 1973 the writer identified S. vesicarium as a component pathogen of the chrysanthemum flower blight complex in New Zealand (Singh, 1973), and more recently Koh (1976) implicated this latter species as the causal agent of an asparagus stem and foliage disease.

In 1973 the writer initiated a taxonomic survey of plant pathogenic stemphylia present in New Zealand. Forty-eight isolates were collected from 10 host species and identification attempted on the basis of shape, dimensions, number of lateral septal constrictions, surface ornamentation, and pigmentation of mature conidia as expressed on culture media. These conidial features were considered valid criteria since they were used by Simmons (1969) to differentiate six species following examination of all available type specimens of known species. Because Simmons (1969) did not define his cultural conditions, in the present study all isolates were initially grown on laboratory PDA at 24C in darkness.

Under these conditions, 13 of the 48 isolates were tentatively identified as S. botryosum, 18 as S. vesicarium and 4 as S. globuliferum,

but in each instance conidial dimensions were less than those given by Simmons. Conidia of the remaining 13 isolates, while typical of the genus Stemphylium, in each instance were so variable in shape and dimensions that they could justifiably have been identified as either S. botryosum or S. vesicarium. These results implied that either the morphological criteria used as the basis for species delimitation were unsatisfactory, and/or that the cultural conditions did not enable sharp definition of each species.

It is now widely accepted that species delimitation within Hyphomycete genera must be based on distinctive morphological criteria. Further, there is acceptance of the fact that expression of morphological characters can be considerably modified by environmental factors, particularly nutrition (Harding, 1975; Kafi & Tarr, 1966; Rangaswami & Sambandam, 1960; Williams, 1959), temperature (Leach & Aragaki, 1970; Ruppel, 1974; Vargas & Wilcoxson, 1969; Williams, 1959), photoperiod (Harter, 1939; Snyder & Hansen, 1941b; Williams, 1959) and pH (Harding, 1975; Tarr & Kafi, 1968; Williams, 1959). Certainly with the genus Stemphylium a range of morphological criteria have been used in defining species (Baverman, 1968; Graham & Zeiders, 1960; Rotem, Cohen & Wahl, 1965; Simmons, 1969; Sobers & Seymour, 1963) but the relative suitability of each has neither been experimentally determined, nor has the influence of component environmental factors on these features been concurrently investigated for more than one species of the genus. Since the present study was primarily concerned with the total question of species delimitation, including a taxonomic survey of plant pathogenic stemphylia present in New Zealand, it was therefore first necessary to define:

(i) those morphological features least prone to environmentally induced variability, and therefore of greatest taxonomic value; and

(ii) the combination of environmental conditions under which these morphological features are best expressed.

Additionally, studies were undertaken of the ultrastructure of conidiogenesis, reversion of conidiophores and juvenile conidia to hyphal-like growth, and the mechanism of ascospore release in Pleospora.

Note

Throughout this thesis the imperfect binomial has been used when referring to isolates, although the perfect states are known. This procedure was adopted because it is the Stemphylium state which is most

commonly encountered in the field, and furthermore, the specific epithets for many Pleospora states genetically connected with Stemphylium are still undecided.

CHAPTER 1

TAXONOMY OF STEMPHYLIUM AND ASSOCIATED PLEOSPORA STATES

1.1 THE GENUS STEMPHYLIUM

1.1.1 EFFECT OF ENVIRONMENTAL FACTORS ON CULTURAL AND MORPHOLOGICAL FEATURES

The present study was primarily concerned with firstly identifying those features of Stemphylium that are relatively stable and sufficiently distinct amongst individuals to be of taxonomic value, and secondly, determining the environmental circumstances under which these diagnostic features are most clearly expressed. In order to achieve these two objectives a series of experiments were conducted examining the effect of growth media, incubation temperature, photoperiod and pH on the gross colony characters and morphological features of conidiophores and conidia.

MATERIALS AND METHODS

As previously stated, in a preliminary survey of New Zealand species of Stemphylium, 48 pathogenic isolates (Table 1) were tentatively categorised into four groups. Grouping was on the basis of shape, dimensions, number of lateral septal constrictions, surface ornamentation and pigmentation of mature conidia as expressed on laboratory PDA at 24C in darkness.

Conidial group 'A' (13 isolates) - Conidia subspherical to subdoliform; verrucose; one constriction; dark greyish-brown to greenish-grey; 16-31 x 12-22µ (av. 24.2 x 17.6µ). Corresponds to S. botryosum, as defined by Simmons (1969).

Conidial group 'B' (4 isolates) - Conidia subspherical to subdoliform; sparsely punctate; one constriction; pale olivaceous-grey to olivaceous-grey; 17-31 x 12-21µ (av. 24.4 x 17.2). Corresponds to S. globuliferum, as defined by Simmons (1969).

Conidial group 'C' (18 isolates) - conidia mainly oval to oblong, a few subspherical to subdoliform; verrucose; with

one to three constrictions; dark greyish-brown to greenish-grey; 23-42 x 12-19µ (av. 32.7 x 16.5µ).
Corresponds to S. vesicarium, as defined by Simmons (1969).

Conidial group 'D' (13 isolates) - conidia oval, oblong, subspherical or subdoliform; verrucose; one to three constrictions; dark greyish-brown to greenish-grey; 21-39 x 11-20µ (av. 31-4 x 17.0µ).
A decision as to the identify of these isolates could not be made; they could justifiably be identified as either S. botryosum or S. vesicarium.

Three representative monosporous isolates from each of the four conidial groups were randomly selected for further study.

The growth media examined were hay decoction agar (HDA), malt agar (MA), 20% V-8 juice agar (20% V-8), 10% V-8 juice agar (10% V-8), 5% V-8 juice agar (5% V-8), potato carrot agar (PCA), potato marmite agar (PMA), and laboratory potato dextrose agar (PDA). Details regarding the composition and preparation of the culture media are presented in Appendix 6.

The influence of six incubation temperatures namely, 12, 16, 20, 24, 27 and 30C on colony characters and morphological features of the 12 isolates was determined.

The importance of photoperiod was determined by comparing incubation in darkness with an 8h photoperiod.

The five pH levels examined were pH 6.0, 6.5, 7.0, 7.5 and 8.0.

In all experiments petri dishes were centrally inoculated with a 7 mm diameter mycelial plug taken from the edge of actively growing cultures. Gross colony characteristics and the intensity of sporulation were determined after seven days growth.

Because conidia at various stages of development differ considerably in shape, dimensions, septation, and pigmentation it was imperative that the morphology of only fully mature conidia be considered. This was achieved by the use of the so-called injury technique (Luttrell, 1963a), which induced synchronous sporulation and therefore enabled preparation of slides with spores at a similar stage of development. The method

TABLE 1. *Stemphylium* isolates, origin and conidial grouping

Host	Isolate	Conidial group *
Asparagus	MU 18	C
	MU 19	C
	MU 20	C
	MU 32	D
	MU 39	C
Carnation	MU 33	A
	MU 34	A
Chrysanthemum	MU 1	D
	MU 2	C
	MU 3	C
	MU 26	D
Lettuce	MU 4	A
	MU 10	A
	MU 11	A
	MU 12	A
	MU 25	A
Lucerne	MU 5	B
	MU 6	C
	MU 7	C
	MU 8	D
	MU 9	A
	MU 23	D
	MU 24	B
	MU 37	C
	MU 38	D
	MU 44	D
	MU 45	A
	MU 47	D
	MU 48	C
Blue lupin	MU 13	C
	MU 28	B
	MU 29	D
	MU 46	B
Tree lupin	MU 14	D
	MU 30	A
	MU 36	A
Russell lupin	MU 27	C
	MU 42	A
	MU 43	A
Onion	MU 17	C
	MU 21	C
	MU 22	C
	MU 40	C
	MU 41	C
Tomato	MU 15	D
	MU 16	C
	MU 31	D
	MU 35	D

* Conidial groups delimited on the basis of conidial features expressed on laboratory PDA at 24C in continuous dark.

involved cutting 1.5cm^2 plugs from the periphery of 5-day old colonies used in the determination of gross colony characters. These plugs were then transferred to the surface of water agar plates. Profuse sporulation occurred along the cut (injured) surface of the plugs, and when it was considered spores were mature (within 24h) slides of conidia and conidiophores were prepared for microscopic examination. With experience one acquired the facility for deciding when in fact conidia were mature.

All conidial dimensions were subjected to a statistical analysis for significance. Due to the great variation in the length of the conidiophores within an isolate in any one treatment a statistical analysis of conidiophore dimensions was not attempted.

The experiments were conducted in two series, as follows:

Series I

(a) The influence of media type.

The 12 isolates were grown on the eight media, pH 6.5, at 24C in darkness.

(b) The influence of the incubation temperature.

The 12 isolates were grown on PDA, pH 6.5, and incubated at the six temperatures in darkness.

(c) The influence of photoperiod.

The 12 isolates were grown on PDA, pH 6.5, at 24C, either in darkness or an 8h photoperiod.

(d) The influence of media pH.

The 12 isolates were grown on plates of PDA adjusted to the five pH levels. All cultures were incubated at 24C in darkness.

In these experiments it was found that the most satisfactory cultural conditions were 5% V-8 juice agar, pH 7.5, incubation temperature of 20C, and an 8h photoperiod.

Series II

In this series of experiments a specific environmental factor was examined by growing the 12 isolates at the optimum for the other three environmental factors, as follows:

(a) The influence of media type.

The 12 isolates were grown on the eight media each adjusted to pH 7.5. The cultures were incubated at 20C under an 8h

photoperiod.

(b) The influence of incubation temperature.

The 12 isolates were grown on 5% V-8 adjusted to pH 7.5, and incubated at the six temperatures under an 8h photoperiod.

(c) The influence of photoperiod.

The 12 isolates were grown on 5% V-8 (pH 7.5) at 20C. The plates were incubated in either darkness or in an 8h photoperiod.

(d) The influence of media pH.

The 12 isolates were grown on plates of 5% V-8 adjusted to the five pH levels. All cultures were incubated at 20C under an 8h photoperiod.

Results are presented for the four experiments of Series II only.

RESULTS

The effect of growth media, incubation temperature, photoperiod, and pH on conidial features and sporulation intensity are summarized in Tables 2-5, and the effects of these four factors on gross colony characters and conidiophore features are presented in Appendices 2-5.

These results reveal that only conidial features were of taxonomic value. Conidiophore features and gross colony characters, although relatively stable in some instances, were insufficiently distinct to enable separation of the four conidial groups. While sporulation intensity, was of no taxonomic assistance the information was of value in subsequent pathogenicity studies.

A. GROWTH MEDIA

(a) Conidial features

(i) Shape. On all media the shape of the great majority of conidia of conidial group 'A' (CGA) and conidial group 'B' (CGB) were subspherical to subdoliform, whereas those of conidial group 'C' (CGC) and conidial group 'D' (CGD) were usually oval to oblong. On PDA and PMA, CGD was exceptional in that approximately 20% of the spores were subspherical to subdoliform. These results applied irrespective of whether the conidia were juvenile or mature, and indicate that for all media, except PDA and PMA, conidial shape is a stable factor distinguishing

CGA and CGB from CGC and CGD.

(ii) Dimensions. Growth media significantly affected conidial length of all isolates (Table 2a-d). However, differences in conidial width were significant only for two isolates each of CGA and CGD, and one isolate of CGC. Conidia of CGA and CGB with the greatest length and width were produced on 5% V-8, and the smallest on PDA. In the case of CGC and CGD the longest conidia were produced on 5% V-8 and the broadest on PDA.

These results indicate that although growth medium will affect conidial dimensions, this morphological feature has value in that it distinguishes CGA and CGB from CGC and CGD. This contrast is most clearly expressed on 5% V-8.

(iii) Length/Width (L/W) Ratio. On all media CGA and CGB had a L/W ratio within the range of 1.4 to 1.6. With CGC and CGD the L/W ratio was greatest (approx. 2.2) on 5% V-8 and smallest (approx. 1.8) on PDA and PMA. That is, particularly on 5% V-8, the L/W ratio of conidia distinguishes CGA and CGB from CGC and CGD.

(iv) Septation. Irrespective of media type the number of longitudinal and lateral septa of all isolates of CGA and CGB was in the range of 1-3 x 3-4(-5). By contrast, the conidial septation of isolates of CGC and CGD were 1-2 x 3-6(-7) except on PDA and PMA where there were 1-2(-3) x 3-5. Thus on any of the test media, other than PDA and PMA, conidial septation distinguishes CGA and CGB from CGC and CGD.

(v) Surface Ornamentation. Growth media had no effect on the surface ornamentation of the conidia of any of the 12 isolates. The conidia of CGA, CGC and CGD were consistently verrucose whereas those of CGB were sparsely punctate. That is, surface ornamentation is a stable character readily distinguishing CGB from CGA, CGC and CGD.

(vi) Pigmentation. The conidia of CGA, CGC and CGD were generally more pigmented (greenish-grey to grey) on the richer media of PDA, PMA, 20% V-8, and 10% V-8, and less pigmented (greyish-brown) on DA, PCA, MA and 5% V-8. On all media conidial pigmentation of CGB was pale olivaceous-grey to pale olivaceous-brown and consistently less intense than those of the other 3 conidial groups. Thus irrespective of media type conidial pigmentation readily distinguishes CGB from CGA, CGC and CGD.

(vii) Number of Lateral Septal Constrictions. On all culture media conidia of CGA and CGB had a median lateral septal constriction,

whereas those of CGC and CGD were two or three constricted, with the exception that on PDA and PMA these latter two conidial groups (more particularly CGD) produced an appreciable number of conidia that were one-constricted. Thus the number of lateral septal constrictions produced by isolates on culture media other than PDA and PMA provides a further criterion for separating CGA and CGB from CGC and CGD.

(b) Intensity of sporulation

All isolates sporulated readily on all culture media but sporulation was most profuse on 20% V-8 and 10% V-8, and noticeably less intense on the other six media.

B. INCUBATION TEMPERATURE

(a) Conidial features (Figs. 1-4)

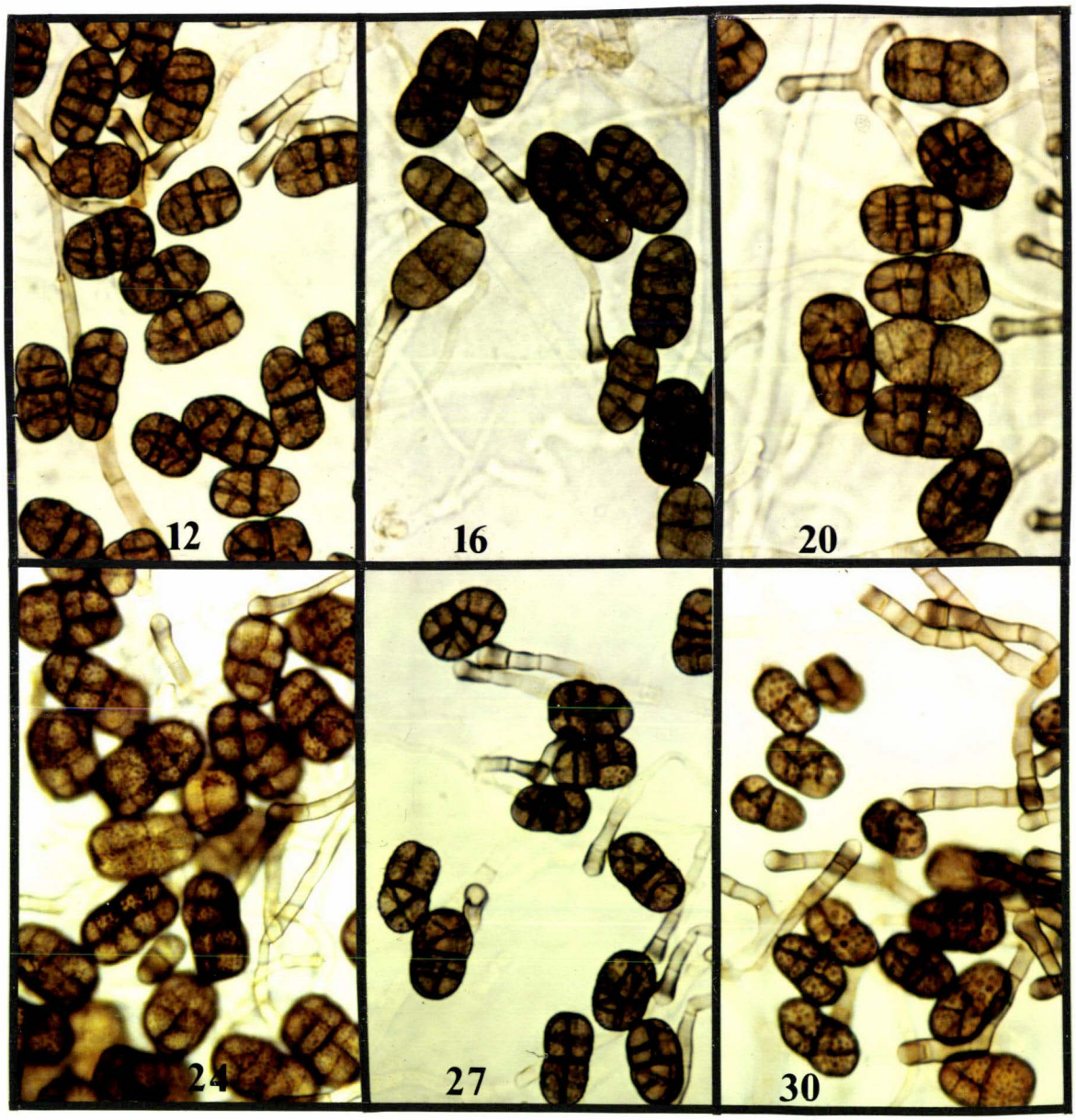
(i) Shape. Incubation temperature did not significantly affect the overall shape of conidia (Figs. 1-4). As in the culture media studies, both juvenile and mature spores of CGA and CGB were essentially subspherical to subdoliform and quite distinct from those of CGC and CGD, which were oval to oblong.

(ii) Dimensions. Incubation temperature significantly affected conidial length and width of all isolates (Tables 3a-d). In CGA and CGB the largest conidia were produced at 20C, the length and width decreasing as the temperature increased or decreased. In CGC and CGD the longest conidia were produced at 20C and the broadest at 12C. Conidial length of these isolates decreased as the temperature increased or decreased from 20C, and the width decreased only as the temperature increased beyond 12C. Thus although conidial dimensions were an unstable feature they were of taxonomic value in distinguishing the relatively long and narrow conidia of CGC and CGD from the almost spherical conidia of CGA and CGB. This contrast was most clearly expressed at 20C.

(iii) L/W Ratio. Temperature had relatively little effect on L/W ratio of conidia. The range for isolates of CGA and CGB was 1.3-1.6, and 1.8-2.2 for CGC and CGD. Because the L/W ratio is a relatively stable feature it is therefore useful for distinguishing CGA and CGB from CGC and CGD.

(iv) Septation. Incubation temperature had an appreciable effect on the number of longitudinal and lateral septa of all isolates and, as would be expected, this was positively correlated with conidial size. Since this applied to isolates of all four conidial groups,

Fig. 1. Effect of incubation temperatures (12, 16, 20, 24, 27 and 30C), on conidial morphology of isolate MU 9, conidial group 'A' (= *Stemphylium botryosum*). The isolate was grown on 5% V-8, pH 7.5, in an 8h photoperiod (x550).


12
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27
30

Fig. 2. Effect of incubation temperatures (12, 16, 20, 24, 27 and 30C), on conidial morphology of isolate MU 24, conidial group 'B' (= Stemphylium globuliferum). The isolate was grown on 5% V-8, pH 7.5, in an 8h photoperiod (x550).

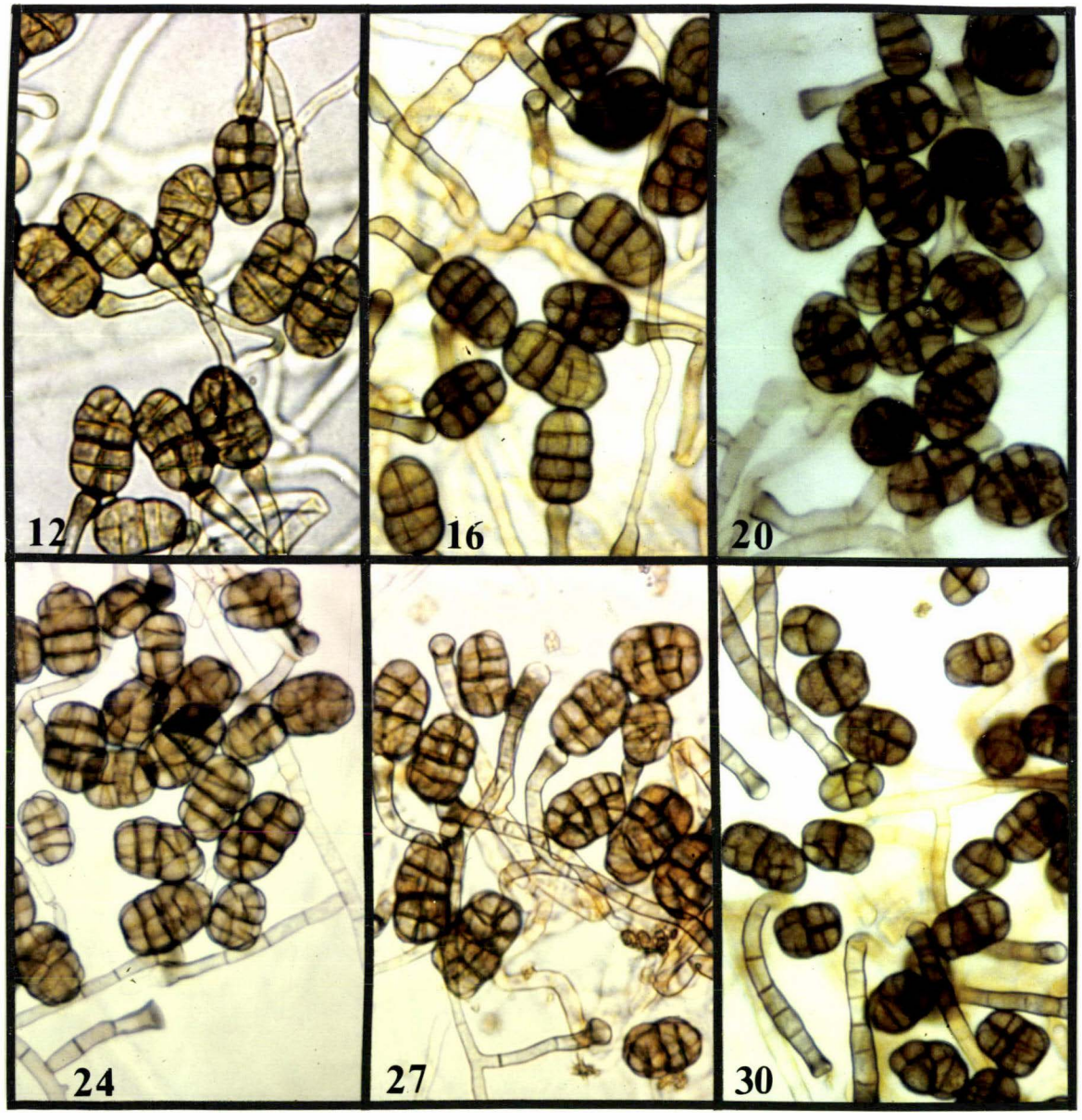
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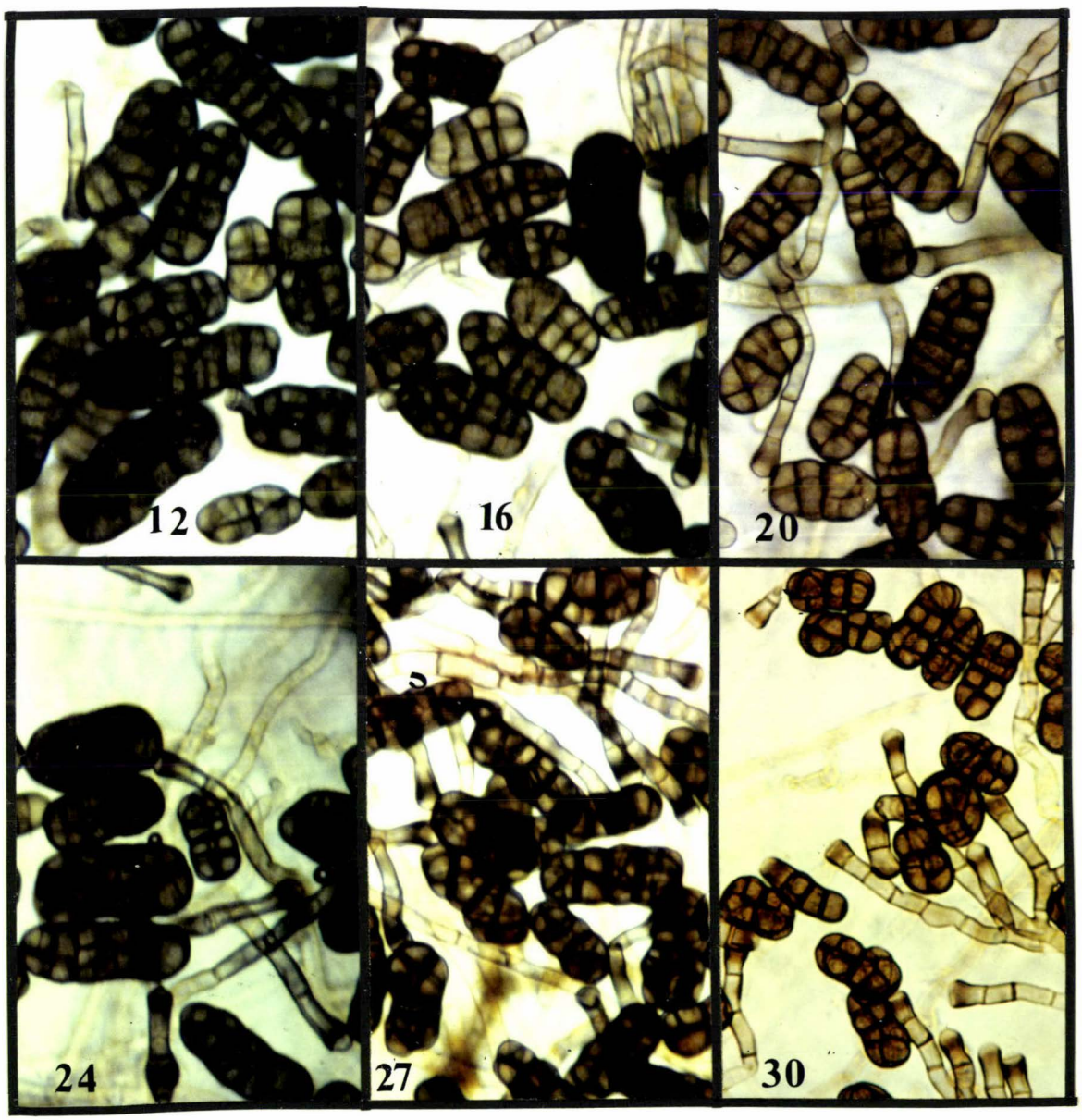
Fig. 3. Effect of incubation temperatures (12, 16, 20, 24, 27 and 30C), on conidial morphology of isolate MU 2, conidial group 'C' (= Stemphylium vesicarium). The isolate was grown on 5% V-8, pH 7.5, in an 8h photoperiod (x550).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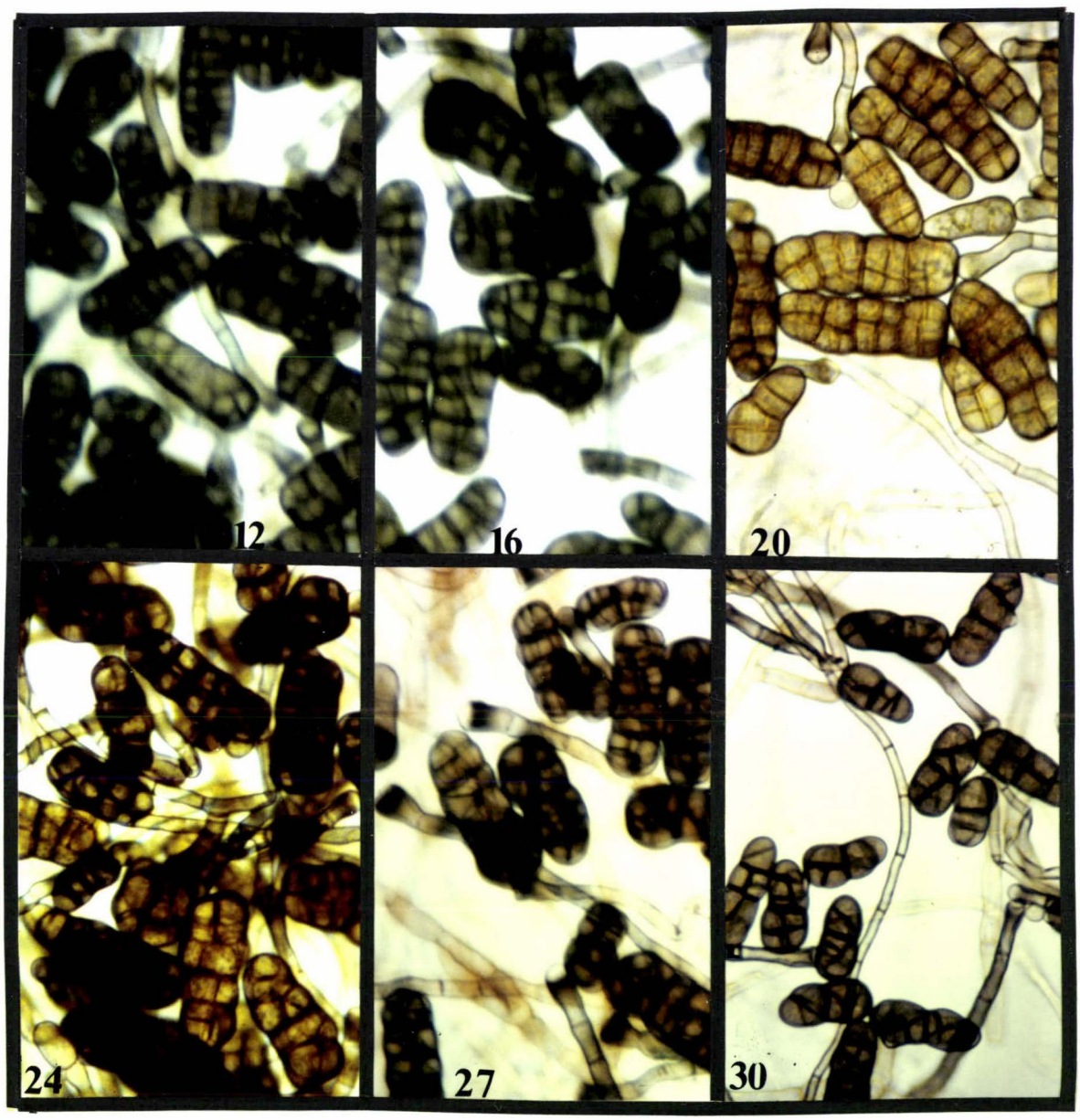
30

Fig. 4. Effect of incubation temperatures (12, 16, 20, 24, 27 and 30C), on conidial morphology of isolate MU 14, conidial group 'D'. The isolate was grown on 5% V-8, pH 7.5, in an 8h photoperiod (x550).


12
16
20
24
27
30

septation in itself was strictly secondary to conidial shape and size as a taxonomic criterion. The number of longitudinal and lateral septa for CGA and CGB at the different temperatures was as follows: 12, 16, 20, 24C (1-3 x 3-4(-5)), 27C (1-3 x 2-3(-4)), 30C (1-2 x 1-3); whilst for CGC and CGD the septation was: 12C (1-2 x 3-6), 16, 20, 24C (1-2 x 3-6(-7)), 27C (1-2 x 3-4(-5)), 30C (1 x 1-3).

(v) Surface Ornamentation. As in the case of culture media, incubation temperature had no effect on the surface ornamentation of conidia. Without exception conidia of CGA, CGC and CGD were verrucose, whereas those of CGB were sparsely punctate.

(vi) Pigmentation. Conidia of all four conidial groups were generally less pigmented at 12, 16 and 20C (brown to greyish-brown for CGA, CGC and CGD and pale olivaceous-brown to pale olivaceous-grey for CGB), than at 24, 27 and 30C (grey to dark greyish-brown for CGA, CGC and CGD, and olivaceous-grey for CGB). Thus at any one temperature the paler conidia of CGB could be readily distinguished from the darker coloured spores of CGA, CGC and CGD.

(vii) Number of Lateral Septal Constrictions. Temperature had no effect on the number of lateral septal constrictions of conidia. At all temperatures the conidia of CGA and CGB only had a median lateral septal constriction, and were readily distinguishable from the predominantly two to three constricted spores of CGC and CGD.

(b) Intensity of sporulation

Conidial production of all isolates was markedly reduced at the temperature limits tested (12C to 30C). At the other four temperatures (16, 20, 24 and 27C) sporulation was abundant to profuse.

C. PHOTOPERIOD

Photoperiod had no appreciable effect on conidial features of any of the 12 isolates, except that incubation in darkness induced both intensification of pigmentation and some conidial distortion (Tables 4a-d). All isolates consistently sporulated better when incubated in an 8h photoperiod than in conditions of continuous darkness.

D. MEDIA pH

The pH of the growth medium had no appreciable effect on conidial features of the 12 isolates (Tables 5a-d). At all pH levels sporulation of all isolates was abundant.

KEY TO TABLES 2-5

Colour

Bn = Brown
Gr = Grey
OGr = Olivaceous-grey
POGr = Pale Olivaceous-grey
POBn = Pale Olivaceous-brown
GrBn = Greyish-brown
GnGr = Greenish-grey
DGrBn = Dark greyish-brown

Abbreviations

Juv. = Juvenile
Mat. = Mature
Long. = Longitudinal
Lat. = Lateral

Ornamentation

Verr = Verrucose
Punc = Punctate

Shape

Ss = Subspherical
Sd = Subdoliform
Ov = Oval
Ob = Oblong

Sporulation Intensity

0 = None
1 = Poor
2 = Moderate
3 = Abundant
4 = Profuse

TABLE 2a Effect of growth media on conidial features and intensity of sporulation of three *Stemphylium* isolates* of conidial group 'A'

Medium	CONIDIAL FEATURES									
Isolate MU 9	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		Sporulation Intensity
	Length	Width					Juv.	Mat.	Long.	Lat.	
HDA	28.4(23.9-34.4)	19.6(15.5-21.9)	1.45	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
MA	29.5(24.0-35.1)	20.3(15.6-23.4)	1.45	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
20% V-8	29.0(23.2-33.8)	19.8(15.2-22.4)	1.46	GnGr/Gr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	4
10% V-8	29.1(23.5-34.1)	20.2(15.9-22.8)	1.44	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	4
5% V-8	29.6(24.3-34.7)	20.4(15.5-23.2)	1.45	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PCA	28.9(22.7-33.4)	19.5(14.9-22.4)	1.48	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PMA	26.6(21.6-32.1)	18.9(14.3-22.6)	1.41	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PDA	25.0(20.2-31.7)	18.3(14.1-22.0)	1.37	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	2
LSD 5%	1.59	1.09									
Isolate MU 11											
HDA	28.9(21.2-34.1)	18.1(14.0-21.8)	1.60	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
MA	27.3(21.7-33.4)	18.6(14.4-22.2)	1.47	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
20% V-8	26.5(18.2-30.6)	19.3(15.6-24.2)	1.37	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	4
10% V-8	28.6(20.8-34.1)	19.0(15.0-23.1)	1.51	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	4
5% V-8	29.9(22.1-34.9)	18.7(14.5-22.4)	1.60	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PCA	27.6(18.2-32.7)	18.3(13.3-21.4)	1.51	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PMA	25.2(17.8-31.8)	17.8(13.1-21.5)	1.42	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PDA	24.8(16.8-31.2)	17.6(13.8-21.2)	1.41	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
LSD 5%	1.86	0.99									
Isolate MU 33											
HDA	27.8(22.4-35.9)	18.1(15.1-21.6)	1.54	Bn/GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
MA	28.4(22.6-36.2)	18.5(15.3-22.1)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
20% V-8	28.2(23.3-36.0)	18.8(15.6-22.4)	1.50	GnGr/Gr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	4
10% V-8	28.6(23.8-35.9)	18.6(15.1-22.5)	1.54	GnGr/Gr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	4
5% V-8	28.9(24.0-36.5)	18.8(15.4-22.7)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PCA	27.6(23.2-35.3)	17.9(14.9-21.2)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PMA	24.8(19.9-31.3)	17.6(14.6-20.9)	1.41	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
PDA	24.4(19.4-30.1)	17.4(14.2-21.1)	1.40	GnGr	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
LSD 5%	1.55	N.S.									

* pH 7.5, incubated at 20C in an 8h photoperiod.

TABLE 2b Effect of growth media on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'B'

Medium	CONIDIAL FEATURES									Sporulation
Isolate MU 5	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape	Septations		Intensity
	Length	Width					Juv. Mat.	Long.	Lat.	
HDA	25.6(19.6-32.7)	18.2(15.3-23.1)	1.41	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
MA	26.8(22.2-33.6)	18.3(14.8-21.8)	1.46	POBn	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
20% V-8	26.4(20.2-32.2)	18.5(13.6-22.7)	1.43	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	4
10% V-8	26.9(21.8-33.7)	18.3(13.3-22.4)	1.47	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	4
5% V-8	27.2(22.6-34.1)	18.4(13.9-22.8)	1.48	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PCA	26.7(20.0-31.9)	18.2(14.0-21.2)	1.47	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PMA	24.8(20.7-32.8)	17.8(14.1-21.6)	1.39	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PDA	23.9(19.8-30.6)	17.6(13.6-21.4)	1.36	POGr	Punc	1	Ss/Sd Ss/Sd	1-2(-3)	3-4 (-5)	2
LSD 5%	1.69	N.S. N.S.								
Isolate MU 24										
HDA	25.8(19.9-33.1)	18.0(14.9-22.6)	1.43	POBn	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
MA	26.4(20.8-34.2)	18.2(14.4-23.0)	1.45	POBn	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
20% V-8	26.1(21.1-33.3)	18.4(14.6-22.9)	1.42	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	4
10% V-8	27.3(22.2-34.3)	18.5(14.3-23.1)	1.48	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	4
5% V-8	27.8(21.9-34.7)	18.6(14.1-23.4)	1.49	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PCA	26.3(20.6-34.1)	18.1(14.0-21.9)	1.45	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PMA	25.2(20.9-31.7)	18.1(14.2-21.6)	1.39	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PDA	24.8(20.0-30.9)	17.8(13.8-21.4)	1.39	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
LSD 5%	1.51	N.S.								
Isolate MU 28										
HDA	27.4(20.0-33.1)	17.7(14.4-21.7)	1.55	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
MA	27.8(21.3-34.2)	17.5(14.0-21.5)	1.59	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
20% V-8	26.9(21.1-33.4)	17.7(13.9-21.7)	1.52	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	4
10% V-8	27.3(20.9-34.3)	17.8(14.0-22.2)	1.53	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	4
5% V-8	28.2(21.4-34.8)	17.8(14.2-22.0)	1.58	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PCA	27.6(20.3-33.9)	17.6(14.4-21.9)	1.57	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PMA	25.1(19.4-31.9)	17.2(13.6-21.5)	1.46	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
PDA	24.8(18.8-31.2)	17.2(13.8-21.4)	1.44	POGr	Punc	1	Ss/Sd Ss/Sd	1-3	3-4 (-5)	3
LSD 5%	0.94	N.S.								

* pH 7.5, incubated at 20C in an 8h photoperiod.

TABLE 2c Effect of growth media on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'C'

Medium	CONIDIAL FEATURES										Sporulation
Isolate MU 2	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		Intensity
	Length	Width					Juv.	Mat.	Long.	Lat.	
HDA	36.2(24.6-48.2)	16.5(12.8 20.4)	2.19	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
MA	34.6(25.4-46.6)	17.1(13.1-21.0)	2.02	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
20% V-8	36.4(24.8-47.9)	17.3(13.6-21.7)	2.10	GnGr/Gr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
10% V-8	36.7(25.8-48.4)	17.0(13.4-20.9)	2.16	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
5% V-8	37.8(26.6-51.5)	16.8(13.1-20.2)	2.25	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
PCA	36.6(25.4-46.7)	16.6(12.8-19.8)	2.20	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
PMA	33.9(23.9-43.1)	18.0(14.3-22.4)	1.88	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5(-7)	3
PDA	33.4(23.6-42.6)	18.1(14.0-22.9)	1.85	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5(-7)	3
LSD 5%	3.00	N.S.									
Isolate MU 19											
HDA	35.4(24.8-41.6)	16.6(13.2-20.5)	2.13	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
MA	36.0(28.1-43.9)	16.8(12.8-20.2)	2.14	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
20% V-8	34.6(26.9-43.0)	17.3(14.3-21.1)	2.00	GnGr/Gr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
10% V-8	35.8(27.6-43.6)	16.9(13.9-20.7)	2.12	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
5% V-8	36.2(28.4-44.2)	16.8(14.0-20.8)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
PCA	35.7(26.4-43.5)	16.6(13.4-19.9)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
PMA	33.8(24.8-41.7)	17.8(13.8-22.4)	1.89	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5(-7)	3
PDA	33.0(24.2-40.1)	18.0(14.3-22.7)	1.83	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5(-7)	3
LSD 5%	2.70	N.S.									
Isolate MU 40											
HDA	35.5(28.1-44.9)	16.6(12.9-19.6)	2.14	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
MA	36.8(28.4-47.0)	16.9(13.3-20.6)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
20% V-8	36.0(28.0-45.4)	17.2(14.1-21.1)	2.09	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
10% V-8	36.9(27.4-46.7)	17.0(13.9-20.9)	2.17	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
5% V-8	36.9(28.7-47.5)	16.9(13.7-20.2)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
PCA	35.7(27.7-45.8)	16.7(13.1-19.8)	2.14	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
PMA	33.4(25.4-42.2)	17.9(14.0-22.4)	1.87	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5(-7)	3
PDA	33.3(25.7-41.7)	17.6(14.2-21.8)	1.89	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5(-7)	2
LSD 5%	1.02	1.03									

* pH 7.5, incubated at 20C in an 8h photoperiod

TABLE 2d Effect of growth media on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'D'

Medium	CONIDIAL FEATURES										Sporulation Intensity
Isolate MU 14	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		
	Length	Width					Juv.	Mat.	Long.	Lat.	
HDA	36.0(27.7-44.0)	16.5(14.8-20.4)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
MA	34.9(27.5-41.8)	17.1(13.2-20.6)	2.04	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
20% V-8	33.8(28.5-44.2)	17.6(14.4-21.4)	1.92	GnGr/Gr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
10% V-8	35.7(28.7-43.6)	17.1(14.0-20.8)	2.09	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
5% V-8	36.9(28.4-44.5)	16.6(13.8-20.1)	2.22	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
PCA	35.2(22.0-42.2)	16.4(14.3-20.4)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
PMA	32.4(21.0-40.8)	18.0(13.2-21.7)	1.80	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5 (-7)	3
PDA	31.9(23.2-39.6)	18.4(14.1-22.2)	1.73	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5 (-7)	3
LSD 5%	2.45	1.1									
Isolate MU 23											
HDA	34.7(24.8-43.7)	16.4(13.3-20.3)	2.12	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
MA	33.1(27.5-41.5)	16.7(12.8-20.6)	1.98	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
20% V-8	35.1(26.4-44.1)	17.0(14.4-21.1)	2.06	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
10% V-8	34.8(28.7-44.7)	17.1(14.1-21.6)	2.04	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
5% V-8	35.7(25.6-45.2)	16.8(13.6-20.2)	2.13	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
PCA	34.8(22.4-42.4)	16.4(14.3-20.4)	2.12	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
PMA	32.4(22.6-39.1)	18.2(14.4-22.2)	1.78	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5 (-7)	3
PDA	32.1(22.2-40.2)	18.2(14.1-22.6)	1.76	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-3	3-5 (-7)	3
LSD 5%	2.45	1.3									
Isolate MU 29											
HDA	34.5(22.2-41.9)	16.5(12.4-19.3)	2.09	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
MA	33.3(22.6-40.1)	16.7(13.2-19.8)	1.99	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
20% V-8	34.4(23.6-41.7)	16.9(13.0-19.6)	2.04	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
10% V-8	34.6(22.8-42.2)	17.1(12.8-19.8)	2.02	GnGr	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
5% V-8	35.0(23.8-42.9)	16.7(12.7-19.6)	2.10	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
PCA	34.3(23.0-41.8)	16.5(12.6-19.4)	2.08	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
PMA	31.5(22.4-40.4)	17.3(13.3-21.9)	1.82	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-2(-3)	3-5 (-7)	3
PDA	31.8(22.7-39.9)	17.8(13.8-22.6)	1.79	GnGr	Verr	1-3	Ov/Ob/Ss	Ov/Ob/Ss	1-3	3-5 (-7)	3
LSD 5%	2.42	N.S.									

* pH 7.5, incubated at 20C in an 8h photoperiod.

TABLE 3a Effect of incubation temperature on conidial features and intensity of sporulation of three *Stemphylium* isolates* of conidial group 'A'

Temperature (°C)	CONIDIAL FEATURES								Sporulation Intensity
	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape	Septations	
Isolate MU 9	Length	Width					Juv. Mat.	Long. Lat.	
12	27.2(23.9-32.2)	20.0(16.3-23.9)	1.36	Bn/GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	2
16	28.9(24.1-32.6)	20.2(15.7-22.8)	1.43	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
20	29.6(24.3-34.7)	20.4(15.5-23.2)	1.45	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
24	26.7(20.4-32.3)	18.3(14.8-22.3)	1.46	DGrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	4
27	25.9(20.1-30.0)	17.4(14.5-21.4)	1.49	DGrBn	Verr	1	Ss/Sd Ss/Sd	1-2 2-3(-4)	4
30	18.9(17.0-20.6)	14.4(11.1-16.8)	1.31	Gr	Verr	1	Ss/Sd Ss/Sd	1-2 1-3	1
LSD 5%	1.40	0.60							
Isolate MU 11									
12	28.6(21.2-32.8)	18.5(15.1-21.8)	1.55	Bn/GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	2
16	29.2(21.6-34.7)	18.7(14.7-22.1)	1.56	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
20	29.9(22.1-34.9)	18.7(14.5-22.4)	1.60	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
24	24.7(21.6-31.3)	17.6(14.0-21.2)	1.40	DGrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	4
27	23.7(20.6-28.7)	15.5(13.8-18.3)	1.53	DGrBn	Verr	1	Ss/Sd Ss/Sd	1-2 2-3(-4)	4
30	22.8(19.4-26.4)	14.8(13.3-17.9)	1.54	Gr	Verr	1	Ss/Sd Ss/Sd	1-2 1-3	1
LSD 5%	1.50	0.98							
Isolate MU 33									
12	26.8(22.7-33.1)	18.4(15.0-21.9)	1.46	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	1
16	28.3(24.3-35.8)	18.6(15.6-22.2)	1.52	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
20	28.9(24.0-36.5)	18.8(15.4-22.7)	1.54	GrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
24	27.4(23.7-35.2)	18.1(14.7-21.0)	1.51	DGrBn	Verr	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	4
27	26.5(20.4-32.7)	16.9(13.7-19.8)	1.57	DGrBn	Verr	1	Ss/Sd Ss/Sd	1-2 2-3(-4)	4
30	18.6(15.6-24.4)	14.0(11.4-16.6)	1.33	Gr	Verr	1	Ss/Sd Ss/Sd	1-2 1-3	1
LSD 5%	1.58	1.02							

* Grown on 5% V-8, pH 7.5 and in an 8h photoperiod.

TABLE 3b Effect of incubation temperature on conidial features and intensity of sporulation of three *Stemphylium* isolates* of conidial group 'B'

Temperature (°C) / Isolate MU 5	CONIDIAL FEATURES								Sporulation Intensity
	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape	Septations	
	Length	Width					Juv. Mat.	Long. Lat.	
12	25.5(20.1-28.4)	17.9(12.8-20.6)	1.42	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	1
16	26.7(21.6-31.4)	18.0(13.6-21.4)	1.48	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
20	27.2(22.6-34.1)	18.4(13.9-22.8)	1.48	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
24	24.9(20.5-32.5)	17.6(13.4-21.7)	1.42	OGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	4
27	24.7(20.6-29.5)	16.7(13.2-20.2)	1.48	OGr	Punc	1	Ss/Sd Ss/Sd	1-2 2-3(-4)	4
30	19.4(15.0-22.2)	13.9(12.3-17.5)	1.40	OGr	Punc	1	Ss/Sd Ss/Sd	1-2 1-3	1
LSD 5%	1.42	0.94							
Isolate MU 24									
12	25.9(19.8-29.2)	18.0(13.6-21.8)	1.44	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	2
16	27.1(21.4-32.6)	18.3(14.3-22.6)	1.48	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
20	27.8(21.9-34.7)	18.6(14.1-23.4)	1.49	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
24	26.4(20.3-33.1)	18.1(13.8-22.2)	1.46	OGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	4
27	25.2(19.6-30.1)	16.5(13.2-20.4)	1.53	OGr	Punc	1	Ss/Sd Ss/Sd	1-2 2-3(-4)	4
30	19.8(15.4-23.4)	13.6(12.2-17.2)	1.46	OGr	Punc	1	Ss/Sd Ss/Sd	1-2 1-3	1
LSD 5%	1.52	1.18							
Isolate MU 28									
12	26.7(20.4-32.1)	17.6(13.4-21.3)	1.52	POBn	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	1
16	27.9(20.9-33.6)	17.9(13.9-21.9)	1.56	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
20	28.2(21.4-34.8)	17.8(14.2-22.0)	1.58	POGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	3
24	26.2(20.7-33.2)	17.4(13.6-21.7)	1.51	OGr	Punc	1	Ss/Sd Ss/Sd	1-3 3-4(-5)	4
27	24.3(19.7-30.1)	16.8(12.4-20.0)	1.45	OGr	Punc	1	Ss/Sd Ss/Sd	1-2 2-3(-4)	4
30	18.8(14.3-21.9)	13.1(11.9-16.9)	1.44	OGr	Punc	1	Ss/Sd Ss/Sd	1-2 1-3	1
LSD 5%	1.57	0.74							

* Grown on 5% V-8, pH 7.5 and in an 8h photoperiod.

TABLE 3c Effect of incubation temperature on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'C'

Temperature (°C)	CONIDIAL FEATURES									Sporulation Intensity
	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		
Isolate MU 2	Length	Width					Juv.	Mat.	Long.	Lat.	
12	34.9(25.5-43.8)	17.2(15.0-21.0)	2.03	Bn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	2
16	36.5(26.1-45.7)	17.0(14.2-20.9)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
20	37.8(26.6-51.5)	16.8(13.1-20.2)	2.25	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
24	34.2(23.4-44.6)	16.2(12.8-19.7)	2.11	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
27	30.7(21.5-36.5)	15.5(11.2-18.5)	1.98	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-4 (-5)	4
30	23.2(20.4-25.5)	11.1(8.9-13.4)	2.09	Gr	Verr	2-3	Ov/Ob	Ov/Ob	1	1-3	1
LSD 5%	3.58	1.12									
Isolate MU 19											
12	34.4(26.5-42.5)	17.3(15.5-21.4)	1.99	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	2
16	35.8(27.1-43.8)	17.1(14.9-21.2)	2.09	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
20	36.2(28.4-44.2)	16.8(14.0-20.8)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
24	34.4(27.0-42.4)	16.6(13.2-20.6)	2.07	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
27	33.1(27.4-38.6)	15.6(13.0-18.8)	2.12	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-4 (-5)	4
30	22.5(19.2-24.8)	11.8(10.4-14.0)	1.91	Gr	Verr	2-3	Ov/Ob	Ov/Ob	1	1-3	1
LSD 5%	2.06	0.96									
Isolate MU 40											
12	34.6(25.0-43.2)	17.6(14.8-20.9)	1.97	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	2
16	36.1(26.4-46.7)	17.3(14.4-20.4)	2.09	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
20	36.9(28.7-47.5)	16.9(13.7-20.2)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
24	35.1(27.6-45.4)	16.4(13.3-19.1)	2.14	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	4
27	30.9(22.8-38.7)	15.8(12.9-18.4)	1.96	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-4 (-5)	4
30	23.7(19.8-26.2)	11.4(9.8-14.1)	2.08	Gr	Verr	2-3	Ov/Ob	Ov/Ob	1	1-3	1
LSD 5%	2.5	0.76									

* Grown on 5% V-8, pH 7.5 and in an 8h photoperiod.

TABLE 3d Effect of incubation temperature on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'D'

Temperature (°C)	CONIDIAL FEATURES									Sporulation Intensity
	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		
Isolate MU 14	Length	Width					Juv.	Mat.	Long.	Lat.	
12	34.2(25.4-42.0)	17.2(15.7-21.2)	1.99	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	2
16	36.3(26.7-44.1)	16.8(14.2-20.4)	2.16	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
20	36.9(28.4-44.5)	16.6(13.8-20.1)	2.22	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
24	32.9(25.5-43.7)	16.2(13.1-19.8)	2.03	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
27	31.6(24.3-37.4)	15.1(12.7-18.1)	2.09	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-4(-5)	4
30	23.1(17.5-26.9)	12.5(10.5-14.2)	1.85	Gr	Verr	2-3	Ov/Ob	Ov/Ob	1	1-3	1
LSD 5%	2.20	0.80									
Isolate MU 23											
12	34.0(24.1-42.6)	17.4(14.8-21.3)	1.95	Bn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	2
16	34.8(25.8-43.4)	17.2(14.1-20.8)	2.02	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
20	35.7(25.6-45.2)	16.8(13.6-20.2)	2.13	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
24	33.3(24.0-41.4)	16.5(13.3-19.5)	2.02	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
27	31.9(22.4-38.4)	15.6(12.2-18.8)	2.05	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-4(-5)	4
30	24.7(21.8-29.0)	12.7(10.0-15.6)	1.94	Gr	Verr	2-3	Ov/Ob	Ov/Ob	1	1-3	1
LSD 5%	2.21	1.05									
Isolate MU 29											
12	33.8(20.9-41.4)	17.6(13.1-20.8)	1.92	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	2
16	34.6(23.4-42.2)	17.1(13.2-20.4)	2.02	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
20	35.0(23.8-42.9)	16.7(12.7-19.6)	2.10	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
24	33.0(22.2-40.7)	16.5(12.4-19.3)	2.00	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	4
27	31.2(21.7-36.9)	15.8(12.1-18.6)	1.97	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-4(-5)	3
30	24.1(19.6-28.4)	12.6(9.8-14.7)	1.91	Gr	Verr	2-3	Ov/Ob	Ov/Ob	1	1-3	1
LSD 5%	2.41	1.01									

* Grown on 5% V-8, pH 7.5 and in an 8h photoperiod.

TABLE 4a Effect of photoperiod on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'A'

Photoperiod	CONIDIAL FEATURES									Sporulation Intensity
Isolate MU 9	Dimensions‡ (µ) Length	Dimensions‡ (µ) Width	L/W Ratio	Colour	Ornamentation	Constrictions	Shape Juv.	Shape Mat.	Septations Long.	Septations Lat.	
8h photoperiod	29.6(24.3-34.7)	20.4(15.5-23.2)	1.45	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
Continuous dark	29.4(23.1-33.9)	20.1(14.7-22.9)	1.46	DGrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	1
Isolate MU 11											
8h photoperiod	29.9(22.1-34.9)	18.7(14.5-22.4)	1.60	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
Continuous dark	29.2(22.6-33.4)	18.9(14.2-23.0)	1.59	DGrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	1
Isolate MU 33											
8h photoperiod	28.9(24.0-36.5)	18.8(15.4-22.7)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
Continuous dark	28.3(23.2-36.0)	18.5(14.9-22.2)	1.53	DGrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	1

* Grown on 5% V-8, pH 7.5 and incubated at 20C.

‡ Statistical analysis using T test show no significant differences in length and width between treatments for any of the three isolates.

TABLE 4b Effect of photoperiod on conidial features and intensity of sporulation of three Stemphylium isolates* on conidial group 'B'

Photoperiod	CONIDIAL FEATURES									Sporulation Intensity
Isolate MU 5	Dimensions‡ (u)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		
	Length	Width					Juv.	Mat.	Long.	Lat.	
8h photoperiod	27.2(22.6-34.1)	18.4(13.9-22.8)	1.48	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
Continuous dark	26.8(21.8-33.9)	18.5(13.5-23.1)	1.45	OGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	1
Isolate MU 24											
8h photoperiod	27.8(21.9-34.7)	18.6(14.1-23.4)	1.49	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
Continuous dark	27.3(22.1-34.0)	18.3(13.7-22.9)	1.49	OGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	1
Isolate MU 28											
8h photoperiod	26.2(21.4-34.8)	17.8(14..-22.0)	1.58	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
Continuous dark	28.5(21.0-35.2)	18.1(14.8-22.8)	1.57	OGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	1

* Grown on 5% V-8, pH 7.5 and incubated at 20C.

‡ Statistical analysis using T test show no significant differences in length and width between treatments for any of the three isolates.

TABLE 4c Effect of photoperiod on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'C'

Photoperiod	CONIDIAL FEATURES									Sporulation Intensity
Isolate MU 2	Dimensions‡ (μ) Length	Dimensions‡ (μ) Width	L/W Ratio	Colour	Ornamentation	Constrictions	Shape Juv.	Shape Mat.	Septations Long.	Septations Lat.	
8h photoperiod	37.8(26.6-51.5)	16.8(13.1-20.2)	2.25	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
Continuous dark	37.3(25.8-50.8)	16.7(13.3-19.8)	2.23	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	1
Isolate MU 19											
8h photoperiod	36.2(28.4-44.2)	16.8(14.0-20.8)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
Continuous dark	35.8(27.9-43.7)	16.6(13.7-20.2)	2.16	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	1
Isolate MU 40											
8h photoperiod	36.9(28.7-47.5)	16.9(13.7-20.2)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
Continuous dark	37.1(28.4-48.2)	17.0(13.4-20.8)	2.18	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	1

* Grown on 5% V-8, pH 7.5 and incubated at 20C.

‡ Statistical analysis using T test show no significant differences in length and width between treatments for any of the three isolates.

TABLE 4d Effect of photoperiod on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'D'

Photoperiod	CONIDIAL FEATURES									Sporulation Intensity
Isolate MU 14	Dimensions‡ (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations	
	Length	Width					Juv.	Mat.	Long.	Lat.
8h photoperiod	36.9(28.4-44.5)	16.6(13.8-20.1)	2.22	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
Continuous dark	36.3(27.8-43.9)	16.6(13.3-20.4)	2.19	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	1
Isolate MU 23										
8h photoperiod	35.7(25.6-45.2)	16.8(13.6-20.2)	2.13	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
Continuous dark	36.1(25.9-46.1)	17.0(13.4-20.9)	2.22	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	1
Isolate MU 29										
8h photoperiod	35.0(23.8-42.9)	16.7(12.7-19.6)	2.10	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
Continuous dark	34.7(23.4-42.2)	16.5(12.2-19.4)	2.10	DGrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	1

* Grown on 5% V-8, pH 7.5 and incubated at 20C.

‡ Statistical analysis using T test show no significant differences in length and width between treatments for any of the three isolates.

TABLE 5a Effect of pH on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'A'

pH	CONIDIAL FEATURES									Sporulation
Isolate MU 9	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		Intensity
	Length	Width					Juv.	Mat.	Long.	Lat.	
6.0	28.9(23.4-33.9)	19.8(14.8-23.1)	1.46	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
6.5	29.4(24.1-34.6)	20.2(15.7-22.8)	1.46	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
7.0	30.1(23.8-35.0)	20.7(16.1-23.5)	1.45	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
7.5	29.6(24.3-34.7)	20.4(15.5-23.2)	1.45	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
8.0	29.3(23.7-34.0)	20.1(15.1-23.0)	1.46	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
LSD 5%	N.S.	N.S.									
Isolate MU 11											
6.0	28.8(22.0-33.9)	17.9(13.6-21.4)	1.61	Bn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
6.5	29.2(21.8-34.1)	18.1(13.8-21.7)	1.61	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
7.0	29.4(22.3-34.2)	18.5(14.6-22.1)	1.59	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
7.5	29.9(22.1-34.9)	18.7(14.5-22.4)	1.60	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
8.0	28.5(22.4-33.7)	18.3(14.1-21.9)	1.56	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
LSD 5%	N.S.	N.S.									
Isolate MU 33											
6.0	27.6(22.8-34.7)	17.8(14.2-21.4)	1.55	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	2
6.5	28.1(23.2-36.0)	18.2(14.7-21.8)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
7.0	28.6(23.4-35.9)	18.5(15.0-22.1)	1.55	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
7.5	28.9(24.0-36.5)	18.8(15.4-22.7)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
8.0	28.4(23.7-35.4)	18.4(15.1-22.3)	1.54	GrBn	Verr	1	Ss/Sd	Ss/Sd	1-3	3-4 (-5)	3
LSD 5%	N.S.	N.S.									

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

TABLE 5b Effect of pH on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'B'

pH	CONIDIAL FEATURES									
Isolate MU 5	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		Sporulation Intensity
	Length	Width					Juv.	Mat.	Long.	Lat.	
6.0	26.4(21.3-32.9)	17.6(13.2-22.0)	1.50	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
6.5	27.1(22.8-33.6)	18.1(13.8-22.4)	1.50	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
7.0	27.5(22.3-34.8)	18.6(14.1-23.1)	1.48	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
7.5	27.2(22.6-34.1)	18.4(13.9-22.8)	1.48	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
8.0	27.0(23.2-34.4)	17.9(13.7-22.5)	1.51	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
LSD 5%	N.S.	N.S.									
Isolate MU 24											
6.0	26.2(20.4-33.3)	17.7(13.4-21.9)	1.48	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
6.5	26.7(21.4-33.9)	18.0(13.7-22.8)	1.48	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
7.0	27.4(20.7-34.1)	18.3(14.3-23.1)	1.50	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
7.5	27.8(21.9-34.7)	18.6(14.1-23.4)	1.49	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
8.0	26.8(20.8-34.0)	18.1(13.9-22.6)	1.48	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
LSD 5%	N.S.	N.S.									
Isolate MU 28											
6.0	27.4(20.9-33.0)	17.6(13.6-21.3)	1.56	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
6.5	27.9(21.8-33.6)	17.3(13.9-22.4)	1.61	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
7.0	28.0(22.2-33.1)	17.6(14.4-22.8)	1.59	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
7.5	28.2(21.4-34.8)	17.8(14.2-22.0)	1.58	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	3
8.0	27.6(20.6-34.2)	17.5(14.3-21.7)	1.58	POGr	Punc	1	Ss/Sd	Ss/Sd	1-3	3-4(-5)	2
LSD 5%	N.S.	N.S.									

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

TABLE 5c Effect of pH on conidial features and intensity of sporulation of three Stemphylium isolates* of conidial group 'C'

pH	CONIDIAL FEATURES									Sporulation Intensity
Isolate MU 2	Dimensions (µ) Length	Dimensions (µ) Width	L/W Ratio	Colour	Ornamentation	Constrictions	Shape Juv.	Shape Mat.	Septations Long.	Septations Lat.	
6.0	36.5(24.9-48.2)	16.4(12.5-19.6)	2.23	Bn/GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
6.5	36.9(25.7-48.6)	16.7(12.8-20.0)	2.21	Bn/GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
7.0	37.3(26.8-49.7)	16.8(13.3-20.6)	2.22	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
7.5	37.8(26.6-51.5)	16.8(13.1-20.2)	2.25	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
8.0	37.1(25.8-48.1)	16.6(12.9-19.9)	2.23	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
LSD 5%	N.S.	N.S.									
Isolate MU 19											
6.0	35.1(26.8-43.6)	16.3(13.4-19.6)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
6.5	35.4(27.1-44.0)	16.5(13.7-20.1)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
7.0	35.9(27.9-43.8)	16.6(13.8-20.3)	2.16	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
7.5	36.2(28.4-44.2)	16.8(14.0-20.8)	2.15	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
8.0	35.8(28.1-43.6)	16.7(13.8-20.5)	2.14	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
LSD 5%	N.S.	N.S.									
Isolate MU 40											
6.0	35.8(27.8-44.7)	16.4(13.2-19.5)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
6.5	36.6(28.4-46.4)	16.7(13.5-19.9)	2.19	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
7.0	36.4(28.0-46.9)	16.7(13.9-20.4)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
7.5	36.9(28.7-47.5)	16.9(13.7-20.2)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
8.0	36.0(26.9-47.1)	16.5(13.4-19.6)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6(-7)	3
LSD 5%	N.S.	N.S.									

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

TABLE 5d Effect of pH on conidial features and intensity of sporulation of three *Stemphylium* isolates* of conidial group 'D'

pH	CONIDIAL FEATURES									Sporulation
Isolate MU 14	Dimensions (µ)		L/W Ratio	Colour	Ornamentation	Constrictions	Shape		Septations		Intensity
	Length	Width					Juv.	Mat.	Long.	Lat.	
6.0	35.8(28.2-43.6)	16.2(13.2-19.3)	2.21	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	2
6.5	36.1(27.7-44.1)	16.4(13.5-19.5)	2.20	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
7.0	36.4(28.0-44.7)	16.6(13.7-19.9)	2.19	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
7.5	36.9(28.4-44.5)	16.6(13.8-20.1)	2.22	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
8.0	36.0(27.8-43.9)	16.5(13.6-19.7)	2.18	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
LSD 5%	N.S.	N.S.									
Isolate MU 23											
6.0	34.8(24.8-43.9)	16.4(13.1-19.6)	2.12	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
6.5	35.3(25.4-44.1)	16.7(13.5-20.0)	2.11	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
7.0	35.1(26.0-45.0)	17.0(14.0-20.7)	2.06	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
7.5	35.7(25.6-45.2)	16.8(13.6-20.2)	2.13	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
8.0	35.2(25.2-44.7)	16.5(12.9-19.8)	2.13	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
LSD 5%	N.S.	N.S.									
Isolate MU 29											
6.0	34.6(22.9-41.1)	16.4(12.5-19.4)	2.11	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
6.5	35.3(23.6-43.2)	16.8(13.3-19.9)	2.10	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
7.0	35.5(24.2-43.6)	16.8(13.5-20.2)	2.11	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
7.5	35.0(23.8-42.9)	16.7(12.7-19.6)	2.10	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
8.0	34.4(22.4-42.4)	16.5(12.4-19.4)	2.08	GrBn	Verr	2-3	Ov/Ob	Ov/Ob	1-2	3-6 (-7)	3
LSD 5%	N.S.	N.S.									

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

Fig. 5. Conidiophores and conidia of isolate MU 9, conidial group 'A' (= *Stemphylium botryosum*) produced on 5% V-8, pH 7.5, at 20C and in an 8h photoperiod. A, conidiophores (x725); B, conidiophores and juvenile conidia (x650); C, mature conidia (x 850).

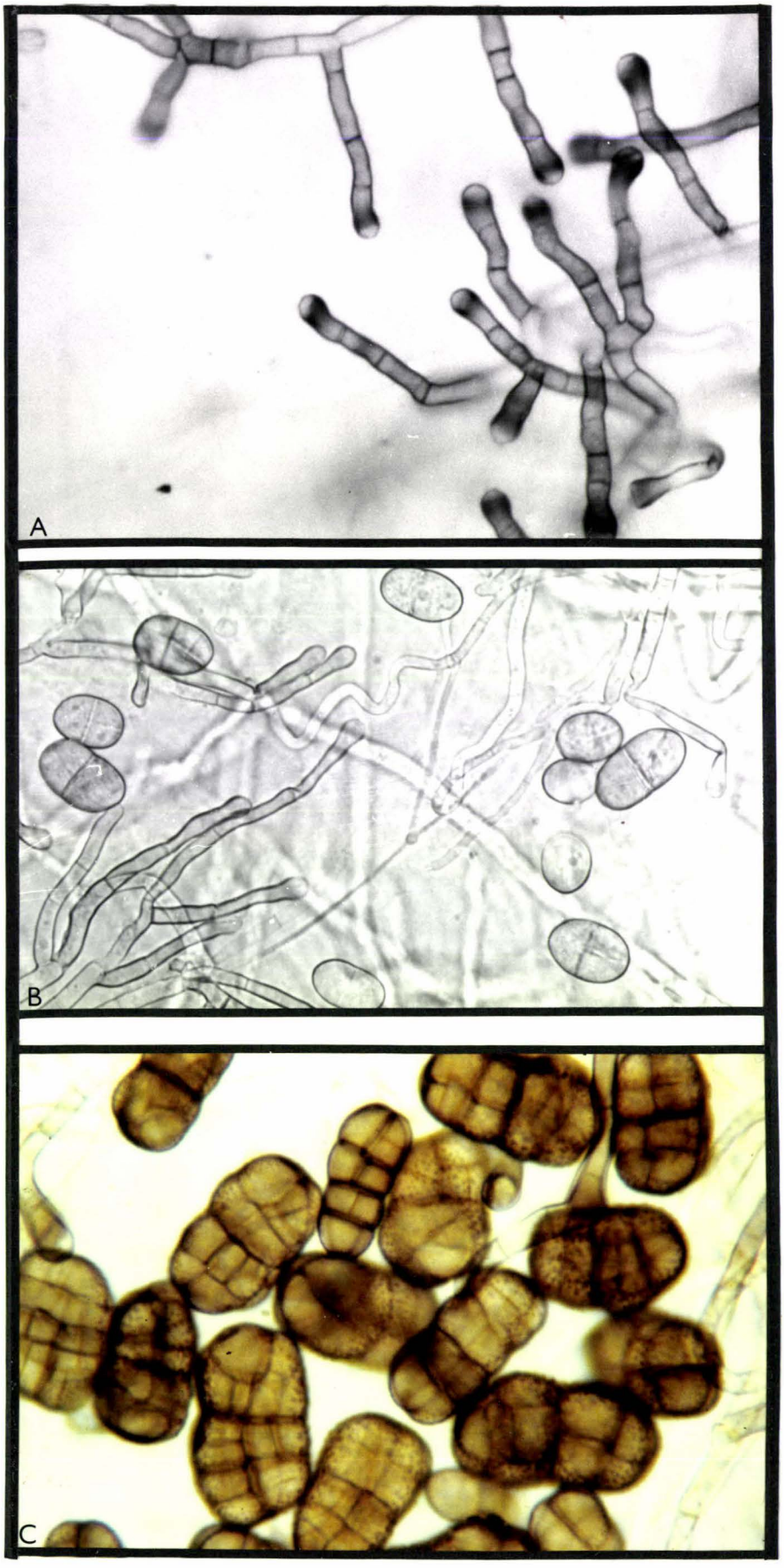

A
B
C

Fig. 6. Conidiophores and conidia of isolate MU 24, conidial group 'B' (= Stemphylium globuliferum) produced on 5% V-8, pH 7.5, at 20C, and in an 8h photoperiod. A, conidiophores (x725); B, conidiophores and juvenile conidia (x650); C, mature conidia (x850).

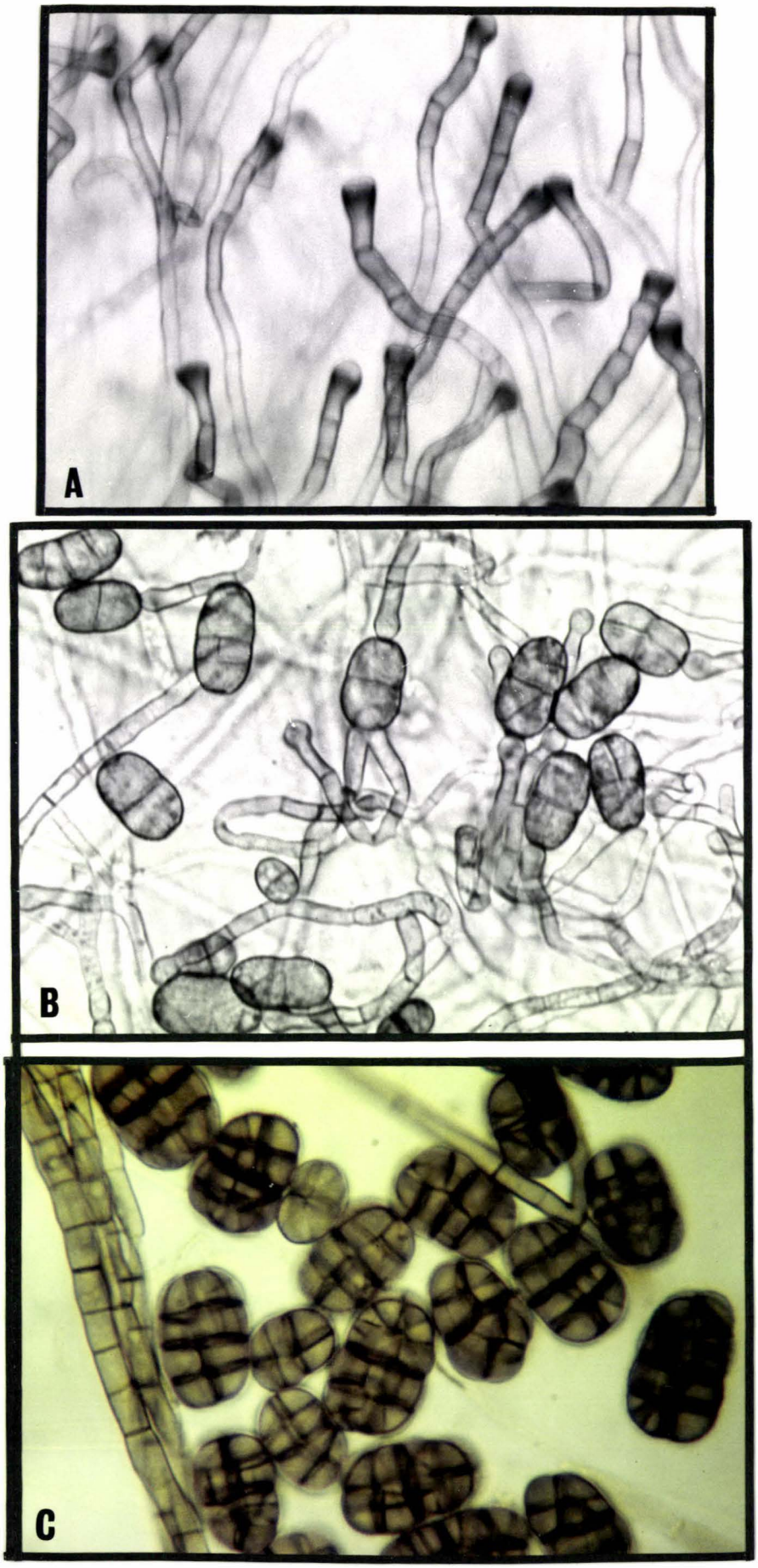

A
B
C

Fig. 7. Conidiophores and conidia of isolate MU 2, conidial group 'C' (= Stemphylium vesicarium) produced on 5% V-8, pH 7.5, at 20C and in an 8h photoperiod. A, conidiophores (x725); B, conidiophores and juvenile conidia (x650); C, conidiophores and mature conidia (x850).

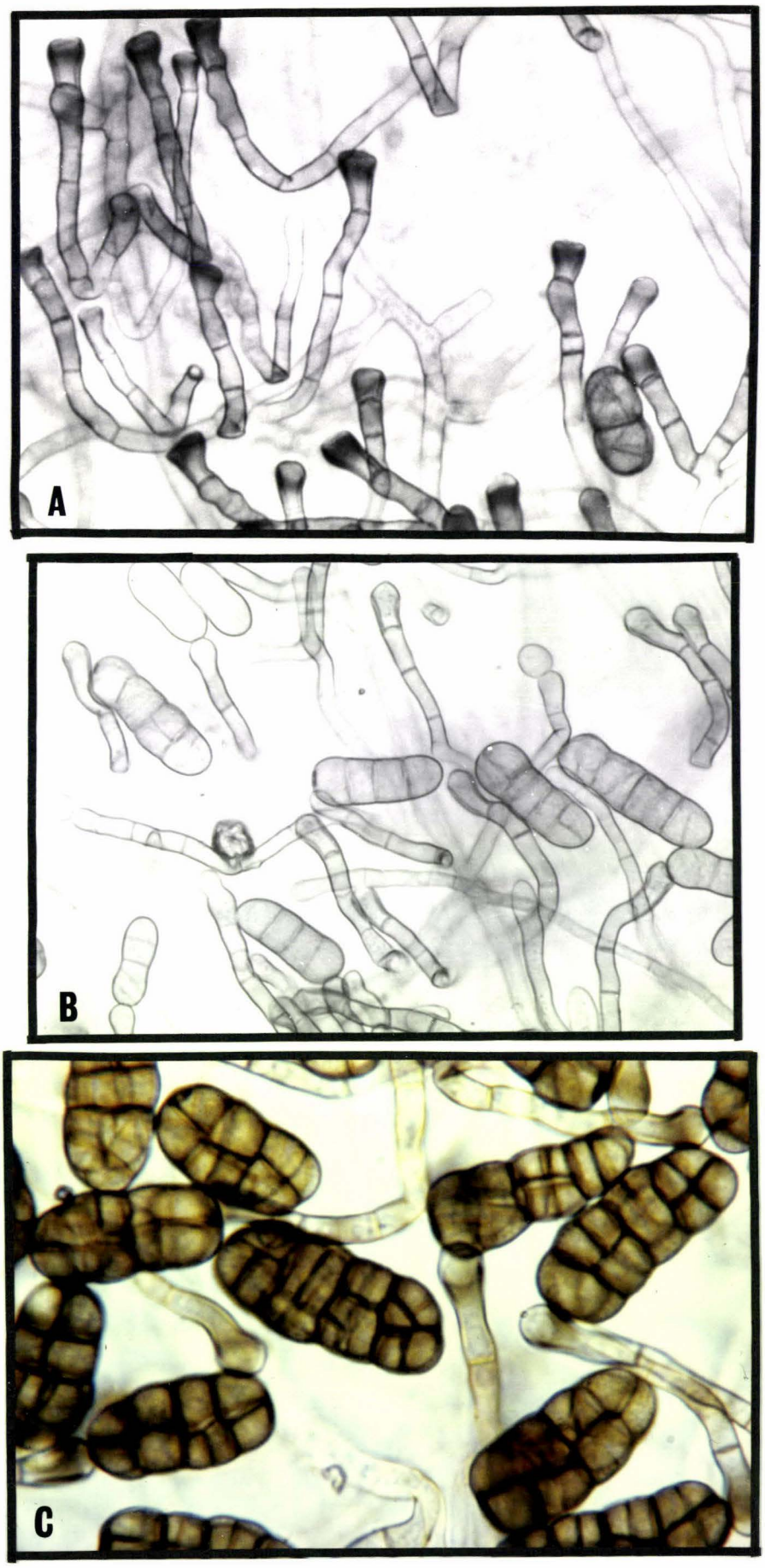
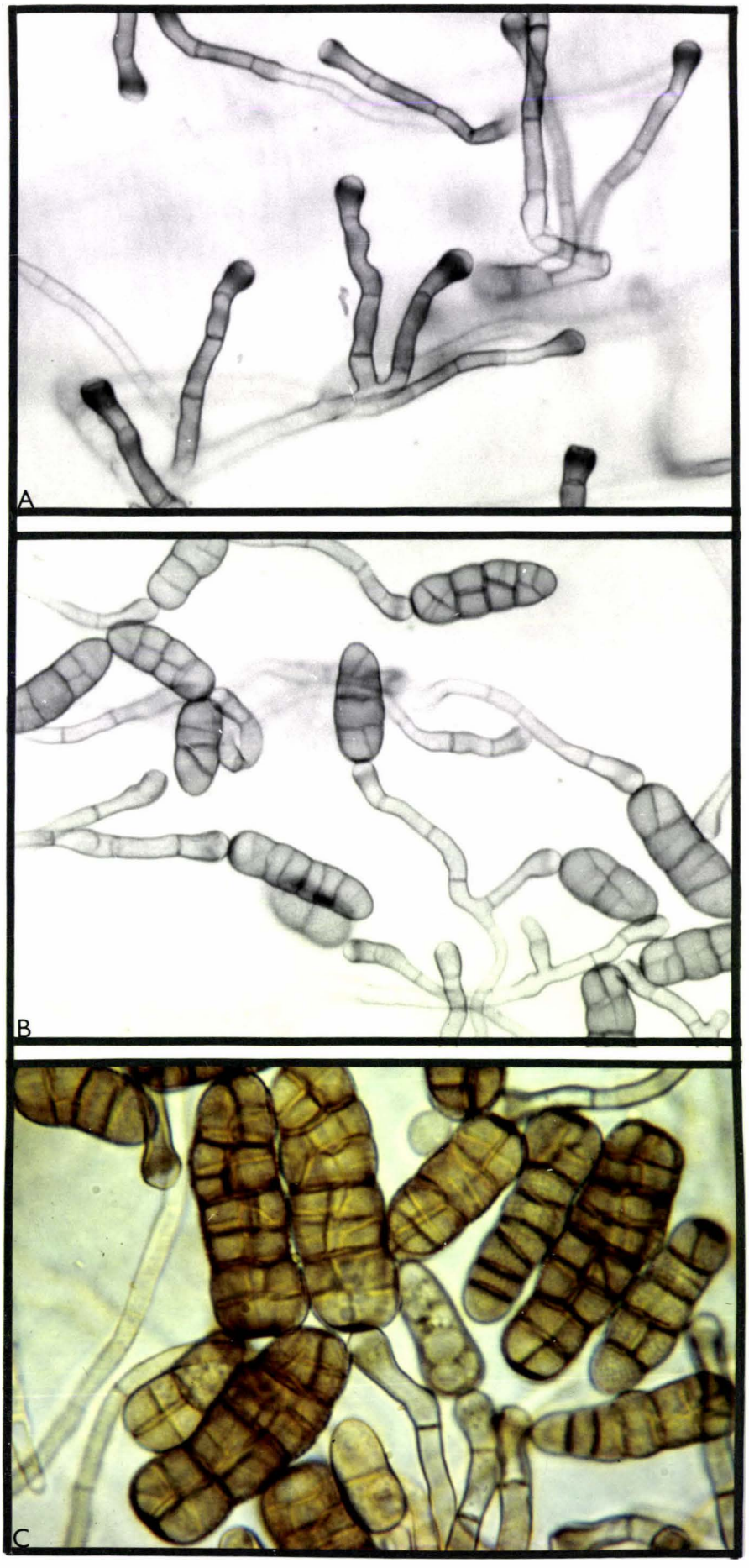

A
B
C

Fig. 8. Conidiophores and conidia of isolate MU 14, conidial group 'D' produced on 5% V-8, pH 7.5, at 20C and in an 8h photoperiod. A, conidiophores (x725); B, conidiophores and juvenile conidia (x650); C, conidiophores and mature conidia (x850).


A
B
C

DISCUSSION

These results show that all conidial features examined were of taxonomic value. Some were relatively stable and distinct and thus enabled ready separation of one or more of the four conidial groups. Included in this category were shape of juvenile and mature conidia, surface ornamentation, number of lateral septal constrictions, and L/W ratio. Additionally, characters such as dimensions, pigmentation, and number of lateral and longitudinal septa, although subject to environmentally induced variability, were still sufficiently distinct under any one set of environmental circumstances as to be of taxonomic significance.

The results further show that of the four environmental factors examined only growth medium, incubation temperature and photoperiod had an appreciable effect on one or more conidial features. However, on any one medium, at any one temperature, and under any one light regime the contrast between conidial groups was sufficient to enable separation. PDA and PMA, and growth of cultures in total darkness were least satisfactory since they induced production of some distorted spores which made segregation more difficult. The most satisfactory environmental conditions for expression of conidial features, which in turn facilitated ready separation of the conidial groups were 5% V-8, pH 7.5, 20C and an 8h photoperiod. Although pH had no significant effect on conidial morphology, pH 7.5 was selected merely because the conidia of most isolates produced at this pH were marginally larger. Unless otherwise stated, these cultural conditions were used through out the study.

It must, however, be emphasised that even under these optimum environmental circumstances no single conidial feature by itself will separate all four conidial groups; only when used in combination is separation possible.

Shape of the juvenile and mature conidia, dimensions, L/W ratio, and number of lateral and longitudinal septa are of very limited value in distinguishing CGA from CGB, the conidia of both being subspherical to subdoliform, approximately 30 x 19µ, 3-4(-5) x 1-3 septate, and with a L/W ratio of between 1.4 to 1.6 (Figs. 5 and 6). However, these features readily separate CGA and CGB from CGC and CGD, which produce predominantly oval to oblong spores, approximately 36 x 17µ, 3-6(-7) x 1-2 septate, and with a L/W ratio of between 2.1 to 2.3 (Figs. 7 and 8).

The number of lateral septal constrictions is an important criterion for distinguishing the one-constricted conidia of CGA and CGB from the mainly 2-3 constricted spores of CGC and CGD (Figs. 5, 6, 7, 8).

Pigmentation and surface ornamentation is useful in distinguishing the sparsely punctate and less intensely pigmented spores of CGB (Fig. 10) from the verrucose and more pigmented conidia of CGA, CGC and CGD (Figs. 9, 11, 12). Since the conidia of CGA and CGB are very similar in shape and size, pigmentation and surface ornamentation are of particular importance in separating these two conidial groups. Because the conidia of CGB are less intensely pigmented and only sparsely punctate, conidial septation appears more sharply defined than in the spores of the other conidial groups (Fig. 6).

Using the above conidial features as taxonomic criteria it was not possible to separate CGD from CGC. This would therefore suggest that the isolates of both these conidial groups belong to one and the same Stemphylium species, which is clearly distinct from the two Stemphylium species represented by CGA and CGB.

As regards the specific identification of the conidial groups the mycological features of CGA, CGB and CGC expressed under the optimum conditions of 5% V-8, pH 7.5, 20C, and an 8h photoperiod provides strong support for the writer's earlier contention that they are S. botryosum, S. globuliferum and S. vesicarium respectively, as defined by Simmons (1969). The isolates of CGD, which prior to this study appeared intermediate between S. botryosum and S. vesicarium, must now be regarded as more closely allied to S. vesicarium.

As stated previously Simmons (1969) demonstrated that these three Stemphylium species each has a distinct Pleospora state. Thus final confirmation that the four conidial groups in fact represent three distinct species would be provided by demonstrating the same Pleospora - Stemphylium connections.

1.1.2 ORNAMENTATION OF CONIDIA AND CONIDIOPHORES

The scanning electron microscope (SEM) is a relatively new tool facilitating detailed study of the surface topography of solid objects. Due largely to its excellent depth of focus and high resolving power it has found a wide range of applications in the field of biology (Echlin, 1968; Vitt & Hamilton, 1974). In mycology the scanning electron microscope has been extensively used for the study of surface ornamentation of spores (Day & Scott, 1973; Jones, 1968; Zogg & Schwinn, 1971).

Because the preceding studies have shown that surface ornamentation of conidia is an important character for separating CGB from the other three conidial groups, a study was conducted to confirm and perhaps extend the light microscopy results.

MATERIALS AND METHODS

The same isolates were used as for the environmental studies (1.1.1). Small blocks of densely sporulating mycelium were cut and fixed in Karnovsky's (1965) formaldehyde - glutaraldehyde fixative for 2.5h. Triton was used as a wetting agent. The blocks were then removed and washed in three changes of distilled water. Excess moisture was removed with blotting paper and the specimen quick-frozen in liquid freon, followed by liquid nitrogen. The blocks were then transferred to super-cooled brass discs in liquid nitrogen and placed in a vacuum overnight. Freeze-dried specimens were glued to metal stubs and coated with ca. 150Å of gold. Observations were made with a Cwikscan 100 field emission scanning electron microscope.

RESULTS AND DISCUSSION

The spores of all the isolates of CGA, CGC and CGD were verrucose (Figs. 9, 11, 12). On young conidia the processes were round and firm (Figs. 9A, 11A, 12A), but as the spores matured the protrusions collapsed and became crater-like (Figs. 9B, 11B, 12B). By contrast, in CGB the processes were sparse and smaller, creating a rather punctate appearance (Fig. 10). Unlike those of the other three conidial groups, the processes on the spores of CGB retained their firmness even when the spores were mature.

The conidiogenous cell of all isolates of the four conidial groups were lightly punctate (Figs. 9A, B, E; 10A, B, D; 11B, D; 12B, D). The ornamentations were less pronounced on young conidiophores.

This study confirms the light microscope observations, again demonstrating that CGB is distinct on this basis, from the other three conidial groups. The observation that the conidia of CGA (which corresponds to S. botryosum) are verrucose is in disagreement with the report of Simmons (1969), who reported conidia of this species are minutely warted or echinulate. Similarly, the results relating to ornamentations of the conidiogenous cell of S. globuliferum are contrary to the findings of Simmons. Observations made with the scanning electron microscope

Fig. 9. Surface ornamentation of conidia and conidiophores of isolate MU 9, conidial group 'A' (= Stemphylium botryosum). A, B & C, scanning electron micrographs (x5500, 4900, 1700 respectively); D & E, light micrographs of conidia (x1600) and conidiogenous cell (x1800) respectively.

Note the punctate nature of the conidiogenous cells and the verrucose surface of the conidia.

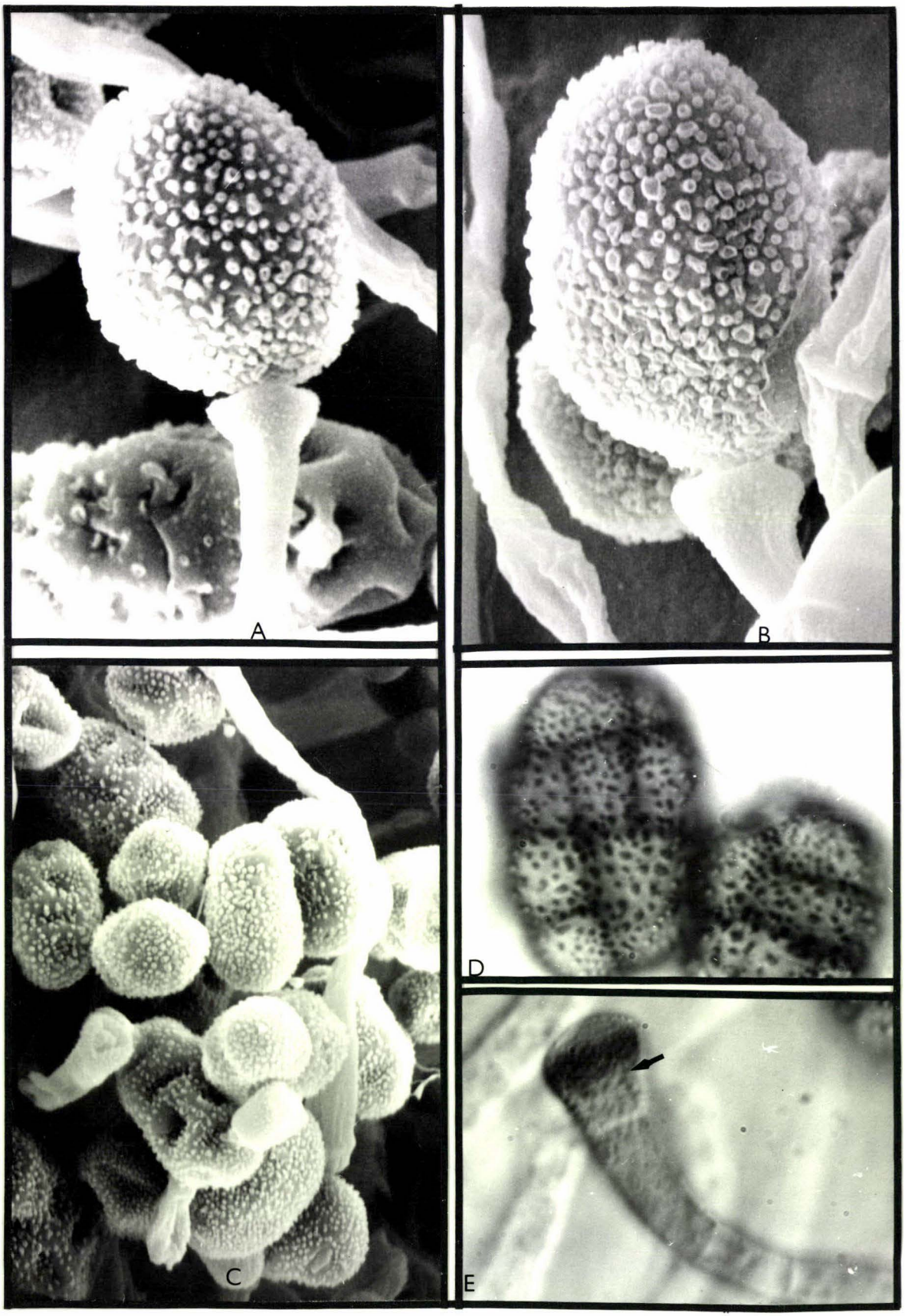

A
B
C
D
E

Fig. 10. Surface ornamentation of conidia and conidiophores of isolate MU 24, conidial group 'B' (= Stemphylium globuliferum). A & B, scanning electron micrographs (x2800, 2700 respectively); C & D, light micrographs of a conidium (x1400) and conidiogenous cell (x1700) respectively.

Note the punctate nature of the conidiogenous cells and the sparsely punctate surface of the conidia.

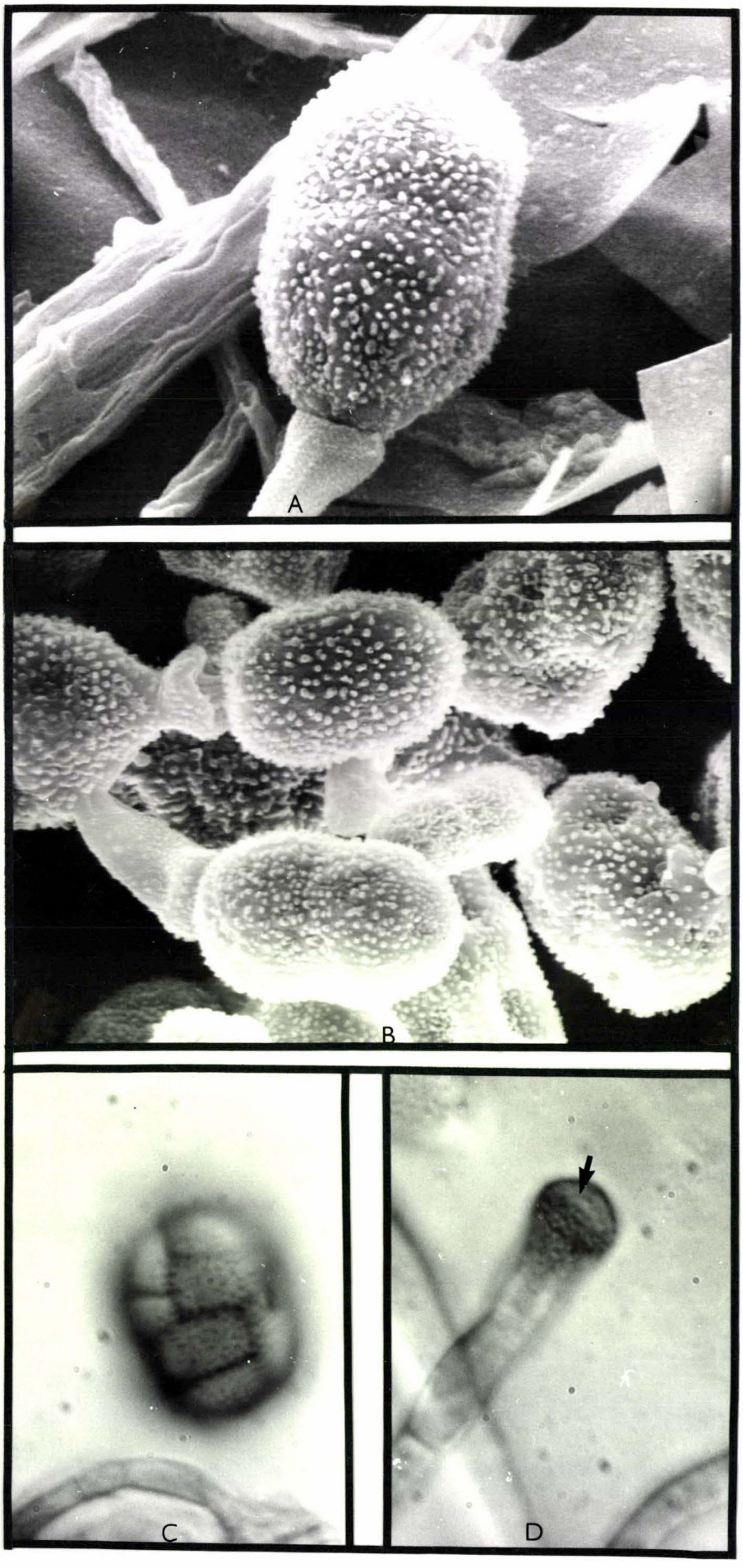
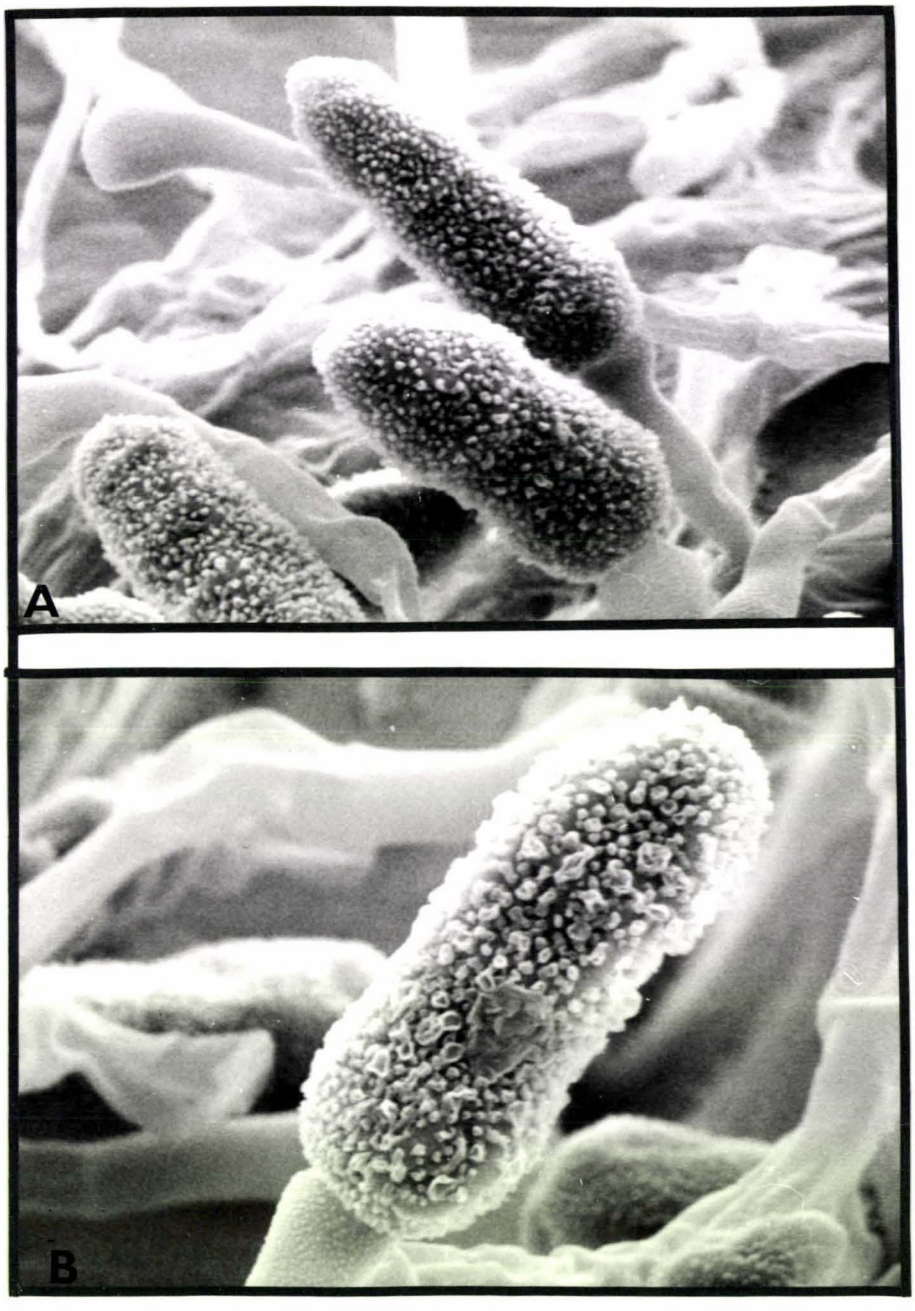

A
B
C
D

Fig. 11. Surface ornamentation of conidia and conidiophores of isolate MU 2, conidial group 'C' (= *Stemphylium vesicarium*). A & B, scanning electron micrographs (x2100, 3100 respectively); C & D, light micrographs of conidia (x1400) and conidiogenous cell (x1700) respectively.

Note the punctate nature of the conidiogenous cells and the verrucose surface of the conidia.


A
B

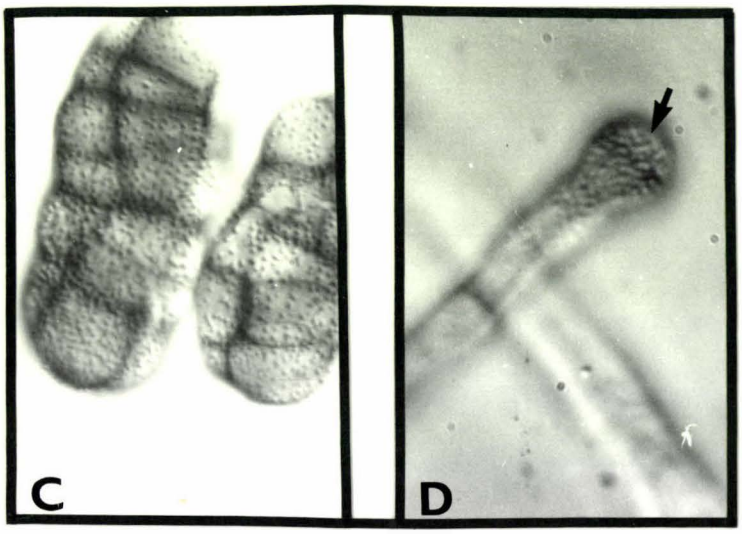
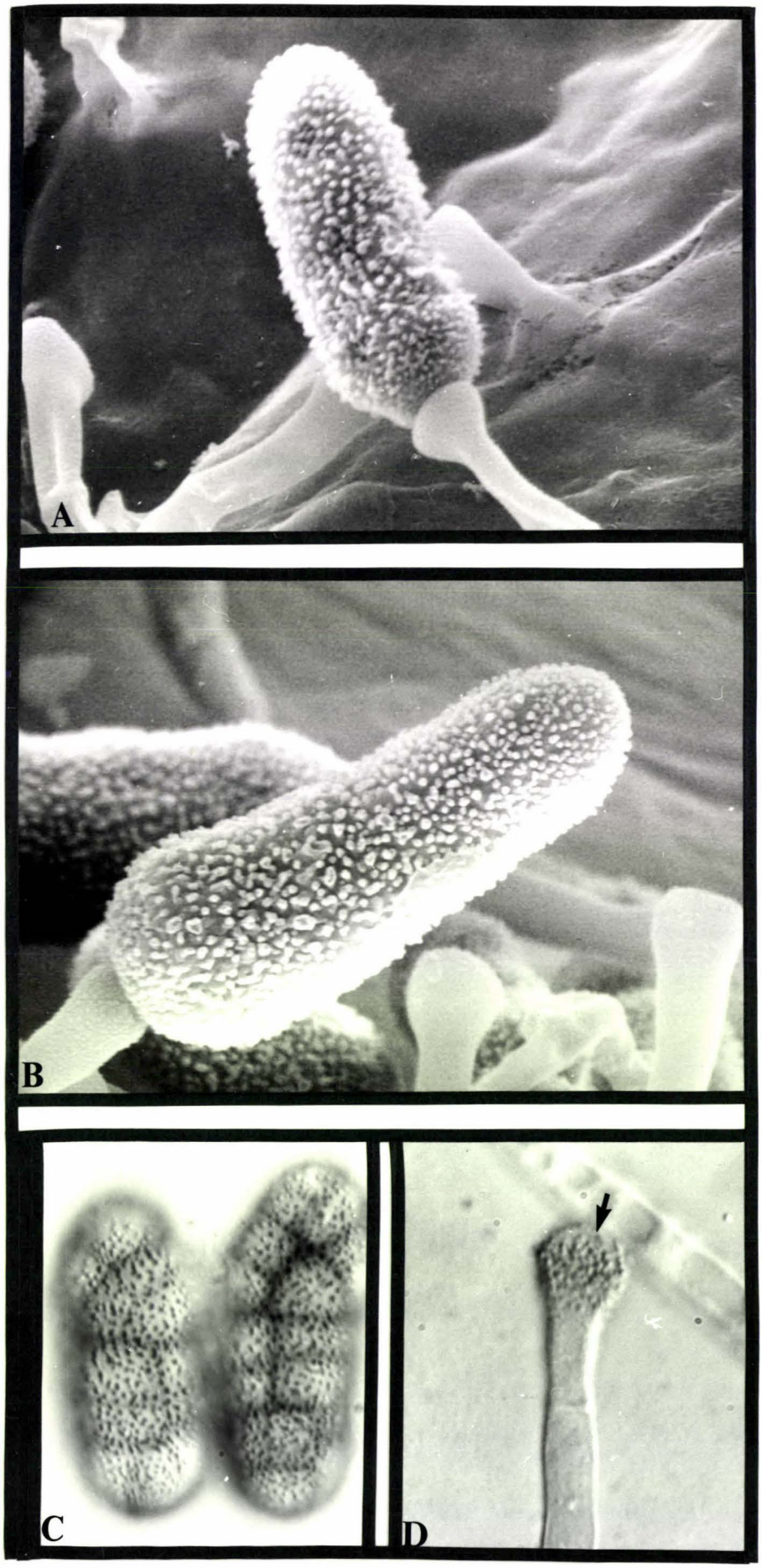

C
D

Fig. 12. Surface ornamentation of conidia and conidiophores of isolate MU 14, conidial group 'D'. A & B, scanning electron micrographs (x2400, 2800 respectively); C & D, light micrographs of conidia (x1400) and conidiogenous cell (x1700) respectively.

Note the punctate nature of the conidiogenous cells and the verrucose surface of the conidia.


A
B
C
D

confirm light microscopic evidence that the conidiogenous cells of CGB isolates (which correspond to *S. globuliferum*) are lightly punctate whereas Simmons concluded they were smooth.

1.1.3 DEVELOPMENT OF CONIDIA

Conidial shape is of considerable taxonomic importance in the delimitation of both genera and species, and this is particularly so in the Hyphomycetes. In the genus *Stemphylium* the environmental studies revealed that shape of mature conidia is a relatively stable feature useful in separating CGA and CGB from CGC and CGD, and that correlated with this character are other spore features such as dimensions, L/W ratio, number of lateral and longitudinal septa and number of lateral septal constrictions. The environmental studies also suggested that the basic overall shape of a mature conidium is expressed at an early stage of development. If correct, this implies that separation of CGA and CGB from CGC and CGD could be achieved by examination of relatively immature conidia. To test this hypothesis studies were undertaken tracing developmental morphology of conidia in the four groups using time-lapse photomicrography.

MATERIALS AND METHODS

Isolates MU 9, MU 24, MU 2 and MU 14 of CGA, CGB, CGC and CGD respectively were used in this study. They were cultured on 5% V-8 and on the fourth day 1.5cm^2 blocks were cut from the periphery of each colony and transferred to a sterile microscope slide. A sterile cover-slip was placed on each culture block and the slides incubated for 6h at 20C in petri dishes lined with moist blotting paper. Slides were then examined using a compound microscope and conidium development recorded photomicrographically.

RESULTS AND DISCUSSION

Profuse conidiophore production occurred along the cut surfaces of each culture block. The conidium initials of all four conidial groups were indistinguishable being spherical, hyaline and aseptate (Figs. 13A-C; 14A-D; 15B; 16A-D). In CGA and CGB the basic spherical shape was maintained through to maturity (Figs. 15 & 14). By contrast conidia of CGC and CGD became obclavate (Figs. 15C-D; 16F & G) and then oval or oblong (Figs. 15E-H; 16H).

In all groups pigmentation increased as the conidia matured.

Fig. 13. Conidium development of isolate MU 9, conidial group 'A' (= *Stemphylium botryosum*). (x775).

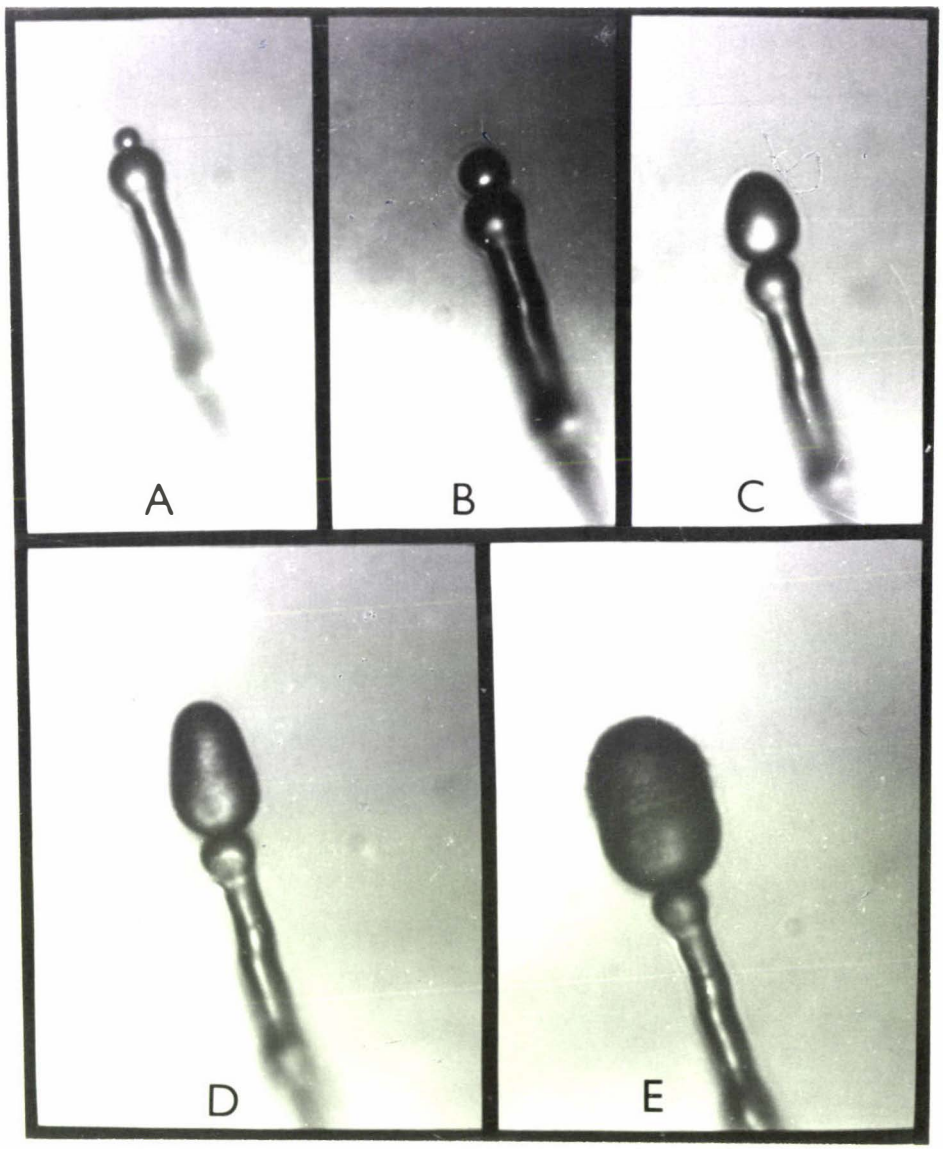

A
B
C
D
E

Fig. 14. Conidium development of isolate I.U 24, conidial group 'B' (= Stemphylium globuliferum). (x750).

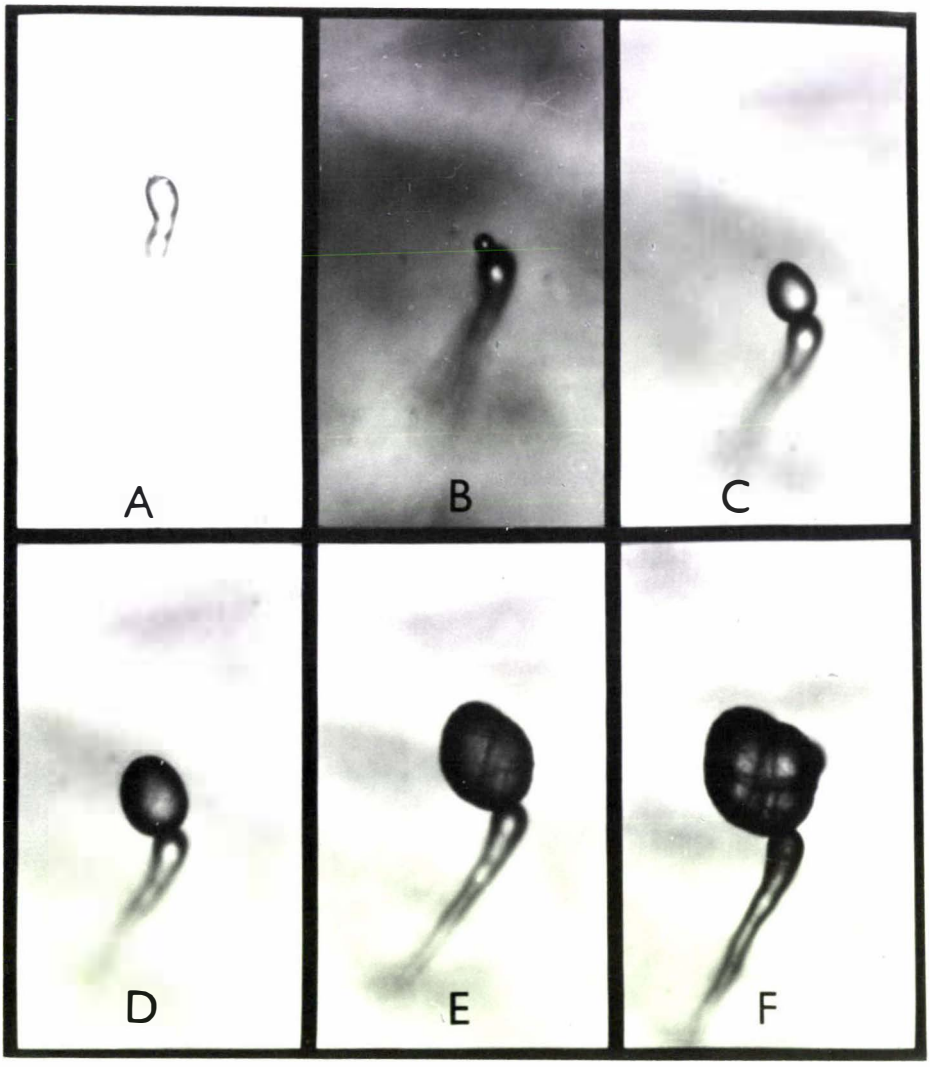

A
B
C
D
E
F

Fig. 15. Conidium development of isolate MU 2, conidial group 'C' (= *Stemphylium vesicarium*). (x650).

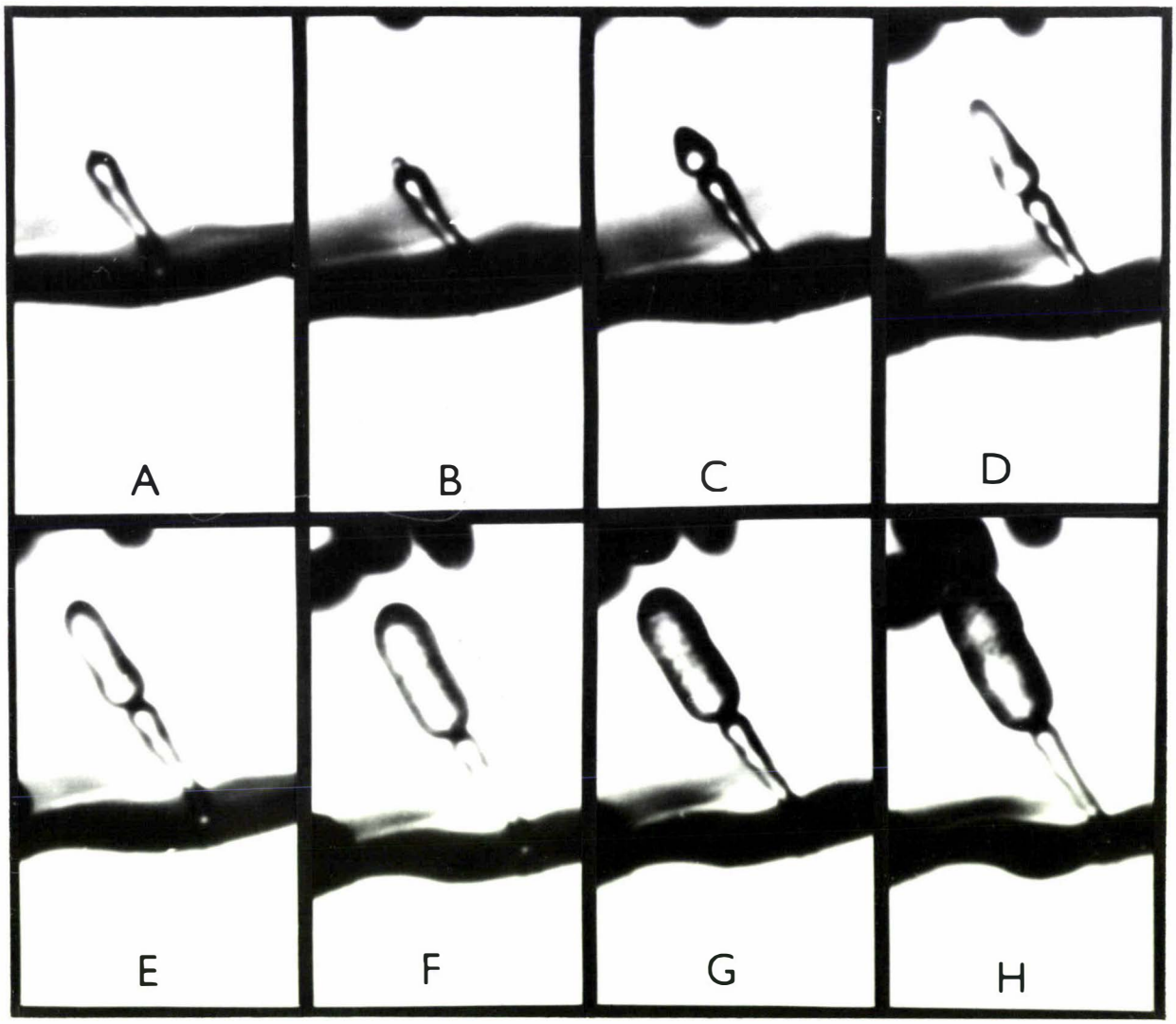
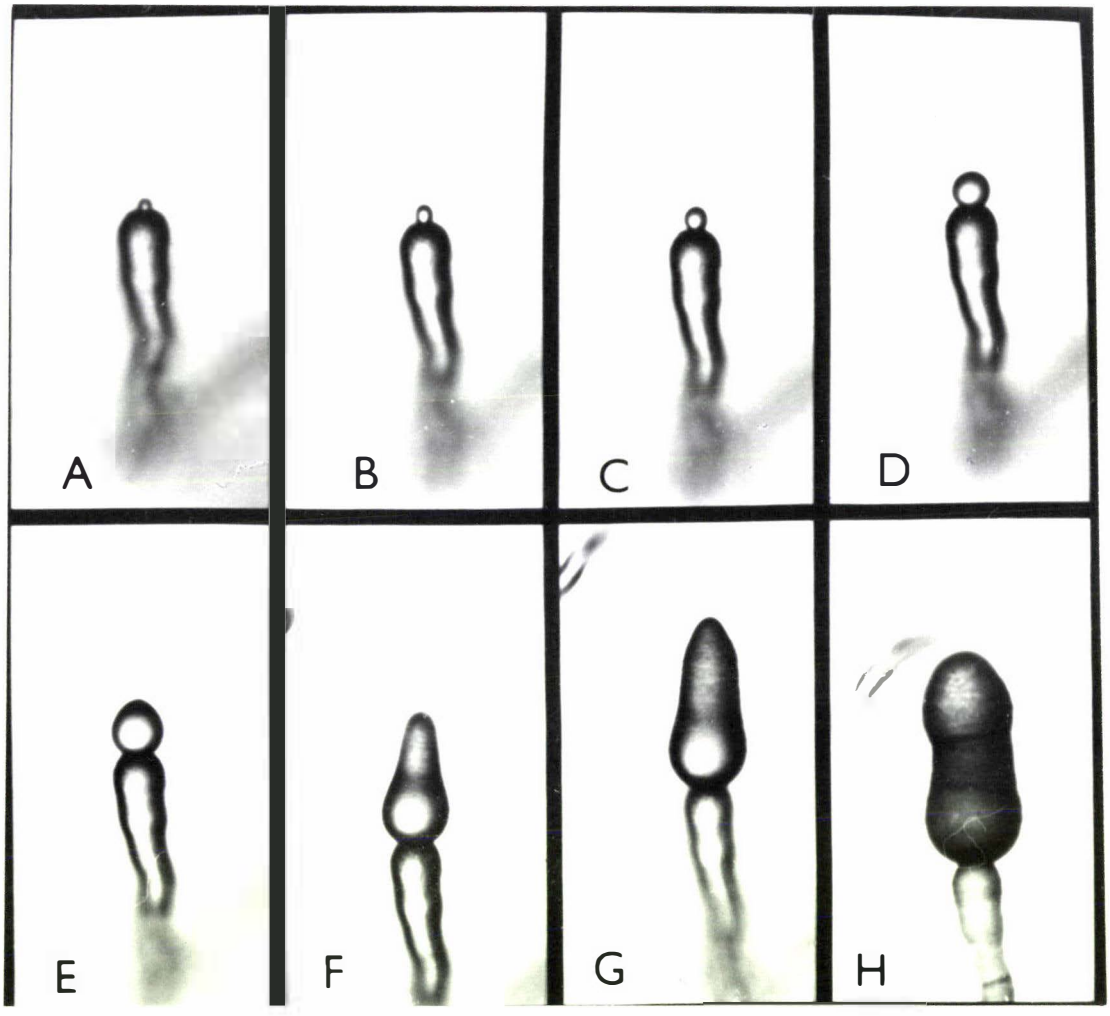

A
B
C
D
E
F
G
H

Fig. 16. Conidium development of isolate MU 14, conidial group 'D' (x 775).


A
B
C
D
E
F
G
H

The first septum was laid laterally dividing the conidium into two approximately equal halves. Subsequent septations were both lateral and longitudinal giving the conidium the characteristic muriform appearance.

These results confirm that shape of mature conidia is expressed at a relatively early stage of development. This in turn means that reliable separation of CGA and CGB from CGC and CGD is possible even with immature conidia.

The similarity between CGC and CGD is further substantiated.

1.2 THE GENUS PLEOSPORA

The genus Pleospora is large, heterogenous, and worldwide in distribution. It is a member of the Loculoascomycetes and is characterised by the production of ascostromata with a central locular cavity in which bitunicate asci bearing muriform, yellowish-brown to golden-brown spores are interspersed amongst pseudoparaphyses.

Over the years an enormous amount of literature has accumulated on the genus; however, the most significant contribution in recent years has been that of Wehmeyer (1961). His detailed study of Pleospora and related genera over several years culminated in a monograph describing approximately 115 Pleospora species. In this monograph Wehmeyer concludes that the developmental sequence and morphology of the ascospores are of primary importance in the delimitation and recognition of species, while details of the ascostromata and asci, although of value, are only of secondary importance. Wehmeyer states that the ascospores of nearly every species has a characteristic mature spore form, sequence of septation, and intensity of coloration. According to Weymeyer, up to the 3-septate stage ascospores follow a more or less similar sequence of septation for all species. Beyond this stage there is divergence in the type and sequence of septum formation, which he uses as the basis for his 'leptosphaeroid', 'vulgaris' and 'herbarum' series. In the 'leptosphaeroid' series the insertion of successive lateral septa takes place mainly in the end cells and the longitudinal septa are laid down almost at random in single cells of the elongating ascospore. The strongly tapered end cells only rarely contain a longitudinal septum.

The 'vulgaris' and 'herbarum' series both develop a longitudinal and lateral septum in one or more of the cells in the form of a cross. Consequently the longitudinal septum is continuous through two cells making them appear in pairs. However, the 'vulgaris' series differs from

the 'herbarum' series in that the latter has additionally a longitudinal or Y-shaped septum in one or both the end cells. Thus a typical herbarum-type ascospore matures at the 7-lateral septum level, although it can also become 8, 9, or more septate by the insertion of quarternary septa.

Wehmeyer studied species of Pleospora with 7 lateral septate ascospores and found that some 96 had the herbarum-type septation. He regarded this feature to be of such major taxonomic importance as to warrant consolidating these into one species, namely P. herbarum. Based on geographical distribution and ascospore pigmentation Wehmeyer further subdivided this species into two varieties, P. herbarum var. herbarum for species of the lowlands with pale yellow to yellow-brown ascospores, and P. herbarum var. occidentalis for upland (mountainous) species with yellow-brown to red-brown or almost opaque ascospores. In the variety 'herbarum' Wehmeyer listed 85 previously described species as synonyms, but acknowledged that within this variety there is wide variation in ascospore morphology and that it may be possible to recognise several distinct taxa.

Simmons (1969) studied the Pleospora states associated with Stemphylium and demonstrated that within Wehmeyer's P. herbarum complex there is in fact variation in ascospore morphology of sufficient magnitude to warrant recognition of five distinct species. More importantly, Simmons established that each produced a distinct Stemphylium species. That is, Simmons' work implies that on identification of a particular Pleospora species one can confidently predict its imperfect Stemphylium state, and vice-versa.

Because all 48 isolates represented in the four conidial groups used in the present study produced mature ascostromata in culture, this provided an ideal opportunity to determine the following:

(i) whether the conidial groups produced distinct Pleospora states, possibly corresponding to Simmons suggested Pleospora-Stemphylium connections;

(ii) temperature influence on morphology of ascostromata, asci, and ascospores. Temperature was the only environmental factor considered because in the imperfect state studies it had the most significant effect on conidial morphology;

(iii) temperature influence on time for ascostromata maturity. This aspect was examined because in routine cultural studies it took up to eight months for the ascostromata to mature when incubated at refrigeration

temperatures (approx. 5C). Temperature has been reported to be particularly critical in the maturation of ascostromata (Leach, 1971; Rotem, Cohen & Wahl, 1965; Whitwell, 1974).

The above three aspects were investigated in a single experiment, as follows:

MATERIALS AND METHODS

The 12 representative conidial isolates from earlier studies were used for this experiment. Ascostromata production was initiated by incubating cultures for 10 days at 25C in an 8h photoperiod. Cultures were then exposed to one of the following six temperature treatments: refrigeration temperature (approx. 5C); 8, 12, 20C; diurnal variations of 8h at 16C/16h at 8C; room temperature (approx. 16 to 26C).

In all instances potato carrot agar (PCA) was used and isolates were grown in both McCartney bottles as well as petri plates. Cultures were examined weekly for the presence of asci and ascospores.

RESULTS

Results are summarised in Tables 6a-d.

(A) Maturation of ascostromata

Mature ascostromata were not produced at either room temperature or 20C. Maturation occurred with all other treatments but was fastest at 12C or with the diurnal variations, and slowest in the refrigerator.

(B) Morphology of ascostromata

Ascostromata of all isolates were globose, black, usually beaked and erumpent. Irrespective of temperature treatment the range in dimensions of the ascostromata was such that conidial groups could not be distinguished.

(C) Morphology of asci

At all temperature treatments the asci of the 12 isolates were hyaline, clavate to cylindrical, with the ascus base claw-like or knob-shaped. The asci of all four conidial groups overlapped in dimensions and were little affected by the temperature.

(D) Morphology of ascospores

(i) Dimensions

Temperature had no significant effect on ascospore dimensions of any isolate. The largest ascospores were produced by isolates of CGB,

the narrowest by CGA and the shortest by CGC and CGD.

(ii) Shape

(a) Juvenile ascospores

At all temperatures the juvenile ascospores of CGA were oblong with rounded ends, and usually constricted at all three initial lateral septa (Fig. 17A), whilst those of CGC and CGD were ellipsoidal, apically conical or rostrate with rounded base, and the penultimate cell broader than the remainder of the spore body (Figs. 19A, 20A). The immature ascospores of CGB were similar in shape to those of CGC and CGD but were much longer and the penultimate cell was less obviously swollen (Fig. 18A).

(b) Mature ascospores

The shape of juvenile ascospores of the conidial groups was generally maintained through to maturity and was unaffected by the temperature at which they were produced. The ascospores of CGA were basically oblong, apex roundly tapered, base rounded or occasionally flat, and constricted at the primary and secondary lateral septa (Fig. 17B). The mature ascospores of CGC and CGD were similar in shape, being ellipsoidal, the apex conical or obtusely pointed and the base gradually tapering to a rounded end. The pronounced swelling between the primary and upper secondary lateral septum resulted in the upper half of the spore being broader than the lower half (Figs. 19B, 20B). These spores resembled those of CGA in being constricted at the primary and secondary lateral septa. The mature ascospores of CGB were intermediate in shape to those of CGC or CGD and CGA, but were longer and broader. They were ellipsoidal or oblong-ellipsoidal, the lower half gently tapering to a rounded base, and the upper half broader and tapering to a rounded subacute apex. The swelling between the primary and upper secondary lateral septum was present but less pronounced than the spores of CGC or CGD (Fig. 18B, C & D).

(iii) Septation

Except in the case of CGB where there was occasionally an eighth lateral septum present, all isolates had 7 lateral septa. The number of longitudinal septa varied with the conidial group, being 1-2(-3) for CGA, 1-3(-4) for CGB, and 1-3 for CGC and CGD.

(iv) Pigmentation

Ascospore pigmentation of all isolates was yellow to yellowish-brown and was unaffected by temperature.

KEY TO TABLES 6a-6d

Colour

Bl = Black
Hy = Hyaline
Y = Yellow
YBn = Yellowish-Brown

Beak

\+ = Beaked

Shape of asci

Cl = Clavate
Cy = Cylindrical

Shape of ascospores

Ob = Oblong
Ellips. = Ellipsoidal

Abbreviations

NM = Not mature
Juv. = Juvenile
Mat. = Mature
Lat. = Lateral
Long. = Longitudinal
N.S. = Not Significant

TABLE 6a Effect of different temperature treatments on ascostromata* maturation and morphological features of the ascostromata, asci and ascospores of three Pleospora isolates of conidial group 'A'

Temperature	ASCOSTROMATA				ASCI				ASCOSPORES					
Isolate MU 9	Time taken to mature (weeks)	Size (µ) range	Colour	Beak	Length (µ)	Width (µ)	Colour	Shape	Length (µ)	Width (µ)	Shape		Septation		Colour
											Juv.	Mat.	Lat.	Long.	
Refrigeration temp. (approx. 5C)	28	248-645	Bl	+	178(141-221)	33(30-35)	Hy	Cl/Cy	38.9(33.9-41.3)	16.9(15.6-18.3)	Ob	Ob	7	1-2(-3)	Y/YBn
Constant 8C	18	251-597	Bl	+	169(134-201)	32(29-35)	Hy	Cl/Cy	39.1(34.7-41.7)	17.0(15.9-18.7)	Ob	Ob	7	1-2(-3)	Y/YBn
Constant 12C	10	232-660	Bl	+	175(148-208)	33(30-36)	Hy	Cl/Cy	39.7(35.5-42.5)	17.1(16.0-18.9)	Ob	Ob	7	1-2(-3)	Y/YBn
8h at 16C/16h at 8C	9	302-693	Bl	+	179(131-226)	33(30-36)	Hy	Cl/Cy	39.6(35.1-42.0)	17.0(15.8-18.6)	Ob	Ob	7	1-2(-3)	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperatures	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 11															
Refrigeration temp. (approx. 5C)	26	268-493	Bl	+	174(158-206)	32(29-35)	Hy	Cl/Cy	38.6(34.4-43.8)	17.1(15.7-19.9)	Ob	Ob	7	1-2(-3)	Y/YBn
Constant 8C	11	310-518	Bl	+	183(161-218)	32(30-35)	Hy	Cl/Cy	38.7(34.0-43.6)	16.8(15.4-19.4)	Ob	Ob	7	1-2(-3)	Y/YBn
Constant 12C	5	280-540	Bl	+	186(160-222)	32(30-35)	Hy	Cl/Cy	38.9(34.2-44.2)	16.9(15.4-19.2)	Ob	Ob	7	1-2(-3)	YBn
8h at 16C/16h at 8C	5	258-569	Bl	+	176(152-201)	33(30-36)	Hy	Cl/Cy	39.1(34.6-44.7)	16.9(15.6-19.4)	Ob	Ob	7	1-2(-3)	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperatures	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 33															
Refrigeration temp. (approx. 5C)	32	284-560	Bl	+	172(148-210)	33(30-35)	Hy	Cl/Cy	38.8(34.8-44.0)	17.1(15.2-18.8)	Ob	Ob	7	1-2(-3)	YBn
Constant 8C	14	298-574	Bl	+	178(150-206)	33(30-36)	Hy	Cl/Cy	39.3(35.1-44.4)	17.0(15.4-18.9)	Ob	Ob	7	1-2(-3)	YBn
Constant 12C	5	300-530	Bl	+	184(138-218)	33(29-36)	Hy	Cl/Cy	39.0(34.8-43.9)	17.2(15.2-19.1)	Ob	Ob	7	1-2(-3)	YBn
8h at 16C/16h at 8C	5	278-563	Bl	+	188(154-221)	33(30-35)	Hy	Cl/Cy	38.6(35.0-43.1)	17.0(14.8-19.0)	Ob	Ob	7	1-2(-3)	YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					

* Produced on PCA and incubated in total darkness.

TABLE 6b Effect of different temperature treatments on ascostromata* maturation and morphological features of the ascostromata, asci and ascospores of three Pleospora isolates of conidial group 'B'

Temperature	ASCOSTROMATA				ASCI				ASCOSPORES						
Isolate MU 5	Time taken to mature (weeks)	Size (µ) range	Colour	Beak	Length (µ)	Width (µ)	Colour	Shape	Length (µ)	Width (µ)	Shape		Septation		Colour
											Juv.	Mat.	Lat.	Long.	
Refrigeration temp. (approx. 5C)	31	210-420	Bl	+	186(141-215)	35(30-38)	Hy	Cl/Cy	44.4(38.2-50.3)	19.3(17.1-22.2)	Ellips.	Ellips.	7(-8)	1-3(-4)	Y/YBn
Constant 8C	18	222-379	Bl	+	194(156-220)	35(30-39)	Hy	Cl/Cy	43.9(37.8-48.9)	19.1(16.8-22.0)	Ellips.	Ellips.	7(-8)	1-3(-4)	YBn
Constant 12C	12	260-510	Bl	+	180(139-211)	34(30-37)	Hy	Cl/Cy	44.1(38.4-49.8)	19.4(17.4-22.6)	Ellips.	Ellips.	7	1-3(-4)	YBn
8h at 16C/16h at 8C	11	280-456	Bl	+	196(161-227)	35(31-38)	Hy	Cl/Cy	44.0(38.0-49.3)	19.6(17.4-22.8)	Ellips.	Ellips.	7(-8)	1-3(-4)	YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 24															
Refrigeration temp. (approx. 5C)	28	262-427	Bl	+	178(142-219)	35(30-39)	Hy	Cl/Cy	44.1(38.6-48.9)	19.6(17.5-22.0)	Ellips.	Ellips.	7	1-3(-4)	YBn
Constant 8C	14	248-464	Bl	+	181(138-222)	35(30-37)	Hy	Cl/Cy	44.0(38.3-48.4)	18.9(16.9-21.7)	Ellips.	Ellips.	7	1-3(-4)	Y/YBn
Constant 12C	12	210-448	Bl	+	194(150-236)	36(31-39)	Hy	Cl/Cy	43.7(38.0-47.2)	19.6(17.7-22.4)	Ellips.	Ellips.	7	1-3(-4)	YBn
8h at 16C/16h at 8C	12	215-431	Bl	+	185(141-231)	34(29-37)	Hy	Cl/Cy	43.8(37.9-47.6)	19.3(17.2-22.1)	Ellips.	Ellips.	7	1-3(-4)	YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 28															
Refrigeration temp. (approx. 5C)	32	208-476	Bl	+	198(147-236)	36(31-40)	Hy	Cl/Cy	43.7(38.1-48.0)	19.4(17.1-22.1)	Ellips.	Ellips.	7(-8)	1-3(-4)	YBn
Constant 8C	17	201-510	Bl	+	200(150-241)	36(31-40)	Hy	Cl/Cy	43.9(37.7-47.7)	19.2(17.4-21.4)	Ellips.	Ellips.	7	1-3(-4)	YBn
Constant 12C	10	241-436	Bl	+	184(146-212)	35(30-39)	Hy	Cl/Cy	44.4(38.2-48.1)	19.4(17.6-21.9)	Ellips.	Ellips.	7(-8)	1-3(-4)	Y/YBn
8h at 16C/16h at 8C	11	224-440	Bl	+	179(139-226)	36(30-39)	Hy	Cl/Cy	44.1(37.9-47.8)	19.6(17.6-22.4)	Ellips.	Ellips.	7	1-3(-4)	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					

* Produced on PCA and incubated in total darkness.

TABLE 6c Effect of different temperature treatments on ascostromata* maturation and morphological features of the ascostromata, asci and ascospores of three Pleospora isolates of conidial group 'C'

Temperature	ASCOSTROMATA				ASCI				ASCOSPORES						
Isolate MU 2	Time taken to mature (weeks)	Size (µ) range	Colour	Beak	Length (µ)	Width (µ)	Colour	Shape	Length (µ)	Width (µ)	Shape		Septation		Colour
											Juv.	Mat.	Lat.	Long.	
Refrigeration temp. (approx. 5C)	26	266-479	Bl	+	172(131-206)	32(29-35)	Hy	Cl/Cy	37.8(34.0-44.6)	18.0(16.1-20.4)	Ellips.	Ellips.	7	1-3	YBn
Constant 8C	19	334-494	Bl	+	178(138-214)	32(29-35)	Hy	Cl/Cy	37.2(33.1-43.7)	18.2(16.3-20.9)	Ellips.	Ellips.	7	1-3	YBn
Constant 12C	7	321-541	Bl	+	185(148-220)	33(29-36)	Hy	Cl/Cy	37.6(34.4-44.3)	17.8(16.2-20.0)	Ellips.	Ellips.	7	1-3	YBn
8h at 16C/16h at 8C	7	318-510	Bl	+	189(147-236)	33(30-36)	Hy	Cl/Cy	37.3(33.8-44.0)	17.9(16.0-20.4)	Ellips.	Ellips.	7	1-3	YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S	N.S					
Isolate MU 19															
Refrigeration temp. (approx. 5C)	30	260-464	Bl	+	168(130-198)	32(29-35)	Hy	Cl/Cy	38.0(34.1-43.0)	17.7(14.9-19.9)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 8C	16	284-510	Bl	+	174(136-210)	33(30-35)	Hy	Cl/Cy	37.9(33.9-43.4)	18.1(15.6-20.6)	Ellips.	Ellips.	7	1-3	YBn
Constant 12C	9	295-556	Bl	+	169(129-196)	33(28-36)	Hy	Cl/Cy	38.3(34.7-44.1)	17.8(15.1-19.6)	Ellips.	Ellips.	7	1-3	YBn
8h at 16C/16h at 8C	9	271-546	Bl	+	177(141-218)	33(30-36)	Hy	Cl/Cy	38.1(34.6-43.7)	18.2(15.5-20.6)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 40															
Refrigeration temp. (approx. 8C)	21	310-490	Bl	+	174(141-221)	33(30-36)	Hy	Cl/Cy	37.7(34.0-43.1)	18.4(15.8-22.8)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 8C	14	300-546	Bl	+	167(139-209)	34(31-37)	Hy	Cl/Cy	38.9(34.4-43.6)	17.9(15.2-21.9)	Ellips.	Ellips.	7	1-3	YBn
Constant 12C	6	275-545	Bl	+	166(132-212)	34(30-36)	Hy	Cl/Cy	37.8(33.9-42.9)	18.2(15.6-22.1)	Ellips.	Ellips.	7	1-3	Y/YBn
8h at 16C/16h at 8C	6	268-494	Bl	+	169(140-216)	33(30-36)	Hy	Cl/Cy	37.6(33.6-43.6)	18.3(16.1-22.4)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					

* Produced on PCA and incubated in total darkness.

TABLE 6d Effect of different temperature treatments on ascostromata* maturation and morphological features of the ascostromata, asci and ascospores of three Pleospora isolates of conidial group 'D'

Temperature	ASCOSTROMATA				ASCI				ASCOSPORES						
Isolate MU 14	Time taken to mature (weeks)	Size (µ) range	Colour	Beak	Length (µ)	Width (µ)	Colour	Shape	Length (µ)	Width (µ)	Shape		Septation		Colour
											Juv.	Mat.	Lat.	Long.	
Refrigeration temp. (approx. 5C)	24	271-510	Bl	+	178(141-209)	33(30-36)	Hy	Cl/Cy	37.8(34.3-43.5)	18.0(16.1-20.7)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 8C	14	226-424	Bl	+	179(146-218)	34(30-37)	Hy	Cl/Cy	38.1(34.9-43.9)	17.9(15.5-20.3)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 12C	8	262-543	Bl	+	175(150-213)	34(31-38)	Hy	Cl/Cy	38.2(34.5-44.5)	17.8(15.6-20.0)	Ellips.	Ellips.	7	1-3	Y/YBn
8h at 16C/16h at 8C	7	310-408	Bl	+	181(149-226)	34(31-37)	Hy	Cl/Cy	37.8(34.1-44.0)	17.8(15.8-20.5)	Ellips.	Ellips.	7	1-3	YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 23															
Refrigeration temp. (approx. 5C)	22	281-526	Bl	+	161(139-198)	34(30-37)	Hy	Cl/Cy	37.9(34.4-43.0)	18.4(16.1-21.7)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 8C	12	246-510	Bl	+	170(141-210)	33(30-36)	Hy	Cl/Cy	38.1(34.7-43.2)	18.2(16.0-21.3)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 12C	7	296-561	Bl	+	159(136-189)	33(30-36)	Hy	Cl/Cy	37.9(34.1-42.9)	18.1(16.0-21.2)	Ellips.	Ellips.	7	1-3	Y/YBn
8h at 16C/16h at 8C	7	271-498	Bl	+	166(132-204)	33(29-37)	Hy	Cl/Cy	38.3(34.6-43.6)	18.1(15.8-20.9)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					
Isolate MU 29															
Refrigeration temp. (approx. 5C)	22	229-576	Bl	+	160(136-201)	33(30-36)	Hy	Cl/Cy	38.1(34.2-43.6)	18.1(15.8-21.4)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 8C	13	261-542	Bl	+	157(130-198)	32(28-35)	Hy	Cl/Cy	37.8(34.0-43.1)	17.8(15.1-20.8)	Ellips.	Ellips.	7	1-3	YBn
Constant 12C	7	317-588	Bl	+	164(142-210)	32(29-35)	Hy	Cl/Cy	38.0(34.1-42.8)	17.9(14.9-20.7)	Ellips.	Ellips.	7	1-3	Y/YBn
8h at 16C/16h at 8C	7	284-531	Bl	+	161(138-206)	33(30-36)	Hy	Cl/Cy	37.7(33.8-42.4)	18.2(15.9-21.7)	Ellips.	Ellips.	7	1-3	Y/YBn
Constant 20C	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Room temperature	NM	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LSD 5%									N.S.	N.S.					

* Produced on PCA and incubated in total darkness.

DISCUSSION

If one accepts Wehmeyer's broad concept of P. herbarum then inclusion of the four conidial groups in this one species would be justified since ascospores matured at the 7-lateral septate level and had the 'herbarum'-type septation. Further, if Wehmeyer's criteria for subdivision within his P. herbarum is accepted, the four conidial groups would be included in the variety 'herbarum', characterised by the spores being yellow to yellowish-brown and confined to the lowlands.

It is now generally agreed by fungal taxonomists that species delimitation should be based on distinctive morphological criteria and that such criteria must be relatively stable under differing environmental conditions. It is clear that Wehmeyer agreed with these principles since he acknowledged that within his P. herbarum var. herbarum - "there is a wide range of variation in spore size, form, colour, and secondary septation, and it will probably be possible to recognise several distinct taxa when this complex group is better known". It would appear that Wehmeyer did not consider the variation to be sufficient and/or distinct enough to differentiate species within his variety 'herbarum'.

In the writer's opinion the degree of variation in ascospore morphology revealed in the present study is of sufficient magnitude to warrant recognition of three distinct Pleospora species, two corresponding each to a conidial group (CGA and CGB), and the third corresponding to two conidial groups (CGC and CGD). The shape both of juvenile and mature ascospores and the dimensions of the latter are the features enabling this morphological differentiation. As in the Stemphylium state, no single feature will separate the three Pleospora species, but in combination the two criteria enable ready distinction, as follows:

Dimensions of mature ascospores readily separates the longer and broader ascospores (approx. 44 x 19.5μ) of the Pleospora state of CGB from the Pleospora states of CGA, CGC and CGD. Since the ascospores of the Pleospora state of CGA were approximately 39 x 17μ and those of CGC and CGD were approximately 38 x 18μ, separation of the Pleospora states of these three conidial groups was not possible.

Juvenile ascospore shape readily distinguishes the Pleospora state of CGA from those of CGB, CGC and CGD. Ascospores of the Pleospora state of CGA are oblong with rounded ends and usually constricted at the three initial lateral septa (Fig. 17A). This shape is quite distinct from that of similar aged ascospores of the Pleospora states of CGB, CGC

and CGD which are ellipsoid with a conical apex and rounded base, and have a characteristically swollen penultimate cell (Figs. 18A, 19A, 20A).

Shape of mature ascospores readily separates the Pleospora state of CGA, which produces oblong ascospores with a roundly tapered apex and rounded to flat base (Fig. 17B), from the Pleospora states of CGC and CGD. These two groups produce ellipsoidal ascospores with a conical or obtusely pointed apex and rounded base (Figs. 19B, 20B). This feature has very limited value in distinguishing CGB, which produces ascospores intermediate in shape between those of CGA, and CGC and CGD (Figs. 18B, C & D).

Using ascospore features as taxonomic criteria it was not possible to distinguish between the Pleospora states of CGC and CGD. This would strongly suggest that both these conidial groups have the same Pleospora state which is clearly distinct from the two Pleospora species associated with CGA and CGB respectively.

A comparison of the ascospore morphology of the above defined three Pleospora species with line drawings and descriptions of the five Pleospora - Stemphylium connections proposed by Simmons (1969) indicates that the Pleospora species associated with CGA and CGB are identical with his for S. botryosum and S. globuliferum respectively, while the Pleospora species connected with CGC and CGD correspond to that of Simmons' S. vesicarium. As regards the specific epithets for these species, apart from P. herbarum, (the Pleospora state of CGA) a decision on the binomial for the other two Pleospora species has not been made. However, from literature it appears that the Pleospora state of CGB fits closely the description of P. armeriae, whilst the Pleospora state of CGC and CGD fits the description of P. allii. These latter two species were tentatively suggested by Simmons for these connections. Confirmation would, however, require detailed examination of type and herbarium material.

Finally, although both 12C and the diurnal temperature cycle of 8h at 16C/16h at 8C resulted in the most rapid maturation of ascostromata, for reasons of convenience 12C was selected for subsequent work. At this temperature the time taken was reduced from 21-32 weeks at refrigerated temperatures to 5-12 weeks.

Fig. 17. Asci and ascospores of isolate MU 9, conidial group 'A' (= *Stemphylium botryosum*/*Pleospora herbarum*), produced on PCA at 12C in darkness. A, ascus with juvenile ascospores (x550); B, ascus with mature ascospores (x550).


A
B

Fig. 18. Asci and ascospores of isolate MU 24, conidial group 'B' (*Stemphylium globuliferum*/*Pleospora* sp.), produced on PCA at 12C in darkness. A, ascus with juvenile ascospores (x550); B, ascus with mature ascospores (x550); C & D, mature ascospores (x550, 850 respectively).

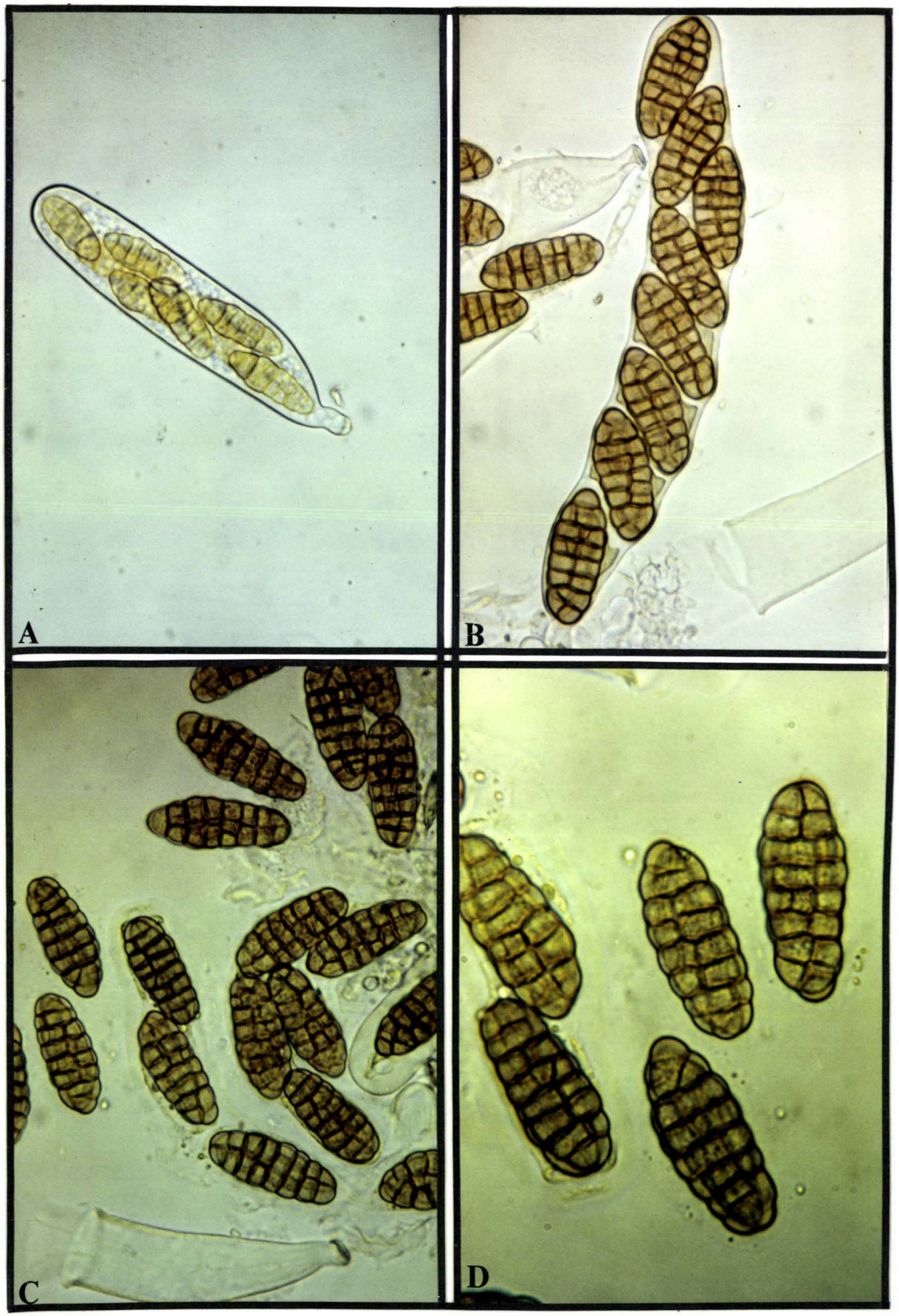

A
B
C
D

Fig. 19. Asci and ascospores of isolate MU 2, conidial group 'C' (*Stemphylium vesicarium*/*Pleospora* sp.), produced on PCA at 12C in darkness. A, asci with juvenile and mature ascospores (x550); B & C, asci with mature ascospores (x550).

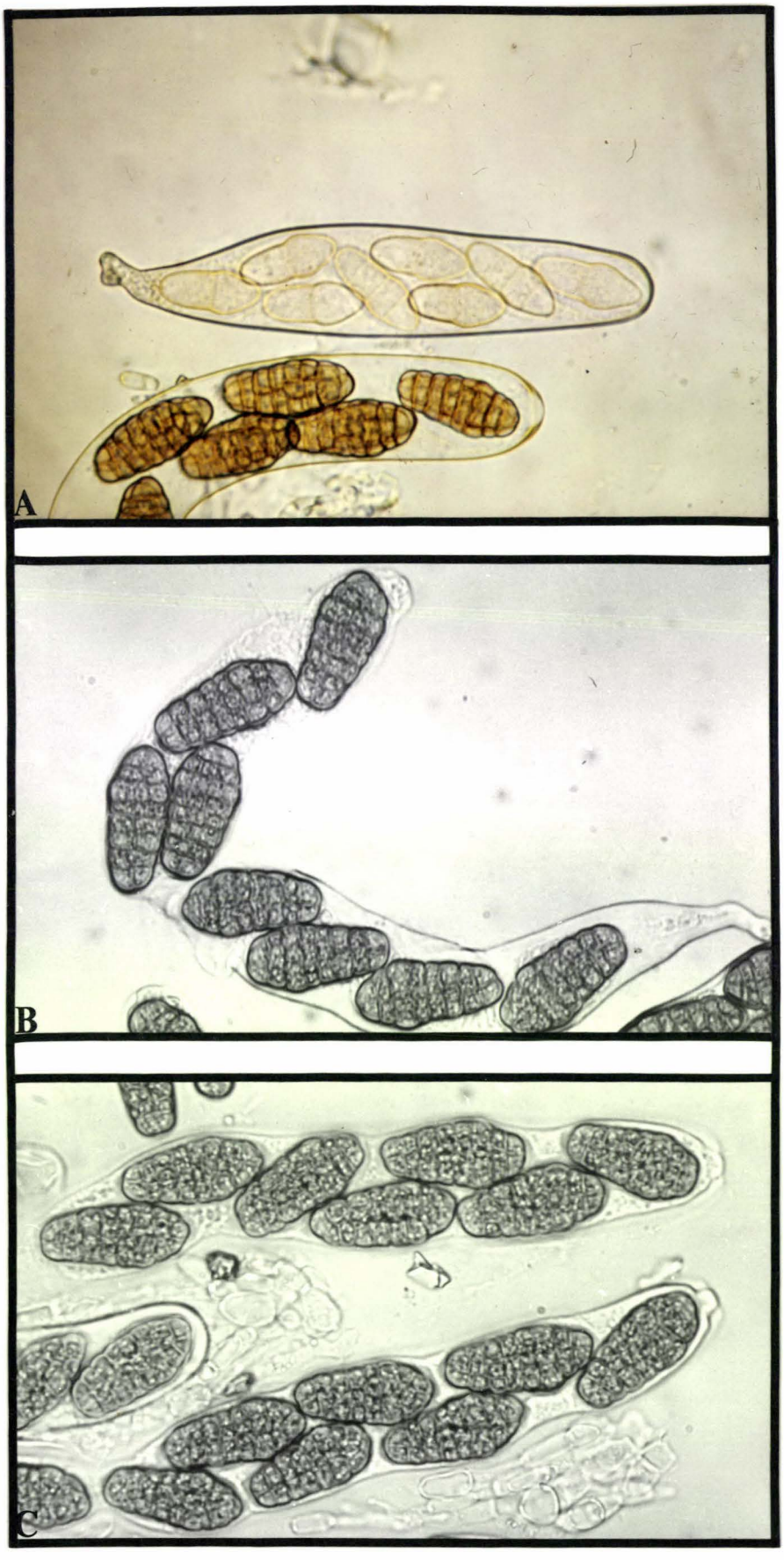
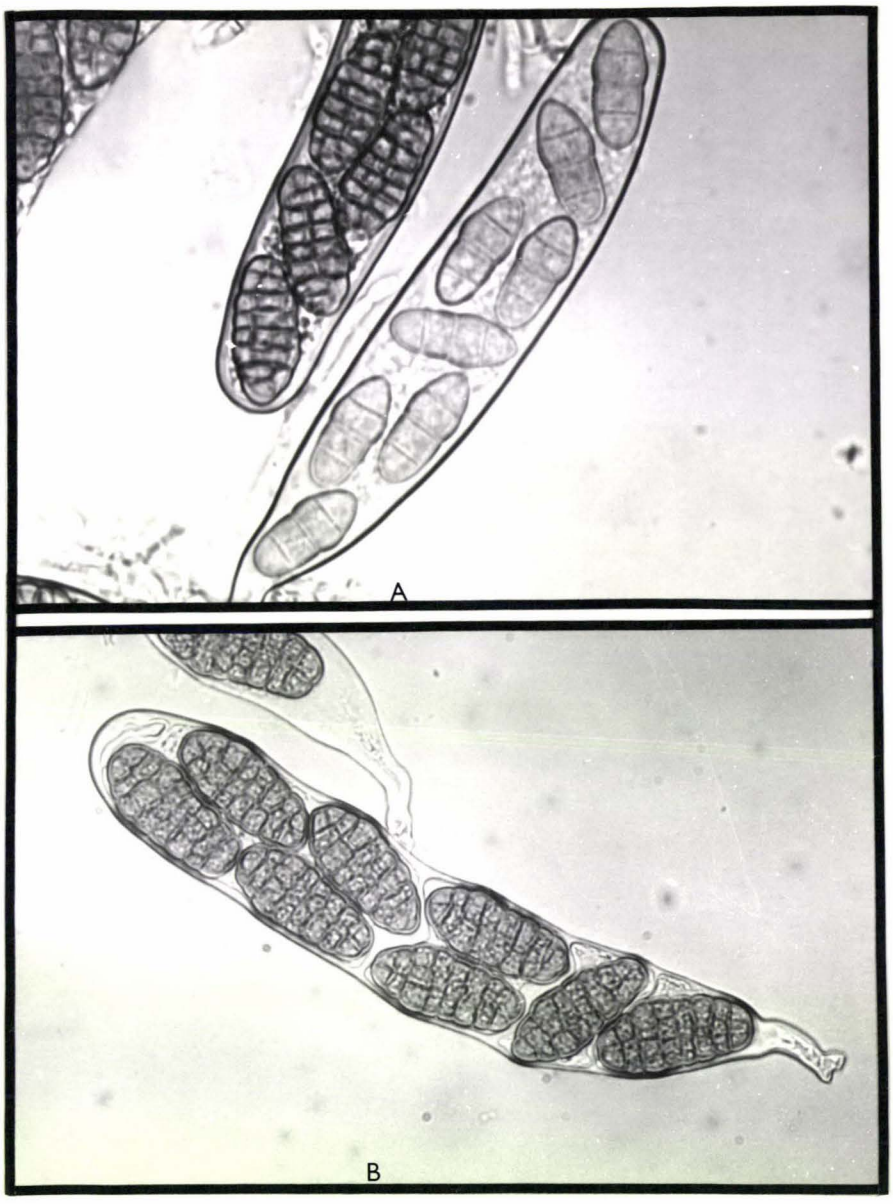
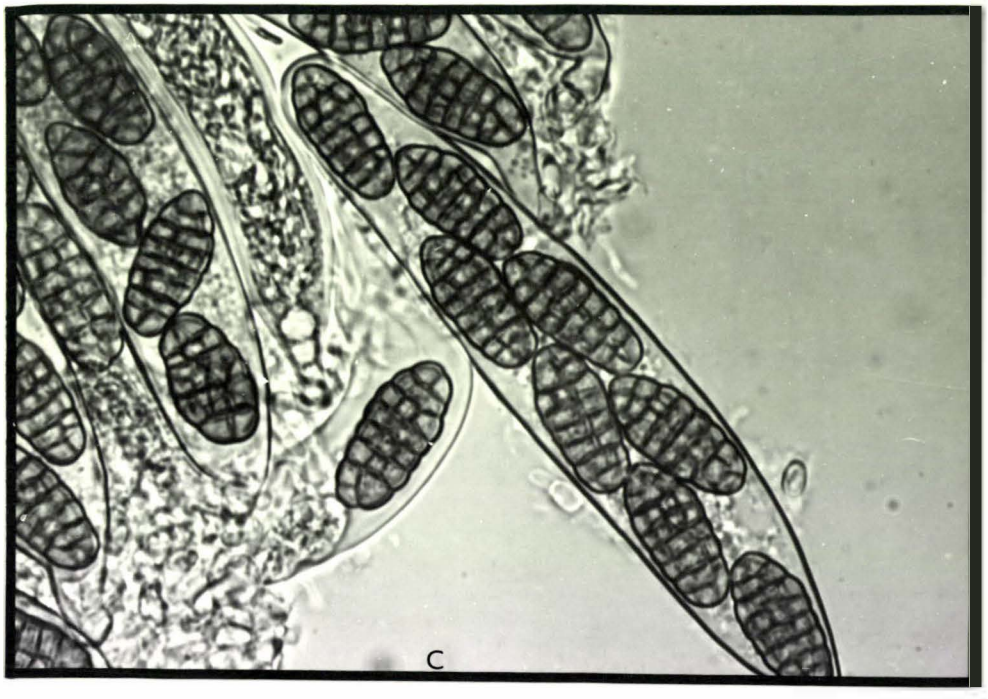

A
B
C

Fig. 20. Asci and ascospores of isolate MU 14, conidial group 'D', produced on PCA and incubated at 12C in darkness.
A, asci with juvenile and mature ascospores (x550);
B, ascus with mature ascospores (x550); C, asci with mature ascospores (x550).


A
B


C

1.3 GENERAL DISCUSSION

The preceding studies confirm that isolates of CGA, CGB and CGC mycologically conform to *S. botryosum*, *S. globuliferum* and *S. vesicarium* respectively. Conidial group 'D', the group presenting the most difficulty when attempting to associate isolates with existing species, is now confirmed as *S. vesicarium*. The three *Stemphylium* species mentioned were each shown to have distinct *Pleospora* states, as follows: *S. botryosum* - *P. herbarum*, *S. globuliferum* - *Pleospora* sp., *S. vesicarium* - *Pleospora* sp., these connections being identical to those reported by Simmons (1969). Such cross-pairing has practical value since identification of *Stemphylium* species enables a reliable prediction of the corresponding *Pleospora* state, and vice-versa. More importantly, however, it demonstrates that it is possible to correlate the phylogenetically based system of classifying *Pleospora* with the form system for *Stemphylium*, and that delimitation of species within one system would be a valuable aid to speciation within the other.

With the exception of Booth (1971, 1975), Muller (1971), Simmons (1969), Shoemaker (1959, 1962) and Tubaki (1958), few mycologists have attempted to find rules for linking the imperfect and perfect systems of fungal classification. The writer agrees with Simmons (1969) that it may be possible to extend the *Stemphylium*-type 'deuteromycetological' approach to other Fungi Imperfecti that have an ascigerous stage. In an ascomycetous genus which has been reported to have more than one imperfect stage e.g. *Ceratocystis* Ellis & Halst, *Chaetosphaeria* Tul., *Leptosphaeria* Ces & de Not. and *Mycosphaerella* Johanson, or conversely in a form genus where a number of ascomycetous genera have been reported e.g. *Drechslera* Ito, *Helminthosporium* Link ex Fr. and *Septoria* Sacc., further studies are required to critically examine these reported connections. It is possible that some of these connections could be erroneous in that either or both generic titles given may be misidentifications. It is also possible that the information obtained from the study of such imperfect-perfect connections may indicate the need for revision of some genera.

Use of the pure culture technique has enabled considerable advances in fungal taxonomy. This is particularly apparent when one considers the role it has played in the resolution of problems involving non-obligate parasites exhibiting pleomorphism, such as occurs with *Stemphylium* - *Pleospora*. The varied cultural requirements of fungi in divergent taxonomic groups precludes standardization of these conditions. However, comparative studies of several species of a single genus should

be conducted concurrently under standardized conditions, which are then fully documented. Such stringent requirements are imperative for accurate identification and for establishment of new species. These requirements have already been stressed by some taxonomists (Booth, 1971; Harding, 1975; Ruppel, 1974; Toussoun & Nelson, 1968).

The diagnostic morphological features of Stemphylium and Pleospora states, together with the environmental conditions under which they are most clearly expressed, are fully documented in the present study. The results indicate that the cultural conditions of PDA, and incubation at 24C in continuous dark, used for initially grouping the 48 isolates into the four conidial groups, were not particularly suitable as they did not allow clear expression of features most suitable for taxonomic purposes. This was especially evident in the case of CGD.

Although it is true that the present day trend in fungal taxonomy is towards consolidation of species, as exemplified by Snyder and Hansen's (1940, 1941a, 1945) treatment of Fusarium Link ex Fr., there is the attendant inherent danger of the species concept becoming so broad that dissimilar individuals are grouped together, thereby obscuring useful mycological information. It has been argued that Snyder and Hansen's system, which is based on the shape of Fusarium macroconidia, is far too broad (Booth, 1971, 1975; Toussoun & Nelson, 1975). In the present study, the writer, in recognising two additional species within Wehmeyer's (1961) P. herbarum complex, has elected to follow a rather narrower species concept, similar to that of Simmons (1969). This is justified since the morphological features of the juvenile and mature ascospores of these two Pleospora species are sufficiently distinct from that of P. herbarum to warrant their recognition as separate entities. However, it is suggested that consolidation may be possible in some other Stemphylium species. A comparative study of S. loti*, S. sarcinaeforme* and S. globuliferum, under the optimum cultural conditions defined, indicated that there is little or no difference in the morphological features of these three species (Fig. 21). Whether the production of a perfect state by S. globuliferum is sufficient justification for retaining this fungus as a separate species is questionable. Similarly, the writer considers that S. lycopersici* and S. solani* may represent one and the the same fungus (Fig. 22). These examples further illustrate the suitability of the environmental conditions that have been selected for studies with Stemphylium.

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* Cultures obtained from Simmons; these have not been reported in New Zealand.

Fig. 21. Conidia of (A) Stemphylium loti; (B) Stemphylium sarcinaeforme; (C) Stemphylium globuliferum, produced on 5% V-8, pH 7.5, at 20C and in an 8h photoperiod (x850).

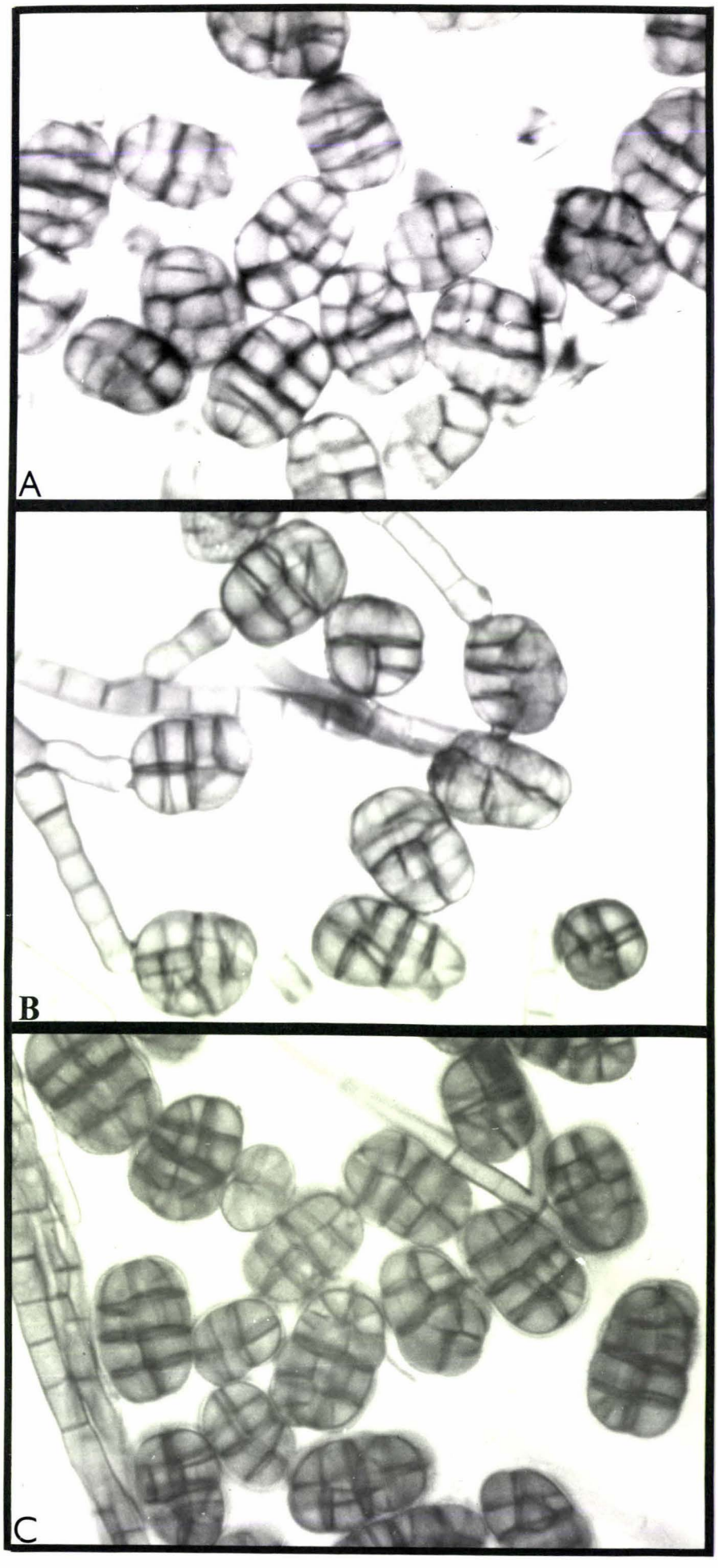
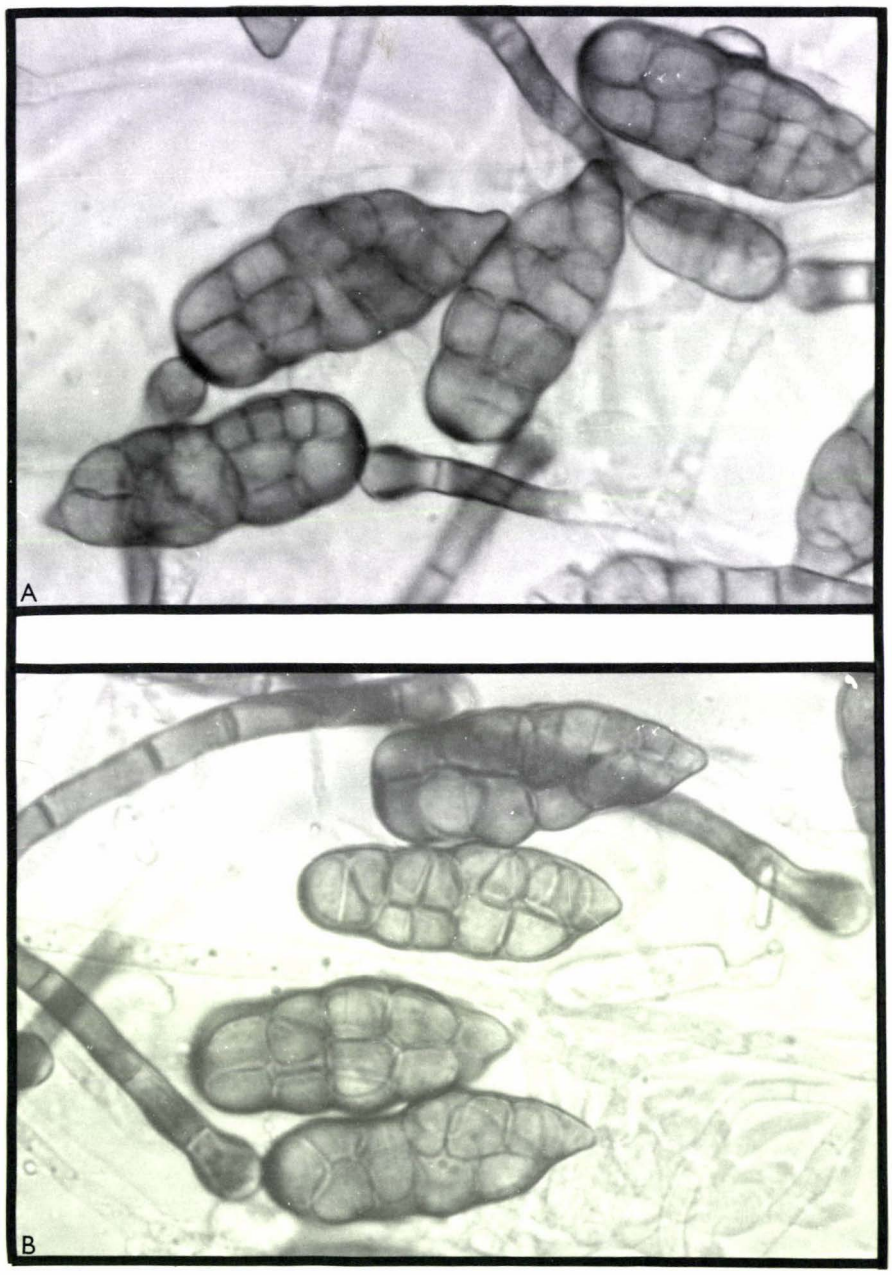

A
B
C

Fig. 22. Conidia of (A) Stemphylium solani; (B) Stemphylium lycopersici produced on 5% V-8, pH 7.5, at 20C and in an 8h photoperiod (x850).


A
B

Mycological Descriptions of the Stemphylium/Pleospora spp.

Descriptions of the asexual features are based upon growth of the fungi on 5% V-8, pH 7.5, and incubation at 20C in an 8h photoperiod. Descriptions of the perfect stage are from cultures on PCA incubated at 12C in the dark.

Stemphylium botryosum

Conidiophores	Hyaline to translucent olivaceous-brown, simple or branched, commonly 1-4 septate, 20-90µ x 4-6µ.
Conidiogenous cell	Olivaceous-grey, lightly punctate, 6.0 - 8.0µ in diameter.
Conidia	Greyish-brown, subspherical, subdoliform or occasionally oval, with 1-3 longitudinal and 3-4(-5) lateral septa, constricted conspicuously at the median lateral septum, verrucose, 22-37 x 14-23µ (av. 29.5 x 19.4µ), L/W ratio 1.4 - 1.6.
Ascostromata	Black, globose, usually beaked, 230-660µ in diameter.
Asci	Hyaline, clavate to cylindrical, bitunicate, base claw-like or knob-like 140-220 x 29-36µ (av. 182 x 33µ).
Ascospores	Yellow to yellowish-brown, oblong with apex roundly tapered and base broadly rounded or flat, 1-2(-3) longitudinal and 7 lateral septa, constricted at the primary and secondary lateral septa, 34-44 x 15-19µ (av. 39.2 x 17.3µ). Juvenile ascospores oblong with obtusely rounded ends and constricted at the initial 3 lateral septa.

Stemphylium globuliferum

Conidiophores	Hyaline to translucent oblivaceous-brown or translucent olivaceous-grey, simple or branched, commonly 1-4 septate, 20-92 x 4-5µ.
Conidiogenous cell	Olivaceous-brown to olivaceous-grey, lightly punctate, 6-9µ in diameter.

Conidia — Pale olivaceous-grey, subspherical, subdoliform or occasionally oval, with 1-3 longitudinal and 3-4(-5) lateral septa, constricted conspicuously at the median lateral septum, sparsely punctate, 21-35 x 14-23µ (av. 27.7 x 18.3µ). L/W ratio 1.5 - 1.6.

Ascostromata — Black, globose, usually beaked, 210-510µ in diameter.

Asci — Hyaline, clavate to cylindrical, bitunicate, base claw-like or knob-like, 150-240 x 30-39µ (av. 186 x 35µ).

Ascospores — Yellow to yellowish-brown, oblong-ellipsoidal or ellipsoidal, base generally rounded and apex tapered to a rounded subacute finish, 1-3(-4) longitudinal and 7(-8) lateral septa, constricted at the primary and secondary lateral septa, slightly swollen between the primary and upper secondary lateral septum, 38-49 x 17-22µ (av. 44.1 x 19.5µ). Juvenile ascospores ellipsoidal, constricted at the median septum, apex conical or rostrate, base rounded, penultimate cell broader than rest of spore body.

Stemphylium vesicarium

Conidiophores — Hyaline to translucent olivaceous-brown, simple or branched, commonly 1-4 septate, 25-90 x 4-6µ.

Conidiogenous cell — Olivaceous-brown to olivaceous-grey, lightly punctate, 6-9µ in diameter.

Conidia — Greyish-brown, mainly oval to oblong, with 1-2 longitudinal and 3-6(-7) lateral septa, constricted at 2-3 of the lateral septa, verrucose 27-52 x 13-21µ (av. 36.9 x 16.8µ).

Ascostromata — Black, globose, usually beaked, 280 - 560µ in diameter.

Asci — Hyaline, clavate to cylindrical, bitunicate, base claw-like or knob-like, 130-220 x 28-36µ (av. 173 x 33µ).

Ascospores

Yellow to yellowish-brown, ellipsoidal, apex conical or obtusely pointed and base gradually tapering to a rounded end, 1-3 longitudinal and 7 lateral septa, usually constricted at the primary and secondary lateral septa, conspicuously swollen between the primary and upper secondary lateral septum resulting in the upper half of the spore being broader than the lower half, 34-44 x 15-22μ (av. 37.9 x 17.9μ). Juvenile ascospores ellipsoidal, constricted at the median lateral septum, apex conical or rostrate and base rounded, penultimate cell broader than rest of spore body.

CHAPTER 2

IDENTIFICATION OF NEW ZEALAND ISOLATES OF PLANT PATHOGENIC STEMPHYLIUM SPECIES

2.1 SURVEY

Following identification of the important taxonomic characters of *Stemphylium* and *Pleospora* and the cultural conditions under which they are best expressed, a taxonomic survey was conducted of field collections of plant pathogenic isolates.

MATERIALS AND METHODS

A total of 166 isolates from 12 different host species (Table 7) were studied. The isolates were obtained from infected host material either collected by the writer or submitted to the Plant Health and Diagnostic Station, Ministry of Agriculture & Fisheries, Auckland, Christchurch and Levin. In most instances isolations were made by transference to agar of conidia from lesions previously subjected to a period of high relative humidity. On those occasions where the tissue plating technique was used, small pieces of infected host tissue were surface disinfected in a 1% sodium hyphochlorite solution for 1 min and plated to 5% V-8 agar in which was incorporated penicillin and streptomycin sulphate, each at the rate of 50µg/ml. Once cultures were established, monosporous isolates were prepared and pathogenicity established. Stock cultures of all isolates were maintained in a mixture of soil and vermiculite (1 : 1) and stored at 5-7C. This technique proved very useful for maintaining the viability of isolates and for preventing cultural degradation, a common problem with stemphylia held as agar slope cultures.

Identification was facilitated by growing isolates at the optimum cultural conditions outlined previously and application of the injury technique to induce uniform sporulation.

RESULTS AND DISCUSSION

The results of the survey are presented in Table 7. Three previously described species namely *S. botryosum*, *S. vesicarium*, and *S. globuliferum*, and a fourth undescribed species herein referred to as *Stemphylium* sp. were isolated. *S. vesicarium* was by far the most common species encountered and on asparagus, onions, chrysanthemums, tomatoes

and peppers it was the only species present. By contrast S. botryosum was the only species associated with lettuce and carnations. S. globuliferum was isolated on only eight occasions and in each instance from the two leguminous hosts, lucerne and blue lupins. The geographical distribution of this species currently appears to be confined to the Auckland and Gisborne area, since seeds and diseased plant material of lucerne and blue lupins from the other growing areas did not yield S. globuliferum.

The legumes were hosts of more than one species of Stemphylium. Besides S. globuliferum, lucerne and blue lupins were also infected by S. vesicarium and S. botryosum, whilst Russell lupins and tree lupins were only parasitized by the latter two species. On lucerne S. vesicarium was more frequently present than S. botryosum whereas on the lupins both were equally common.

One of the S. botryosum isolates (MU 80) from lucerne, which was found to be a weak pathogen, showed certain divergent tendencies in spore production. Frequently the entire bulbous conidiogenous cell was "blown-out" to form a conidium, the latter eventually becoming delineated from the conidiophore by the formation of a septum at its base (Fig. 23). When mature such spores were generally released with the conidiogenous cell still attached to the base. This type of spore production has also been reported for Drechslera sorokiniana by Subramanian and Jain (1964) and Luttrell (1963). Approximately 20% of the conidia in this isolate were formed in this manner, whilst the remainder were typical of normal Stemphylium spore production.

The new species of Stemphylium was isolated from annual phlox, on which it was strongly pathogenic and the only Stemphylium sp. found. Mature conidia were oblong, subdoliform or subspherical, 24-42µ x 15 - 24µ (av. 33.1 x 20.7µ), L/W ratio approximately 1.6, with usually 3 - 5 lateral and 1 - 3 longitudinal septa (Fig. 24). Besides having a pronounced constriction at the median transverse septum, many of the spores were also commonly constricted at one or two of the other transverse septa. They were dark brown to greyish-brown in colour, and the surface ornamentation was verrucose (Figs. 24 & 25). Juvenile conidia were subspherical to oval. Conidiophores were simple or branched, hyaline to translucent olivaceous-brown, 3 - 5 septate and measured 20 - 90µ x 4 - 6µ. The conidiogenous cell was bulbous, lightly punctate (Fig. 25), reddish brown to greyish-brown and 6 - 10µ in diameter. Ascostromata were black, globose or subglobose, beaked when mature, and 300 - 600µ in diameter.

TABLE 7 Dimensions of the conidia, and ascospores of plant pathogenic *Stemphylium* species from different hosts* examined in the survey

Host	Common name of host	*Stemphylium* species	No. of isolates	DIMENSIONS (µ)			
				Conidium		Ascospore	
				Range	Mean	Range	Mean
Asparagus officinalis L.	asparagus	*S. vesicarium*	12	26-53 x 13-21	36.9 x 17.0	33-46 x 15-21	37.7 x 17.8
Allium cepa. L.	onion	*S. vesicarium*	14	26-50 x 14-21	36.8 x 17.1	32-44 x 15-23	37.9 x 18.2
Capsicum frutescens L.	red pepper	*S. vesicarium*	6	25-45 x 13-20	34.3 x 16.9	33-43 x 14-21	37.2 x 17.8
Chrysanthemum morifolium Ramat.	chrysanthemum	*S. vesicarium*	15	26-50 x 13-21	36.4 x 16.7	33-44 x 14-20	38.3 x 17.8
Dianthus caryophyllus L.	carnation	*S. botryosum*	6	24-36 x 15-23	29.8 x 19.8	34-43 x 16-20	39.4 x 17.1
Lactuca sativa L.	lettuce	*S. botryosum*	20	20-36 x 13-24	29.1 x 18.8	33-47 x 15-20	39.1 x 17.3
Lupinus angustifolius L.	blue lupin	*S. botryosum*	7	20-37 x 13-23	29.4 x 19.3	34-45 x 15-20	39.2 x 17.2
		S. globuliferum	3	21-35 x 14-23	27.9 x 18.4	36-49 x 17-23	43.9 x 19.7
		S. vesicarium	7	26-49 x 13-20	34.9 x 16.8	34-45 x 15-22	37.8 x 18.0
Lupinus arboreus L.	tree lupin	*S. botryosum*	7	21-37 x 14-23	28.7 x 18.8	33-44 x 15-21	38.7 x 17.3
		S. vesicarium	8	26-49 x 13-20	35.7 x 17.0	33-46 x 15-21	37.9 x 18.1
Lupinus polypyllus L.	Russell lupin	*S. botryosum*	5	22-38 x 14-23	28.9 x 18.8	33-44 x 15-20	39.0 x 17.2
		S. vesicarium	7	24-46 x 13-20	35.4 x 16.9	32-45 x 15-22	37.7 x 18.1
Lycopersicon esculentum Mill.	tomato	*S. vesicarium*	15	23-45 x 13-20	34.1 x 16.7	33-43 x 14-21	37.4 x 17.9
Medicago sativa L.	lucerne	*S. botryosum*	7	22-36 x 13-23	29.0 x 19.0	33-44 x 15-20	39.3 x 17.2
		S. globuliferum	5	21-35 x 14-23	28.1 x 18.3	36-49 x 17-23	44.3 x 19.5
		S. vesicarium	12	25-51 x 12-20	35.9 x 16.7	34-46 x 15-23	38.0 x 18.2
Phlox drummondii Hook.	Annual phlox	*Stemphylium sp.*	10	24-42 x 15-24	33.1 x 20.7	41-66 x 19-28	54.6 x 24.7

* Pathogenicity was demonstrated for all isolates.

Fig. 23. Stemphylium botryosum (isolate MU 80); production of conidia by "blowing-out" of the entire bulbous conidiogenous cell. A, juvenile conidia (x550); B & C mature conidia (x700, 725 respectively). Arrow indicates a detached conidium with the conidiogenous cell attached to its base.

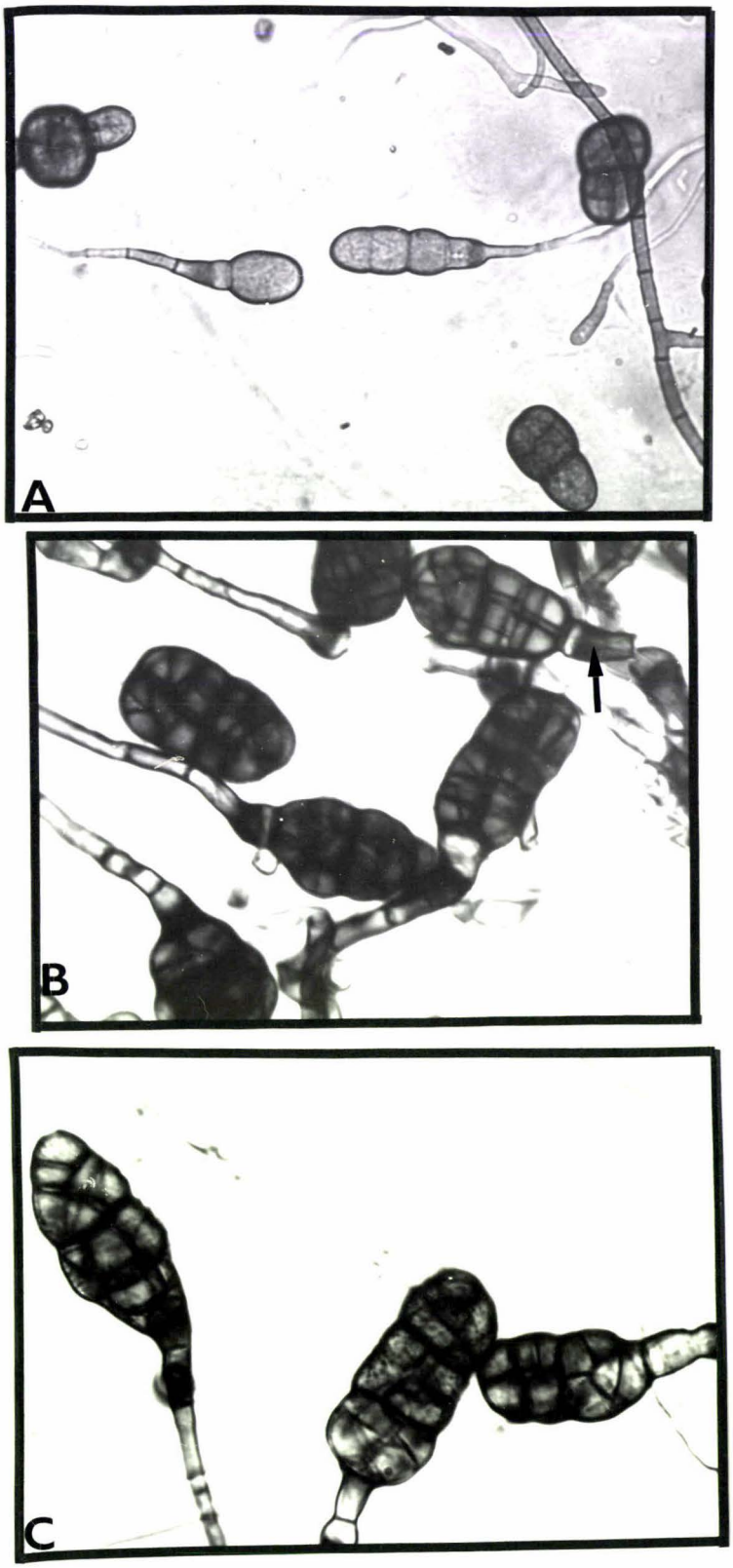

A
B
C

Fig. 24. Conidiophores and conidia of a Stemphylium sp. isolated from annual phlox. The fungus was grown on 5% V-8, pH 7.5 and incubated at 20C in an 8h photoperiod. A, conidiophores (x725); B, conidiophores and juvenile conidia (x650); C, mature conidia (x850).

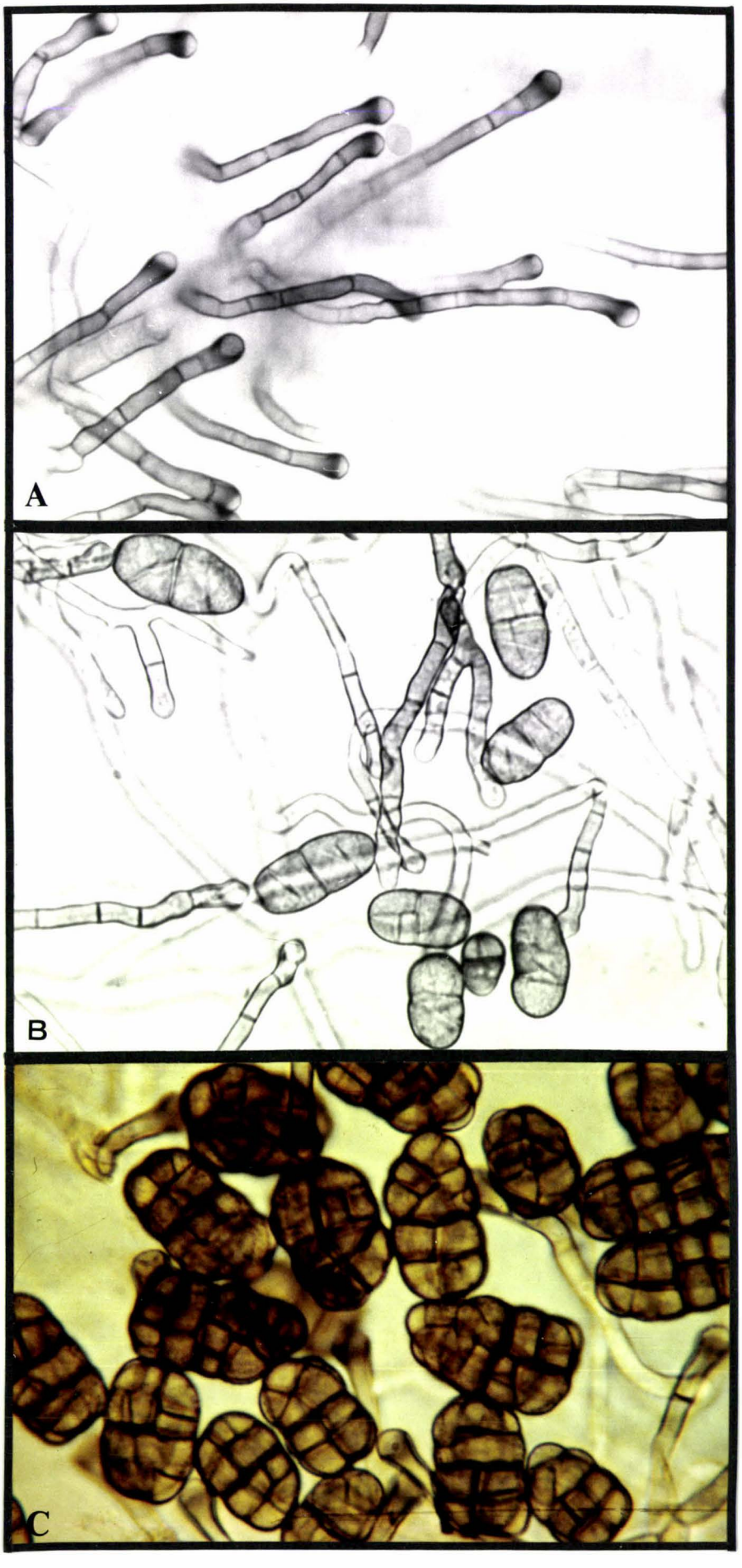

A
B
C

Fig. 25. Surface ornamentation of conidia and conidiophores of a Stemphylium sp. isolated from annual phlox. A & B, scanning electron micrographs (x3900); C & D, light micrographs of conidia (x1400) and conidiogenous cell (x1700) respectively.

Note the punctate nature of the conidiogenous cell and the verrucose surface of the conidia.

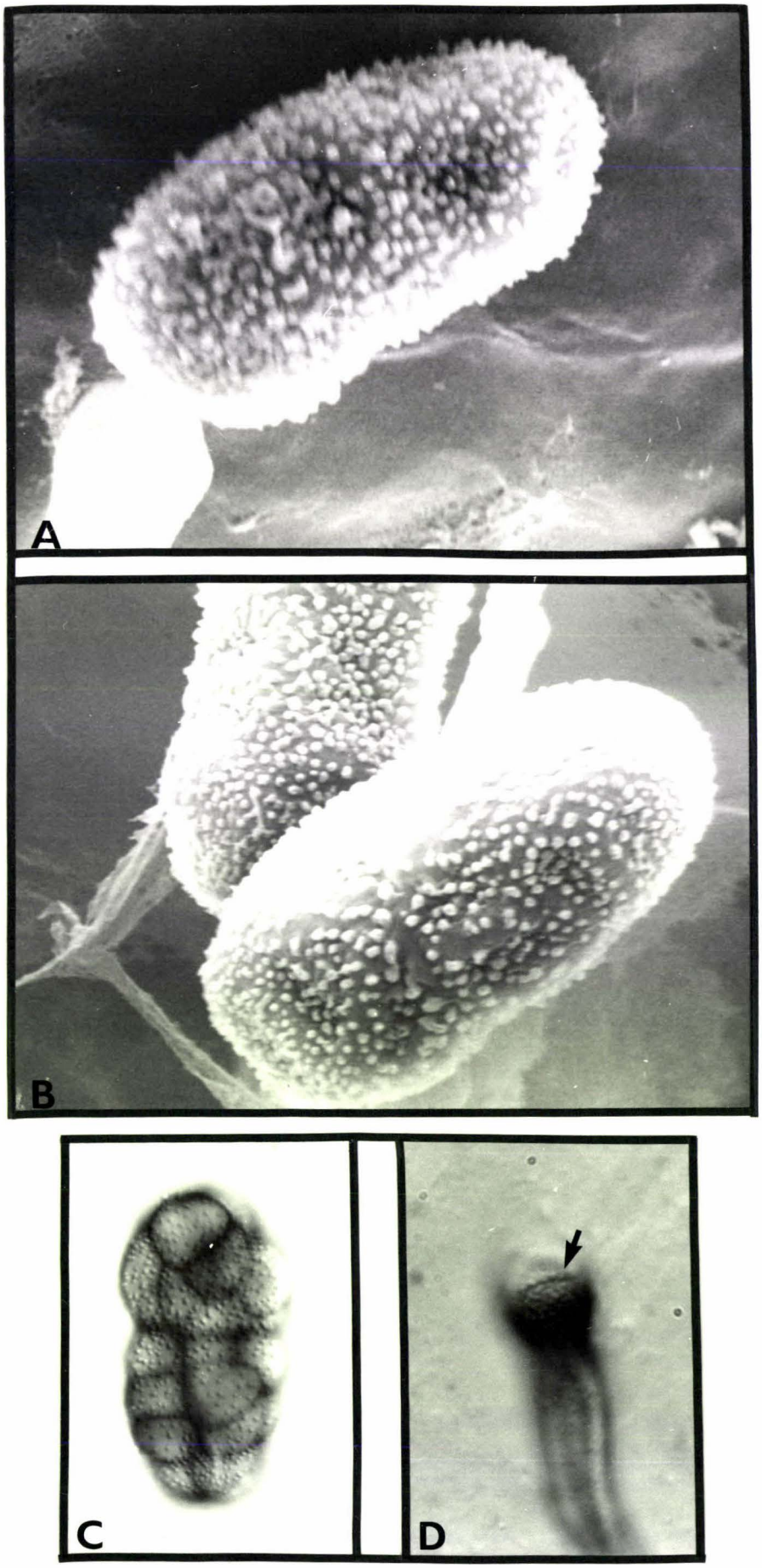
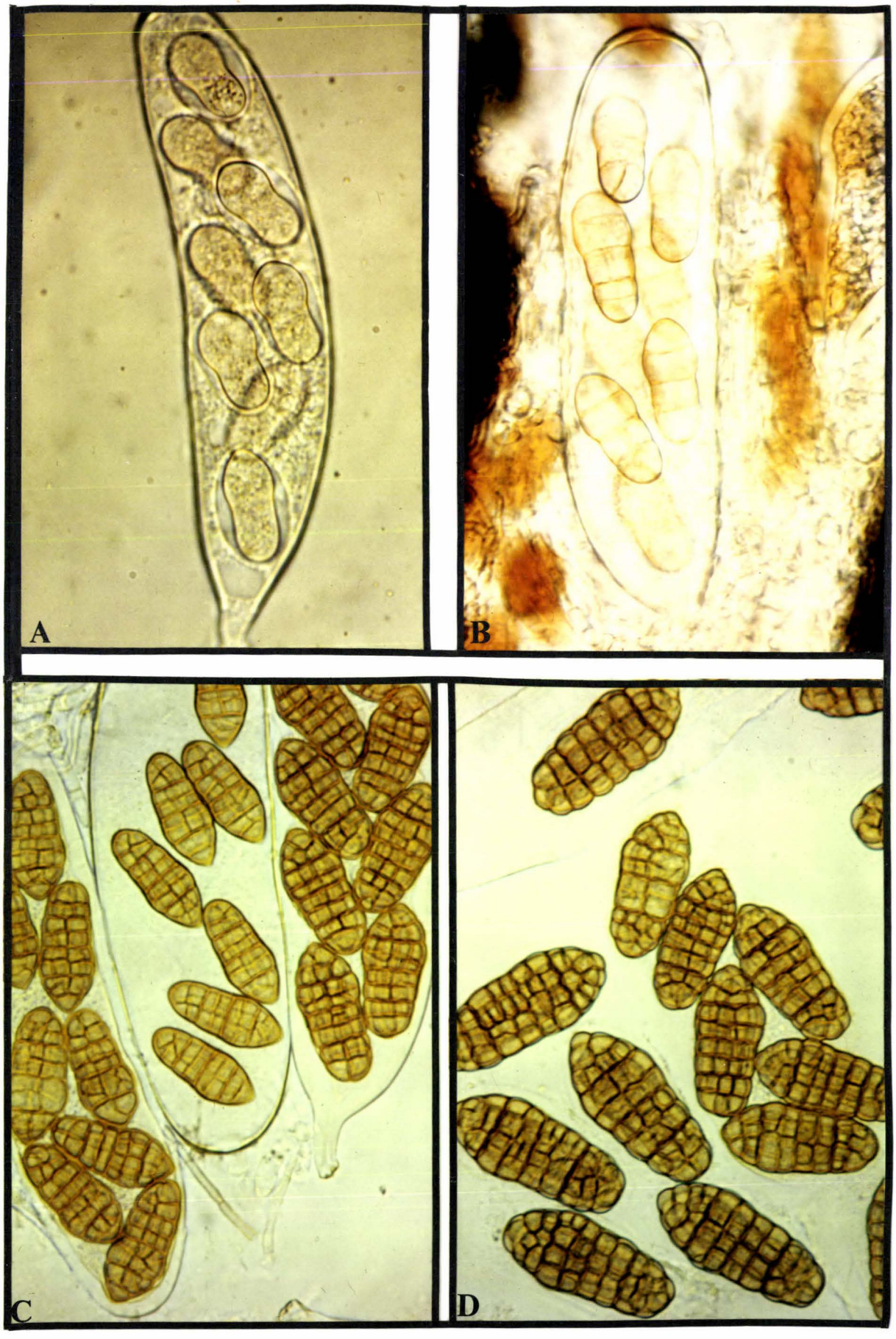

A
B
C
D

Fig. 26. Asci and ascospores of a Stemphylium sp./Pleospora sp. isolated from annual phlox. Cultured on PCA at 12C in darkness. A, ascus with one-septate ascospores (x550); B, ascus containing ascospores with three or four lateral septa (x550); C, asci with both immature and mature ascospores (x475); D, mature ascospores (x550).


A
B
C
D

The asci were hyaline, broadly clavate to broadly cylindrical or even ellipsoidal, tapered to a knob-like or claw-like base, and measured 150 - 280μ x 39 - 75μ (Fig. 26). Mature ascospores were 41 - 66μ x 19 - 28μ (av. 54.6 x 24.7μ), biseriately arranged, oblong-ellipsoid, with the upper portion broader and acutely pointed, lower portion tapering to a rounded base, yellowish-brown to golden-brown, with 9-13 lateral and 3-4 longitudinal septa, and constricted at the primary, secondary, and occasionally at the tertiary transverse septa (Fig. 26C, D). The juvenile ascospores at the one-septate stage were hyaline, basically oblong, with the apical cell broader and almost spherical and the basal cell cylindrical and tapering to a rounded base (Fig. 26A). At the three-septate stage the penultimate cell of juvenile ascospores was markedly broader than the remainder of the spore body (Fig. 26B).

Although the Stemphylium sp. from annual phlox resembles S. botryosum in the general shape of the conidia, and S. vesicarium in the presence of 1 - 3 lateral septal constrictions, it can be readily distinguished from these two species on the basis of the conidial dimensions. Furthermore, ascospores of the phlox isolate differ considerably in shape during development, and dimensions from the ascospores of the Pleospora state of either S. botryosum or S. vesicarium, or for that matter any of the Stemphylium spp. described by Simmons in 1969. No decision has been made on the specific epithet for the Stemphylium or Pleospora state, although study of Wehmeyer's (1961) monograph on the genus Pleospora suggests that Pleospora gigaspora Karst., Pleospora rainierensis Wehm., or Pleospora njegusensis Bubak should be considered as possibilities.

2.2 CROSS-PATHOGENICITY STUDIES

Physiological specialization of plant pathogenic fungi has been used by various workers to supplement morphological taxonomy, and Stemphylium species are no exception. Padhi and Snyder (1954) found that S. botryosum from lettuce displayed selective pathogenicity to lettuce and would not infect onions, carrots, beans, tomatoes, and sweet potatoes. Similarly host specialization has been demonstrated for isolates of S. botryosum from tomatoes (Bashi, Rotem & Wahl, 1973; Rotem, Cohen & Wahl, 1965) and peppers (Baverman, 1968). The primary purpose of the present investigations was to obtain further information concerning the pathogenicity and host range of the Stemphylium isolates in New Zealand.

MATERIALS AND METHODS

Cross-pathogenicity tests were conducted on nine host species namely, asparagus, chrysanthemum, lucerne, onion, tomato, pepper, annual phlox, lettuce, and blue lupin. The 39 isolates of Stemphylium used were as follows:

(i) 21 isolates of S. vesicarium, 3 each from onion, tomato, pepper, asparagus, chrysanthemum, lucerne and blue lupin.

(ii) 9 isolates of S. botryosum, 3 each from lettuce, lucerne, and blue lupin.

(iii) 6 isolates of S. globuliferum, 3 each from lucerne and blue lupin.

(iv) 3 isolates of Stemphylium sp. from annual phlox.

Test plants were grown in a peat-sand-gravel medium (5 : 4 : 1) enriched with nutrients. Except in the case of chrysanthemums where shoot cuttings were used, plants were raised from seeds. Lucerne, onion and asparagus were grown 3 plants/14cm pot, the remainder at 2 plants/pot. Chrysanthemums were inoculated when the flowers were fully open whereas the other plants were inoculated when they were 6-8 weeks old.

Inoculum was produced on 10% V-8 by incubating cultures for 8 days at 24C in an 8h photoperiod. A conidial suspension of ca. 250,000 spores/ml was prepared in sterile distilled water to which Tween 80 was added (4 drops/250 ml) to improve wettability. Each isolate was tested on all nine plant species. An atomizer gun operating at a pressure of 30 p.s.i. was used to spray the plants to run-off. Controls consisted of uninoculated plants sprayed with sterile distilled water containing Tween 80. To facilitate infection plants were covered with a plastic bag for 48h to maintain high humidity and then transferred to walk-in controlled climate rooms. The environmental conditions in the room were as follows:

(i) air temperature 20C $\pm$ 0.3C

(ii) relative humidity 95% $\pm$ 5%

(iii) light regime 180 Wm^{-2} (400-700nm), obtained with 4 x 1000 W Sylvania "metal-arc" high pressure discharge lamps, together with 4 x 1000 W Philips tungsten iodide lamps. Intensity was measured with an Eppley pyranometer and Schott R.G.8 filter system. A 12h photoperiod with an abrupt light-dark change was used.

All pathogenicity tests were replicated three times. Results were recorded after 7 days using a disease rating system as follows: 0 = no infection; 1 = trace infection; 2 = moderate infection; 3 = severe infection; 4 = very severe infection.

Using the same system, cross-pathogenicity tests were also conducted between the three Lupinus species, namely Lupinus angustifolius L. (blue lupin), Lupinus aboreus L. (tree lupin) and Lupinus polypyllus L. (Russell lupin).

RESULTS

Results summarised in Table 8 show that S. botryosum from lettuce and the Stemphylium sp. from annual phlox were the only host specific isolates. Although a few ray florets of one chrysanthemum flower showed some lesioning when inoculated with isolate MU 68 from phlox, these results could not be repeated. None of the isolates from the other hosts were pathogenic to lettuce or phlox.

The isolates of S. vesicarium from pepper and tomato were very similar in that besides being cross-pathogenic they were also pathogenic to chrysanthemum.

All three isolates of S. vesicarium from asparagus were moderately pathogenic to chrysanthemum and lucerne, and mildly pathogenic to onion. Asparagus was not infected by any of the isolates from other hosts, including isolates of S. vesicarium from the three host species mentioned previously on which asparagus isolates of S. vesicarium were pathogenic.

The chrysanthemum and onion isolates of S. vesicarium were not only cross-pathogenic but also caused mild to moderate infection on lucerne. Of the plant species tested, chrysanthemum proved more susceptible to infection by the Stemphylium species, but this greater susceptibility was probably due to the tenderness of the floral tissues.

The isolates of S. vesicarium, S. botryosum, and S. globuliferum from lucerne and blue lupin were all cross-pathogenic. S. vesicarium isolates from these two leguminous hosts were also mildly pathogenic to chrysanthemum. Furthermore, all three isolates of this species from lucerne and one isolate (MU 13) from blue lupin were pathogenic to onion.

Separate cross-pathogenicity tests conducted with Russell lupin, tree lupin and blue lupin indicated that S. botryosum and S. vesicarium from these three hosts were cross-pathogenic and that S. globuliferum

TABLE 8 Results of cross-inoculation of 39 *Stemphylium* isolates on 9 different hosts

Isolate	Source Host	*Stemphylium* species	Disease Rating* on Test Plants								
			asparagus	chrysanthemum	onion	pepper	tomato	lettuce	phlox	lucerne	blue lupin
MU 18	asparagus	*S. vesicarium*	4	2	1	0	0	0	0	2	0
MU 32	"	"	3	2	1	0	0	0	0	2	0
MU 59	"	"	4	2	1	0	0	0	0	2	0
MU 2	chrysanthemum	*S. vesicarium*	0	4	1	0	0	0	0	2	0
MU 26	"	"	0	4	2	0	0	0	0	2	0
MU 83	"	"	0	4	1	0	0	0	0	1	0
MU 21	onion	*S. vesicarium*	0	2	3	0	0	0	0	1	0
MU 77	"	"	0	2	4	0	0	0	0	2	0
MU 99	"	"	0	2	4	0	0	0	0	1	0
MU 70	pepper	*S. vesicarium*	0	1	0	3	3	0	0	0	0
MU 100	"	"	0	1	0	3	3	0	0	0	0
MU 124	"	"	0	1	0	3	3	0	0	0	0
MU 15	tomato	*S. vesicarium*	0	1	0	3	4	0	0	0	0
MU 31	"	"	0	1	0	3	3	0	0	0	0
MU 75	"	"	0	1	0	3	3	0	0	0	0
MU 4	lettuce	*S. botryosum*	0	0	0	0	0	4	0	0	0
MU 54	"	"	0	0	0	0	0	4	0	0	0
MU 159	"	"	0	0	0	0	0	4	0	0	0
MU 65	phlox	*Stemphylium sp.*	0	0	0	0	0	0	3	0	0
MU 68	"	"	0	0	0	0	0	0	3	0	0
MU 109	"	"	0	0	0	0	0	0	3	0	0
MU 6	lucerne	*S. vesicarium*	0	2	2	0	0	0	0	4	2
MU 8	"	"	0	1	1	0	0	0	0	4	3
MU 44	"	"	0	1	1	0	0	0	0	4	2
MU 61	"	*S. botryosum*	0	0	0	0	0	0	0	3	3
MU 80	"	"	0	0	0	0	0	0	0	4	3
MU 152	"	"	0	0	0	0	0	0	0	3	3
MU 5	"	*S. globuliferum*	0	0	0	0	0	0	0	4	3
MU 24	"	"	0	0	0	0	0	0	0	4	2
MU 113	"	"	0	0	0	0	0	0	0	4	3
MU 13	blue lupin	*S. vesicarium*	0	1	1	0	0	0	0	3	4
MU 29	" "	"	0	1	0	0	0	0	0	2	3
MU 51	" "	"	0	1	0	0	0	0	0	2	3
MU 63	" "	*S. botryosum*	0	0	0	0	0	0	0	2	4
MU 144	" "	"	0	0	0	0	0	0	0	2	4
MU 154	" "	"	0	0	0	0	0	0	0	1	3
MU 28	" "	*S. globuliferum*	0	0	0	0	0	0	0	2	4
MU 46	" "	"	0	0	0	0	0	0	0	3	3
MU 142	" "	"	0	0	0	0	0	0	0	2 - 3	

* Disease rating: 0 = no infection; 1 = trace infection; 2 = moderate infection; 3 = severe infection; 4 = very severe infection.

from blue lupin was also moderately pathogenic to Russell lupin and tree lupin.

DISCUSSION

The results indicate that host specialization in Stemphylium is relatively uncommon. This result is in contrast to Dingley's (1969) report that strains of the fungus that are primary pathogens are usually host specific.

In the literature there are anomalies regarding interpretation of the term host specialization as it relates to Stemphylium. Baverman (1968) and Sivan & Barkai-Golan (1976) investigating the pathogenicity of S. botryosum from pepper and lettuce respectively, reported the fungus to be host specific and proceeded to use the trinomial system of nomenclature, despite the fact that the fungus infected other members of the same host family. If the concept of host specialization is to be meaningful, and as intended (Cowan, 1968; Lapage et al., 1976) its use be confined to pathogens that are restricted to one host species then the reports of Baverman and Sivan & Barkai-Golan must be disregarded.

2.3 GENERAL DISCUSSION

The recognition in New Zealand of four species of Stemphylium, namely S. botryosum, S. globuliferum, S. vesicarium and Stemphylium sp. is in contrast to the traditionally held view that all isolates of the genus in this country are S. botryosum (Dingley, 1969).

The results of the survey indicate that S. vesicarium is by far the most common species present in this country. Furthermore, this is the first report of S. vesicarium as a pathogen on tomato, pepper, lucerne, blue lupin, tree lupin and Russell lupin. The study also confirms the writer's (1973) and Koh's (1975) earlier reports of S. vesicarium as a pathogen causing chrysanthemum flower blight and asparagus stem and foliage blight, respectively.

Suzui's (1973) recent claim that stemphylium leaf spot of asparagus is caused by S. botryosum is questionable. On the basis of his descriptions, line drawings, and photographs of conidia the pathogen appears to be more typical of S. vesicarium than S. botryosum.

The isolation of three species of Stemphylium from lucerne may be of more than passing concern to the lucerne industry of New Zealand. Observations made in the Manawatu district during the period 1973-1975

indicate that the disease is very common in spring and autumn, and causes severe defoliation when the crop is held longer than the usual 4-6 weeks between grazing or cutting. It is therefore essential that any programme aimed at producing disease resistant lucerne varieties should recognise the occurrence of three Stemphylium spp. pathogenic to the crop.

The apparent localization of S. globuliferum in the Auckland and Gisborne areas may be indicative of this species being only recently introduced into New Zealand, and as yet has not become widely distributed. Although cross-pathogenicity tests have shown that this species from blue lupin can infect Russell lupin and tree lupin, the results of the survey and many later isolations suggests that in the field S. globuliferum occurs only on blue lupin.

Finally, because the lettuce fungus exhibits host specialization the proposal of Padhi & Snyder (1954) to use the trinomial Stemphylium botryosum f. sp. lactucum is endorsed. This demonstration of host specificity is in disagreement with Slade (1961) who studied the stemphylium leaf spot disease of lettuce in New Zealand and claimed the fungus could also infect Solanum nigrum L. In the present study repeated inoculation tests using three S. botryosum isolates from lettuce failed to infect S. nigrum.

KEY TO PLANT PATHOGENIC STEMPHYLIUM SPECIES IN NEW ZEALAND BASED ON MORPHOLOGICAL FEATURES OF CONIDIA AND ASCOSPORES

A[1] Conidia predominantly oval to oblong, greyish-brown, 2-3 constricted, 27-52 x 13-21μ (av. 37 x 17μ), ascospores ellipsoidal, 34-44 x 15-22μ (av. 38 x 18μ) ____ *S. vesicarium*

A[2] Conidia predominantly subspherical to subdoliform

B[1] Conidia verrucose, 1 constricted, greyish-brown, 22-37 x 15-23μ (av. 30 x 19μ), ascospores oblong, 34-44 x 15-19μ (av. 39 x 17μ) ____ *S. botryosum*

B[2] Conidia sparsely punctate, 1 constricted, pale olivaceous-brown to pale olivaceous-grey, septation sharply defined, 21-35 x 14-23μ (av. 28 x 18μ), ascospores ellipsoidal, 38-49 x 17-22μ (av. 44 x 20μ) ____ *S. globuliferum*

B[3] Conidia verrucose, 1-3 constricted, dark brown to greyish-brown, 24-42 x 15-24μ (av. 33 x 21μ), ascospores ellipsoidal, 41-66 x 19-28μ (av. 55 x 25μ) ____ *Stemphylium* sp. ex phlox

CHAPTER 3

SUPPLEMENTARY MYCOLOGY

3.1 ULTRASTRUCTURE OF CONIDIOGENESIS IN STEMPHYLIUM BOTRYOSUM

In 1953 Hughes proposed a system of classifying the Hyphomycetes according to the manner of conidium production. He outlined eight basic methods by which a conidium can be formed and assigned each of these to a taxonomic section. Subsequently a great deal of interest has been shown in fungal ontogeny and considerable progress and understanding of the Hyphomycetes has been achieved. Section VI in which Hughes classified Stemphylium has in recent years been somewhat controversial. According to Hughes the fungi included in this section are characterized by the presence of minute pores in the conidiogenous cell through which the conidia arise as protoplasmic protrusions. However, fine structure studies suggest that there could in fact be two different modes of conidium production in this section:

(i) holoblastic, involving a simple blowing out of all wall layers of the conidiogenous cell, as in Stemphylium (Carroll & Carroll, 1971) and Ulocladium (Carroll & Carroll, 1974);

(ii) tretic, involving the extrusion of the inner wall of the conidiogenous cell through a preformed channel in the outer wall, as in Drechslera (Cole, 1973) and Alternaria (Campbell, 1969).

Luttrell (1963) found from light microscopy of conidium production in Drechslera sorokiniana (Sacc.) Subram. (= Helminthosporium sorokiniana (Sacc.) in Sorok.) that temperature influenced the mode of conidium production. At 31C the conidium originated as a swelling of the entire tip of the conidiogenous cell, whereas when it was grown at 20C it originated through a pore in the wall of the conidiogenous cell. Production of D. sorokiniana conidia with differing modes of ontogeny, sometimes on the same conidiophore, have also been reported by Subramanian (1964, 1972) who suggested that both "nature" and "nurture" may affect conidium ontogeny and morphogenesis.

While conducting scanning electron microscopic studies of the surface topography of conidiophores and conidia of Stemphylium, the writer frequently observed a discrete pore in the apical region of a fully developed conidiogenous cell (Fig. 27A, B, C). This pore could be readily

differentiated from one that was formed after abstriction of the conidium in that it was smaller in diameter and smoother in outline as compared to the cup-like or crater-like appearance of the latter (Fig. 30B). In some instances it was also possible to observe the outgrowth of the conidium initial through this pore (Fig. 27C, D), possibly indicating tretic type conidium development. As these observations were contrary to those of Carroll and Carroll (1971) who reported a holoblastic type development for the fungus, ultrastructural studies were conducted to elucidate the cell-wall relationship between the conidium and the conidiogenous cell.

MATERIALS AND METHODS

S. botryosum (isolate MU 11) was grown on 5% V-8 at either 20C or 30C for 4 days in an 8h photoperiod. Small agar blocks with densely sporulating mycelium were cut from the fungal colony and fixed in 2% glutaraldehyde buffered in 0.1M sodium cacodylate at pH 7.2 for 2h. A drop of Triton was added as a wetting agent. Specimens were then washed four times in the same buffer, post-fixed in 1% osmium tetroxide buffered in 0.1M cacodylate buffer (pH 7.4) for 5h, washed in 3 changes of distilled water and soaked overnight in 0.5% aqueous uranyl acetate. After washing in 3 changes of distilled water, and dehydrating in an ethanol series the specimens were infiltrated and embedded in Spurr's resin (1969). Specimens were sectioned with a diamond knife on a LKB ultramicrotome, double stained with uranyl acetate followed by lead acetate, and examined with a Philips EM 200 electron microscope.

Some conidiophores and conidia were also prepared for scanning electron microscopy using the technique previously described (see 1.1.2).

RESULTS

The longitudinal sections through the junction of conidiogenous cell and conidium in Figure 28 show that all wall layers of the conidiogenous cell and the conidium are continuous during conidium production. Unlike D. sorokiniana (Luttrell, 1963) incubation temperature does not affect the mode of conidium production because the fungus grown at 30C produced conidia in exactly the same manner as those produced at 20C.

When the conidium is fully developed a septum is formed at its base (Fig. 28C). At about the same time a triangular, electron dense Woronin body (Cole, 1973) can also be seen at the junction between the conidium and conidiogenous cell (Fig. 28C). The Woronin body presumably helps to plug the septal pore at the base of the conidium, thereby

inhibiting the passage of cytoplasmic organelles but allowing the free flow of nutrient molecules. Furthermore, during abstriction it may help to seal the conidial pore. Conidium abstriction is believed to occur by centripetal separation, at the basal septum of the conidium (Fig. 29A, B). On detachment a crater-like scar is left at the base of the conidium (Fig. 30A), and the conidiogenous cell collapses inwards and becomes cup-shaped (Fig. 30B).

Conidiophore proliferation occurs either from the side of the conidiogenous cell and below the terminal conidium (Fig. 31A, C), or directly through the pore formed in the conidiogenous cell after abstriction of the conidium (Fig. 31B, D). In the former, cell wall layers of the conidiogenous cell are continuous with the newly formed secondary conidiophore (Fig. 31C), whilst in the latter it is presumed that proliferation originates from the basal septum of the conidiogenous cell (Fig. 31D).

DISCUSSION

Results of the ultrastructural studies of *S.botryosum* confirm the earlier report of Carroll & Carroll's (1971) that conidiogenesis in this fungus is of the holoblastic type. Since all wall layers of the conidiogenous cell are continuous with the conidium during conidiogenesis, the pore, as seen under the scanning electron microscope prior to conidium production, may in fact be a narrow channel formed in the superficial layer of melanin deposits through which the juvenile conidium emerges. If such is the case Figure 27 would be more correctly interpreted as a conidium initial emerging through a pore dissolved in the superficial melanin layer.

The claims for tretic conidiation in *Drechslera sorokiniana* (Cole, 1973) and *Alternaria brassicicola* (Schw.) Wiltshire (Campbell, 1969) are questionable. Following examination of Cole's (1973) and Campbell's (1969) transmission electron micrographs the writer agrees with Carroll & Carroll (1974) that conidiogenesis in this two fungi is holoblastic rather than tretic, as reported. The primary wall indicated in Campbell's Figure 17 can be interpreted as a superficial layer of pigment deposition which has been enzymically dissolved, and through which the conidium has developed holoblastically. Similarly, in *D. sorokiniana*, Cole's Figures 6-12, depicting early tetroconidium production could also be interpreted as an off-median or glancing section of the conidiogenous cell and conidium. The primary wall indicated in

these figures could be interpreted as a superficial layer of pigment deposits.

The writer agrees with Carroll & Carroll (1974) that because tretic conidiation has not been convincingly demonstrated for any Hyphomycete fungus, the use of this term should be discontinued until experimental evidence in support of the concept is provided.

Fig. 27. Scanning electron micrographs of conidiogenous cells of Stemphylium botryosum. A, B & C, pore-like (P) structure at distal end of conidiogenous cell (x9000, 7000, 14000 respectively). D & E, early stages of conidium (Cn) development (14000, 7700 respectively).

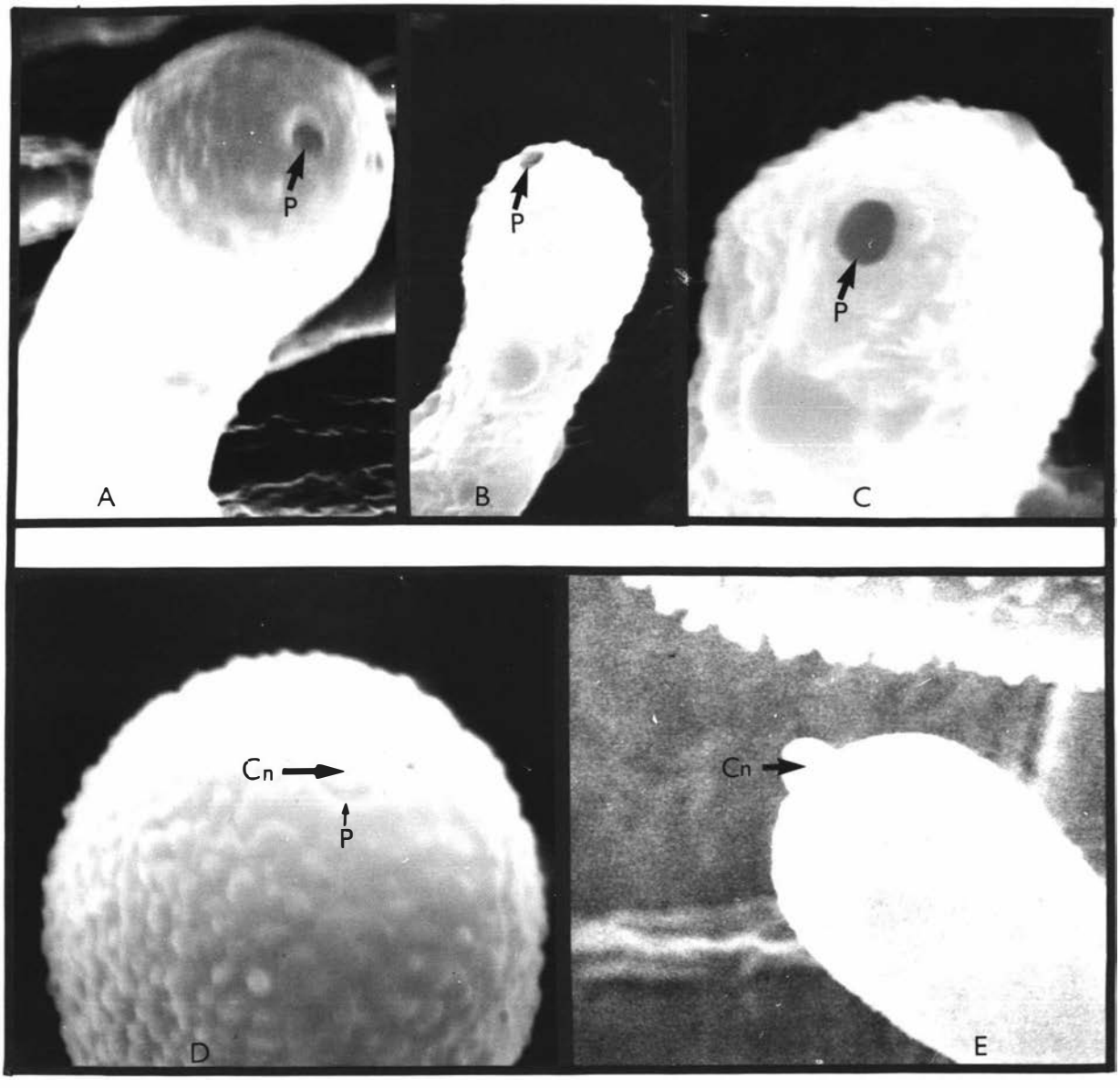
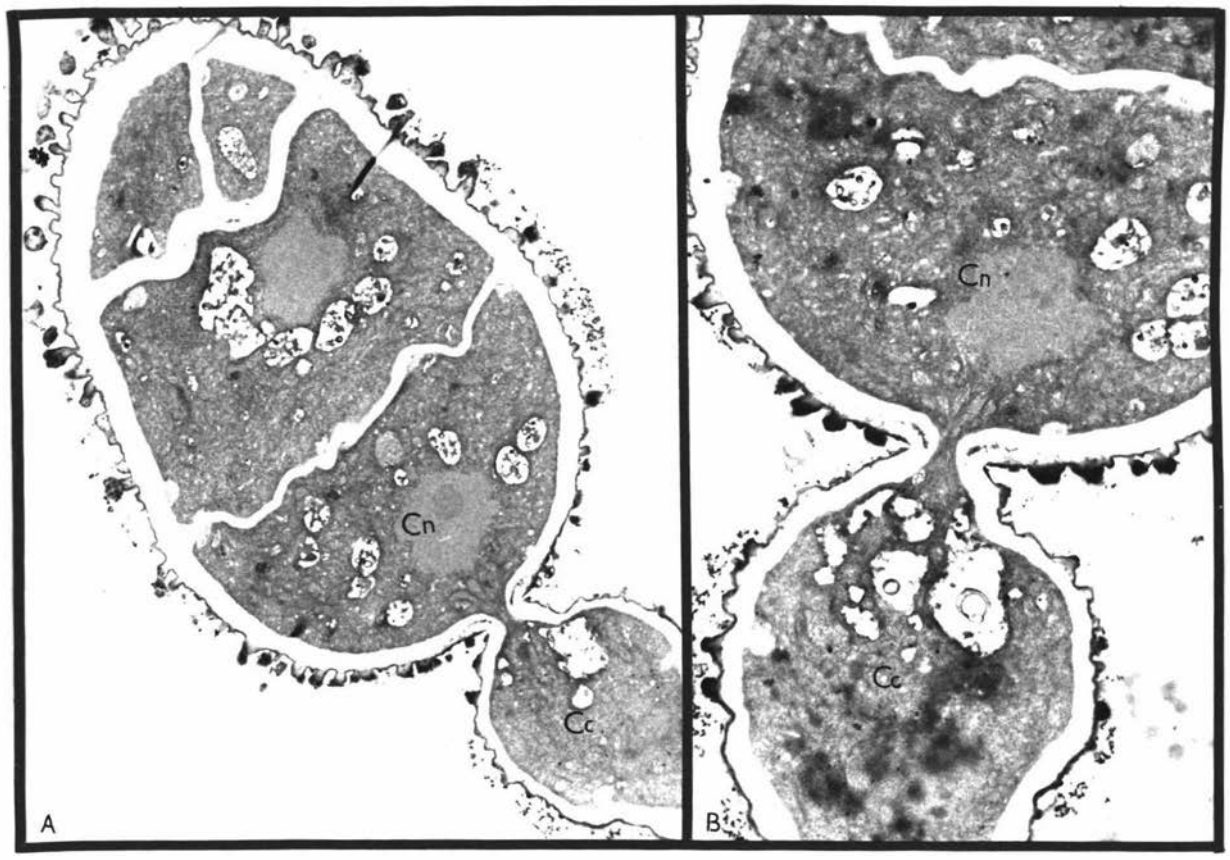

P
A
P
B
P
C
Cn
P
D
Cn
E

Fig. 28. Longitudinal section through the junction between conidiogenous cell (Cc) and conidium (Cn) of *Stemphylium botryosum*. A, section through a developing conidium (x6400); B, close-up of 'A' (x10,500); C, section through a mature conidium showing the formation of septum (S) and the presence of a Woronin body (W) in the vicinity of the septum (x17,500).

Note the continuity of the wall layers of the conidiogenous cell and conidium.


Cn
Cc
A
Cn
Cc
B

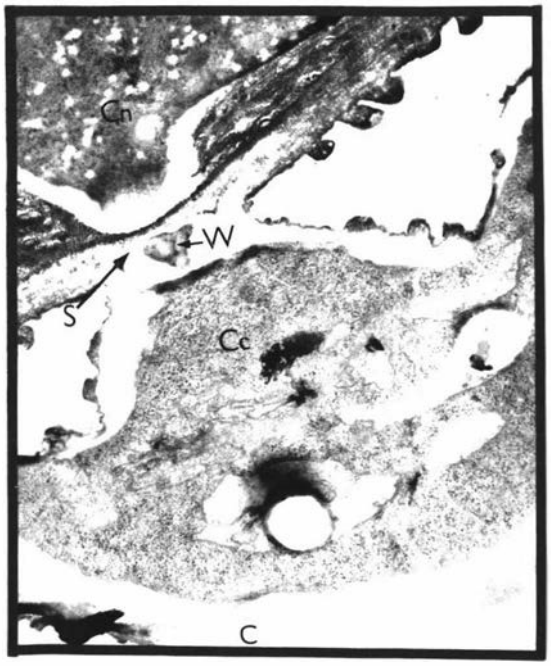
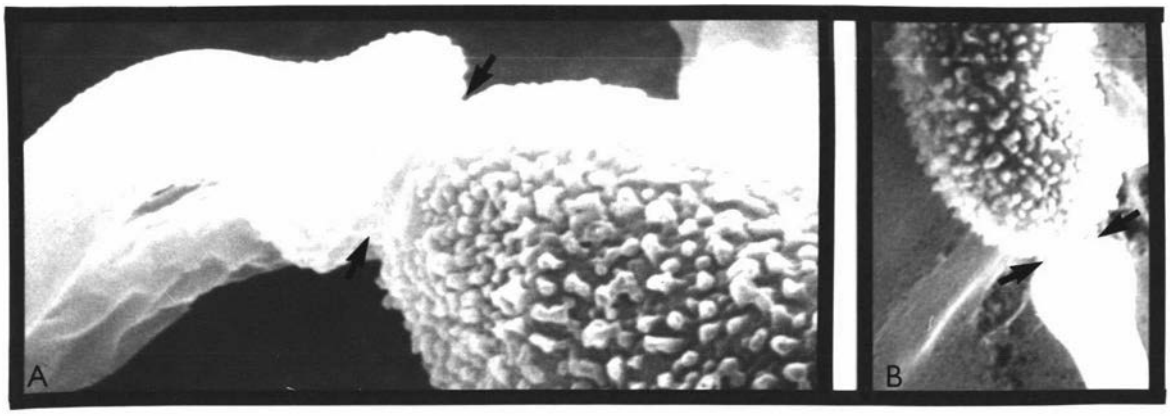

Cn
W
S
Cc
C

Fig. 29. Scanning electron micrographs of Stemphylium botryosum showing conidium abstriction. A (x7000); B (x3000).

Arrows indicate the line of secession.

Fig. 30. Scanning electron micrographs of Stemphylium botryosum after abstriction of conidium. A, abstriction scar at the base of conidium (x5000); B, crater-like depression of the conidiogenous cells after conidium release (x4600).


A
B

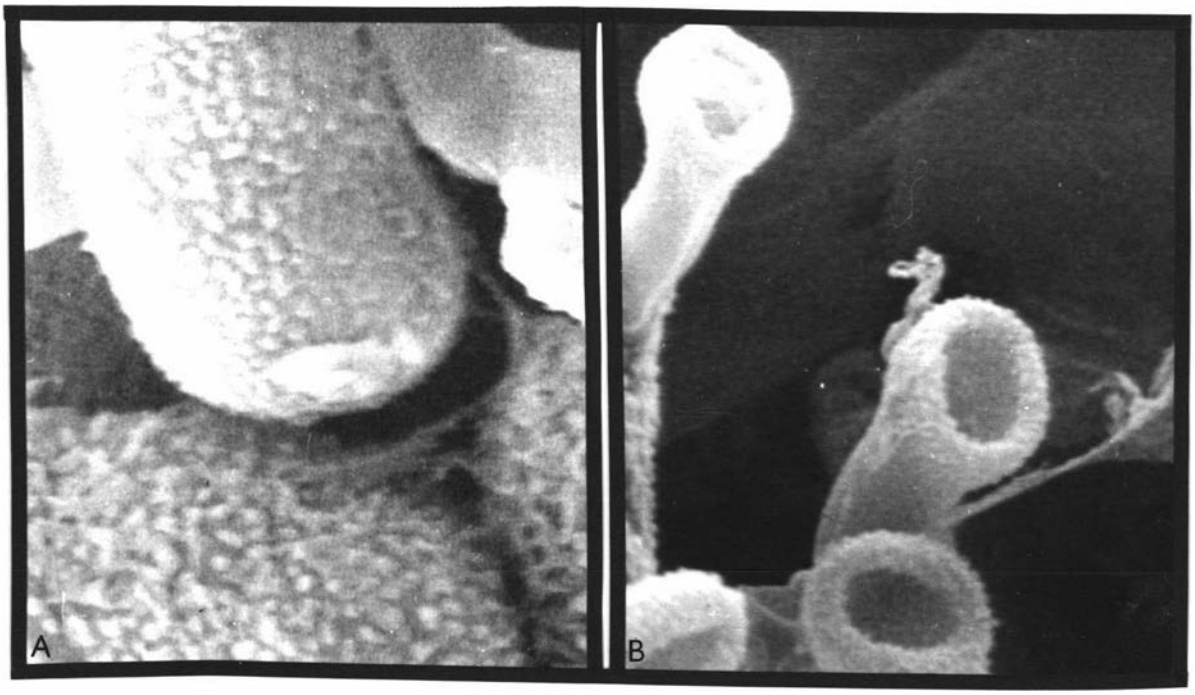
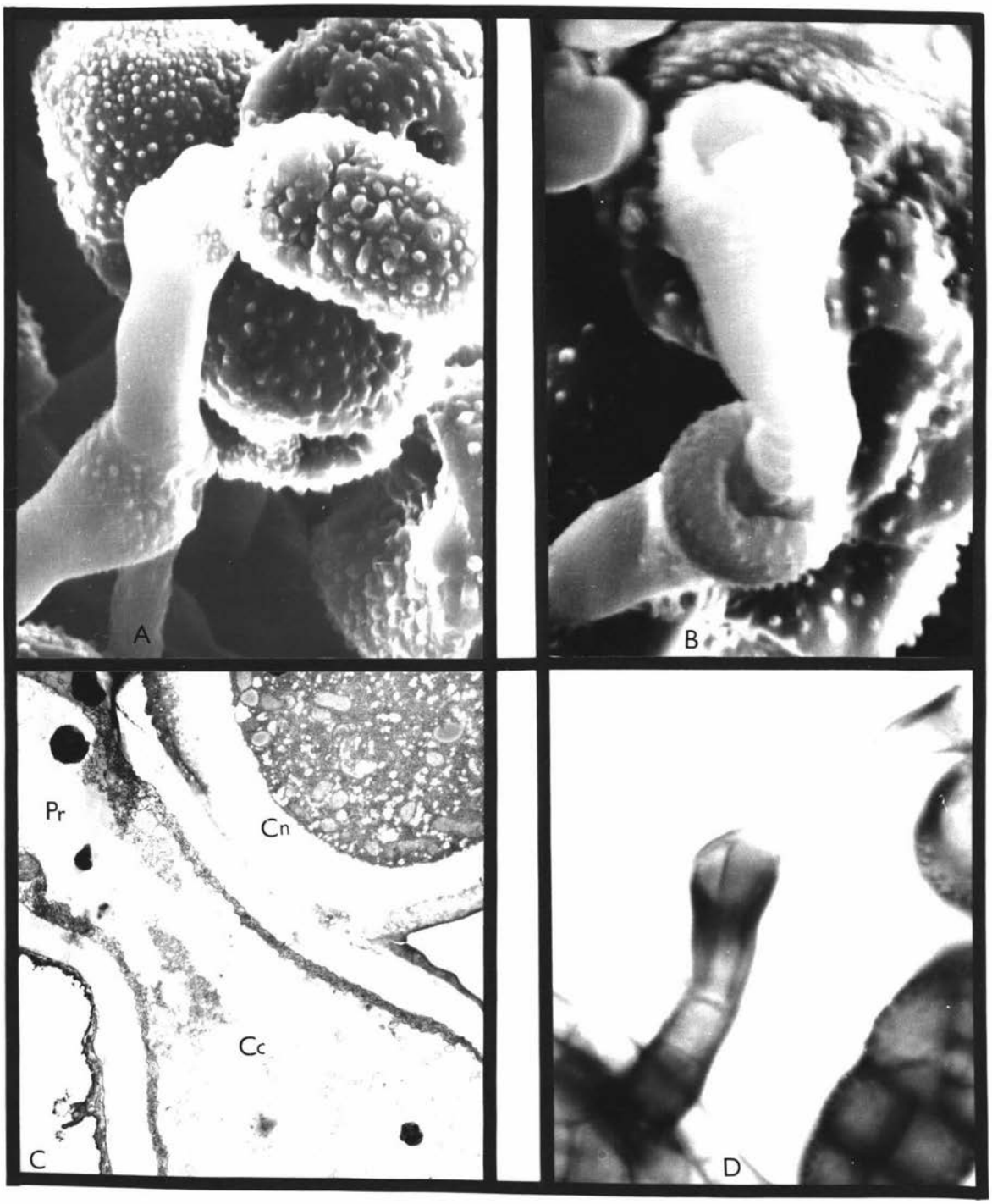

A
B

Fig. 31. Conidiophore proliferation of Stemphylium botryosum.
A & C, scanning electron micrograph (x3500) and longitudinal section (x9300) respectively, showing proliferation as an extension of the conidiogenous cell. Note the continuity of wall layers between conidiogenous cell and the proliferating extension. B & D, scanning electron micrograph (x5200) and light micrograph (x1200) respectively, showing proliferation through the pore in the conidiogenous cell formed after abstriction of the conidium.

Cc = conidiogenous cell; Cn = conidium, Pr = proliferation of conidiogenous cell.


A
B
Pr
Cn
Cc
C
D

3.2 REVERSION OF CONIDIOPHORES AND JUVENILE CONIDIA TO HYPHAL-LIKE GROWTHS

While determining temperature effects on Stemphylium cultures it was observed that at either 27 or 30C and an 8h photoperiod isolates frequently exhibited hyphal-like regrowth of the conidiophores. This regrowth was generally accompanied by photoperiodic inhibition of conidium production. The phenomenon has previously been reported for Alternaria (Aragaki, Nishimoto & Hylin, 1973; Kumagai & Oda, 1969b; Lukens, 1963). Aragaki, Nishimoto & Hylin (1973) interpreted this regrowth of Alternaria tomato (Cke.) Weber as reversion of conidiophores to vegetative or non-reproductive growth. They observed this development occurred in cultures incubated at temperatures above 25C in light lacking the near-ultraviolet (NUV) wavelength, and also following the flooding of cultures with water.

To investigate this phenomenon further, experiments were conducted to examine the process of conidiophore reversion and determine more precisely the factors responsible for its occurrence.

MATERIALS AND METHODS

Cultures of S. botryosum (isolate MU 4) were grown for 5 days on 5% V-8 under continuous fluorescent light at 27C. To induce full development of conidiophores the cultures were subsequently exposed to a 4h period of darkness at the same temperature. Following this they were divided into nine groups and each group subjected for 18h to one of nine treatments. Unless otherwise stated the incubation temperature during the treatments was 27C.

(i) NUV light (40W Philips BLB tube)
(ii) blue* light (480 nm)
(iii) green* light (521 nm)
(iv) yellow* light (583 nm)
(v) red* light (662 nm)
(vi) fluorescent light filtered through Mylar Type W (proprietary plastic, du Pont) to remove wavelengths below 400 nm.
(vii) fluorescent light at 16C
(viii) fluorescent light at 20C
(ix) flooded with sterile distilled water; half the plates then incubated in continuous fluorescent light and the remainder in darkness.

* Wavelengths obtained by filtering the light of two 40W fluorescent light tubes through 'Depal' monochromatic interference filters, manufactured by Jena Glarwerk Schott & Gen; Mainz.

RESULTS

Exposure of cultures to a 4h dark period prior to the various treatments resulted in a variety of conidiophore development. As evidenced by the presence of the bulbous conidiogenous cell, the majority of the conidiophores were fully developed and typical of the genus Stemphylium (Fig. 32A), although a few developed further and produced a young conidium. In addition there were other conidiophores which had still to develop the bulbous conidiogenous cell (Fig. 32B). The existence of these different stages of development within one culture facilitated examination of the effect of different treatments on conidiophores at the various developmental stages.

A considerable number of the conidiophores in cultures exposed to blue light showed hyphal-like regrowth, with only traces of conidium development. By contrast cultures exposed to green, yellow, and red light produced abundant conidia and no regrowth of conidiophores. In NUV light conidiophore regrowth was absent and conidium production was only sparse.

Reversion of conidiophores to hyphal-like growth was very common in cultures exposed to light filtered through Mylar or in cultures flooded with sterile distilled water. Regrowth of conidiophores by physical contact with water occurred regardless of whether the cultures were incubated in dark or light, and as reported by Aragaki, Nishimoto & Hylin (1973) this was an extremely effective method of inducing regrowth.

Reversion of conidiophores, whether induced by light or by physical contact with water, occurred irrespective of their stage of development. When the conidiophores were fully developed the apex of the bulbous conidiogenous cell became conical, eventually elongating into a hyphal-like structure (Fig. 33A). Conidiophores which were still not fully developed initially became pointed at the apex and then gradually developed into slender hyphae (Fig. 33B). In instances where juvenile conidia were already formed prior to exposure to the different treatments, the developing conidia were either aborted and the conidiogenous cell reverted to hyphal-like growth (Fig. 34), or the apices of the conidia became nipple-shaped (Fig. 35A) and then began to grow like a germ-tube (Fig. 35B, C). With this type of development, conidia which had developed beyond a certain stage (3 or more septa) seldom reverted but rather matured normally. Occasionally the hyphal-like growth originated from the basal septum of the conidiogenous cell, grew right through the conidium and emerged at the apex (Fig. 36).

When cultures exhibiting conidiophore reversion were again incubated in the dark for 4h or more at 27C, either the entire vegetative regrowth or a few apical cells became more pigmented, assumed the role of a conidiophore and produced a conidium terminally (Figs. 35C & 37). If, however, Stemphylium cultures with secondary conidiophores were again flooded or exposed to blue light (480 nm) or fluorescent light lacking wavelengths less than 400 nm they would again revert to fine aerial hyphae. By alternating these conditions it was possible to produce a conidiophore with a series of bulbous nodular swellings without conidium production (Fig. 38).

Cultures incubated in continuous fluorescent light at either 16C or 20C (treatments vii & viii) exhibited no inhibition of conidium production. Conidiophore reversion was very uncommon, with only a few showing any evidence of regrowth.

DISCUSSION

As in A. tomato, photoinduced vegetative regrowth of both conidiophores and juvenile conidia of S. botryosum at temperatures of 27C and above is dependent upon blue light in the absence of the NUV wavelength. When both these wavelengths are present simultaneously, as in fluorescent light, conidiophores and juvenile conidia remain unchanged. Physical contact with water is also a very effective means of causing reversion. However, it appears that S. botryosum contrasts with A. tomato (Aragaki, Nishimoto & Hylin, 1973) and A. solani (Lukens, 1963) in that inhibition of sporulation of the reverted conidiophores is only temporary.

It is now known that blue light is responsible for many photoresponses, including stimulation of spore discharge (Callaghan, 1969; Walkey & Harvey, 1967), inhibition of sporulation (Aragaki, Nishimoto & Hylin, 1973; Honda & Sakamoto, 1968; Tan, 1974), induction of sporulation (Bjornsson, 1959; Kumagai & Oda, 1969a). These responses are believed to be correlated with photoreceptor pigments such as carotenoids and flavoproteins present in the fungal systems (Carlile, 1970; Tan & Epton, 1973).

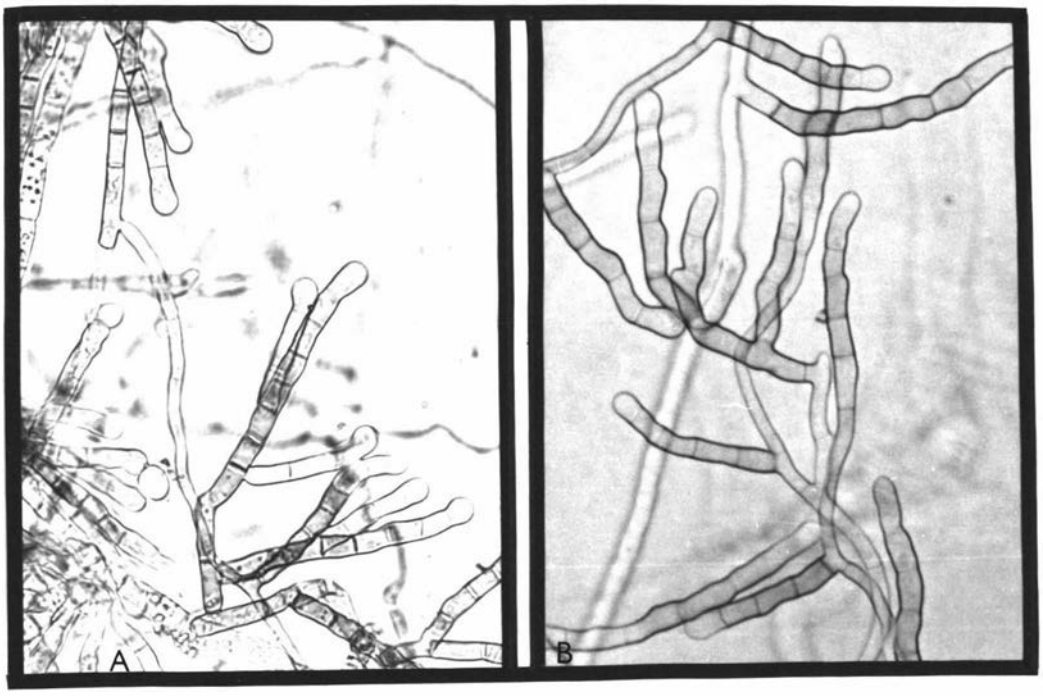
The results of the present study as they relate to blue-light induced vegetative regrowth of conidiophores and juvenile conidia, suggests the presence of such a photoreceptor. The fact that reversion will only occur when the NUV wavelength is absent and at high temperatures (27C or above) indicates that the photoreceptor pigment involved is extremely

precise and sensitive.

Because the conidiophores and conidia are aerial structures, their reversion to vegetative growth when flooded is believed to be due to a contact stimulus with water, somewhat similar to the germination process.

Fig. 32. Stages in conidiophore development of Stemphylium botryosum. The fungus was grown on 5% V-8 and initially incubated in continuous fluorescent light at 27C for 5 days and then exposed to a 4h period of darkness. A, conidiophores fully developed as evidenced by the bulbous conidiogenous cells (x575); B, conidiophores lacking the bulbous conidiogenous cells (x575).

Fig. 33. Stages in vegetative reversion of Stemphylium botryosum conidiophores after exposure of cultures to Mylar-filtered light at 27C. A, vegetative regrowth (V) of fully developed conidiophores after a 3h exposure (x575); B, vegetative regrowth of conidiophores lacking the bulbous conidiogenous cell, after 5h exposure (x575).


A
B

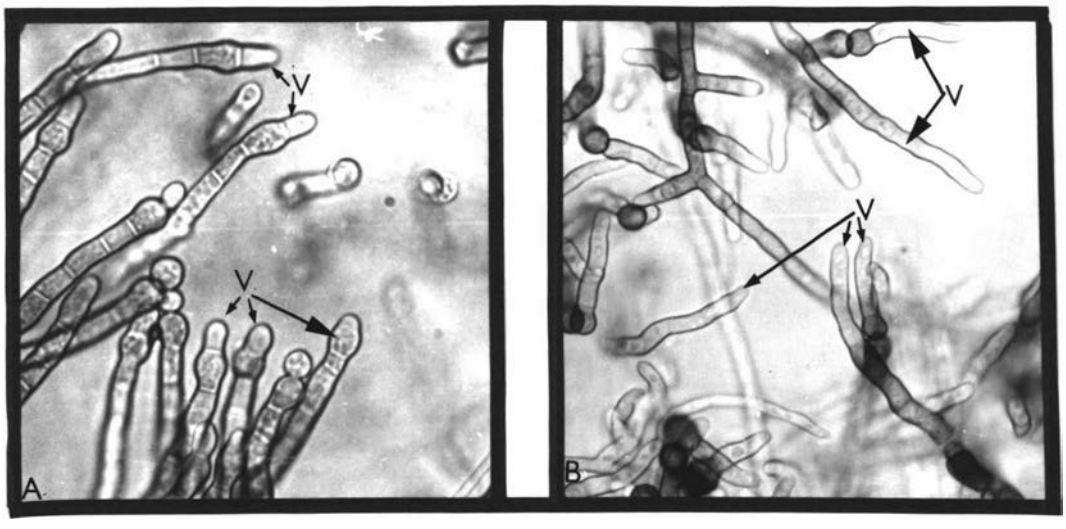
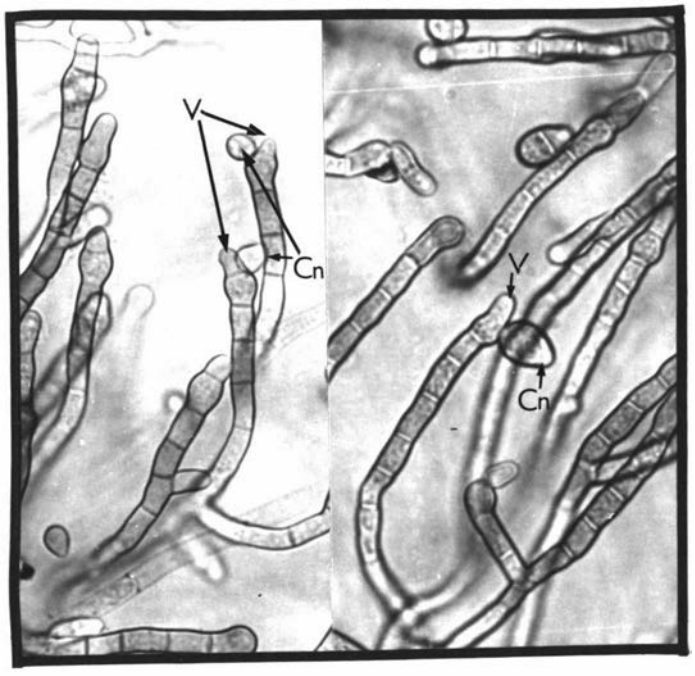

V
V
A
V
V
B

Fig. 34. Conidiophores of Stemphylium botryosum with vegetative regrowth (V) and aborted conidia (Cn) after 5h exposure of cultures to Mylar-filtered light at 27C (x575).

Fig. 35. Vegetative regrowth (V) of juvenile conidia of Stemphylium botryosum after exposure of cultures to Mylar-filtered light at 27C. A, conidium beginning to revert after 3h exposure (x575); B, conidia showing vegetative regrowth after 10h exposure (x575); C, vegetative reversion of a conidium in light followed by production of a further conidium when returned to darkness (x575).


V
Cn
V
Cn

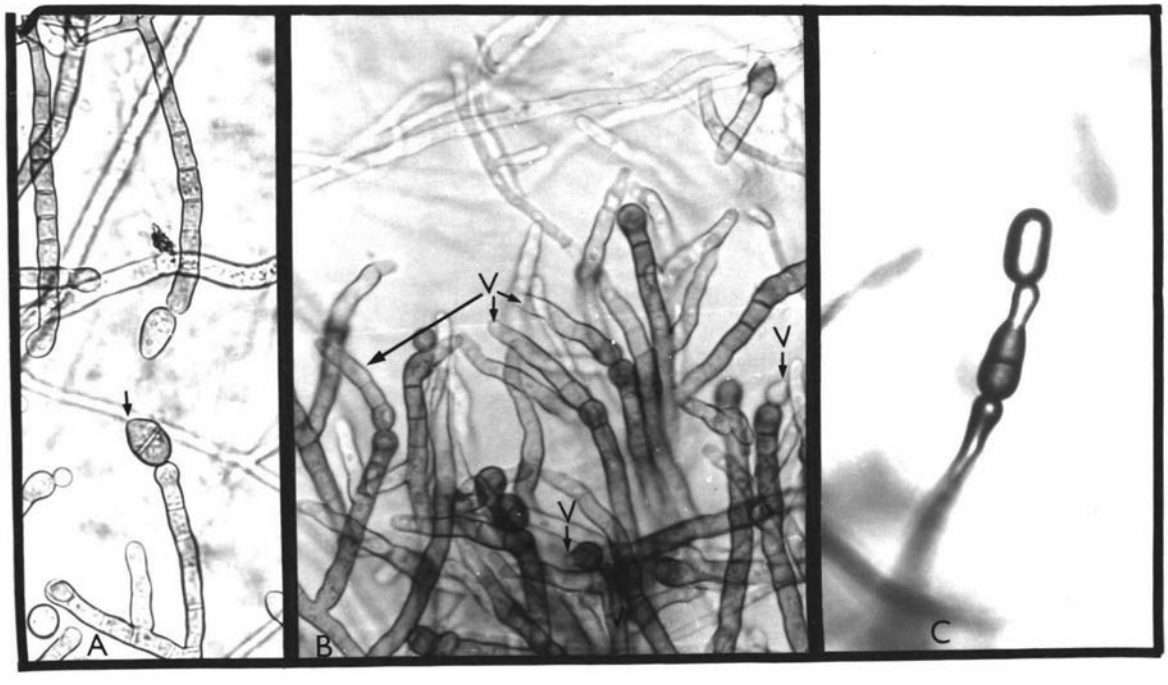
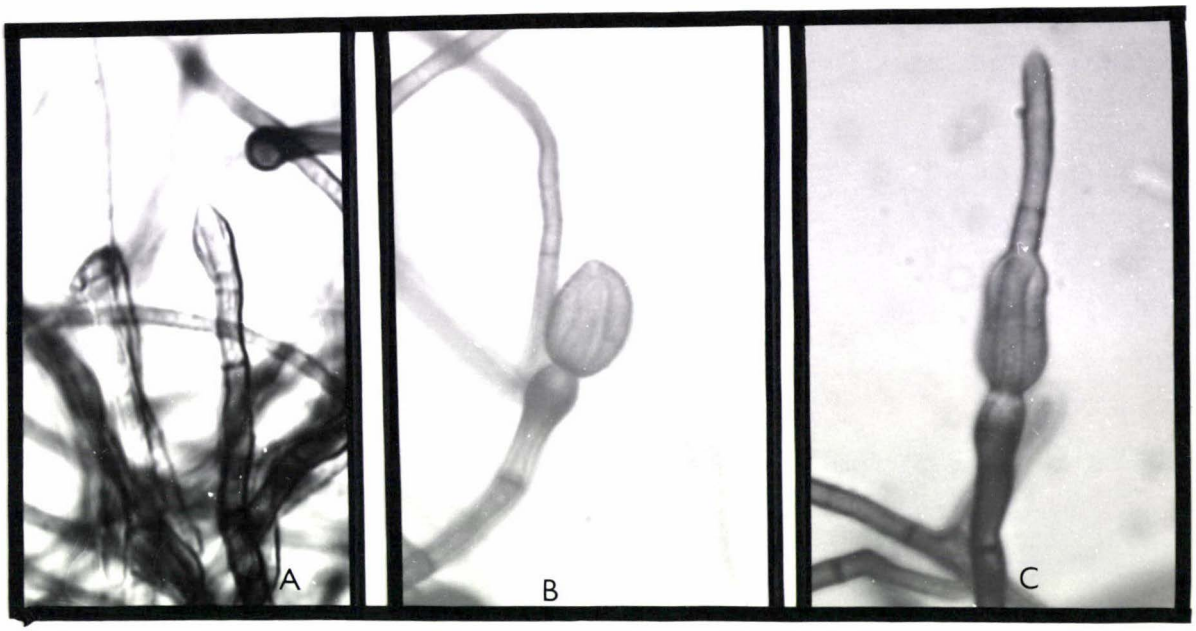

A
V
V
V
B
C

Fig. 36. Vegetative regrowth of Stemphylium botryosum conidiophores. A, regrowth occurring from basal septum of the conidiogenous cell (x800); B & C, hyphal-like regrowth of conidiophore growing through a juvenile conidium (x800).

Fig. 37. Transformation of the hyphal-like regrowth of Stemphylium botryosum conidiophores to functional conidiophores. A & B, transformation of upper half of the vegetative regrowth into a functional conidiophore (x500); C & D, transformation of the entire vegetative regrowth into a functional conidiophore (x500).


A
B
C

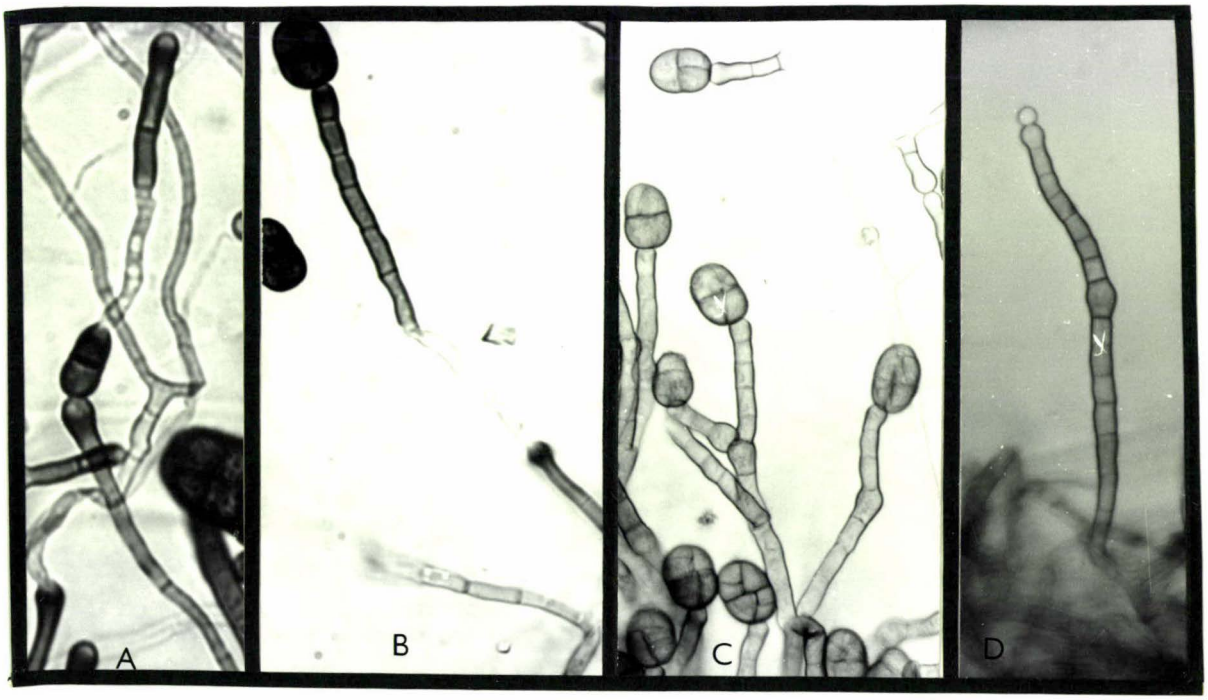
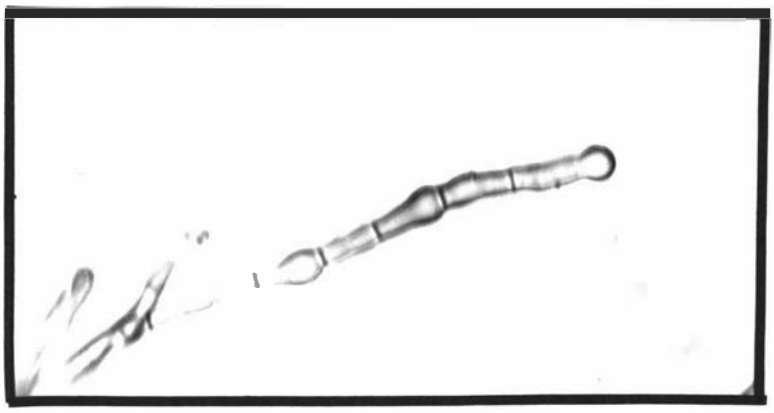
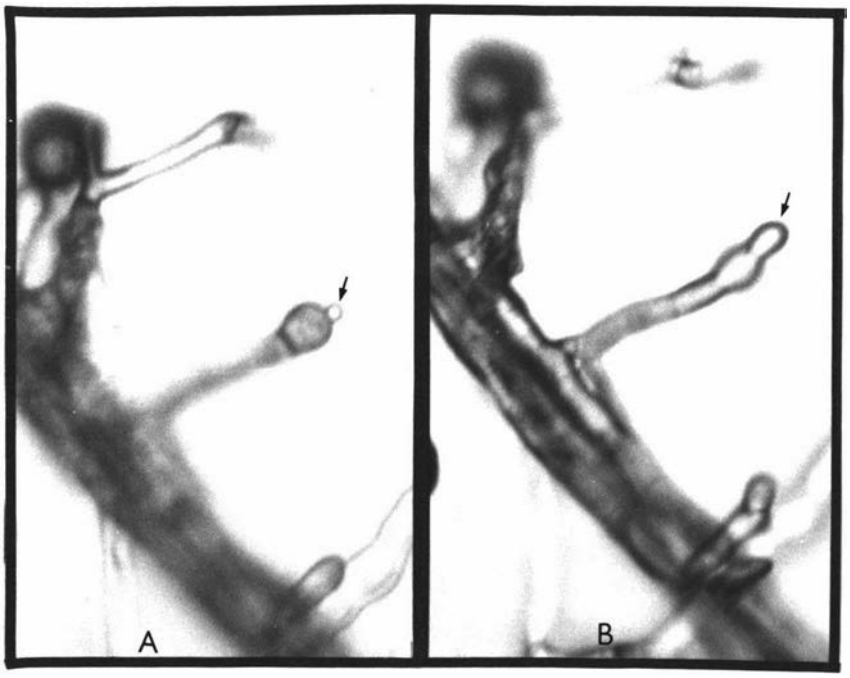

A
B
C
D

Fig. 38. Nodular swellings of a Stemphylium botryosum conidiophore induced by manipulation of photoperiod (x650). At no stage were conidia produced during this treatment.

Fig. 39. Transformation of a Stemphylium botryosum conidium initial to a vegetative hypha induced by blue light (480 nm) at 27C. A, conidium initial (x650); B. conidium initial transformed into a vegetative hypha (x650).




A
B

3.3 SPORE DISCHARGE MECHANISM IN PLEOSPORA

Because spores are propagules of new life it is essential that they are effectively released and disseminated if the species is to be successful. Some fungi have no special adaptations for spore discharge, the mature spores either remaining on the conidiophores or in ruptured fruiting bodies until removed by air currents or other agencies. However, in many other fungi special and often intricate mechanisms are involved (Brodie, 1951; Buller, 1922, 1934).

Ingold (1933) outlined four types of spore discharge in the Pyrenomycetes;

(i) ascus unitunicate, remains attached to the perithecium during spore release e.g. Podospora curvula (de Bary) Niessl;

(ii) ascus bitunicate, remains attached to perithecium during spore release e.g. Sporormia intermedia Auersw.;

(iii) perithecium long-necked, ascus becomes detached from perithecium when spores mature e.g. Ceratostomella ampullasca (Cooke.) Sacc.;

(iv) ascus non-explosive type, deliquesces when mature e.g. Chaetomium Kunze ex Fr.

Pringsheim (quoted from Hodgetts, 1917) working with Pleospora scirpicola (D.C.) Karst. (= Sphaeria scirpi Rab.) reported that the ascus remains attached to the base of the ascostroma during spore release and the ascospores are liberated singly through a pore in the apex of the endoascus.

A method quite different from that described for P. scirpicola has been reported for P. herbarum by Atanasoff (1919) and Smith (1940). Both authors imply that asci are liberated from the ascocarp, and provide evidence that the ascospores are released from the sides of the ascus after the rupture of both the inner and outerwall.

The fact that two quite different methods of spore discharge have been reported within the genus Pleospora prompted study of the spore discharge mechanism in this fungus.

MATERIALS AND METHODS

Pleospora herbarum isolate MU 11 and *Pleospora* sp. (*Stemphylium vesicarium*) isolate MU 2 were grown on PCA impregnated with sterile pea straw. To induce the production of large numbers of ascostromata the cultures were incubated for 12 days at 25C in an 8h photoperiod. After this initial induction phase cultures were transferred to 12C and incubated in darkness to induce maturation. Observations were made at weekly intervals. By the sixth week, when most of the ascostromata were mature, pieces of pea straw bearing the mature ascostromata were removed from the agar and immersed in water for ca. 20 min. They were then fixed laterally to a slide and examined under the low power of the compound microscope.

RESULTS

The spore release mechanism in the two *Pleospora* spp. was identical. Absorption of moisture by the endoascus resulted in a build up of osmotic pressure within the ascus. Because the outer wall of the ascus is inelastic, the increased pressure caused it to rupture at a weak point located near the distal end. The elastic endoascus then expanded considerably, occasionally up to twice its normal size (Fig. 42). Because these developments occurred within the ascostroma they could only be observed microscopically when the ascostromata were delicately squashed in a drop of water and a group of asci separated from the wall material. The greatly elongated endoascus emerged through the ostiole (Fig. 40) usually protruding about one to two spore lengths beyond the aperture. A conspicuous nipple-like invagination was present at the apex of the endoascus (Fig. 43) into which the distal end of the uppermost spore became firmly appressed. A pore eventually developed in this region and the first spore was discharged. Immediately after release of the first spore the ascus contracted a short distance due to the reduction of pressure within the ascus. Simultaneously the second spore was forced into the pore which it plugged until it too was released. Contraction of the ascus occurred after the liberation of each spore. Although there was generally a short interval between the release of successive spores, a spore frequently followed its predecessor so rapidly that it appeared as though they were ejected together. Occasionally two or three spores were discharged in quick succession and then the process was interrupted for some considerable time, presumably until the pressure within the ascus built up again causing

then the remainder of the spores to be discharged. If, however, the endoascus was unable to attain the pressure required to discharge the remaining spores then it gradually dehydrated and collapsed, eventually being drawn back into the ascostroma. Sometimes the endoascus over-elongated, to the extent that the internal pressure was unable to increase to the level required to discharge the spores (Fig. 41). In such instances no pore had been observed to develop, suggesting therefore that the pore is not chemically induced but rather the result of a mechanical process. Immediately after the eighth spore had been discharged the endoascus collapsed and was retracted permanently into the ascostroma. Shortly afterwards another ascus emerged through the ostiole and the same process was repeated. When all the mature ascospores within the ascostroma had been released the process was temporarily suspended until more spores attained maturity.

The narrowness of the ascostroma neck canal permitted the passage of only one ascus at a time. However, it was observed that if the neck was dissected from the ascostroma body and the ascostroma briefly exposed to free moisture, up to three asci simultaneously emerged through the now broadened ostiole. This indicates that within a mature ascostroma there can be more than one ascus elongating at any one time.

The ascospores were surrounded by a mucilaginous sheath (Fig. 44) which presumably helped in reducing friction between the spore and the pore while the former was squeezing through. In the case of released spores the mucilage may slow the rate of spore dehydration and also assist adherence to objects.

DISCUSSION

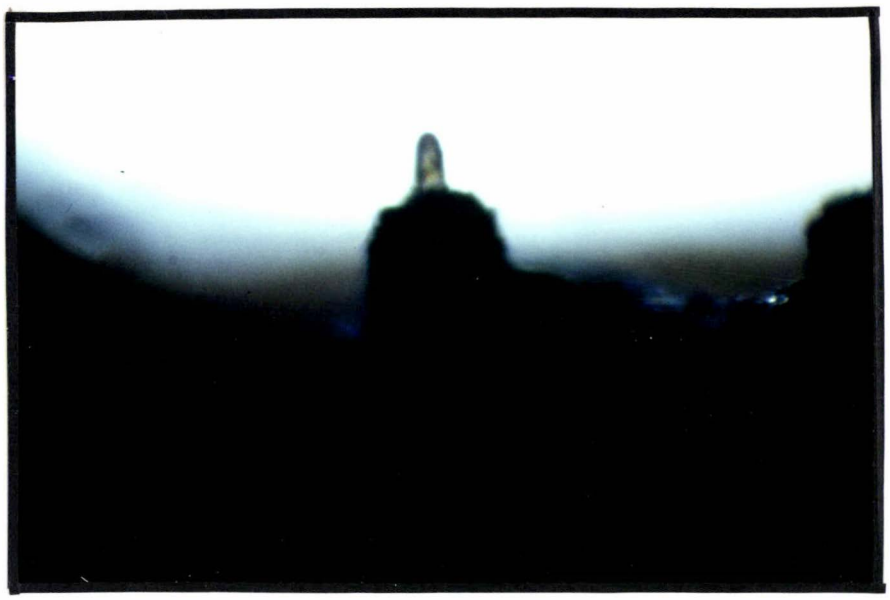
This study shows that the ascospore discharge mechanism in *P. herbarum* and *Pleospora* sp. (*S. vesicarium*) is similar to that reported for *P. scirpicola* (Hodgetts, 1917), *Sporormia intermedia* (Ingold, 1933, 1971) and *Venturia rumicis* (Desm.) Wint. (Kerr, 1961). Atanasoff's (1919) and Smith's (1940) account of spore discharge as it occurs in *P. herbarum* can be questioned. Their conclusions are based on observations of asci and ascospores released from ascostromata in a drop of water. One can speculate that detachment of asci from the ascostroma base, and lateral release of ascospores from asci were a consequence of pressure applied to the ascostroma wall, a situation quite alien to what would occur in the field. Further, the presence of a definite nipple-like invagination in the apical region of the endoascus in Atanasoff's

(1919) and Smith's (1940) illustrations indicate that P. herbarum is adapted to spore release through the apical region of the ascus, and not from the sides as was reported by them.

Fig. 40. Ascostroma and protruding ascus of Pleospora herbarum (x200).

Fig. 41. An overelongated ascus of Pleospora herbarum protruding from the ascostroma (x200).

Note, due to overelongation, the internal pressure of the ascus is unable to increase to the level required to discharge the spores.



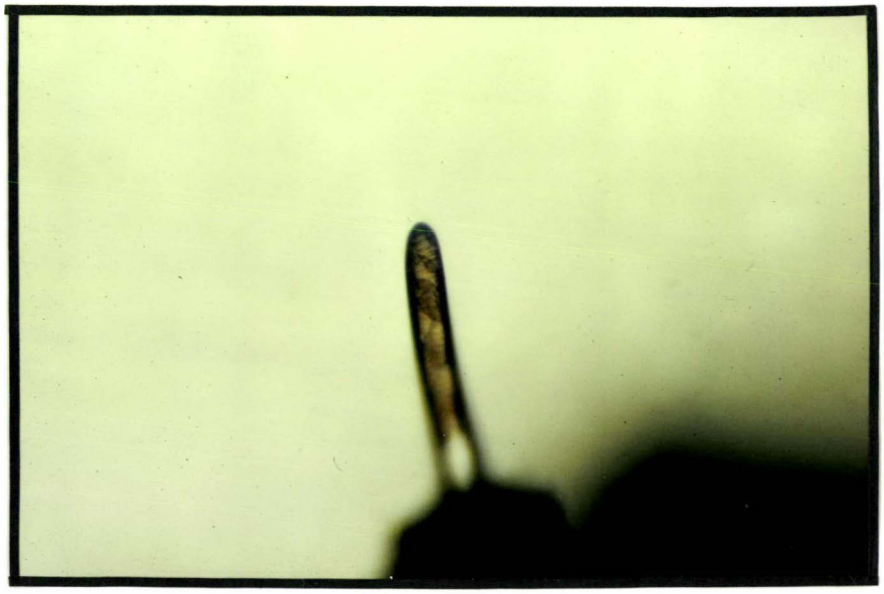
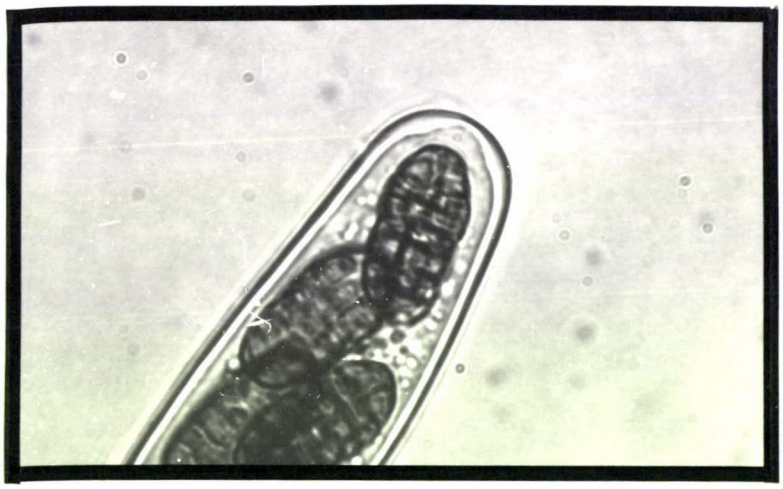
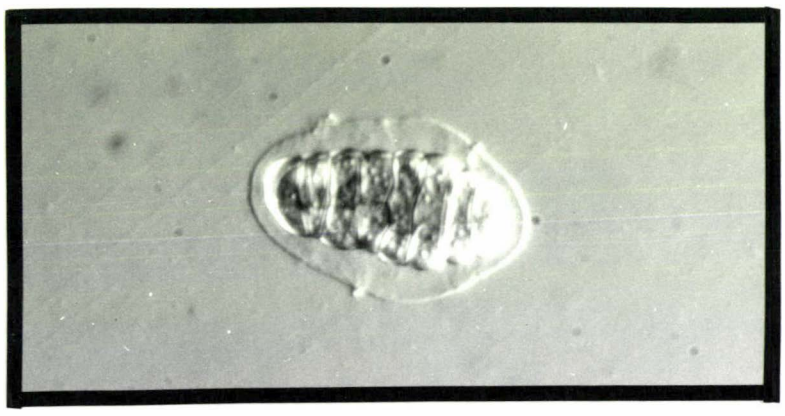


Fig. 42. A group of asci of Pleospora herbarum, some with outerwall ruptured (x200).

Fig. 43. Bitunicate ascus tip of Pleospora sp. (= Stemphylium vesicarium) showing the nipple-like invagination of endoascus (x650).

Fig. 44. An ascospore of Pleospora sp. (= Stemphylium vesicarium) surrounded by a mucilaginous sheath (x800).





APPENDIX 1

A PREVIOUSLY UNDESCRIBED CHAIN-FORMING SPECIES OF STEMPHYLIUM

Recently the writer isolated a fungus from decomposing hay straw which conformed to Stemphylium conidiophore and conidial features, but differed from previously described species in that conidia were produced in short chains of up to four spores. Monoconidial isolations to 5% V-8 yielded greenish-grey colonies typical of the genus Stemphylium. Conidiophores were solitary or sparingly branched, with a swollen conidiogenous cell. Conidiophore proliferations were infrequent.

Conidial chains were a common feature (Fig. 45) and were clearly visible under the dissecting microscope. These chains were very fragile, requiring great care to keep them intact when preparing microscopic mounts. Conidia varied greatly in shape and size. The basal conidium was generally oblong to cylindrical whereas the others were usually broader, being subspherical, obpyriform, ovoid, ellipsoidal or subdoliform.

On culture media stromatic bodies were produced which were indistinguishable from immature ascostromata of Stemphylium species. However even after four months incubation on PCA at 12C there was no evidence of differentiation of the stromatic body contents.

Conidial chains were produced in acropetal succession. That is, one conidium gave rise to a second conidium directly from the unmodified apical region, the youngest conidia being at the apex of the chain. This method of spore production is to be contrasted with reports of secondary conidial production within the genus Stemphylium (Singh, 1973; Sobers & Seymour, 1963) involving formation of a short secondary conidiophore from the apex of a maturing conidium, which in turn produces a further conidium.

Repeated pathogenicity tests of tomato, lucerne, blue lupin, lettuce, chrysanthemum, barley and ryegrass were unsuccessful.

There is need for comment on the generic status of the fungus. One may argue that the fact of conidial production being catenulate precludes inclusion of the fungus in the genus Stemphylium. However, it is the writer's opinion that whether the spores are borne singly or in chains is of secondary importance compared with morphological features of the conidiophores and conidia. That is, the apically swollen conidiophores and the oval or subangular muriform conidia provide sufficient

Fig. 45. Conidiophores and conidia of a Stemphylium sp. isolated from hay straw. Produced on 5% V-8, pH 7.5, at 20C with an 8h photoperiod. A (x850); B(x550); C(x200).

Note the production of conidia in chains and the bulbous conidiogenous cell.


A
B


C

justification for recognition as *Stemphylium*. Confirmation would be provided by maturation of stromatic bodies producing asci and ascospores typical of the genus *Pleospora*.

MYCOLOGICAL DESCRIPTION

Colonies	greenish-grey, zoned, circular, effuse.
Conidiophores	solitary to sparingly branched, 2-5 septate, up to 75µ long, 4-6µ wide, hyaline to translucent olivaceous-brown.
Conidiogenous cells	bulbous, olivaceous-brown to olivaceous-grey, lightly punctate, 7-10µ in diameter.
Conidia	usually in chains of up to 4 spores produced acropetally; olivaceous-grey to olivaceous-brown; verrucose; oval, oblong, subspherical, obpyriform, ovoid, subdoliform or ellipsoidal; apex often conical, base rounded; with 3-6 lateral and 1-2(-3) longitudinal septa; constricted at 1-3 of the major lateral septa; 24.2 - 49.5 x 13.1 - 20.4µ (average 35.2 x 16.1µ).
Stromata	black, globose, up to 500µ in diameter, possibly immature ascostromata.

KEY OF APPENDICES 2-5

Colour

H = Hyaline
Gr = Grey
GnGr = Greenish-grey
GrBn = Greyish-brown
GrW = Greyish-white
TOGr = Translucent Olivaceous-grey
TOBn = Translucent Olivaceous-brown
OBn = Olivaceous-brown
OGr = Olivaceous-grey
DGnGr = Dark greenish-grey

Shape

Cir = Circular
Irr = Irregular

Zonation

Z = Zoned
NZ = Not Zoned

Ornamentation

Punc = Punctate

APPENDIX 2a Effect of growth media on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'A'

Medium	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 9	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter	Ornamentation
HDA	GnGr	Cir	Z	H/TOGr	1-4	20-79 x 4.1-5.3	OGr	6.2-8.6	Punc
MA	GnGr	Cir	Z	H/TOBn	1-4	25-84 x 4.1-5.2	OGr	5.9-8.0	Punc
20% V-8	Gr	Cir	Z	TOGr	1-4	24-80 x 4.4-5.3	OGr/Gr	6.0-8.3	Punc
10% V-8	Gr	Cir	Z	TOGr	1-4	28-84 x 4.0-5.3	OGr	6.3-7.9	Punc
5% V-8	OGr/GnGr	Cir	Z	H/TOBn	1-4	21-90 x 3.9-5.4	OGr	6.3-8.1	Punc
PCA	OGr/GnGr	Cir	Z	H/TOBn	1-4	26-93 x 4.2-5.6	OGr	6.0-7.8	Punc
PMA	DGnGr	Cir	Z	TOGr	1-4	18-82 x 4.0-5.4	OGr/Gr	6.3-8.0	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	24-89 x 4.1-5.3	OGr/Gr	6.1-8.2	Punc
Isolate MU 11									
HDA	GnGr	Cir	Z	H/TOGr	1-4	26-82 x 3.9-5.3	OGr	5.9-8.0	Punc
MA	GnGr	Cir	NZ	H/TOBn	1-4	20-80 x 4.1-5.6	OGr	6.3-7.9	Punc
20% V-8	Gr	Cir	Z	TOGr	1-4	18-72 x 4.1-5.4	OGr/Gr	6.0-8.9	Punc
10% V-8	Gr	Cir	Z	TOGr	1-4	24-81 x 4.1-5.1	OGr	6.4-8.4	Punc
5% V-8	OGr	Cir	Z	H/TOBn	1-4	20-78 x 4.0-5.2	OGr	6.1-8.1	Punc
PCA	OGr	Cir	Z	H/TOBn	1-4	26-84 x 4.0-5.4	OGr	6.3-8.4	Punc
PMA	DGnGr	Cir	NZ	TOGr	1-4	25-76 x 4.3-5.4	OGr	6.6-7.9	Punc
PDA	DGnGr	Cir	NZ	TOGr	1-4	22-89 x 4.2-5.3	OGr/Gr	6.1-7.6	Punc
Isolate MU 33									
HDA	GnGr	Cir	Z	H/TOBn	1-4	29-87 x 4.1-5.4	OGr	6.3-7.6	Punc
MA	GnGr	Cir	Z	H/TOBn	1-4	21-83 x 4.3-5.6	OGr	5.9-8.4	Punc
20% V-8	DGnGr	Cir	Z	TOGr	1-4	18-84 x 3.9-5.5	OGr/Gr	6.4-8.3	Punc
10% V-8	GnGr	Cir	Z	TOGr	1-4	26-86 x 4.1-5.3	OGr	6.1-7.8	Punc
5% V-8	OGr	Cir	Z	H/TOBn	1-4	24-90 x 4.2-5.6	OGr	6.0-8.0	Punc
PCA	OGr	Cir	Z	H/TOBn	1-4	22-82 x 4.1-5.6	OGr	6.6-7.6	Punc
PMA	DGnGr	Cir	Z	H/TOGr	1-4	24-89 x 4.0-5.4	OGr	6.4-7.9	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	23-84 x 4.4-5.3	OGr/Gr	6.1-8.4	Punc

* pH 7.5, incubated at 20C in an 8h photoperiod.

APPENDIX 2b Effect of growth media on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'B'

Medium	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 5	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
HDA	GrBn	Cir	Z	H/TOBn	1-5	26-81 x 4.1-5.3	OBn	6.2-7.9	Punc
MA	OGr	Cir	Z/NZ	H/TOBn	1-5	28-84 x 3.9-5.4	OBn	6.6-8.2	Punc
20% V-8	GnGr	Cir	Z	TOGr	1-4	22-87 x 4.2-5.4	OGr	6.1-8.8	Punc
10% V-8	GnGr	Cir	Z	H/TOGr	1-4	24-91 x 4.2-5.3	OGr	6.3-8.0	Punc
5% V-8	OGr	Cir	Z	H/TOBn	1-4	22-90 x 4.2-5.3	OBn	6.6-8.6	Punc
PCA	OGr	Cir	Z	H/TOGr	1-4	24-89 x 4.0-5.6	OGr	6.0-8.0	Punc
PMA	GnGr	Cir	NZ	H/TOGr	1-4	26-80 x 4.4-5.4	OGr/Gr	6.4-8.4	Punc
PDA	Gr	Cir		TOGr	1-4	29-86 x 4.1-5.4	OGr/Gr	6.5-8.9	Punc
Isolate MU 24									
HDA	GrBn	Cir	Z	H/TOBn	1-4	21-84 x 4.1-5.4	OBn	6.0-8.1	Punc
MA	GrBn	Cir	NZ	H/TOBn	1-4	22-81 x 4.1-5.4	OBn	6.0-8.4	Punc
20% V-8	GnGr	Cir	Z	TOGr	1-4	28-89 x 4.4-5.3	OGr	5.9-8.0	Punc
10% V-8	GnGr	Cir	Z	TOGr	1-4	24-84 x 4.0-5.4	OGr	6.1-8.7	Punc
5% V-8	OGr	Cir	Z	H/TOBn	1-4	18-92 x 3.9-5.4	OGr	5.8-7.7	Punc
PCA	OGr	Cir	Z	H/TOGr	1-4	20-86 x 4.4-5.6	OGr	6.1-8.1	Punc
PMA	GnGr	Cir	Z	TOGr	1-4	23-89 x 4.3-5.4	OGr	6.6-7.9	Punc
PDA	GnGr	Cir	NZ	TOGr	1-4	21-90 x 4.0-5.5	OGr	6.3-7.8	Punc
Isolate MU 28									
HDA	OGr	Cir	Z	H/TOBn	1-4	22-81 x 4.2-5.6	OBn	6.0-7.8	Punc
MA	OGr	Cir	Z/NZ	H/TOBn	1-4	26-85 x 4.1-5.3	OBn	6.4-8.9	Punc
20% V-8	GnGr	Cir	Z	TOGr	1-4	28-84 x 4.4-5.2	OGr	6.1-8.6	Punc
10% V-8	GnGr	Cir	Z	TOGr	1-4	21-88 x 4.0-5.4	OGr	6.2-8.2	Punc
5% V-8	OGr	Cir	Z	H/TOGr	1-4	20-94 x 4.4-5.1	OGr	6.5-8.0	Punc
PCA	OGr	Cir	Z	H/TOGr	1-4	23-82 x 4.4-5.3	OGr	6.2-8.8	Punc
PMA	GnGr	Cir	Z/NZ	TOGr	1-4	26-83 x 4.3-5.2	OGr	6.3-8.3	Punc
PDA	GnGr	Cir	Z/NZ	TOGr	1-4	21-89 x 4.1-5.2	OGr	6.4-8.0	Punc

* pH 7.5, incubated at 20C in an 8h photoperiod.

APPENDIX 2c Effect of growth media on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'C'

Medium	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 2	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
HDA	OGr	Cir	Z	H/TOBn	1-4	21-80 x 4.0-5.3	OBn	6.4-8.1	Punc
MA	OGr	Cir	Z	H/TOBn	1-4	19-84 x 4.1-5.5	OBn	6.0-8.4	Punc
20% V-8	GnGr/Gr	Cir	Z	TOGr	1-4	24-76 x 4.4-5.2	OGr	6.6-7.9	Punc
10% V-8	GnGr/Gr	Cir	Z	TOGr	1-5	23-89 x 4.0-5.7	OGr	6.1-8.5	Punc
5% V-8	GnGr	Cir	Z	H/TOBn	1-4	25-83 x 4.4-5.6	OGr	6.7-9.1	Punc
PCA	GnGr	Cir	Z	H/TOGr	1-5	21-78 x 3.9-5.4	OGr	6.4-8.5	Punc
PMA	DGnGr	Cir	Z	TOGr	1-4	22-84 x 4.0-5.3	OGr	6.2-9.0	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	24-86 x 4.1-5.3	OGr	6.0-8.9	Punc
Isolate MU 19									
HDA	OGr	Cir	Z	H/TOBn	1-4	24-81 x 4.0-5.3		6.3-7.9	Punc
MA	OGr	Cir	Z	H/TOBn	1-4	22-88 x 4.1-5.6	OGr	6.2-8.2	Punc
20% V-8	Gr	Cir	Z	TOGr	1-4	20-83 x 4.1-5.4	OGr	6.6-8.1	Punc
10% V-8	Gr	Cir	Z	H/TOGr	1-4	28-86 x 3.9-5.4	OGr	6.1-8.0	Punc
5% V-8	GnGr	Cir	Z	H/TOBn	1-4	26-88 x 4.1-5.3	OGr	6.3-8.4	Punc
PCA	GnGr	Cir	Z	H/TOBn	1-4	18-84 x 4.4-5.2	OGr	6.0-8.7	Punc
PMA	DGnGr	Cir	Z	TOGr	1-4	26-81 x 4.1-5.6	OGr	6.0-8.6	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	23-73 x 4.2-5.2	OGr	6.4-8.4	Punc
Isolate MU 40									
HDA	OGr	Cir	Z	H/TOBn	1-4	29-81 x 4.1-5.4	OBn	6.0-8.1	Punc
MA	OGr	Cir	Z	H/TOBn	1-4	28-75 x 4.3-5.3	OBn	6.3-8.6	Punc
20% V-8	Gr	Cir	Z	TOGr	1-4	31-86 x 4.1-5.2	OGr	6.4-8.9	Punc
10% V-8	Gr	Cir	Z	H/TOBn	1-4	24-88 x 4.4-5.4	OGr	5.9-9.1	Punc
5% V-8	GnGr	Cir	Z	H/TOBn	1-4	28-78 x 4.2-5.2	OGr	6.7-9.3	Punc
PCA	GnGr	Cir	Z	H/TOBn	1-4	22-84 x 3.9-5.3	OGr	6.1-8.8	Punc
PMA	DGnGr	Cir	Z	TOGr	1-4	28-80 x 4.0-5.3	OGr	6.4-8.6	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	26-84 x 4.1-5.2	OGr	6.3-8.9	Punc

* pH 7.5, incubated at 20C in an 8h photoperiod

APPENDIX 2d Effect of growth media on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'D'

Medium	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 14	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
HDA	GrBn	Cir	Z	H/TOBn	1-5	26-74 x 3.9-5.4	OBn	6.5-8.0	Punc
MA	GrBn	Cir	Z	H/TOBn	1-4	28-81 x 4.1-5.4	OBn	6.6-8.6	Punc
20% V-8	OGr	Cir	Z	TOBn	1-4	18-86 x 4.3-5.6	OGr	6.1-8.4	Punc
10% V-8	GnGr	Cir	Z	H/TOBn	1-4(-5)	22-84 x 4.0-5.3	OGr	6.0-9.1	Punc
5% V-8	OGr	Cir	Z	H/TOBn	1-4(-5)	20-84 x 4.0-5.4	OGr	6.6-8.4	Punc
PCA	OGr	Cir	Z	H/TOBn	1-5	26-81 x 4.2-5.2	OGr	6.4-8.9	Punc
PMA	DGnGr	Cir	Z	TOGr	1-4	24-74 x 4.6-5.4	OGr	6.1-8.6	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	20-79 x 4.3-5.3	OGr	6.3-8.3	Punc
Isolate MU 23									
HDA	OGr	Cir	Z	H/TOBn	1-4	24-78 x 4.1-5.6	OBn	6.3-9.1	Punc
MA	OGr	Cir	Z	H/TOBn	1-4	21-82 x 4.1-5.4	OBn	6.0-8.2	Punc
20% V-8	DGnGr	Cir	Z	TOBn	1-4	26-80 x 4.3-5.3	OGr	6.4-8.8	Punc
10% V-8	Gr	Cir	Z	H/TOBn	1-4	24-84 x 4.2-5.3	OGr	6.6-8.6	Punc
5% V-8	GnGr	Cir	Z	H/TOBn	1-4	21-76 x 4.1-5.2	OGr	6.3-8.9	Punc
PCA	GnGr	Cir	Z	H/TOBn	1-4	23-79 x 4.4-5.4	OGr	6.0-8.4	Punc
PMA	DGnGr	Cir	Z	TOGr	1-4	20-86 x 3.9-5.3	OGr	6.1-8.6	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	18-80 x 4.4-5.5	OGr	6.4-8.8	Punc
Isolate MU 29									
HDA	OGr	Cir	Z	H/TOBn	1-4	28-88 x 4.4-5.6	OGr	5.9-8.1	Punc
MA	OGr	Cir	Z	H/TOBn	1-4	26-81 x 4.3-5.4	OGr	6.3-8.6	Punc
20% V-8	Gr	Cir	Z	TOGr	1-4	31-83 x 4.0-5.7	OGr	6.0-8.4	Punc
10% V-8	Gr	Cir	Z	H/TOBn	1-5	24-79 x 4.2-5.5	OGr	6.3-8.0	Punc
5% V-8	GnGr	Cir	Z	H/TOBn	1-4	23-88 x 4.4-5.4	OGr	6.2-7.8	Punc
PCA	DGnGr	Cir	Z	H/TOBn	1-4	26-84 x 4.4-5.3	OGr	6.4-8.7	Punc
PMA	DGnGr	Cir	Z	H/TOBn	1-4	20-80 x 4.0-5.6	OGr	6.3-7.9	Punc
PDA	DGnGr	Cir	Z	TOGr	1-4	24-83 x 4.1-5.6	OGr	6.0-8.4	Punc

* pH 7.5, incubated at 20C in an 8h photoperiod.

APPENDIX 3a Effect of incubation temperature on colony conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'A'

Temp. (°C)	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 9	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
12	OGr	Cir	Z/NZ	H/TOBn	1-4	24-78 x 4.1-5.6	OBn	6.0-8.4	Punc
16	GrBn	Cir	Z/NZ	H/TOBn	1-4	20-81 x 4.3-5.3	OBn	6.3-8.7	Punc
20	OGr/GnGr	Cir	Z/NZ	H/TOBn	1-4	21-90 x 3.9-5.4	OGr	6.3-8.1	Punc
24	GnGr	Cir	Z/NZ	H/TOGr	1-5	23-89 x 4.0-5.2	OGr	6.0-8.4	Punc
27	GnW	Cir	Z/NZ	TOGr	1-5	26-84 x 4.0-5.3	OGr	6.6-8.0	Punc
30	GnW	Cir/Irr	NZ	TOGr	1-4(-5)	28-91 x 4.2-5.2	OGr	6.4-7.9	Punc
Isolate MU 11									
12	OGr	Cir	Z	H/TOBn	1-4	20-74 x 4.4-5.4	OBn	6.0-8.4	Punc
16	OGr	Cir	Z	H/TOBn	1-4	18-81 x 4.4-5.6	OBn	6.4-8.6	Punc
20	OGr	Cir	Z	H/TOBn	1-4	20-78 x 4.0-5.2	OGr	6.1-8.1	Punc
24	GnGr	Cir	Z	H/TOGr	1-4	21-84 x 4.2-5.2	OGr	6.4-8.3	Punc
27	Gr	Cir	Z	TOGr	1-5	26-84 x 4.0-5.4	OGr	5.9-8.7	Punc
30	GrW	Irr	NZ	TOGr	1-5	24-89 x 3.9-5.3	OGr	6.0-8.2	Punc
Isolate MU 33									
12	OGr	Cir	Z/NZ	H/TOBn	1-4	18-74 x 4.6-5.8	OBn	5.9-8.4	Punc
16	OGr	Cir	Z/NZ	H/TOBn	1-4	24-78 x 4.4-5.4	OBn	6.4-8.7	Punc
20	OGr	Cir	Z/NZ	H/TOBn	1-4	24-90 x 4.2-5.6	OGr	6.0-8.0	Punc
24	GnGr	Cir	Z/NZ	H/TOGr	1-4	22-84 x 4.4-5.6	OGr	6.4-8.2	Punc
27	Gr	Cir	Z/NZ	TOGr	1-5(-6)	28-90 x 4.1-5.3	OGr	6.3-8.1	Punc
30	GrW	Cir/Irr	NZ	TOGr	1-5(-6)	26-94 x 4.0-5.4	OGr	6.0-8.1	Punc

* Grown on 5% V-8, pH 7.5 and incubated in an 8h photoperiod.

APPENDIX 3b Effect of incubation temperature on colony conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'B'

Temp. (°C)	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 5	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
12	OGr	Cir	Z	H/TOBn	1-4	20-80 x 4.4-5.6	OBn	6.6-8.2	Punc
16	OGr	Cir	Z	H/TOBn	1-4	21-82 x 4.3-5.4	OBn	6.3-8.3	Punc
20	OGr	Cir	Z	H/TOBn	1-4	22-90 x 4.2-5.3	OBn	6.6-8.6	Punc
24	OGr/GnGr	Cir	Z	H/TOGr	1-4	24-84 x 4.1-5.4	OGr	6.3-8.4	Punc
27	GrW	Cir	Z	TOGr	1-5	24-89 x 3.9-5.3	OGr	6.4-8.1	Punc
30	GrW	Irr	NZ	TOGr	1-5	26-94 x 4.0-5.4	OGr	6.0-7.9	Punc
Isolate MU 24									
12	OGr	Cir	Z	H/TOBn	1-4	26-81 x 4.4-5.6	OBn	6.4-8.1	Punc
16	OGr	Cir	Z	H/TOBn	1-4	20-79 x 4.5-5.3	OBn	6.0-8.3	Punc
20	OGr	Cir	Z	H/TOBn	1-4	18-92 x 3.9-5.4	OGr	5.8-7.7	Punc
24	GnGr	Cir	Z	H/TOGr	1-4	20-82 x 4.0-5.2	OGr	6.0-8.6	Punc
27	GnGr	Cir	Z/NZ	TOGr	1-5	21-86 x 4.3-5.4	OGr	5.9-8.0	Punc
30	GrW	Irr	NZ	TOGr	1-5	26-94 x 4.0-5.3	OGr	6.4-8.4	Punc
Isolate MU 28									
12	OGr	Cir	Z	H/TOBn	1-4	21-82 x 4.4-5.6	OBn	6.4-8.4	Punc
16	OGr	Cir	Z	H/TOBn	1-4	23-78 x 4.3-5.4	OBn	6.3-7.8	Punc
20	OGr	Cir	Z	H/TOGr	1-4	20-94 x 4.4-5.1	OGr	6.5-8.0	Punc
24	OGr	Cir	Z	TOGr	1-4	19-79 x 4.0-5.2	OGr	6.1-8.2	Punc
27	GnGr	Cir	Z	TOGr	1-5(-6)	24-88 x 3.9-5.1	OGr	6.4-8.4	Punc
30	GrW	Irr	NZ	TOGr	1-5	26-96 x 3.9-5.2	OGr	6.0-8.1	Punc

* Grown on 5% V-8, pH 7.5, and incubated in an 8h photoperiod.

APPENDIX 3c Effect of incubation temperature on colony conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'C'

Temp. (°C)	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 2	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
12	OGr	Cir	Z	H/TOBn	1-4	20-78 x 4.6-5.4	OBn	6.3-8.4	Punc
16	OGr	Cir	Z	H/TOBn	1-4	22-81 x 4.3-5.3	OBn	6.6-8.6	Punc
20	GnGr	Cir	Z	H/TOBn	1-4	25-83 x 4.4-5.6	OGr	6.7-9.1	Punc
24	DGnGr	Cir	Z	H/TOGr	1-4	23-85 x 4.4-5.5	OGr	6.4-8.3	Punc
27	Gr	Cir	Z	TOGr	1-5	26-79 x 4.1-5.3	OGr	6.5-9.0	Punc
30	GrW	Cir/Irr	NZ	TOGr	1-5(-6)	18-86 x 4.1-5.2	OGr	6.2-8.4	Punc
Isolate MU 19									
12	OGr	Cir	Z	H/TOBn	1-4	28-81 x 4.4-5.6	OBn	6.4-8.8	Punc
16	OGr	Cir	Z	H/TOBn	1-4	24-86 x 4.2-5.3	OBn	6.0-8.2	Punc
20	GnGr	Cir	Z	H/TOBn	1-4	26-88 x 4.1-5.3	OGr	6.3-8.4	Punc
24	DGnGr	Cir	Z	H/TOBn	1-5	24-79 x 4.0-5.4	OGr	5.9-8.6	Punc
27	Gr	Cir	Z	TOGr	1-5	28-89 x 4.0-5.3	OGr	6.1-9.0	Punc
30	GrW	Irr	NZ	TOGr	1-5(-6)	30-94 x 4.1-5.2	OGr	6.1-8.4	Punc
Isolate MU 40									
12	OGr	Cir	Z	H/TOBn	1-4	22-80 x 4.2-5.4	OBn	6.6-8.4	Punc
16	OGr	Cir	Z	H/TOBn	1-4	20-84 x 4.4-5.4	OBn	6.4-8.6	Punc
20	GnGr	Cir	Z	H/TOBn	1-4	28-78 x 4.2-5.2	OBn	6.7-9.3	Punc
24	Gr	Cir	Z	H/TOGr	1-4	21-80 x 4.3-5.3	OGr	6.3-9.0	Punc
27	DGnGr	Cir	Z	TOGr	1-5(-6)	26-84 x 3.9-5.4	OGr	6.5-8.8	Punc
30	GrW	Cir/Irr	NZ	TOGr	1-5(-6)	31-88 x 4.0-5.3	OGr	6.1-8.4	Punc

* Grown on 5% V-8, pH 7.5, and incubated in an 8h photoperiod.

APPENDIX 3d Effect of incubation temperature on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'D'

Temp. (°C)	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 14	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
12	OGr	Cir	Z	H/TOBn	1-4	18-78 x 4.4-5.6	OBn	6.4-8.1	Punc
16	OGr	Cir	Z	H/TOBn	1-4	24-82 x 4.1-5.3	OBn	6.3-7.9	Punc
20	OGr	Cir	Z	H/TOBn	1-4(-5)	20-84 x 4.0-5.4	OGr	6.6-8.4	Punc
24	DGnGr	Cir	Z	TOGr	1-5	26-88 x 4.0-5.4	OGr	6.0-8.3	Punc
27	Gr	Cir	Z	TOGr	1-5(-6)	30-92 x 4.4-5.3	OGr	6.2-8.4	Punc
30	GrW	Cir	NZ	TOGr	1-5(-6)	28-90 x 4.0-5.3	OGr	6.3-8.0	Punc
Isolate MU 23									
12	OGr	Cir	Z	H/TOBn	1-4	18-84 x 4.1-5.6	OBn	6.1-8.6	Punc
16	OGr	Cir	Z	H/TOBn	1-4	24-80 x 4.1-5.6	OBn	6.4-8.3	Punc
20	GnGr	Cir	Z	H/TOBn	1-4	21-76 x 4.1-5.2	OGr	6.3-8.9	Punc
24	DGnGr	Cir	Z	H/TOGr	1-4(-5)	20-79 x 4.3-5.4	OGr	6.0-8.4	Punc
27	GrW	Cir	Z	TOGr	1-5	28-84 x 3.9-5.2	OGr	6.2-8.2	Punc
30	GrW	Cir/Irr	NZ	TOGr	1-5(-6)	26-88 x 4.0-5.3	OGr	6.0-8.0	Punc
Isolate MU 29									
12	OGr	Cir	Z	H/TOBn	1-4	24-79 x 4.4-5.6	OBn	6.2-8.4	Punc
16	OGr	Cir	Z	H/TOBn	1-4	26-82 x 4.3-5.6	OBn	6.1-8.1	Punc
20	GnGr	Cir	Z	H/TOBn	1-4	23-88 x 4.4-5.4	OGr	6.2-7.8	Punc
24	DGnGr	Cir	Z	TOGr	1-5	24-84 x 3.9-5.3	OGr	5.9-8.3	Punc
27	Gr	Cir	Z	TOGr	1-5	26-91 x 4.2-5.4	OGr	6.0-8.4	Punc
30	GrW	Irr	NZ	TOGr	1-5(-6)	24-88 x 4.1-5.2	OGr	6.0-7.8	Punc

* Grown on 5% V-8, pH 7.5, and incubated in an 8h photoperiod.

APPENDIX 4a Effect of photoperiod on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'A'

Photoperiod	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 9	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
8h photoperiod	OGr/GnBn	Cir	Z	H/TOBn	1-4	21-90 x 3.9-5.4	OGr	6.3-8.1	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	24-86 x 4.1-5.3	OGr	6.0-8.4	Punc
Isolate MU 11									
8h photoperiod	OGr	Cir	Z	H/TOBn	1-4	20-78 x 4.0-5.2	OGr	6.1-8.1	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	26-81 x 3.9-5.4	OGr	6.4-8.0	Punc
Isolate MU 33									
8h photoperiod	OGr	Cir	Z	H/TOBn	1-4	24-90 x 4.2-5.6	OGr	6.0-8.0	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	21-82 x 4.0-5.4	OGr	6.1-8.4	Punc

* Grown on 5% V-8, pH 7.5 and incubated at 20C.

APPENDIX 4b Effect of photoperiod on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'B'

Photoperiod	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 5	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
8h photoperiod	OGr	Cir	Z	H/TOBn	1-4	22-90 x 4.2-5.3	OBn	6.6-8.6	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	26-84 x 4.1-5.3	OGr	6.4-8.2	Punc
Isolate MU 24									
8h photoperiod	OGr	Cir	Z	H/TOBn	1-4	18-92 x 3.9-5.4	OGr	5.8-7.7	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	21-86 x 4.0-5.3	OGr	6.0-7.9	Punc
Isolate MU 28									
8h photoperiod	OGr	Cir	Z	H/TOGr	1-4	20-94 x 4.4-5.1	OGr	6.5-8.0	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	24-86 x 4.2-5.2	OGr	6.3-8.4	Punc

* Grown on 5% V-8, pH 7.5, and incubated at 20C

APPENDIX 4c Effect of photoperiod on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'C'

Photoperiod	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 2	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
8h photoperiod	GnGr	Cir	Z	H/TOBn	1-4	25-83 x 4.4-5.6	OGr	6.7-9.1	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	23-86 x 4.1-5.3	OGr	6.3-8.8	Punc
Isolate MU 19									
8h photoperiod	GnGr	Cir	Z	H/TOBn	1-4	26-88 x 4.1-5.3	OGr	6.3-8.4	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	22-81 x 4.3-5.5	OGr	6.1-8.3	Punc
Isolate MU 40									
8h photoperiod	GnGr	Cir	Z	H/TOBn	1-4	28-78 x 4.2-5.2	OBn	6.7-9.3	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	22-84 x 4.0-5.3	OGr	6.4-8.9	Punc

* Grown on 5% V-8, pH 7.5, and incubated at 20C.

APPENDIX 4d Effect of photoperiod on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'D'

Photoperiod	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 14	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
8h photoperiod	OGr	Cir	Z	H/TOBn	1-4(-5)	20-84 x 4.0-5.4	OGr	6.6-8.4	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4(-5)	26-87 x 4.1-5.3	OGr	6.3-8.2	Punc
Isolate MU 23									
8h photoperiod	GnGr	Cir	Z	H/TOBn	1-4	21-76 x 4.1-5.2	OGr	6.3-8.9	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	22-81 x 4.0-5.3	OGr	6.4-8.6	Punc
Isolate MU 29									
8h photoperiod	GnGr	Cir	Z	H/TOBn	1-4	23-88 x 4.4-5.4	OGr	6.2-7.8	Punc
Continuous dark	GnGr	Cir	NZ	H/TOGr	1-4	20-76 x 4.2-5.4	OGr	6.0-8.1	Punc

* Grown on 5% V-8, pH 7.5, and incubated at 20C.

APPENDIX 5a Effect of pH on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'A'

pH	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 9	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
6.0	OGr	Cir	Z	H/TOBn	1-4	26-89 x 4.3-5.6	OBn	6.2-8.4	Punc
6.5	OGr	Cir	Z	H/TOBn	1-4	22-88 x 4.4-5.4	OGr	6.4-8.0	Punc
7.0	OGr	Cir	Z	H/TOBn	1-4	26-82 x 4.1-5.5	OGr	6.1-8.6	Punc
7.5	OGr/GnGr	Cir	Z	H/TOBn	1-4	21-90 x 3.9-5.4	OGr	6.3-8.1	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	24-86 x 4.0-5.3	OGr	6.0-8.9	Punc
Isolate MU 11									
6.0	OGr	Cir	Z	H/TOBn	1-4	23-84 x 3.9-5.4	OGr	6.0-8.2	Punc
6.5	OGr	Cir	Z	H/TOBn	1-4	26-78 x 4.0-5.5	OGr	6.2-8.4	Punc
7.0	OGr	Cir	Z	H/TOBn	1-4	21-80 x 4.1-5.4	OGr	5.9-7.8	Punc
7.5	OGr	Cir	Z	H/TOBn	1-4	20-78 x 4.0-5.2	OGr	6.1-8.1	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	26-81 x 4.3-5.6	OGr	6.4-8.3	Punc
Isolate MU 33									
6.0	OGr	Cir	Z	H/TOBn	1-4	20-86 x 4.4-5.3	OGr	6.0-8.7	Punc
6.5	OGr	Cir	Z	H/TOBn	1-4	26-96 x 4.1-5.5	OGr	5.9-8.1	Punc
7.0	OGr	Cir	Z	H/TOBn	1-4	25-88 x 4.0-5.3	OGr	6.2-8.6	Punc
7.5	OGr	Cir	Z	H/TOBn	1-4	24-90 x 4.2-5.6	OGr	6.0-8.0	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	26-84 x 4.1-5.3	OGr	6.1-8.4	Punc

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

APPENDIX 5b Effect of pH on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'B'

pH	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 5	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
6.0	OGr	Cir	Z	H/TOBn	1-4	23-78 x 4.4-5.6	OBn	6.6-8.9	Punc
6.5	OGr	Cir	Z	H/TOBn	1-4	26-82 x 3.9-5.2	OBn	6.0-8.4	Punc
7.0	OGr	Cir	Z	H/TOBn	1-4	28-86 x 4.4-5.3	OBn	6.3-8.2	Punc
7.5	OGr	Cir	Z	H/TOBn	1-4	22-90 x 4.2-5.3	OBn	6.6-8.6	Punc
8.0	GnGr	Cir	Z	H/TOBn	1-4	24-84 x 4.1-5.5	OGr	6.1-8.0	Punc
Isolate MU 24									
6.0	OGr	Cir	Z	H/TOBn	1-4	24-90 x 4.4-5.6	OBn	6.6-8.3	Punc
6.5	OGr	Cir	Z	H/TOBn	1-4	18-86 x 4.3-5.2	OBn	6.1-7.9	Punc
7.0	OGr	Cir	Z	H/TOBn	1-4	22-84 x 4.1-5.4	OBn	6.0-8.4	Punc
7.5	OGr	Cir	Z	H/TOBn	1-4	18-92 x 3.9-5.4	OGr	5.8-7.7	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	24-86 x 3.9-5.5	OGr	6.1-8.2	Punc
Isolate MU 28									
6.0	OGr	Cir	Z	H/TOBn	1-4	26-86 x 4.1-5.3	OBn	6.0-8.0	Punc
6.5	OGr	Cir	Z	H/TOBn	1-4	22-79 x 4.4-5.5	OBn	6.4-7.9	Punc
7.0	OGr	Cir	Z	H/TOBn	1-4	26-84 x 4.3-5.2	OGr	6.1-8.3	Punc
7.5	OGr	Cir	Z	H/TOGr	1-4	20-94 x 4.4-5.1	OGr	6.5-8.0	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	28-82 x 4.3-5.4	OGr	6.3-8.4	Punc

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

APPENDIX 5c Effect of pH on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'C'

pH	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 2	Colour	Shape	Zonation	Colour	Septation	Dimensions (µ)	Colour	Diameter (µ)	Ornamentation
6.0	GnGr	Cir	Z	H/TOBn	1-4	22-81 x 4.1-5.6	OBn	6.1-8.9	Punc
6.5	GnGr	Cir	Z	H/TOBn	1-4	31-86 x 4.4-5.5	OBn	6.4-8.3	Punc
7.0	GnGr	Cir	Z	H/TOBn	1-4	28-88 x 4.3-5.5	OGr	6.3-8.7	Punc
7.5	GnGr	Cir	Z	H/TOBn	1-4	25-83 x 4.4-5.6	OGr	6.7-9.1	Punc
8.0	DGnGr	Cir	Z	H/TOGr	1-4	26-91 x 4.3-5.4	OGr	6.5-8.4	Punc
Isolate MU 19									
6.0	GnGr	Cir	Z	H/TOBn	1-4	26-81 x 4.1-5.2	OBn	6.2-8.1	Punc
6.5	GnGr	Cir	Z	H/TOBn	1-4	29-88 x 4.3-5.5	OBn	6.6-8.8	Punc
7.0	GnGr	Cir	Z	H/TOBn	1-4	31-80 x 4.6-5.3	OGr	6.0-8.5	Punc
7.5	GnGr	Cir	Z	H/TOBn	1-4	26-88 x 4.1-5.3	OGr	6.3-8.4	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	28-79 x 4.0-5.2	OGr	6.1-8.9	Punc
Isolate MU 40									
6.0	GnGr	Cir	Z	H/TOBn	1-4	24-78 x 4.1-5.4	OBn	6.5-8.9	Punc
6.5	GnGr	Cir	Z	H/TOBn	1-4	26-89 x 3.9-5.4	OBn	6.1-8.4	Punc
7.0	GnGr	Cir	Z	H/TOBn	1-4	22-84 x 4.0-5.3	OBn	6.6-8.8	Punc
7.5	GnGr	Cir	Z	H/TOBn	1-4	28-78 x 4.2-5.2	OBn	6.7-9.3	Punc
8.0	GnGr	Cir	Z	H/TOGr	1-4	26-80 x 4.3-5.4	OGr	6.4-8.4	Punc

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

APPENDIX 5d Effect of pH on colony, conidiophore and conidiogenous cell features of three *Stemphylium* isolates* of conidial group 'D'

pH	COLONY			CONIDIOPHORE			CONIDIOGENOUS CELL		
Isolate MU 14	Colour	Shape	Zonation	Colour	Septation	Dimensions (u)	Colour	Diameter (µ)	Ornamentation
6.0	GnGr	Cir	Z	H/TOBn	1-4	24-86 x 4.1-5.4	OGr	6.1-8.2	Punc
6.5	GnGr	Cir	Z	H/TOBn	1-4	26-80 x 3.9-5.3	OGr	6.0-7.8	Punc
7.0	GnGr	Cir	Z	H/TOBn	1-4	22-88 x 3.8-5.2	OGr	6.5-8.0	Punc
7.5	GnGr	Cir	Z	H/TOBn	1-4	20-84 x 4.0-5.4	OGr	6.6-8.4	Punc
8.0	DGnGr	Cir	Z	H/TOGr	1-4	26-78 x 4.1-5.6	OGr	6.3-8.1	Punc
Isolate MU 23									
6.0	GnGr	Cir	Z	H/TOBn	1-4	26-84 x 4.2-5.3	OBn	6.0-8.4	Punc
6.5	GnGr	Cir	Z	H/TOBn	1-4	20-79 x 3.9-5.3	OGr	6.1-8.8	Punc
7.0	GnGr	Cir	Z	H/TOBn	1-4	22-80 x 4.0-5.3	OGr	6.0-8.4	Punc
7.5	GnGr	Cir	Z	H/TOBn	1-4	21-76 x 4.1-5.2	OGr	6.3-8.9	Punc
8.0	DGnGr	Cir	Z	H/TOGr	1-4	26-81 x 4.3-5.4	OGr	6.4-8.6	Punc
Isolate MU 29									
6.0	GnGr	Cir	Z	H/TOBn	1-4	24-84 x 4.1-5.4	OGr	6.4-8.0	Punc
6.5	GnGr	Cir	Z	H/TOBn	1-4	29-89 x 4.1-5.3	OGr	6.1-7.9	Punc
7.0	GnGr	Cir	Z	H/TOBn	1-4	26-83 x 4.0-5.6	OGr	6.6-8.4	Punc
7.5	GnGr	Cir	Z	H/TOBn	1-4	23-88 x 4.4-5.4	OGr	6.2-7.8	Punc
8.0	DGnGr	Cir	Z	H/TOGr	1-4	26-81 x 4.2-5.3	OGr	6.0-8.1	Punc

* Grown on 5% V-8, and incubated at 20C in an 8h photoperiod.

APPENDIX 6

COMPOSITION AND PREPARATION OF CULTURE MEDIA

The ingredients and method of preparation of the different media are presented. In all cases the media were sterilized by autoclaving in 250ml flasks at 15 p.s.i. for 20 minutes.

(i) Laboratory potato dextrose agar (PDA)

Potatoes (peeled and sliced)	200g
Agar (Davis)	12g
Dextrose	10g
Distilled water	1000ml

The sliced potatoes were cooked gently for approximately one hour in 500ml of distilled water, after which they were filtered through cheesecloth. The agar and dextrose were melted in 500ml of distilled water and the potato filtrate then added and autoclaved.

(ii) Potato marmite agar (PMA)

Potatoes (peeled and sliced)	200g
Agar (Davis)	20g
Dextrose	20g
Marmite	1g
Distilled water	1000ml

The peeled and sliced potatoes were cooked gently for approximately one hour in 500ml of distilled water and then filtered through cheesecloth. The filtrate was then added to the agar, dextrose and marmite melted in 500ml of distilled water and autoclaved.

(iii) Malt agar (MA)

Malt extract	20g
Agar (Davis)	25g
Distilled water	1000ml

The malt extract was cooked in 500ml of distilled water for half hour and then filtered through cheesecloth. The filtrate was then added to the agar melted in 500ml of distilled water and autoclaved.

(iv) Potato carrot agar (PCA)

Potato (peeled and sliced)	20g
Carrot (peeled and sliced)	20g
Agar (Davis)	20g
Distilled water	1000ml

The sliced potatoes and carrots were cooked gently for approximately one hour in 500ml of distilled water, and then filtered through cheesecloth. The agar was melted in 500ml of distilled water and the potato-carrot filtrate added and autoclaved.

(v) Hay diffusion agar (HDA)

Decomposing hay	50g
Distilled water	1000ml
Infusion filtrate	1000ml
K_2HPO_4	2g
Agar (Davis)	15g

The hay was cooked gently in 1000 ml of distilled water for one hour and then filtered through cheesecloth. The infusion filtrate was made up to 1000ml and to it was added the agar and K_2HPO_4, and then autoclaved.

(vi) 20% V-8 juice agar (20% V-8)

V-8 juice	200ml
Agar (Davis)	15g
$CaCO_3$	3g
Distilled water	800ml

The agar was melted in the distilled water and to it was added the V-8 juice and $CaCO_3$, and then autoclaved.

(vii) 10% V-8 juice agar (10% V-8)

V-8 juice	100ml
Agar (Davis)	15g
$CaCo_3$	2g
Distilled water	900ml

Preparation was the same as for 20% V-8.

(viii) 5% V-8 juice agar (5% V-8)

V-8 juice agar	50ml
Agar (Davis)	15g
$CaCO_3$	2g
Distilled water	950ml

Preparation was the same as for 20% V-8.

REFERENCES

ARAGAKI, M. 1962. Quality of radiation inhibitory to sporulation of Alternaria tomato. Phytopathology 52: 1227-1228.

ARAGAKI, M., K.M. NISHIMOTO & J.W. HYLIN. 1973. Vegetative reversion of conidiophores in Alternaria tomato. Mycologia 65: 1205-1210.

ATANASOFF, D. 1919. A novel method of ascospore discharge. Mycologia 11: 125-128.

BARNETT, H.L. & B.B. HUNTER. 1972. Illustrated genera of imperfect fungi. Burgess Publishing Co., Minneapolis, Minnesota. 241pp.

BARRON, G.L. 1972. The genera of Hyphomycetes from soil. Robert E. Krieger Publishing Co. Inc., Huntington, N.Y. 364 pp.

BASHI, E., J. ROTEM & J. PUTTER. 1973. Effect of wetting duration and of the environmental factors, on the development of Stemphylium botryosum f. sp. lycopersici in tomatoes. Phytoparasitica 1: 87-94.

BAVERMAN, S.W. 1968. A new leaf spot of pepper incited by Stemphylium botryosum f. sp. capsicum. Phytopathology 58: 1164-1167.

BJORNSSON, I.P. 1959. Response of certain fungi particularly Trichoderma sp. to light. Jr. Washing. Acad. Sci. 49: 317-323.

BOLLE, P.C. 1924. Die durch Schwarzepilze (Phaeodictyae) erzeugten Pflanzenkrankheiten. Meded. phytopath. Lab. Willie Commelin Scholten, VII.

BOOTH, C. 1962. Taxonomy and the Fusarium problem. Phytopathology News 8: 57-58.

BOOTH, C. 1971. The genus Fusarium. Commonwealth Mycological Institute, Kew, England. 237pp.

BOOTH, C. 1975. The present status of Fusarium taxonomy. Ann. Rev. Phytopathol. 13: 83-94.

BRODIE, H.J. 1951. The splash-cup dispersal mechanism in plants. Can. Jr. Bot. 29: 224-234.

BULLER, A.H.R. 1922. Researches on fungi. Vol. II. Longmans, Green and Co., London.

BULLER, A.H.R. 1934. Researches on fungi. Vol. VI. Longmans, Green and Co., London.

CALLAGHAN, A.A. 1969. Light and spore discharge in Entomophthorales. Trans. Brit. Mycol. Soc. 33: 87-97.

CAMPBELL, R. 1969. An electron microscope study of spore structure and development in Alternaria brassicola. J. Gen. Microbiol. 54: 381-392.

CARLILE, M.J. 1970. The photoresponses of fungi. In Photobiology of microorganisms. Edited by Halldas, P., Wiley-Interscience, a division of John Wiley & Sons Ltd., London. pp. 309-344.

CARROLL, F.E. & G.C. CARROLL. 1971. Fine structural studies on "poroconidium" formation in Stemphylium botryosum. In Taxonomy of Fungi Imperfecti. Edited by Bryce Kendrick. Univ. Toronto Press. pp. 75-89.

CARROLL, F.E. & G.C. CARROLL. 1974. A fine structure of conidium initiation in Ulocladium atrum. Can. J. Bot. 52: 443-446.

CHILTON, S.J.P. 1942. Some pathogenic fungi occurring in the seed of red and subterranean clover. Phytopathology 32: 738-739.

COLE, G.T. 1973. Ultrastructure of conidiogenesis in Drechslera sorokoniana. Can. J. Bot. 51: 629-638.

COWAN, S.T. 1968. A dictionary of microbial taxonomic usage. Oliver and Boyd Ltd., Tweedale Court, Edinburgh. 118pp.

DAY, P.R. & K.J. SCOTT. 1973. Scanning electron microscopy of fresh material of Erysiphe graminis f. sp. hordei. Physiol. Pl. Pathology 3: 433-435.

DESHPANDE, K.B. & K.S. DESHPANDE. 1965. A new species of Stemphylium. Mycopathol. Mycol. Appl. 27: 203-204.

DINGLEY, J.M. 1969. Record of plant diseases in New Zealand. N.Z. Dep. Sci. Ind. Res. Bull. No. 192. 298pp.

DOUGLAS, D.R. 1972. Occurrence of Ulocladium consortiale as an associate fungus of Alternaria solani in potato tuber blight. Pl. Dis. Reptr. 56: 308-309.

DRECHSLER, C. 1923. Some graminicolous species of Helminthosporium. I. J. Agr. Res. 24: 641-740.

ECHLIN, P. 1968. The use of scanning reflection electron microscope in the study of plant and microbial material. J. R. Micr. Soc. 88: 407-418.

ELLIS, M.B. 1962. Dematiaceous Hyphomycetes: III. Commonwealth Mycol. Inst. Mycol. Paper 82: 1-55.

ELLIS, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, England. 608pp.

FINDLAY, R.M. 1970. A study of flower blight complex of chrysanthemums (Chrysanthemum morifolium Ramate). Postgraduate Diploma thesis for Hort. Sci. Massey Univ., New Zealand.

GRAHAM, J.H. 1953. A disease of birdsfoot trefoil caused by a new species of Stemphylium. Phytopathology 43: 577-579.

GRAHAM, J.H. 1957. A Stemphylium disease of ladino white clover. Phytopathology 47: 213-215.

GRAHAM, J.H. & K.E. ZEIDERS. 1960. Pathogenicity and morphology of some leguminicolous and related species of Stemphylium. Phytopathology 50: 757-760.

GROVES, J.W. & A.J. SKOLKO. 1944. Notes on seed-borne fungi. I. Stemphylium. Can. J. Res. 22: 190-199.

HALL, J.A. & A. SIVANESAN. 1972. Cochliobolus state of Curvularia cymbopogonis. Trans. Brit. Mycol. Soc. 59: 314-317.

HANNON, C.I. & G.F. WEBER. 1955. A leaf spot of tomato caused by Stemphylium floridanum sp. nov. Phytopathology 45: 11-16.

HARDING, H. 1975. Effect of pH and sucrose concentration on conidium size and septation in four Bipolaris species. Can. J. Bot. 53: 1457-1464.

HARRISON, Y.H. 1970. Scanning electron microscopy of fresh leaves of Pinguicula. Science 167: 172-174.

HARTER, L.L. 1939. Influence of light on the length of the conidia in certain species of Fusarium. Amer. Jr. Bot. 26: 234-243.

HODGETTS, W.J. 1917. On the forcible discharge of spores of Leptosphaeria acuta. New Phytologist 16: 139-146.

HOES, J.A., G.W. BRUEHL & C.G. SHAW. 1965. A new Pseudostemphylium. Mycologia 57: 904-912.

HONDA, Y. & M. SAKAMOTO. 1968. On the blue light inhibition of sporulation in Helminthosporium oryzae. Ann. Phytopath. Soc. Japan. 34: 328-335.

HOSFORD, R.M. et al. 1969. Studies of Leptosphaeria avenaria f. sp. triticea on wheat in North Dakota. Pl. Dis. Reptr. 53: 378-381.

HUGHES, S.J. 1953. Conidiophores, conidia, and classification. Can. J. Bot. 31: 577-659.

HYNES, H.J. 1937. Species of Helminthosporium and Curvularia associated with root-rot of wheat and other graminaceous plants. J. & Proc. Roy. Soc. New South Wales 70: 378-391.

INGOLD, C.T. 1933. Spore discharge in the Ascomycetes. I. Pyrenomycetes. New Phytologist 32: 178-196.

INGOLD, C.T. 1971. The liberation of spores in Ascomycetes. In Fungal spores, their liberation and dispersal. Clarendon Press. Oxford, England. 302 pp.

JACKSON, C.R. 1961. A flower rot symptom associated with Stemphylium ray speck of chrysanthemum. Pl. Dis. Reptr. 45: 798.

JONES, D. 1968. Surface features of fungal spores as revealed in a scanning electron microscope. Trans. Brit. Mycol. Soc. 51: 608-610.

JONES, F.R. & J.L. WEIMER. 1938. Stagnospora leaf spot and root rot of forage legumes. J. Agr. Res. 57: 791-812.

KAFI, A. & S.A.J. TARR. 1966. Growth, sporulation and conidial characteristics of five graminicolous species of Helminthosporium. I. Effect of nutrients. Trans. Brit. Mycol. Soc. 49: 327-337.

KARNOVSKY, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell. Biol. 27: 137A-138A.

KERR, J.E. 1961. The life history and taxonomic position of Venturia rumicis (Desm.) Wint. Trans. Brit. Mycol. Soc. 44: 465-486.

KOH, H.L. 1975. Identity of the causal organism of Stemphylium stem and foliage disease of asparagus in New Zealand. Postgraduate Diploma thesis for Agr. Sci. Massey Univ., New Zealand.

KUMAGAI, T. &. Y. ODA. 1969a. An action spectrum for photoinduced sporulation in the fungus Trichoderma viride. Plant and Cell Physiol. 10: 387-392.

KUMAGAI, T. & Y. ODA. 1969b. Blue and near ultraviolet reversible photoreaction in conidial development of the fungus Alternaria tomato. Developm. Growth Different. 11: 130-142.

LAPAGE, S.P. et al. 1976. International code of nomenclature of bacteria 1976. American Society for Microbiology, Washington, D.C. 180 pp.

LEACH, C.M. 1967. Interaction of near-ultraviolet light and temperature on sporulation of the fungi Alternaria, Cercosporella, Fusarium, Helminthosporium and Stemphylium. Can. J. Bot. 45: 1999-2016.

LEACH, C.M. 1968. An action spectrum for light inhibition of the "terminal phase" of photosporogenesis in the fungus Stemphylium botryosum. Mycologia 60: 532-546.

LEACH, C.M. 1971. Regulation of perithecium development and maturation in Pleospora herbarum by light and temperature. Trans. Brit. Mycol. Soc. 57: 295-315.

LEACH, C.M. & M. ARAGAKI. 1970. Effect of temperature on conidium characteristics of Ulocladium chartarum and Stemphylium floridanum. Mycologia 62: 1071-1076.

LUKENS, R.J. 1963. Photo-inhibition of sporulation in Alternaria solani. Amer. Jr. Bot. 50: 720-724.

LUTTRELL, E.S. 1957. Helminthosporium nodulosum and related species. Phytopathology 47: 540-548.

LUTTRELL, E.S. 1963a. Taxonomic criteria in Helminthosporium. Mycologia 55: 643-674.

LUTTRELL, E.S. 1963b. A Trichometasphaeria perfect stage for Helminthosporium causing a leaf spot of Dactyloctenium. Phytopathology 53: 281-285.

MRAK, E.M. & L. BONAR. 1938. The effect of temperature on asci and ascospores in the genus Debaryomyces. Mycologia 30: 182-186.

MULLER, E. 1971. Imperfect-perfect connections in Ascomycetes. In Taxonomy of Fungi Imperfecti. Edited by Bryce Kendrick, Univ. Toronto Press. pp. 184-201.

MULLER, E. & M. TOMASEVIC. 1957. Kulturversuche mit einigen Arten der Gattung Leptosphaeria Ces. et de Not. Phytopath. Z. 29: 287-294.

NEERGARD, P. 1945. Danish species of Alternaria and Stemphylium. Einar Munksgaard. Copenhagen. 560pp.

NELSON, R.R. 1960. Cochliobolus intermedius the perfect state of Curvularia intermedia. Mycologia 52: 775-778.

NELSON, R.R. 1964. The perfect stage of Helminthosporium spiciferum. Mycologia 56: 198-201.

NELSON, R.R. 1965. The perfect stage of Helminthosporium pedicellatum. Mycologia 57: 665-668.

PADHI, B. & W.C. SNYDER. 1954. Stemphylium leaf spot of lettuce. Phytopathology 44: 175-180.

PAUL, A.R. 1972. Pyrenophora erythrospila sp. nov. the perfect state of Drechslera erythrospila. Trans. Brit. Mycol. Soc. 59: 97-102.

PETRAK, F. 1950. Stemphylium dichroum Petr. n. spec. Sydowia (Ann. Mycol.) 4: 585-587.

PLUMB, R.T. & R.H. TURNER. 1972. Scanning electron microscopy of Erysiphe graminis. Trans. Brit. Mycol. Soc. 59: 149-150.

RAGHAVENDRA RAO, N.N. & M.S. PAVGI. 1975. Stemphylium leaf blight on onion. Mycopathologia 56: 113-118.

RANGASWAMI, G. & C.N. SAMBANDAM. 1960. The influence of substrate on spore size of Alternaria melongenae. Phytopathology 50: 486-488.

ROBERTS, D.A. & C.H. WARD. 1956. Diseases of forage crops before the first harvest of hay in New York 1956. Pl. Dis. Reptr. 40: 807-809.

ROTEM, J. 1968. Field trials for the control of Stemphylium botryosum f. sp. lycopersici in tomatoes. Israel. Jnl. Agric. Ress 18: 87-88.

ROTEM, J., Y. COHEN & I. WAHL. 1965. A new tomato foliage disease in Israel caused by Stemphylium botryosum. Can. J. Plant Sci. 46: 265-270.

RUPPEL, E.G. 1974. Factors affecting conidial dimensions of a Drechslera species. Mycologia 66: 803-807.

SHOEMAKER, R.A. 1959. Nomenclature of Drechslera and Bipolaris, grass parasites segregated from 'Helminthosporium'. Can. J. Bot. 37: 879-887.

SHOEMAKER, R.A. 1962. Drechslera Ito. Can. J. Bot. 40: 809-836.

SIMMONS, E.G. 1967. Typification of Alternaria, Stemphylium, and Ulocladium. Mycologia 59: 67-92.

SIMMONS, E.G. 1969. Perfect states of Stemphylium. Mycologia 61: 1-26.

SINGH, G. 1973. Flower blight of chrysanthemums: the causal fungi and their control. M. Agr. Sci. Thesis. Massey Univ., New Zealand. 152pp.

SINGH, G. & K.S. MILNE. 1974a. Laboratory evaluation of fungicides against fungi causing flower blight of chrysanthemums. N.Z. Journal of Experimental Agriculture 2: 181-183.

SINGH, G. & K.S. MILNE. 1974b. Field evaluation of fungicides for the control of chrysanthemum flower blight. N.Z. Journal of Experimental Agriculture 2: 185-188.

SIVAN, J. &.R. BARKAI-GOLAN. 1976. Stemphylium botryosum f. lactucae - its development in vitro and its pathogenicity to lettuce leaves. Ann. appl. Biol. 82: 421-432.

SLADE, D.A. 1961. A study of the leaf spot disease of lettuce caused by Stemphylium botryosum Wallr. M. Agr. Sci. Thesis. Massey Univ. New Zealand.

SMITH, O.F. 1940. Stemphylium leaf spot of red clover and alfalfa. J. Agr. Res. 61: 831-846.

SMITH, H.C. & B.C. SUTTON. 1964. Leptosphaeria maculans the ascogenous state of Phoma lingam. Trans. Brit. Mycol. Soc. 47: 159-165.

SNELL, W.A. & E.A. DICK. 1971. A glossary of mycology. Harvard Univ. Press, Cambridge, Mass. 181pp.

SNYDER, W.C. & H.N. HANSEN. 1940. The species concept in Fusarium. Amer. Jr. Bot. 27: 64-67.

SNYDER, W.C. & H.N. HANSEN. 1941a. The species concept in Fusarium, with reference to section Martiella. Amer. Jr. Bot. 28: 738-742.

SNYDER, W.C. & H.N. HANSEN. 1941b. The effect of light on taxonomic characters in Fusarium. Mycologia 33: 581-591.

SNYDER, W.C. & H.N. HANSEN. 1945. The species concept in Fusarium, with reference to Discolor and other sections. Amer. Jr. Bot. 32: 657-666.

SNYDER, W.C. & H.N. HANSEN. 1954. Variation and speciation in the genus Fusarium. N.Y. Acad. Sci. Ann. 60: 16-23.

SOBERS, E.K. 1965. A form of Stemphylium floridanum pathogenic to species of Kalanchoe. Phytopathology 55: 1313-1316.

SOBERS, E.K. & C.P. SEYMOUR. 1963. Stemphylium leaf spot of Echeveria, Kalanchoe and Sedum. Phytopathology 53: 1443-1446.

SPURR, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. ultrastruct. Res. 26: 31-43.

SUBRAMANIAN, C.V. 1961. Stemphylium and Pseudostemphylium. Curr. Sci. 30: 423-424.

SUBRAMANIAN, C.V. 1972. Conidium ontogeny. Curr. Sci. 41: 619-624.

SUBRAMANIAN, C.V. & B.L. JAIN. 1964. Conidiophore types in Cochliobolus sativus. Trans. Brit. Mycol. Soc. 47: 613-618.

SUZUI, T. 1973. Stemphylium leaf spot (Stemphylium botryosum Wallr.) on asparagus plants. Ann. Phytopath. Soc. Japan. 39: 364-366.

TAMMEN, J. 1963. Stemphylium ray speck of chrysanthemum. Phytopathology 53: 749-754.

TAN, K.K. 1974. Blue-light inhibition of sporulation in Botrytis cinerea. J. Gen. Microbiol. 82: 191-200.

TAN, K.K. & H.A.S. EPTON. 1973. The effect of light on the growth and sporulation of Botrytis cinerea. Trans. Brit. Mycol. Soc. 61: 147-157.

TARR, S.A.J. & A. KAFI. 1968. Growth, sporulation and conidial characteristics of five graminicolous species of Helminthosporium. II. Effect of nitrogen and pH. Trans. Brit. Mycol. Soc. 51: 771-777.

TATE, K.G. 1968. Identity, taxonomy and seed-borne aspects of the gray leaf spot organisms on blue lupin. M.Agr.Sc. Thesis. Massey Univ., New Zealand. 123pp.

TATE, K.G. 1970. A foliage disease of blue lupin caused by Stemphylium botryosum Wallr. N.Z. Jl. agric. Res. 13: 710-716.

TOUSSOUN, T.A. & P.E. NELSON. 1968. A pictorial guide to identification of Fusarium species according to the taxonomic system of Snyder & Hansen. Univ. Park, Pennsylvania State Univ. Press. Pennsylvania. 51pp.

TOUSSOUN, T.A. & P.E. NELSON. 1975. Variation and speciation in fusaria. Ann. Rev. Phytopathol. 13: 71-82.

TUBAKI, K. 1958. Studies on the Japanese Hyphomycetes. V.J. Hattori Bot. Lab. 20: 142-244.

VARGAS, Jr. J.M. & R.D. WILCOXSON. 1969. Some effects of temperature and radiation on sporulation by Helminthosporium dictyoides on agar media. Phytopathology 59: 1706-1712.

VITT, D.H. & C.D. HAMILTON. 1974. A scanning electron microscope study of the spores and selected peristomes of the North American Encalyptaceae (Musci). Can J. Bot. 52: 1973-1981.

WALKEY, D.G.A. & R. HARVEY. 1967. Spore discharge rhythms in Pyrenomycetes. III. Ascospore production and the quantitative and qualitative influences of light on spore discharge in Sordaria macrospora. Trans. Brit. Mycol. Soc. 50: 241-249.

WEBER, G.F. 1930. Gray leaf spot of tomato caused by Stemphylium solani sp. nov. Phytopathology 20: 513-518.

WEBSTER, J. 1969. The Pleospora state of Stemphylium triglochinicola. Trans. Brit. Mycol. Soc. 53: 478-482.

WEHMEYER, L.E. 1961. A world monograph of the genus Pleospora and its segregates. Univ. Michigan Press, Ann Arbor. 451pp.

WELLS, H.D. et al. 1956. Two Stemphylium diseases of blue lupine. Pl. Dis. Reptr. 40: 803-806.

WHITWELL, P.D. 1974. Studies on Stemphylium leaf spot and Leptosphaerulina pepper spot, two foliage diseases of lucerne. M. Agr. Sci. Thesis, Massey Univ., New Zealand. 147pp.

WILLIAMS, C.N. 1959. Spore size in relation to culture conditions. Trans. Brit. Mycol. Soc. 42: 213-222.

WILTSHIRE, S.P. 1938. The original and modern conception of *Stemphylium*. Trans. Brit. Mycol. Soc. 21: 211-239.

ZOGG, H. & F.J. SCHWINN. 1971. Surface structure of spores of the Ustilaginales. Trans. Brit. Mycol. Soc. 57: 403-410.

ERRATA

P.4, line 9 and p.114, line 43, 'Neergard' should read 'Neergaard'.

P.6, line 14, 'five septate' should read '5-septate'.

P.43, line 4, 'is useful' should read 'are useful'.

P.92, line 31, 'this two fungi' should read 'these two fungi'.

P.112, line 22, 'youngest conidia' should read 'youngest conidium'.