STUDIES ON A SEED-BORNE SEPTORIA DISEASE OF PHLOX DRUMMONDII HOOK.

by

Hugh Trevor Wenham

A THESIS

Presented at
Massey University
in partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

November 1966

ACKNOWLEDGMENTS

I am indebted to Professor R.G. Thomas, Mr J.D. Sargent and Dr J.S. Yeates for acting as supervisors.

I also wish to thank:

Mr A.M. Bryant for assistance with the statistical analyses.

Miss D. Scott and Miss C. Mitchell for preparation of the illustrations.

Mrs M. Wood for preparing Figure 3.

Professor E.E. Butler, Department of Plant Pathology, University of California for many helpful discussions on the subject of taxonomy.

Dr R.T.J. Clarke for helpful criticism of the manuscript.

CONTENTS

LIST OF PLATES

LIST OF FIGURES

LIST OF TABLES

PREFACE

The genus Phlox (family Polemoniaceae) comprises some 60 annual and perennial species, all of which are indigenous to the North American continent. The only annual species in cultivation is Phlox drummondii Hook. which is very popular as an ornamental in temperate climates. Seed of the wild form of this species was sent to England from Texas by Drummond in 1835, and later that year was described by W.J. Hooker as Phlox drummondii sp.nov. (Hay, 1937). Today the species has been extensively modified by domestication and dozens of named, true breeding varieties differing in stature, colour, size and shape of flowers are listed in trade catalogues.

In New Zealand P. drummondii is very popular in home gardens as a spring bedding species and for many years has been one of the main selling lines of commercial nurseries specialising in the production of annual seedlings. In the Manawatu first sowings are usually made early in September and thereafter at two week intervals through until November. The seed is mainly of European origin, with small importations from the United States and Canada. P. drummondii seed is not commercially produced in New Zealand.

During the spring of 1953 the author investigated a severe outbreak in two Palmerston North nurseries of a foliage disease of pricked-out P. drummondii seedlings which had been experienced in the Manawatu for several years, but had not previously been as severe. The symptoms of the disease were severe leaf lesioning with the production of pycnidia on necrotic tissues. Microscopic examination revealed large numbers of pycnidiospores typical of the form-genus Septoria and on the basis of spore and pycnidial dimensions the pathogen was identified as Septoria drummondii Ell. and Ev. This fungus had previously been recorded in New Zealand on P. drummondii by Brien (1939).

Several features associated with the disease and its development in the two nurseries suggested the possibility of seed being the source of primary inoculum, viz:

(a) a characteristic of the genus Septoria is that conidia are not adapted to wind dispersal, spread of spore inoculum being strictly localised and largely dependent on water splashing;

(b) a perfect stage, involving the production of air-borne sexual spores had not been reported for Septoria isolates from Phlox spp;

(c) both nurseymen followed the practice of steam sterilising all soil used in seed sowing and pricking-out mixes;

(d) where the same seed line was used in both nurseries in no instance were the resultant seedlings infected in one nursery but not in the other;

(e) several Septoria diseases are known to be seed-borne.

A Septoria disease of P. drummondii was first recorded in North America by Ellis and Everhart (1892). Subsequent literature has been confined to brief reports of the disease as a new record for an area or to brief descriptions of symptoms in established plantings with suggestions for control by the application of fungicides. All references to the disease are of a semi-popular nature, there being no detailed studies of the morphology, taxonomy, etiology and epidemiology of the pathogen and the disease it causes.

During the period 1954-56 a study satisfied the writer that the disease was in fact seed-borne and caused by Septoria drummondii Ell. and Ev. A short paper was prepared at that time and submitted for publication in "Plant Pathology", a quarterly journal published for the Ministry of Agriculture, Fisheries and Food, England. In a reply dated 7 January 1957, the Editor of the journal advised that the paper had been referred to W.C. Moore, then Director of the Plant Pathology Laboratory, Harpenden, England, who:

1. considered the evidence provided was insufficient for a first assertion that the disease was seed-borne; and

2. commented that more than one species of Septoria has been reported as pathogenic to P. drummondii and that the assumption that S. drummondii Ell. and Ev. was in this instance the pathogen was unwarranted.

This work is a study of the disease and its causal organism, with particular emphasis on the two contentious issues, namely, the seed-borne nature of the disease, and taxonomy of the pathogen. As no detailed study had previously been made of the disease itself, considerations of field survival of the pathogen, mode of secondary spread, and host-parasite relations have also been included.

I TAXONOMY OF THE PATHOGEN

A. Introduction

Although the present study is primarily concerned with the Septoria disease of P. drummondii a decision as to the correct nomenclature of the causal organism could not be reached without consideration of the total problem of the taxonomy of Septoria species pathogenic to the genus Phlox.

Four species of the form-genus Septoria have been recorded as foliage pathogens of Phlox drummondii, namely:

Septoria phlogis Sacc. & Speg.

Septoria divaricata Ell. & Ev.

Septoria drummondii Ell. & Ev.

Septoria vogliniana Sacc. & Trott.

I. S. phlogis was originally described in 1879 by Saccardo on the perennial species Phlox paniculata Linn. His description of the disease and the causal organism at that time is as follows:

Septoria phlocis Sacc. & Speg. (Michelia, 1879, Vol.1, p.184).

Maculis subcircularibus minutis albis, late fuligineo-rufo-marginatis; perithecciis sparsis paucis, punctiformibus, lenticularibus, 150-200 /u diam., pertusis, laxe ochraceo parenchymaticis; spermatiis bacillaribus, flexuosis, 40-60 x 1-2 /u, tenuiter 1-3 septatis, hyalinis. Hab. - in foliis Phlocis paniculatae, a Conegliano, Italiae, October 1877 (Spegazzini).

Translation:

Spots small, more or less circular and white, later becoming smoky to brownish-red at the margin. Pycnidia scattered, few in number, punctate, lenticular, 150-200 /u in diameter, ostiolate, ochraceous, loosely pseudoparenchymatous in texture. Spores rod-shaped, flexuous, 40-60 x 1-2 /u, hyaline and usually 1-3 septate. On foliage of Phlox paniculata, from Conegliano, Italy, October 1877 (Spegazzini).

In the above original description the species epithet was given as "phlocis" but later in his Sylloge Fungorum, Saccardo (1884) modified the spelling to S. phlogis, by which name the organism has since been known. In the International Rules of Botanical Nomenclature (Lanjouw et al., 1961), under the section on Orthography of Names and Epithets it is stated that —— "the original spelling of a name or epithet must be retained, except for typographic or orthographic errors". The spelling "phlocis" could be a typographic error by the printer, the letter 'g' being incorrectly considered 'c', or orthographic if Saccardo did not at the time know or remember the genitive of Phlox. A typographic error can be presumed since the spelling on the envelope containing the type material (Plate 1), considered to be in the writing of Saccardo (Hughes, pers. comm.), is clearly 'phlogis'. In either case the correction to S. phlogis is justified since the intended sense was obviously 'of Phlox'.

The disease was later identified in North America on Phlox divaricata Linn. by Trelease (1885) who described the spots as up to 5 mm in diameter and often confluent. Pycnidia were numerous and the colorless, filiform pycnidiospores measured 30–40 x 2 /u, and were usually 2 septate.

Martin (1887) recorded what he considered to be the same disease on P. divaricata, and described the spots as amphigenous, olivaceous below, dirty white above, 1-3 mm in diameter or, by confluence larger, with a purplish shaded border. Pycnidia were numerous, epiphyllous, lenticular, dull black and 100–120 /u in diameter, and the spores were 18–30 x 0.75 - 1 /u, faintly nucleolate, nearly straight and hyaline.

Grove (1935) reported the species in England and recorded pycnidia as 100–200 /u in diameter, and spores 1-3 septate, 40–60 x 1-2 /u.

In a brief note Pape (1935) reported the pathogen as prevalent in

Germany and described pycnidia as black and spherical, produced in the centre of lesions, 100–200 /u in diameter, and containing curved, 1–5 celled hyaline spores measuring 40–60 x 1–2 /u.

The above is the total recorded mycological information on S. phlogis but the host range has been enlarged to include P. drummondii. P. reptans, and P. virginiaca (Pape, 1935), P. alba (Grove, 1935), and P. glaberrina (Greene, 1953).

II. Ellis and Everhart (1889) reported a Septoria foliage disease of the perennial species P. divaricata which they believed to be identical with that described earlier by Martin (1887) on this host. However, they considered features of spores and pycnidia to be different from S. phlogis and elevated the fungus to new species status, naming it Septoria divaricata spec. nov. Their description of the disease and causal fungus is as follows:

> Septoria divaricata Ell. & Ev. (Jour. Mycology, 1889, Vol.5, p.151).
>
> "Spots white, spores 15–35 x 1 /u, mostly 20–25 /u long, nearly straight, very faintly nucleolate, non-septate".
>
> "On living leaves of P. divaricata, Lyndonville, New York, May, 1889".

The only other mycological account of this species is that by Grove (1935) who records pycnidia as 100–200 /u in diameter, and spores aseptate, 18–30 x 0.75 – 1 /u, but variable in length, mostly 20–25 /u. The organism has since been reported on P. paniculata (Seymour, 1929), P. drummondii (Toms, 1949) and P. amoena (Greene, 1951).

III. Ellis and Everhart (1892) erected a further species, Septoria drummondii spec. nov. citing it as the causal organism of a foliage disease of the annual species P. drummondii. Their description is as follows:

Septoria drummondii Ell. & Ev. (Jour. Mycology, 1892, Vol.7, p.133).

"Differs from S. divaricata on P. divaricata in the pycnidia being scattered thickly over the entire surface of the leaf, and not being on any definite spots. Pycnidia black, sub-prominent, 100 /u in diameter. Spores nearly straight, nucleate, 35-50 x 1.5-2.0 /u, rather narrower at one end.

On leaves of P. drummondii, London, Canada, September, 1891".

This binomial has since been widely accepted for the Septoria species on P. drummondii but has never been used for Septoria infections of perennial Phlox species, the implication being, presumably, that S. drummondii is not pathogenic to perennial species of Phlox.

IV. Voglino (1907) erected the species Septoria longispora spec. nov. on leaves of P. drummondii from northern Italy. His original Latin diagnosis and translation is as follows:

Septoria longispora Vogl. (Atti R. Acad. Sc. Torino XLIII, 1907-08).

"Maculis exaridis indeterminatis, irregularibus, saepe confluentibus, castaneis, picnidiis epiphyllis, minutis, innato-prominulis, olivaceo-fuscis, sphaeroideis, 100-130-150 /u latis; sporulis cylindricis, subflexuosis, utrinque rotundatis, hyalinis, distincte 5-9 septatis, 70-80-120 x 3 /u.

Hab.- in foliis Phlogis drummondii, quae maxime vexantur (Torino, Lucento, novembre 1907).

Maximtudine sporarum Septoria phlogis Sacc. & Speg. and S. divaricata Ell. & Ev. bene distincta".

Translation:

"Spots are indeterminate and somewhat dry and irregular, often confluent, chestnut coloured; pycnidia on upper side of leaf and minute, immersed becoming erumpent, olivaceous to smokey, spherical, 100-130-150 /u in diameter. Conidia cylindrical and subflexuous, somewhat rounded at the ends, hyaline, distinctly 5-9 septate, from 70-80-120 x 3 /u.

On leaves of Phlox drummondii, from North Italy, November 1907. Readily distinguished from S. phlogis and S. divaricata by the size of spores".

In his Sylloge Fungorum, Saccardo (1913) changed the binomial to Septoria vogliniana Sacc. & Trott. as the species epithet "longispora" had previously been used for a species of Septoria on Convolvulus arvensis.

Although spore dimensions and septation would suggest a clearly defined species the only other mention of this fungus is by Oudemans (1923) who lists it under the name of S. vogliniana as a record on P. drummondii, and citing Voglino's original paper as his authority. That is, this species is known only from the original diagnosis.

Other than the above cited references the only reported study of a Septoria disease of Phlox is that by Bond (1941), who gives a descriptive, semi-popular account of a Septoria leafspot of P. drummondii in Ceylon. In considering the identity of the pathogen Bond concluded that it was similar to S. drummondii, and in view of its occurrence on the same host provisionally applied that name to it. He commented however, that the wide variability in the spores of the Ceylon fungus (17-66.5 x 1.5-3 /u, mean 43 x 2 /u), and the fact that S. drummondii as originally published was more or less intermediate between S. phlogis and S. divaricata suggested that the distinction between these three species was hypothetical, and that they should therefore be combined. Grove (1935) had already questioned the species distinction by stating - "S. phlogis is doubtless nothing more than an advanced stage of this fungus (S. divaricata). It is said to differ by having white spots and flexuous, 1-3 septate spores, 40-60 x 1-2 /u, but this difference is no greater than can often be found between mature and immature specimens on the same plant". Support for Bond's suggestion was provided by Saville (1952) who noted that —— "there are various records of Septoria leaf spots on this plant (P. drummondii) under S. drummondii, which is certainly synonymous with S. phlogis; or under S. divaricata, which supposedly has much smaller spores. Specimens suggest that there is

a complete integration in spore size".

There is no evidence of the perfect stage being found for any of the above species of Septoria. However Oudemans (1901) erected the ascomycetous species Leptosphaeria phlogis spec. nov. on leaves of Phlox decussata (= P. paniculata). The original Latin description and translation is as follows:

> Leptosphaeria phlogis Oud. spec. nov. - on leaves of Phlox decussata cultivated at Dedemsvaart, 10 November, 1898. Sent by Prof. Ritzema Bos.
>
> "Perithecia parva, sparsa. Asci cylindraceo - clavati, curvuli, sessile, 46 x 9.3 /u. Sporidia disticha, cylindracea, curvula, ad polos obtusa, 3 septata, loculo penultimo antico ceteris ampliore, fuscidula, 23-25 x 4-5 /u".
>
> Translation:
>
> "Perithecia small and scattered. Asci cylindrical-clavate, curved, sessile, 46 x 9.3 /u. Ascospores 2- ranked, cylindrical, curved, blunt at both ends, 3 septate, the penultimate anterior cell larger than the others and somewhat darkened, 23-25 x 4-5 /u".

According to Grove (1935), - "S. phlogis is said to have an ascophorous stage, Leptosphaeria phlogis", - but there appears to be no justification for this latter statement. Nowhere in the literature is there any suggestion of a relationship between these two fungi. Furthermore, other than the above original record and description by Oudemans (1901) there is in fact, no evidence of this organism subsequently being found.

The above review reveals the unsatisfactory state of knowledge regarding the taxonomy and nomenclature of Septoria species pathogenic to the genus Phlox. It is apparent that in each instance the four species were erected solely on the basis of study of one collection, with the criteria for taxonomic differentiation being host specificity, slight differences in symptom expression and features of pycnidia and pycnidiospores (Table 1). In view

TABLE 1

Distinguishing Characters in the Original

Descriptions of Four Species of Septoria on Phlox spp.

Pathogen	Authority	Host Species	Spots on Foliage	Pycnidia	Spores.
S. phlogis	Saccardo (1879)	P. paniculata	Small and more or less circular. White at first later becoming smoky to brownish red at margin.	Scattered, few in number punctate lenticular, ostiolate, clear brown in colour. Size 150-200 /u.	Filiform, flexuous, hyaline. Usually 1-3 Septate. Size 40-60 x 1-2 /u.
S. divaricata	Ellis and Everhart (1889)	P. divaricata	Olivaceous below, whitish above 1-3 mm diam. or larger by confluence, with purplish border. Round in shape.	Epiphyllous, numerous, erumpent dull black. Size 100-120/u.	Filiform, merely straight, aseptate. Size 18-30 x 0.75 - 1 /u.
S. drummondii	Ellis and Everhart (1892)	P. drummondii		Densely gregarious over entire leaf surface, not confined to definite spots. Black, subprominent. Size 100 /u.	Straight or slightly bent, acicular. Size 35-50 x 1.5 - 2 /u.
S. vogliniana	Voglino (1907)	P. drummondii	Indeterminate and irregular, often confluent, chestnut coloured.	Epiphyllous, minute, immersed becoming erumpent olivaceous to smoky; spherical, 100-130-150 /u diam.	Cylindrical and sub-flexuous hyaline, distinctly 5-9 septate, 70-80 -120 x 3 /u.

of the expressed belief that only one real species may be involved and in the absence of critical studies pertaining to this subject the author has attempted to resolve the problem as follows:

(i) type collections and other herbarium material was assembled and critically examined; and

(ii) the alleged species in field collections from several countries were compared with particular attention given to variability of morphological characters both on host material and in pure culture, and the extent of host specificity.

B. Consideration of Herbarium Material

1. Type Collections

The type material of three of the four erected species of Septoria on Phlox spp. was assembled and examined. S. phlogis was obtained from the Institute ed Orto Botanico Della Universita di Padova, Italy, and S. divaricata and S. drummondii from the New York Botanical Garden, New York. In each case the material was examined for symptoms, prevalence of pycnidia, shape, septation and dimensions of pycnidiospores. All measurements were made after mounting in lactophenol cotton blue.

The type material of S. vogliniana was not examined. Several European institutions considered likely to hold Voglino's original collection were approached, but the material could not be located. For this reason, plus the fact that field material of S. vogliniana has since never been located no further consideration is given this species in the present study.

(a) Septoria phlogis Sacc. & Speg.

The material consisted of fragments of two leaves enclosed in a small envelope to which was affixed a small piece of note paper with writing

and a sketch of 4 spores and a pycnidium (Plate 1). The leaf material exhibited numerous distinct, small (1-3 mm), circular spots with white centres and a darkened border. All tissue pieces were carefully examined and found to be quite devoid of pycnidia. The surface of several of the larger lesions, considered more likely to have pycnidia present, were punctured as though pycnidia previously present had been removed by earlier examiners. That is, in its present state the leaves were quite useless as providing a means of re-determining the mycological features of the fungus.

(b) Septoria divaricata Ell. & Ev.

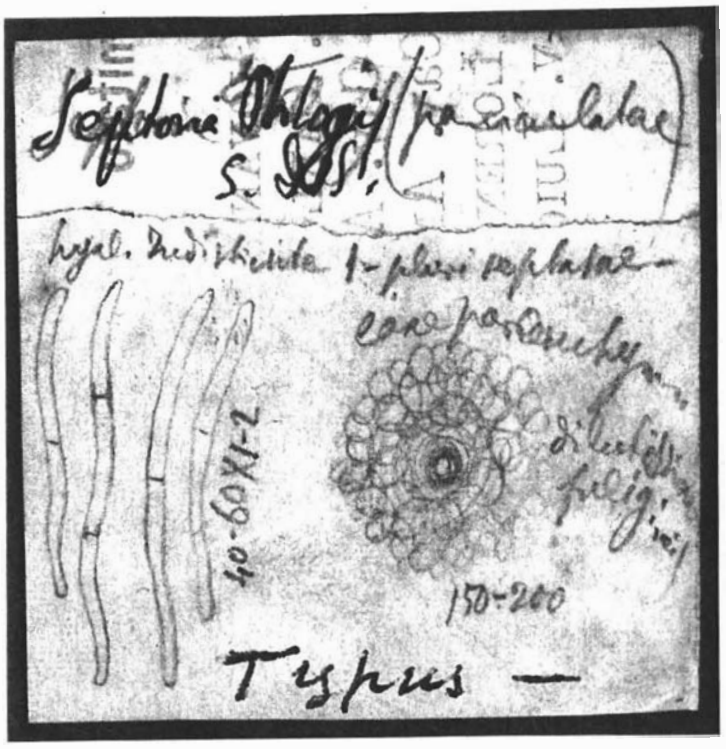
The material consisted of five infected leaves contained in an envelope (Plate 2). The information on the envelope additional to that in the original description stated that pycnidia were 90-112 /u in diameter, erumpent, and present on the upper side of the leaf.

Each leaf exhibited several circular lesions up to 4 mm in diameter, mostly on the upper surface, with pycnidia present on both leaf surfaces but only on the larger lesions. Pycnidia were erumpent, several with cirrhi still present. Five pycnidia were mounted separately and measured, and the dimensions of 100 spores from each recorded (Table 2). Shape of the spores ranged from straight to flexuous, and several were observed to be 3 septate. The number of cells present in the majority of spores could not be determined because of the density of the spores contents.

The observations of Ellis and Everhart were thus inaccurate, as pycnidia were present on both leaf surfaces and spores were variable in shape and in some cases quite definitely septate.

(c) Septoria drummondii Ell. & Ev.

The material consisted of parts of 4 leaves enclosed in a captioned



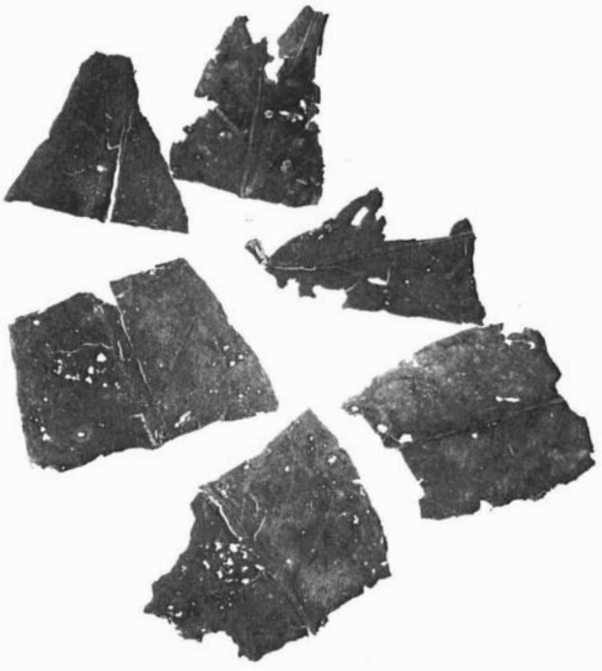


Plate 1 Type material of Septoria phlogis Sacc. & Speg.
Top - inscription on the envelope cover
Bottom - contained leaves

Plate 2 **Type material of Septoria divaricata Ell. & Ev.**

Top - inscription on the envelope cover

Bottom - contained leaves, and inscription on the inside of the envelope

TABLE 2 Dimensions of pycnidiospores and pycnidia from type material of S. divaricata Ell. & Ev.

Pycnidium No.	Pycnidiospore Dimensions [a.] (in microns)			Pycnidium Diameter (in microns)
	Length		Width	
	Range	Mean	Range	
1	19.2-32.0	27.2 ± 0.18	1.0-2.5	105
2	20.8-40.0	30.4 ± 0.31	1.0-2.5	102
3	20.8-33.6	28.4 ± 0.39	1.0-2.5	96
4	30.4-45.8	38.4 ± 0.77	1.0-2.5	141
5	22.4-35.2	28.8 ± 0.28	1.0-2.5	120
Overall	19.2-45.8	30.6 ± 2.01	1.0-2.5	116

a. Sample size of 100 spores

envelope (Plate 3). Several large, irregular lesions were present on both surfaces of each leaf specimen. Pycnidia were brown to black in colour, erumpent, and crowded on the lesioned areas, more particularly on the upper leaf surfaces. Cirrhi were not observed. Symptoms appeared identical to the Septoria disease of P. drummondii present in the Manawatu. Eight pycnidia were mounted separately and measured, and the dimensions of 100 spores from each recorded (Table 3). Pycnidiospores were acicular, straight to flexuous and some were observed to be septate. The 3 septate condition was most common, but spores with one and 2 septa were located. More than 3 septa were not observed. The percentage of spores in each septate class could not be determined as the septa in approximately 20% of the spores were obscured by the dense cell contents.

(d) Discussion

The type material was examined with a view to determining the accuracy of the original descriptions and evaluating the collections considered by Ellis and Everhart to warrant the erection of two further species.

In all three cases symptoms were as originally described. On P. paniculata and P. divaricata lesions were very similar, being small, circular and typical of the disease as it develops on P. paniculata in the Manawatu. Symptoms on P. drummondii were identical with those of the disease on this host in New Zealand and the large irregular areas of dead tissue with crowded conspicuous pycnidia were quite distinct from those on the other host species. However, symptom differences in themselves provide little of taxonomic significance, particularly when three host species are involved as in this case. It has been demonstrated many times that disease characters on the one host species vary with the variety involved and with the age of host tissues, and are also subject to variation with climatic changes. Further,

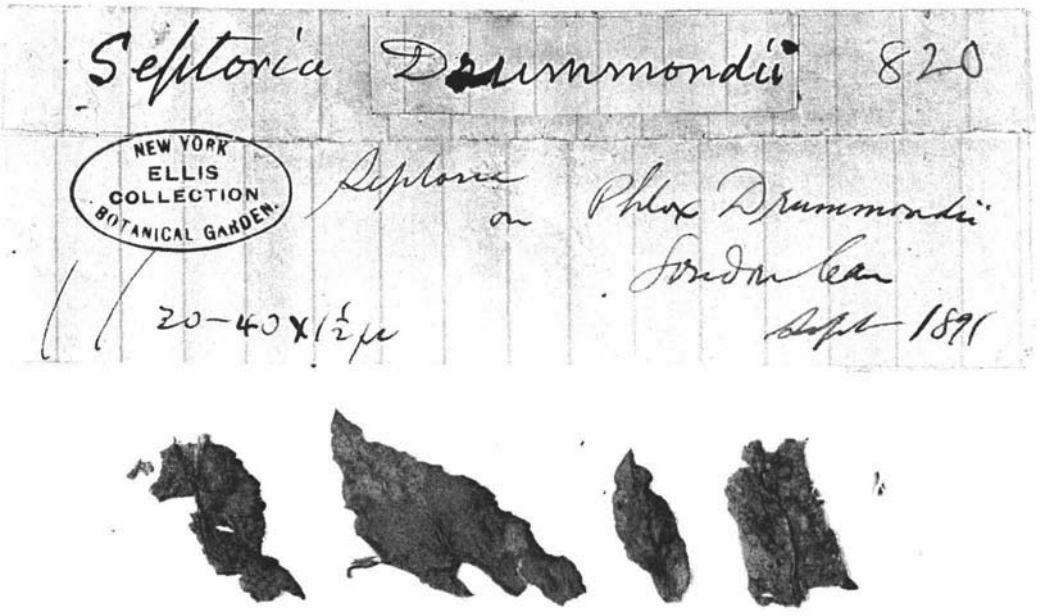


Plate 3 Type material of Septoria drummondii Ell. & Ev. Leaf material, and attached inscription

TABLE 3 Dimensions of pycnidiospores and pycnidia from type material of S. drummondii Ell. & Ev.

Pycnidium No.	Pycnidiospore Dimensions [a.] (in microns)			Pycnidium Diameter (in microns)
	Length		Width	
	Range	Mean	Range	
1	17.6–35.2	25.6 ± 0.31	1.0–2.5	75
2	20.8–36.8	27.2 ± 0.41	1.0–2.5	84
3	17.6–48.0	35.2 ± 0.39	1.0–2.5	90
4	25.6–43.2	36.8 ± 0.53	1.0–2.5	93
5	22.4–41.6	33.6 ± 0.47	1.0–2.5	78
6	25.6–43.2	38.4 ± 0.55	1.0–2.5	105
7	17.6–46.4	35.2 ± 1.02	1.0–2.5	102
8	19.2–43.2	32.0 ± 0.34	1.0–2.5	96
Overall	17.6–48.0	33.0 ± 1.6	1.0–2.5	90

a. Sample size of 100 spores

since the development of lesions is a reaction on the part of the host to the presence of the pathogen and not an attribute of the fungus itself it is at least questionable whether symptoms warrant consideration as a criterion for delimiting species. Certainly in this case symptoms are of no significance in the matter of whether more than one morphological species of pathogen is involved.

Contrary to the claim of Ellis and Everhart, S. divaricata and S. drummondii could not be separated on the basis of spore morphology. According to the original descriptions spores of S. divaricata are aseptate, but in all preparations of this species septa were unquestionably present in some spores, and in this regard were identical with those of S. drummondii. Spore shape was a highly variable feature, ranging from straight to distinctly curved in all spore preparations of both species.

As regards spore dimensions, whereas Ellis and Everhart recorded a range in length of 18-30 /u for S. divaricata and 35-50 /u for S. drummondii (Table 1) in the present study they were found to be 19.2 - 45.8 /u and 17.6 - 48.0 /u respectively. Spore width in both collections was 1.0 - 2.5 /u. That is, there was considerable overlapping in the range of spore length thus making separation of these two species on this basis quite impossible. Likewise the diameter of pycnidia from the two collections so overlapped as to be valueless in diagnosis (Tables 2 and 3).

The absence of pycnidia on the type material of S. phlogis prevented morphological comparison with the Ellis and Everhart collections. Assuming Saccardo's figures to be correct then his pycnidial diameters (150-200 /u) are considerably greater than those found for both S. divaricata (96-141 /u) and S. drummondii (75-105 /u), and his range in spore length (40-60 /u), although overlapping that of the other two species (17.6-48.0 /u) nevertheless

indicates a mean average distinctly greater than that of the other fungi. However, this in itself is not sufficient grounds for regarding Saccardo's S. phlogis as a distinct species. The type material of all three species each represent a single field collection and therefore do not take into account either stage of maturity or field variability. Further collections of each under diverse environmental conditions could well have produced complete integration in pycnidial diameter and spore size.

The only firm conclusion that re-examination of the type material allows is that S. divaricata and S. drummondii are morphologically indistinguishable.

2. Other Herbarium Material

(a) Material Studied

Eight collections of Septoria species on Phlox drummondii were received from the Herbarium of the University of California, Berkeley, of which five were purported to be S. drummondii, two S. divaricata, and one S. phlogis (Table 4). Each collection was examined for symptoms, prevalence of pycnidia, and shape, septation and dimensions of pycnidiospores. In each case slide preparations of spores were prepared by rupturing several pycnidia in lactophenol cotton blue.

(b) Results and Discussion

Although there were differences in the size, shape and prevalence of lesions these were no more diverse than could be found on infected P. drummondii plants in the Manawatu during any one season. Specimens number 3 and 4 (Table 4) were least typical in that all lesions were small and circular, but in each case with many erumpent, golden-brown to black pycnidia present. In the remaining collections, lesions ranged from small

TABLE 4

Comparison of Spore Dimensions and Pycnidium Diameter of Herbarium Specimens on *Phlox drummondii* and Labelled as *S. drummondii*, *S. divaricata* and *S. phlogis*

Code	Labelled as	Date of Collection	Place of Collection	Collector	Identified By	SPORE DIMENSIONS				PYCNIDIA DIAMETER		
						No. of Spores	LENGTH Range	LENGTH Mean	Width	No. Measured	Range	Mean
1.	*S. drummondii*	Sept. 1897	London, Canada.	J. Dearness	J. Dearness	120	32.0-59.2	44.8 ± 0.43	1.5-3.0	15	75-125	95 ± 4.45
2.	*S. drummondii*	Apr. 1936	Gainesville, Florida.	G.F. Weber	G.F. Weber	100	28.8-44.8	36.8 ± 0.26	1.5-2.5	15	70-120	90 ± 6.11
3.	*S. drummondii*	May, 1900	Lake City, U.S.A.	H. Harold Hume	H. Harold Hume	100	20.8-40.0	32.0 ± 0.34	1.5-2.5	10	75-130	95 ± 6.78
4.	*S. drummondii*	Mar. 1963	Gainesville, Florida.	W.B. Tisdale	Edmon West	70	20.8-44.8	28.8 ± 0.22	1.5-2.5	22	65-115	80 ± 5.61
5.	*S. drummondii*	Apr. 1957	Kansas, U.S.A.	O.H. Elmer	C.T. Rogerson	70	20.8-38.4	32.0 ± 0.18	1.5-2.5	20	80-130	105 ± 4.19
6.	*S. divaricata*	Jan. 1940	Ontario, Calif.	K.F. Baker	K.F. Baker	100	25.6-41.6	32.0 ± 0.23	1.5-2.5	15	70-110	85 ± 5.83
7.	*S. divaricata*	May, 1956	Jackson City Kansas.	C.T. Rogerson	C.T. Rogerson	70	14.4-33.6	22.4 ± 0.36	1.5-2.0	15	75-115	90 ± 5.91
8	*S. phlogis*	?	?	Petrak	Petrak	100	28.8-48.0	38.4 ± 2.11	1.5-3.0	15	60-155	120 ± 4.63

Note: An analysis of variance gave no significant difference in the mean length of pycnidiospores from different specimens (Appendix 2).

circular areas approximately 3 mm in diameter through to extensive irregular areas of dead tissue densely covered with conspicuous pycnidia. A feature of all collections was the prevalence of pycnidia, particularly on the upper surface of leaves.

Nothing distinctive was observed as regards spore shape and septation. Spore shape was very variable ranging in all collections from straight to distinctly curved and bent. Plate 4 shows pycnidiospores representative of the three species and serves to illustrate the similarity in gross appearance of the spores. Some spores in all collections were observed to be septate but the dense nature of the cell contents in many cases prevented an assessment of the degree of septation.

The dimensions of spores and pycnidia from each collection are presented in Table 4.

It is difficult to determine why the collections were originally identified as either S. drummondii, S. divaricata or S. phlogis. As the host in each instance was P. drummondii, host specificity is ruled out, and symptoms in no way served to differentiate the three species. It would appear therefore, that spore dimensions were the most important single criterion considered. However, specimens 3 and 4 were both labelled as S. drummondii but on the basis of spore dimensions identification as S. divaricata would be more justified. As mentioned earlier, lesions on both these specimens were small and this fact coupled with the relatively small size of the spores suggests early-stage infection. The spore measurements from Petrak's collection (specimen 8) suggests this species to be also incorrectly identified; the range in spore length of 28.8 - 48.0 /u and mean of 28.4 /u warrants the fungus being identified as S. drummondii rather than S. phlogis.

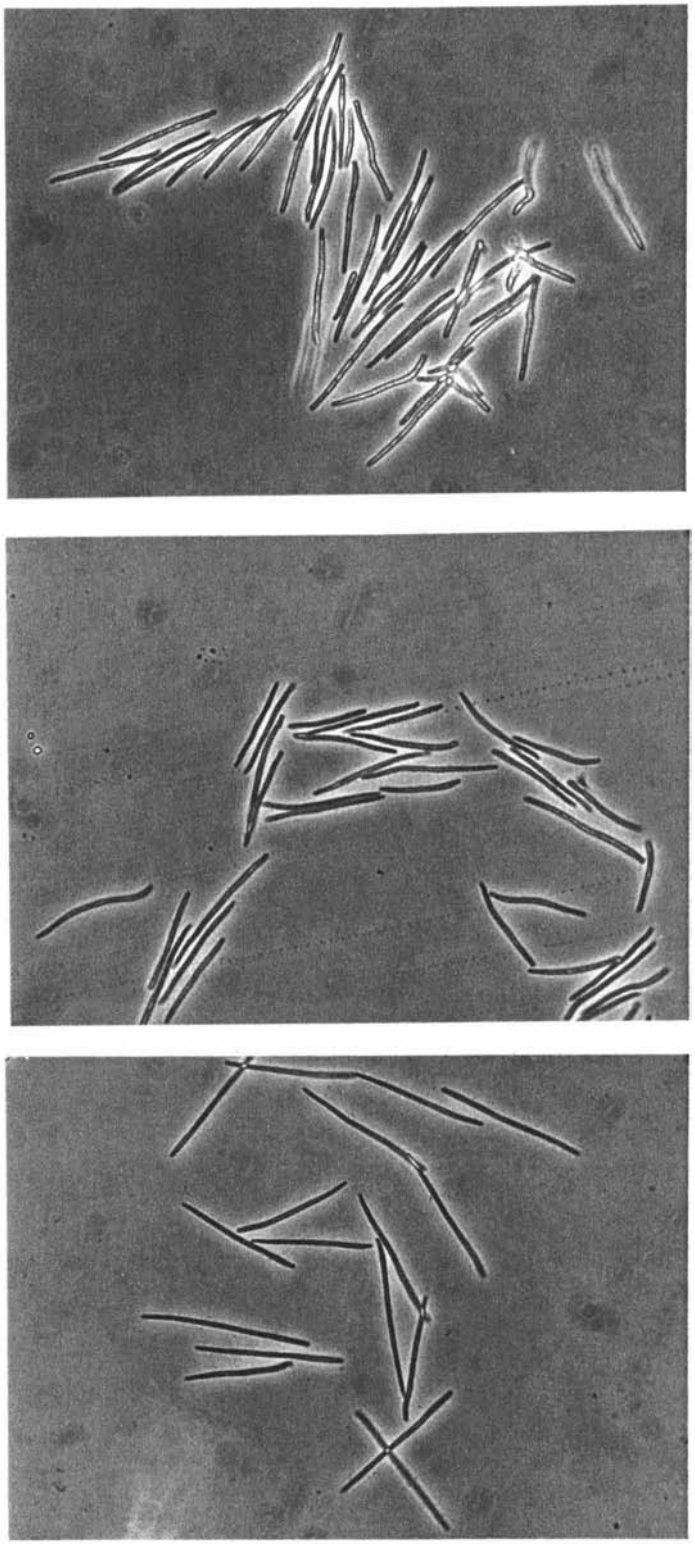


Plate 4 Photomicrograph (phase contrast) of pycnidiospores from herbarium collections of P. drummondii (see Table 4).

Top - S. phlogis
Middle - S. divaricata
Bottom - S. drummondii

C. Study of Field Collections

Currently all three species epithets are in use for Septoria infections of Phlox. Thus according to Large (1957) - "British material all fits S. drummondii Ell. & Ev. and S. phlogis Sacc. & Speg. does not occur here". In Holland, according to Briejer (1965) - "only S. phlogis Sacc. & Speg. is recorded on Phlox species". In recent Annual Reports of the Canadian Plant Disease Survey (1952, 1960, 1961) all three species are recorded, although according to Thompson (1964) - "the species of Septoria most frequently reported as the cause of Septoria leaf spot in Canada is S. divaricata Ell. & Ev." In New Zealand Brien (1939) recorded S. drummondii Ell. & Ev. on P. drummondii, and S. phlogis Ell. & Ev. on P. paniculata.

Infected field material representative of the three species of pathogen as present in the United Kingdom, Holland, Canada and New Zealand was assembled, and comparative studies made to determine whether separation was in fact possible on the basis of criteria generally regarded as of taxonomic significance. Features considered were disease symptoms, and morphology of the pathogen in host tissues.

1. Materials and Methods

Collections of the three alleged species of pathogen were available as follows:

(a) Septoria phlogis Sacc. & Speg.

1. In view of the seed-borne nature of the disease in P. drummondii (Part III) it may be assumed that seedling infections in New Zealand arising from use of imported infected seed are caused by the Septoria species considered present in the source country. Each year infected material was available from crops raised from infected seed imported from Holland, where only S. phlogis is recorded on Phlox species (Briejer, 1965).

In the tables which follow these collections are coded as H.dr.sd.

2. Five collections of infected P. paniculata labelled as S. phlogis were received from Dr D. Leblond, Division of Research, Department of Agriculture, Quebec, Canada (coded as Q.pan).

3. Two collections of infected P. paniculata labelled as S. phlogis were received from Dr Briejer, Plant Disease Service, Holland (coded as H.pan).

4. The Septoria disease of P. paniculata is very prevalent in the Manawatu, the majority of commercial and home garden plantings examined over several years being infected to some extent. S. phlogis is the only species recorded on this host in New Zealand (Brien, 1939). Such collections are coded as M. pan.

(b) Septoria divaricata Ell. & Ev.

Three collections of infected P. paniculata labelled as S. divaricata were received from Dr R.G. Atkinson, Canadian Department of Agriculture, Saanichton, British Columbia (coded as B.C.pan).

(c) Septoria drummondii Ell. & Ev.

Diseased P. drummondii plants arising from use of infected seed imported from England may be assumed to be caused by S. drummondii, since this is the only species recorded in the United Kingdom (Large, 1957). Such collections are coded as U.K. dr.Sd.

In studying spore features only conidia released from pycnidia were used as they were considered more likely to be mature. Leaves were subjected to high humidity in petri-dishes for 36 hours and cirrhi transferred to drops of lactophenol cotton blue. That is, all spore preparations were a blend of several cirrhi. Lactophenol cotton blue was preferred as a stain as it was

found to prevent swelling of the spores and accentuated the presence of septa.

In determining pycnidial dimensions small pieces of infected leaves were left in absolute alcohol and glacial acetic acid (1:1) to encourage removal of chlorophyll, gently boiled for a few minutes in lactophenol, cooled, left in a saturated aqueous solution of chloral hydrate until clear, and mounted in lactophenol cotton blue. By first clearing tissues in this way the total diameter of pycnidia rather than just the exposed surface area could be measured.

The morphology of pycnidia in leaves was determined from serial sections prepared by the paraffin method. Leaves were killed and fixed in formalin: acetic acid: alcohol, dehydrated with tertiary butyl alcohol, embedded in paraffin, cut with a rotary microtome, and mounted in lactophenol cotton blue.

2. Results

All collections on the one host species, irrespective of source, were very similar to each other but as a group differed somewhat from those on the other host species. Accordingly symptoms and pathogen morphology are considered on the basis of host species involved, rather than species of pathogen purported to be present. However, data relating to the dimensions of pycnidiospores and pycnidia are also presented for each of the three species of *Septoria*.

(a) *Phlox drummondii*

(i) Symptoms

The disease first attracts attention in Manawatu commercial nurseries after seedlings have been pricked-out and before the boxes are transferred to cold frames (Plates 5 & 6). Earliest evidence is the development of small, circular, diffuse, chlorotic spots approximately 3 mm

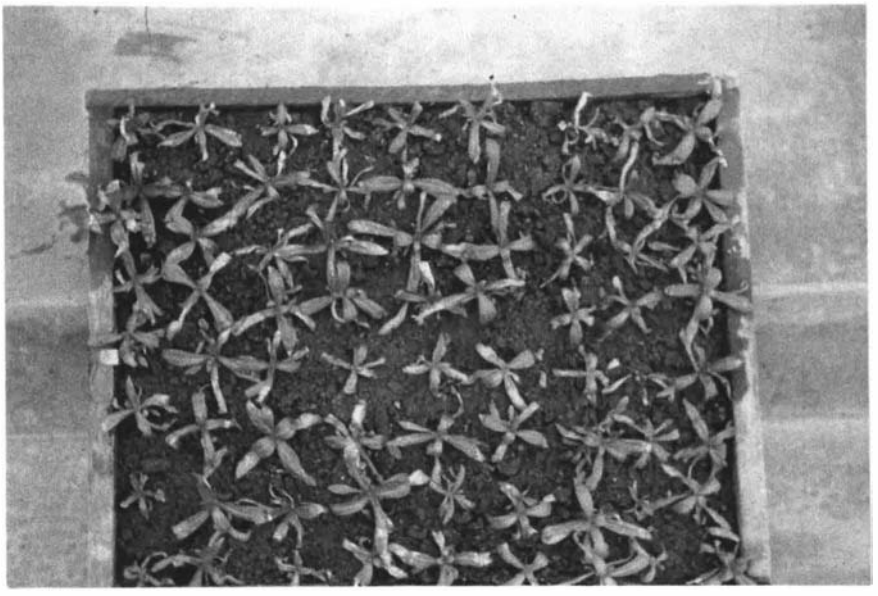


Plate 5 Box of commercially raised P. drummondii seedlings showing severe infection.

Plate 6 Severe infection in pricked-out, commercially raised P. drummondii seedlings.

in diameter which within three days become distinct, light brown lesions on the surface of which, crowded brown pycnidia are readily discernible. With the enlargement and coalescing of lesions the dead tissues lighten in colour and pycnidia darken and stand out prominently against the lighter background (Plate 7). Spore horns (cirrhi) may be macroscopically discernible on close examination. At this stage lesions are irregular in shape, appearing more as dead patches and frequently extending to the tip and edges of leaves. Usually the lower leaves are more severely infected and soon become curled and brittle and die. Some abscission of dead leaves occurs but this is not a characteristic feature of the disease. Only rarely has seedling death been observed, but because practically all plants in a box become infected as a result of secondary spread the over-all effect is extreme unsightliness and pronounced stunting, and complete plantings are commonly discarded at this stage.

Nurserymen have learned to check the disease to some extent by limiting the application of water and reducing humidity by transferring plants to cold frames as soon as possible. In this way some plants are salvaged for sale. Such plants have been studied in the field and further symptom development observed. It is most noticeable that the extent of subsequent disease development and spread is a direct reflection of climatic conditions. If the late spring and summer is dry there is little progress in the disease but after periods of rain more lesions rapidly appear on new growth. These frequently remain small and discrete and only after continued dull wet weather do they enlarge and coalesce. At the height of summer, lesions are noticeably different from those developed at the seedling stage. There is frequently no sharp demarkation between dead and living tissues. The centres of lesions may be dull white, passing through golden brown areas to living but

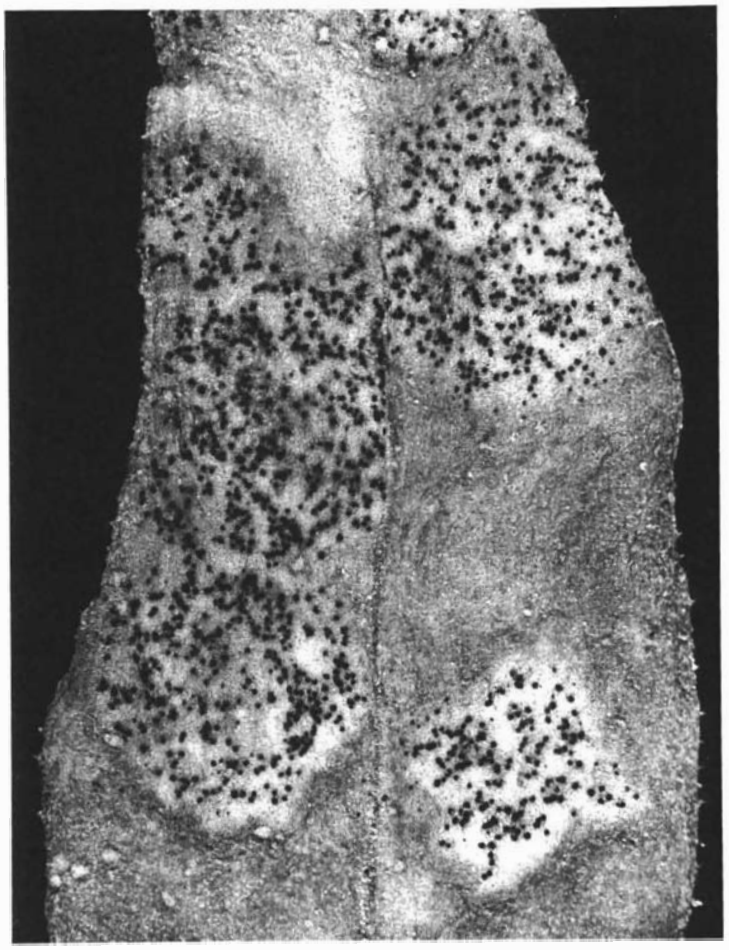


Plate 7

Infected leaves from *P. drummondii* seedlings, showing nature of the lesions and profuse production of pycnidia.

yellowed, chlorotic tissues. Pycnidia are usually less dark. In other cases there may be some purplish-black concentric circling which separates necrotic areas from the chlorotic tissues (Plate 8). The lesions usually extend through to the lower leaf surface, but pycnidia production there is considerably less.

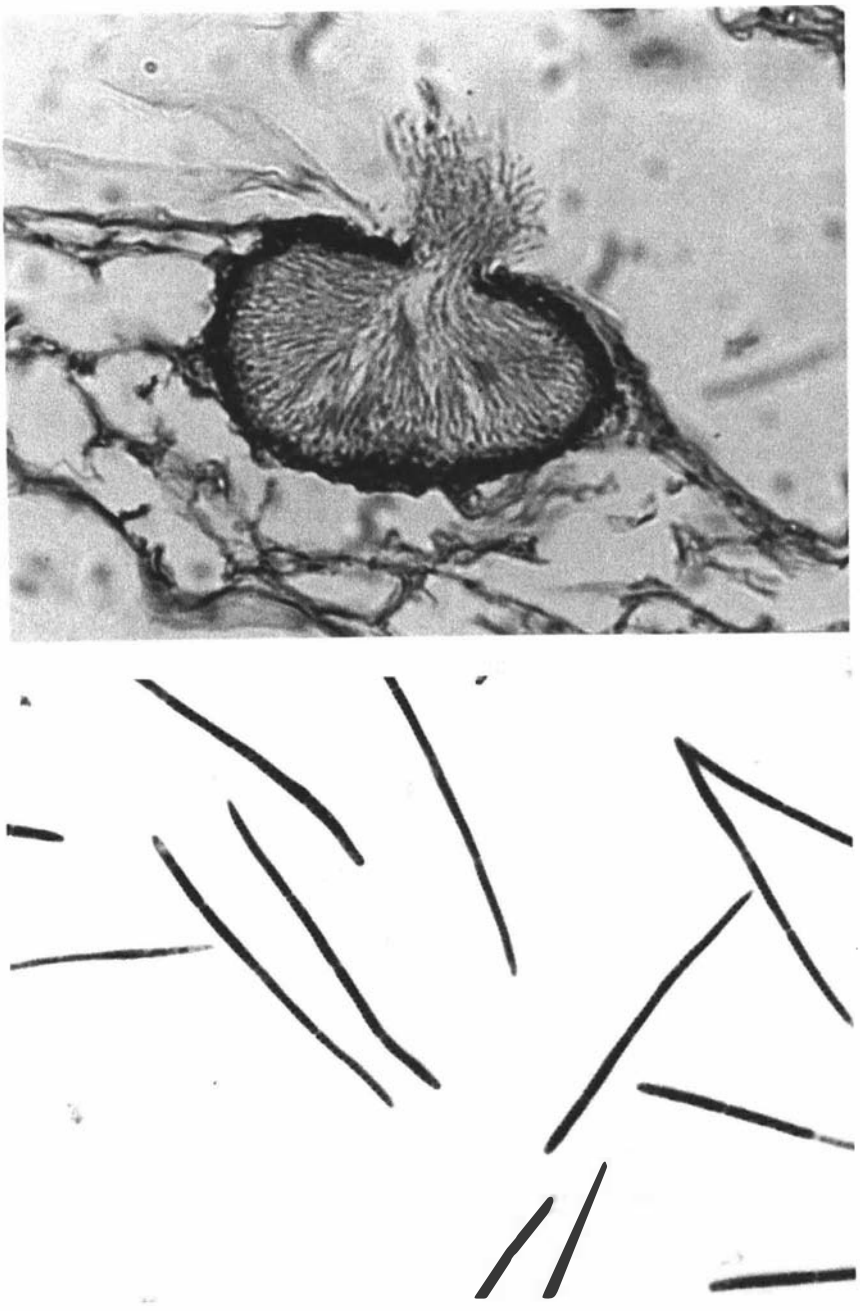
Lesions on the leaves constitute the most striking disease symptom in garden plantings, but lesions with pycnidia are not uncommon on stems, petioles, peduncles and sepals (Plates 39,40 & 44). Lesions or pycnidia have not been observed on the walls of ovaries.

The above account applies to the Septoria leaf spot disease of P. drummondii irrespective of seed source. There was no variation in the pattern of disease development on this host to suggest more than species of pathogen was involved.

(ii) Pathogen Morphology

Pycnidia are produced only on necrotic tissues. They are densely crowded, at first light brown, later darkening with maturity and then clearly visible to the naked eye (Plate 7). In shape they are mostly globose but sometimes slightly pyriform, immersed at first, later becoming erumpent, but never superficial. The ostiole is round, occasionally oval, usually clearly visible, and in diameter about one quarter that of the pycnidium. The pycnidial wall is smooth and composed of three to eight layers of very dense pseudoparenchymatous cells which are isodiametric to elongate in shape. Pycnidiospores are produced from short, hyaline, sporogenous cells lining the inner wall of the pycnidium and come free to fill the cavity of the pycnidium (Plates 9 & 10). It would appear that pressure of the large numbers of spores filling pycnidia orientates spores towards the ostiole. Cirrhi are frequently found exuded from ostioles. They are

Plate 8 Infected leaves collected in late summer from naturally infected P. drummondii plants.



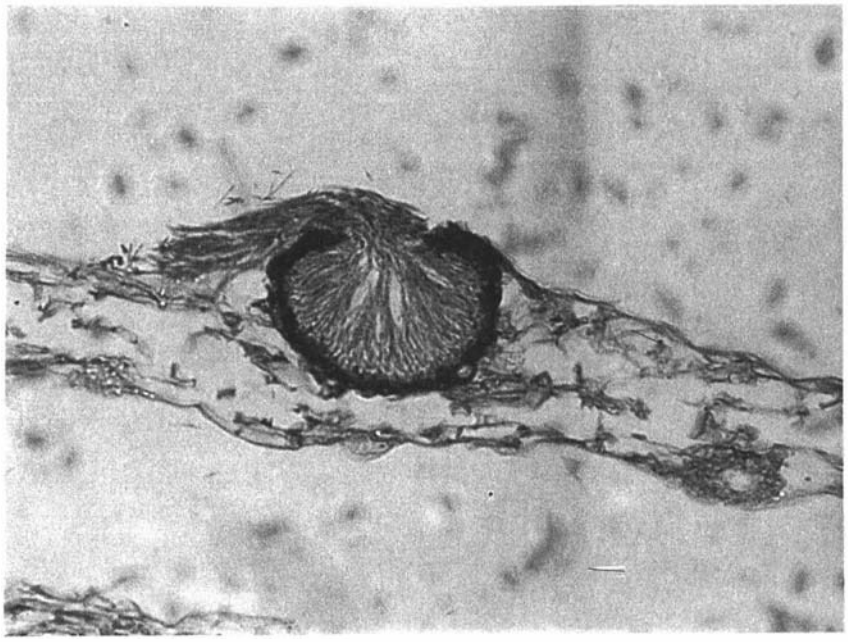
(a)

(b)

Plate 9 Asexual reproductive structures of Septoria sp. on P. drummondii seedlings developed from infected United Kingdom seed:

(a) transverse section of a pycnidium;

(b) pycnidiospores.



(a)

(b)

Plate 10 Asexual reproductive structures of Septoria sp. on P. drummondii seedlings developed from infected Dutch seed:

(a) transverse section of a pycnidium;

(b) pycnidiospores.

light gold in colour, vary considerably in length, but usually are long, curled and twisted, often adhering to neighbouring spore horns. When cirrhi are transferred to drops of water spores immediately separate from each other. The pycnidiospores are hyaline, guttulate, filiform straight to slightly curved, slightly attenuated at one end, with rounded ends, and indistinctly septate (Plates 9 & 10).

The dimensions of pycnidiospores and pycnidia were recorded from 12 Manawatu collections of P. drummondii (coded as M.dr). The collections for each year were made from different home garden plantings, and in all cases the country of origin of the seed was unknown. Since S. drummondii is the species of pathogen considered present in New Zealand (Brien, 1939) for the purpose of discussion this identification is assumed. The results are presented in Table 5. The importance of collection date on both spore length and pycnidia diameter was evaluated in an analysis of variance (Appendices 3 & 4). In no instance was there a significant difference.

There are reports of the morphology of Septoria spp. being appreciably affected by changes in environmental conditions (Beach, 1919; Hughes, 1949; Sprague, 1944). The following experiment was conducted to determine the extent of variation in spore length and pycnidia diameter as revealed by repeated collections from known infections throughout one growing season.

In the spring of 1961 two outbreaks of the disease originating from use of imported infected Dutch and English P. drummondii seed were followed from the time first symptoms appeared in seed boxes through until plants were mature the following autumn. Each seed line was sown thinly in four boxes of sterilised soil and the emerging seedlings permitted to develop without subsequent pricking-out until mature some months later. After hardening-off in cold frames the two series of boxes were set out well spaced from each

TABLE 5 Dimensions of pycnidiospores and pycnidia from 12 Manawatu collections of P. drummondii; seed of unknown origin in each instance (see Appendices 3 & 4)

Collection Date	Pycnidiospore Length[a.] (in microns)			Pycnidia Diameter (in microns)	
	Range	Mean	No Measured	Range	Mean
7.3.56	24.7–60.8	46.5 ± 0.63	23	70.3–140.5	102.2 ± 2.41
7.3.56	22.8–57.0	41.7 ± 0.33	36	66.5–142.4	96.4 ± 3.36
7.3.56	17.1–30.4	22.9 ± 0.53	25	47.5–123.4	73.1 ± 2.93
Overall	17.1–60.8	37.0 ± 4.5		47.5–142.4	90.6 ± 6.9
10.10.57	24.7–43.9	34.6 ± 0.61	43	49.4–119.6	66.6 ± 4.10
10.10.57	19.0–41.8	31.2 ± 0.41	19	55.1–134.8	86.9 ± 3.71
Overall	19.0–43.9	32.9 ± 5.5		49.4–134.8	76.8 ± 8.5
19.4.58	24.7–49.4	37.8 ± 0.83	26	49.4–112.0	75.4 ± 4.63
19.4.58	24.7–58.9	54.1 ± 0.87	25	47.4–117.7	69.9 ± 3.22
19.4.58	30.4–66.5	46.2 ± 1.11	50	60.8–132.9	100.5 ± 1.98
Overall	24.7–66.5	46.0 ± 4.5		47.5–132.9	81.9 ± 6.9
19.5.62	22.8–47.5	33.6 ± 0.44	25	68.4–142.4	94.7 ± 3.43
19.5.62	19.0–45.6	31.0 ± 0.38	25	57.0–119.6	87.0 ± 3.50
19.5.62	17.1–39.9	26.4 ± 0.26	25	55.1–123.4	91.3 ± 4.04
19.5.62	19.0–43.7	29.9 ± 0.71	25	49.4–127.2	86.9 ± 2.16
Overall	17.1–47.5	30.2 ± 3.9		49.4–142.4	90.0 ± 6.0

[a.] Each collection of 100 spores.

Pycnidiospore width throughout ranged between 1.5 – 2.5 μ.

other in a farm area distant from other ornamental plantings. Beginning in October 1961, pycnidia diameter, spore length and percentage of spores in the various septate classes were recorded, first using infected cotyledons, then primary leaves from plants four weeks later when boxes were in cold frames. The study was continued at four week intervals until May 1962, at which time plants were dying off.

The dimensions of spores and pycnidia are shown in Tables 6 and 7, and information on the extent of spore septation in the two series is recorded in Table 8. An analysis of variance of pycnidiospore length and pycnidia diameter revealed there was no significant difference between the two series, and no significant difference between the repeated collections within each series (Appendices 5 & 6).

The data from Tables 5,6 and 7 covering all P. drummondii infections are presented in summarised form in Table 9.

(b) Phlox paniculata

(i) Symptoms

In the Manawatu, first symptoms appear in the spring on newly developed leaves as minute, circular, uniformly reddish-brown spots which may be present in numbers sufficient to give a distinctive speckled appearance to the young foliage (Plate 11). Over 200 lesions each less than 2 mm diameter may be present on young leaves less than 3 inches long. With leaf maturation the lesions enlarge, usually remain circular and frequently reach 8 mm diameter without change in color. On the older lower leaves the necrotic spots commonly coalesce and the centres may lighten to pale brown or dull white (Plate 12). In the advanced stages leaves droop and finally abscise. Severely diseased plants are stripped of their lower leaves, noticeably

TABLE 6 Comparison of spore length from P. drummondii plants raised from imported infected Dutch and English seed; collections taken at four week intervals (see Appendix 5).

Collection Date	Length in Microns [a.]			
	Dutch Seed		U.K. Seed	
	Range	Mean	Range	Mean
17.10.61	27.7–49.4	40.4 ± 0.58	19.0–39.9	28.7 ± 0.56
13.11.61	24.7–49.4	40.2 ± 0.47	22.8–47.5	41.2 ± 0.32
12.12.61	19.0–41.8	30.4 ± 0.44	22.8–51.3	42.8 ± 0.41
9.1.62	19.0–45.6	31.7 ± 0.38	22.8–43.7	33.3 ± 0.46
6.2.62	20.9–38.0	31.9 ± 0.71	26.6–38.0	31.5 ± 0.88
6.3.62	20.9–38.0	29.2 ± 0.46	22.8–41.8	34.7 ± 0.74
4.4.62	19.0–41.8	31.0 ± 0.54	19.0–36.1	26.6 ± 0.83
1.5.62	20.9–43.7	33.2 ± 0.59	24.7–47.5	30.2 ± 0.41
Overall	19.0–49.4	33.5 ± 1.78	19.0–51.3	33.6 ± 1.78

a. Pycnidiospore width throughout ranged from 1.5 – 2.5 /u.

Each collection of 100 spores.

TABLE 7 Comparison of pycnidial diameter from P. drummondii plants raised from imported Dutch and United Kingdom seed; collections taken at four week intervals (see appendix 6)

Collection Date	Diameter in Microns [a.]			
	Dutch Seed		U.K. Seed	
	Range	Mean	Range	Mean
17.10.61	53.2-123.4	94.3 ± 2.66	51.3-127.2	89.1 ± 3.83
13.11.61	66.5-131.0	111.7 ± 3.41	57.0-136.7	112.3 ± 4.24
12.12.61	57.0-127.2	81.7 ± 2.71	47.5-140.5	93.7 ± 4.48
9.1.62	47.5-102.6	76.2 ± 1.41	55.1-129.1	95.2 ± 3.63
6.2.62	58.9-132.9	95.9 ± 2.7	47.5-115.8	83.3 ± 3.47
6.3.62	68.4-138.6	107.4 ± 3.4	60.8-129.1	101.3 ± 5.01
4.4.62	51.3-121.5	86.0 ± 5.4	51.3-108.3	77.7 ± 3.43
1.5.62	49.4-115.8	83.5 ± 4.7	53.2-127.2	102.6 ± 3.67
Overall	47.5-138.6	92.1 ± 3.2	47.5-140.5	94.4 ± 3.2

a. Sample size of 25 pycnidia in each case.

TABLE 8 Septation of spores from P. drummondii plants raised from infected Dutch and English seed; collections taken at 4 week intervals

Collection Date	Percentage of Spores in Various Septate Classes [a.]													
	Dutch Seed							U.K. Seed						
	0	1	2	3	4	5	Not Determined [b.]	0	1	2	3	4	5	Not Determined [b.]
17.10.61	-	7	2	78	-	2	11	-	5	-	84	-	3	4
13.11.61	-	4	14	72	1	2	7	1	10	6	76	-	3	4
12.12.61	1	15	1	61	-	1	21	2	14	6	65	1	-	12
9.1.62	1	16	9	56	2	-	16	4	9	4	69	3	2	9
6.2.62	1	9	1	81	-	4	4	-	21	1	62	-	1	15
6.3.62	2	4	21	59	-	1	13	4	9	3	59	1	2	22
4.4.62	-	7	10	64	-	2	17	-	11	2	74	-	2	11
1.5.62	2	8	7	75	1	7	-	4	8	3	74	1	3	7

a. Sample size of 100 spores in each case.

b. Percentage of spores in sample where septa could not be discerned.

TABLE 9 Pycnidiospore and pycnidia dimensions from *Septoria* infections of *P. drummondii*; collected data from Tables 5,6 and 7

Collections	Pycnidiospore Length (in microns)		Pycnidia Diameter (in microns)	
	Range of Sample Means	Overall Means	Range of Sample Means	Overall Means
U.K.dr.sd	19.0-51.3	33.6	47.5-140.5	94.4
H.dr.sd	19.0-49.4	33.5	47.5-138.6	92.1
M.dr	17.1-66.5	34.4	47.5-142.4	85.9

Plate 11 Infected shoot of P. paniculata showing field symptoms (Manawatu collection).

Plate 12 Early (top) and late (bottom) stages in the development of symptoms on *P. paniculata* (Manavatu collections).

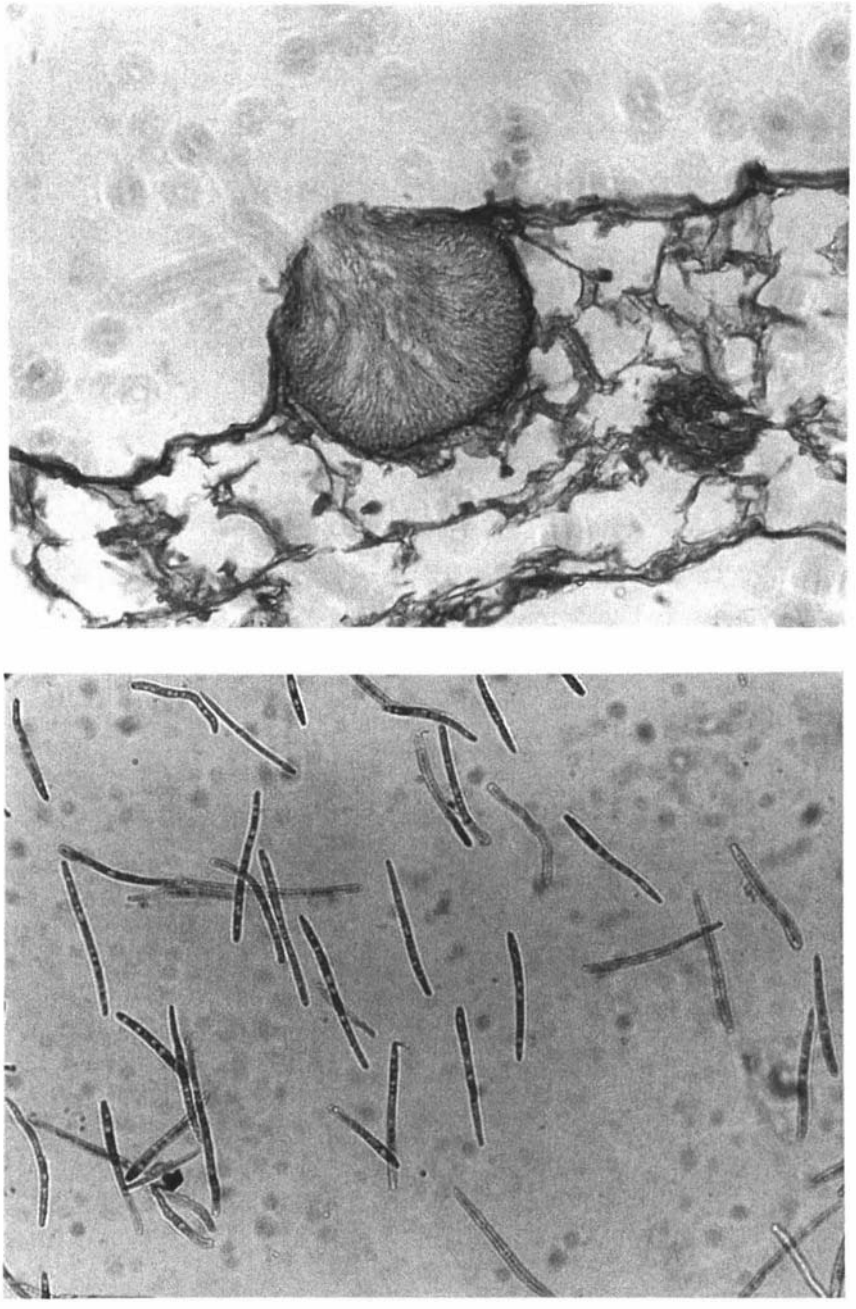
stunted and flower poorly. Unlike Septoria infections of P. drummondii, pycnidia are not macroscopically discernible but may be located on both leaf surfaces with the aid of a hand lens.

The collections of P. paniculata received from Holland and Canada exhibited symptoms essentially similar to those of infected plants in New Zealand, as described above.

(ii) Pathogen Morphology

On all collections the production of pycnidia was scanty and confined to small areas of large lesions on the older leaves, and their presence difficult to locate even with the aid of a steroscopic microscope. This was partly because they are usually golden-brown rather than black when mature and thus do not stand out prominently, but mainly because of their deep location in host tissues. Usually they are immersed, rarely erumpent, with little more than the ostiole area penetrating the leaf surface. The presence of pycnidia was best revealed by subjecting severely infected leaves to high humidity in petri-dishes for at least 36 hours, by which time light-colored, twisted cirrhi could be clearly seen. The structure of pycnidia in microtome sections was essentially similar to those on P. drummondii infections in that the pycnidial wall was smooth, firm, pseudoparenchymatous, and composed of four to six layers of elongate cells. The wall cells were conspicuously less dense how, ever, causing the pycnidia to be dark brown rather than black when mature. The filiform pycnidiospores were mostly flexuous, occasionally straight, often prominently guttulate, and indistinctly septate (Plates 13,14,15 & 16).

With many P. paniculata collections it was found that severely infected fallen leaves exposed to high humidity in petri-dishes for three or more days developed a rather distinctive type of pycnidium in groups of up to twenty.



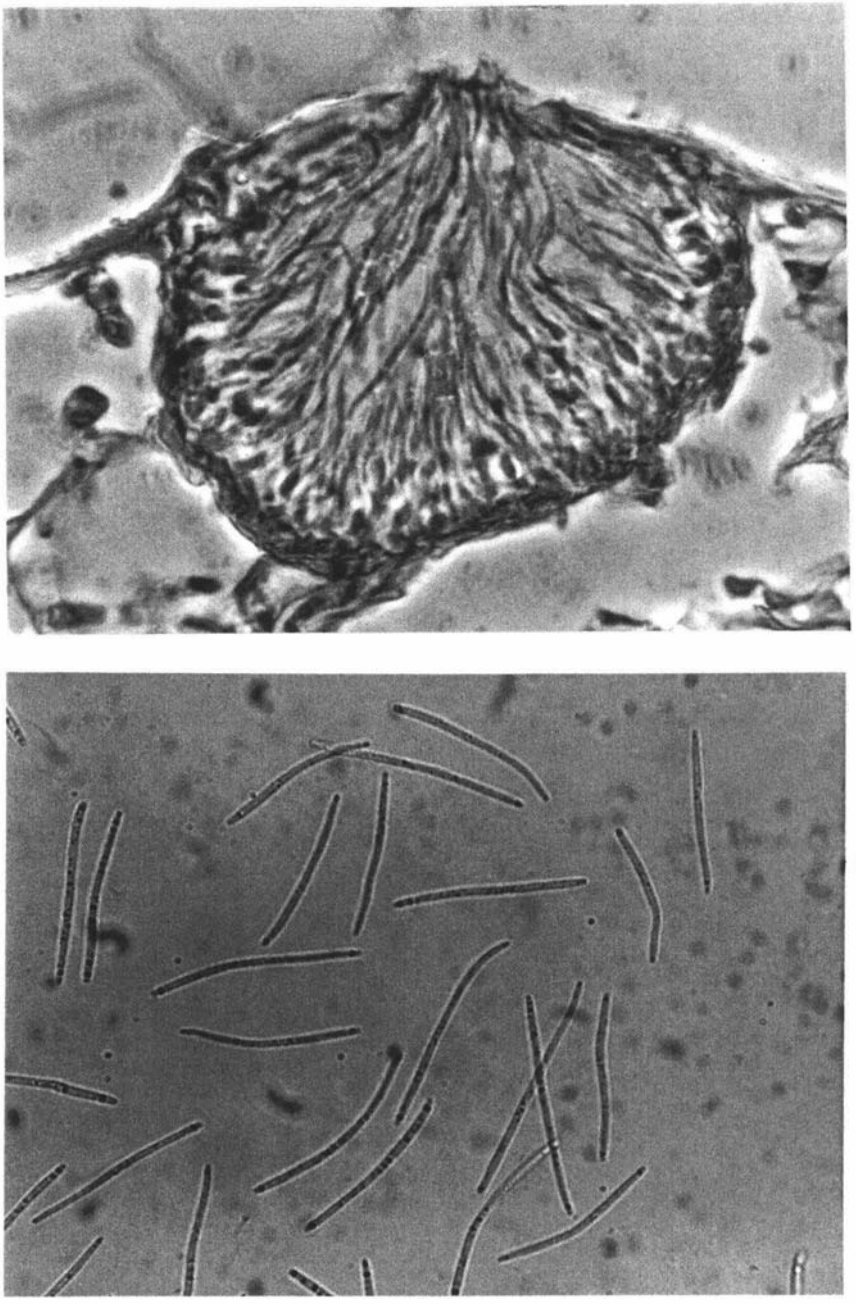
(a)

(b)

Plate 13 Asexual reproductive structures of Septoria sp. on P. paniculata (Manawatu collection).

(a) transverse section of a pycnidium;

(b) pycnidiospores.



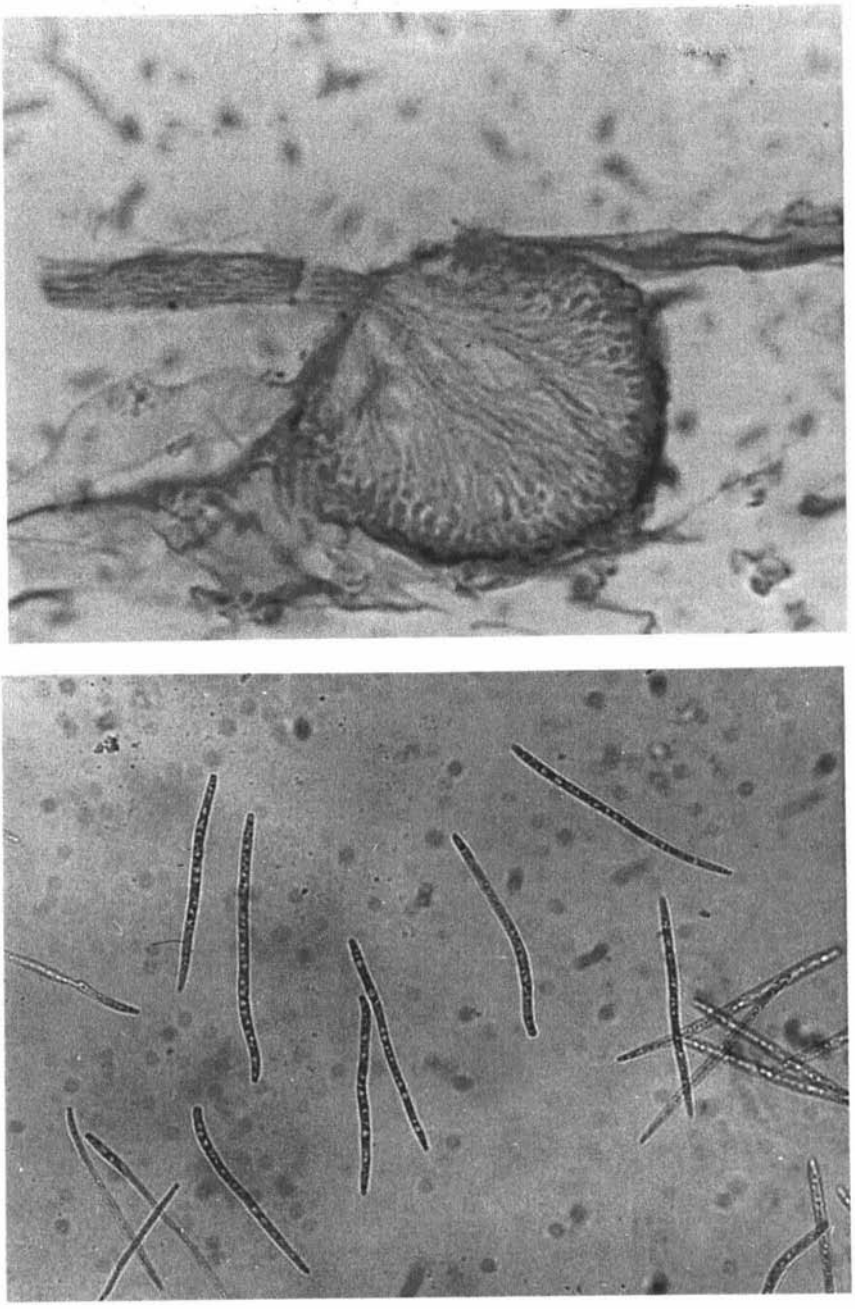
(a)

(b)

Plate 14 Asexual reproductive structures of Septoria sp. on P. paniculata (received from Holland as S. phlogis).

(a) transverse section of a pycnidium;

(b) pycnidiospores.



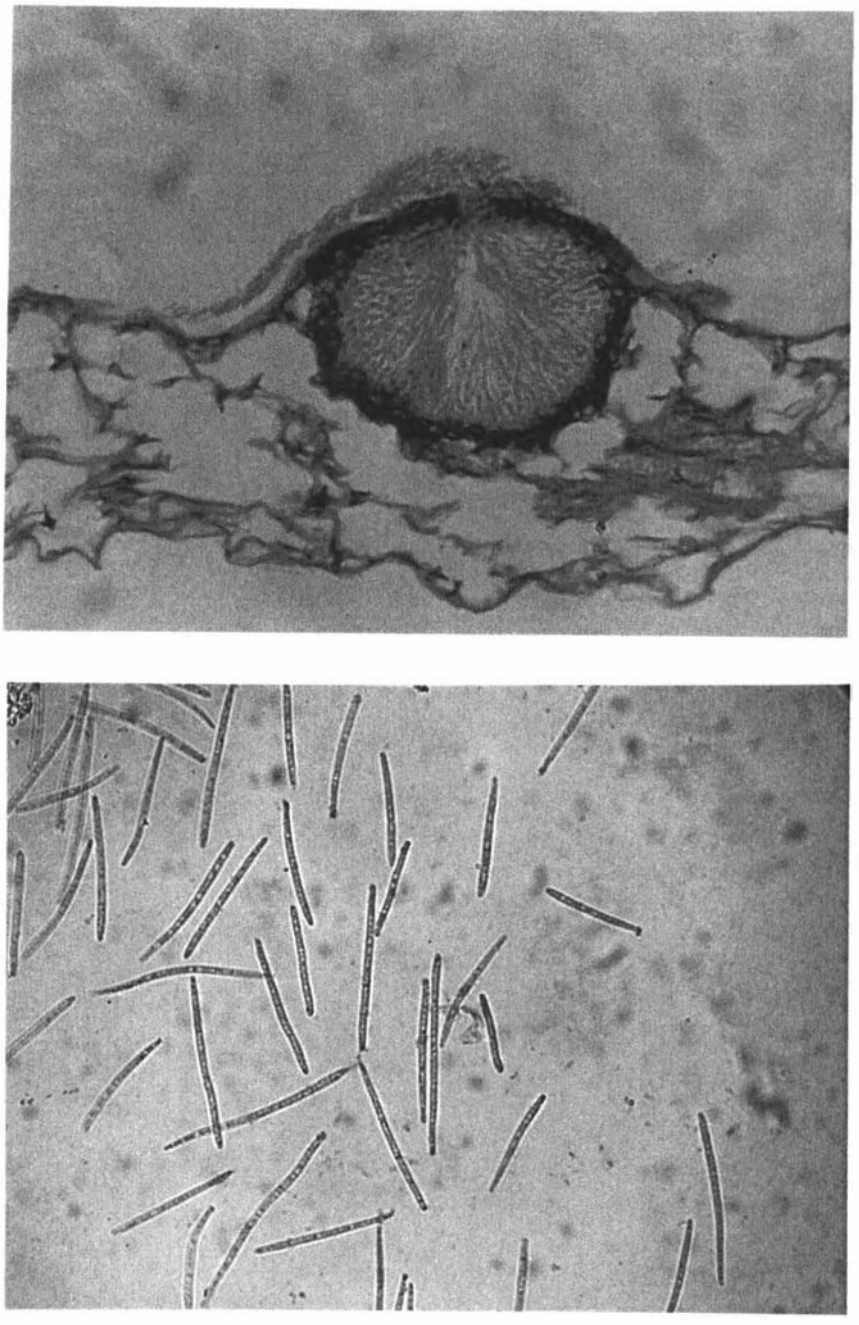
(a)

(b)

Plate 15 Asexual reproductive structures of Septoria sp. on P. paniculata (received from Quebec, Canada as S. phlogis).

(a) transverse section of a pycnidium;

(b) pycnidiospores.



(a)

(b)

Plate 16 Asexual reproductive structures of Septoria sp. on P. paniculata (received from British Columbia, Canada as S. divaricata).

(a) transverse section of a pycnidium;

(b) pycnidiospores.

These were at first light brown and immersed, but became clearly erumpent and gradually darkened to black. They finally became totally superficial, at which stage they could be readily flicked free with a seeker needle leaving a distinct depression in the leaf surface. Cirrhi were distinctive, being relatively short and only slightly curled. Microscopic examination of cirrhi from such pycnidia revealed scolecosporous pycnidiospores typical of the genus Septoria and essentially similar to those released from immersed pycnidia. Isolates to agar were in no way atypical and proved pathogenic in P. paniculata inoculations.

The dimensions of pycnidiospores and pycnidia were recorded for 14 collections of P. paniculata made in the Manawatu over 4 years (coded as M.pan), the collections for each year being from different home garden plantings. The data are presented in Table 10. The importance of collection date on both spore length and pycnidium diameter was evaluated in an analysis of variance (Appendices 7 & 8). In neither case were there significant differences. The percentage of spores in the various septate classes for five of the above Manawatu collections in presented in Table 11.

Data relating to the dimensions of pycnidiospores and pycnidia from P. paniculata specimens received from Holland, Quebec and British Columbia are presented in Tables 12,13 and 14.

The data from Tables 10,12,13 and 14 covering all P. paniculata collections are presented in summarised form in Table 15.

All collections from both P. drummondii and P. paniculata were grouped according to origin and the groups compared by an analysis of variance. The group means and their standard errors are presented in Table 16. Significant differences were determined using a "t" test (Appendices 9 & 10).

In Figures 1 and 2 data on pycnidiospore length and pycnidial diameter

TABLE 10 Dimensions of pycnidiospores and pycnidia from 14 collections of *P. paniculata* in the Manawatu

Collection Date	Pycnidiospore Length[a.] (in microns)			Pycnidia Diameter (in microns)	
	Range	Mean	No Measured	Range	Mean
15.11.61	22.8–57.0	42.1 ± 0.98	15	76.0–238.3	187.4 ± 2.4
15.11.61	19.0–45.6	30.8 ± 0.63	23	91.2–221.2	143.1 ± 1.36
15.11.61	22.8–41.8	32.8 ± 1.22	12	68.4–179.4	127.4 ± 3.11
15.11.61	20.9–38.0	29.1 ± 0.34	25	57.0–161.4	131.0 ± 2.83
Overall	19.0–57.0	33.7 ± 2.0		57.0–238.3	147.2 ± 9.7
7.5.62	19.0–39.9	31.5 ± 0.26	25	66.5–217.4	124.8 ± 1.16
7.5.62	20.9–43.7	32.8 ± 0.49	25	70.3–274.4	156.1 ± 0.98
7.5.62	20.9–39.9	30.7 ± 0.38	20	85.5–196.5	113.6 ± 3.14
7.5.62	22.8–47.5	35.1 ± 0.41	20	74.1–213.6	123.5 ± 2.61
Overall	19.0–47.5	32.5 ± 2.0		66.5–274.4	129.5 ± 9.7
15.10.63	22.8–43.7	34.2 ± 0.31	25	68.4–132.9	104.5 ± 3.88
15.10.63	22.8–43.7	32.8 ± 0.29	25	68.4–200.3	114.0 ± 3.41
15.10.63	20.9–47.5	33.1 ± 0.44	35	81.7–213.6	127.6 ± 3.45
4.9.65	28.5–49.4	37.7 ± 0.36	25	70.3–183.2	138.4 ± 2.70
4.9.65	22.8–60.5	44.7 ± 0.38	25	89.3–285.7	145.4 ± 2.96
4.9.65	22.8–43.7	34.6 ± 0.42	25	91.2–238.3	127.7 ± 3.14
Overall	22.8–60.5	39.0 ± 2.4		70.3–285.7	137.2 ± 11.2

[a.] Each collection of 100 spores.

Pycnidiospore width throughout ranged from 1.5 - 2.5 µ.

TABLE 11 Septation of spores from 5 collections of P. paniculata in the Manawatu [a].

Collection Date	Percentage of Spores in Various Septate Classes						
	0	1	2	3	4	5	Not Determined [b]
15.11.61	4	12	3	64	1	4	12
15.11.61	2	12	4	73	1	-	8
7.5.62	4	20	4	54	-	1	17
4.9.65	5	12	-	68	1	-	14
4.9.65	-	3	2	79	2	3	11

[a] Sample size of 100 spores at each collection.

[b] Percentage of spores in sample where septa could not be discerned.

TABLE 12 Dimensions of pycnidiospores and pycnidia from two collections of P. paniculata from Holland; specimens received as S. phlogis

Collection Number	Pycnidiospore Length[a.] (in microns)			Pycnidia Diameter (in microns)	
	Range	Mean	No Measured	Range	Mean
1	30.4-73.6	46.4 ± 0.41	25	72.0-288.3	138.6 ± 2.43
2	27.2-68.8	51.2 ± 0.34	20	83.2-257.9	164.1 ± 1.71
Overall	27.2-73.6	48.8		72.0-288.3	151.4

[a.] Pycnidiospore width throughout ranged from 1.5 - 3.0 /u

Sample size of 100 spores in each case.

TABLE 13 Dimensions of pycnidiospores and pycnidia from five collections of *P. paniculata* from Quebec, Canada; specimens received as *S. phlogis*

Collection Number	Pycnidiospore Length [a.] (in microns)			Pycnidia Diameter (in microns)	
	Range	Mean	No Measured	Range	Mean
1	28.8–59.2	51.4 ± 0.63	25	89.6–256.0	124.8 ± 2.46
2	25.6–64.0	54.2 ± 1.21	25	81.6–227.2	147.0 ± 3.17
3	27.2–65.6	55.8 ± 0.41	35	73.6–286.4	133.6 ± 4.00
4	28.8–65.6	55.8 ± 0.57	25	97.6–233.6	153.1 ± 2.31
5	24.0–56.0	48.0 ± 0.43	50	84.8–224.0	163.4 ± 3.46
Overall	24.0–65.6	53.0		73.6–286.4	144.4

[a.] Pycnidiospore width throughout ranged from 1.5 - 3.0 μ.

Sample size of 100 spores in each case.

TABLE 14 Dimensions of pycnidiospores and pycnidia from three collections of P. paniculata from British Columbia, Canada; specimens received as S. divaricata

Collection Number	Pycnidiospore Length [a.] (in microns)			Pycnidia Diameter (in microns)	
	Range	Mean	No Measured	Range	Mean
1	24.0-54.4	38.6 ± 0.70	30	62.4-227.2	131.7 ± 1.75
2	19.2-49.6	33.9 ± 0.47	30	70.4-235.2	136.6 ± 2.21
3	20.8-56.0	36.1 ± 0.22	25	78.4-228.6	127.5 ± 1.82
Overall	19.2-56.0	36.2		62.4-235.2	131.9

[a.] Pycnidiospore width throughout ranged from 1.5 - 3.0 /u.

Sample size of 100 spores in each case.

TABLE 15 Pycnidiospore length and pycnidia diameter from *Septoria* infections of *P. paniculata*; collected data from Tables 10,12,13 & 14

Collections	Pycnidiospore Length (in microns)		Pycnidia Diameter (in microns)	
	Range of Sample Means	Overall Means	Range of Sample Means	Overall Means
M.pan	19.0-60.5	34.4	57.0-285.7	133.2
Q.pan	24.0-65.6	53.0	73.6-286.4	144.4
H. pan	27.2-73.6	48.8	72.0-288.3	151.4
B.C.pan	19.2-56.0	36.2	62.4-235.2	131.9

TABLE 16 Means and standard errors of means of seven group collections from P. drummondii and P. paniculata (see Appendices 9 & 10)

Group Origins	Pycnidiospore Length (in microns)			Pycnidium Diameter (in microns)		
	Mean	S.E.	Signif. of Diff.	Mean	S.E.	Signif. of Diff.
M.dr	36.3	± 1.7	A	85.9	± 4.4	A
H.dr.sd	33.5	± 2.1	A	92.1	± 5.4	A
U.K.dr.sd	33.6	± 2.1	A	94.4	± 5.4	A
M.pan	34.4	± 1.6	A	133.2	± 4.1	B
H.pan	48.8	± 4.2	B	151.4	±10.8	B
Q.pan	53.0	± 2.7	B	144.4	± 6.8	B
B.C.pan	36.2	± 3.5	A	131.9	± 8.8	B

Note - those means with a letter in common are not significantly different. Means with no letter in common are significantly different at the 1% level.

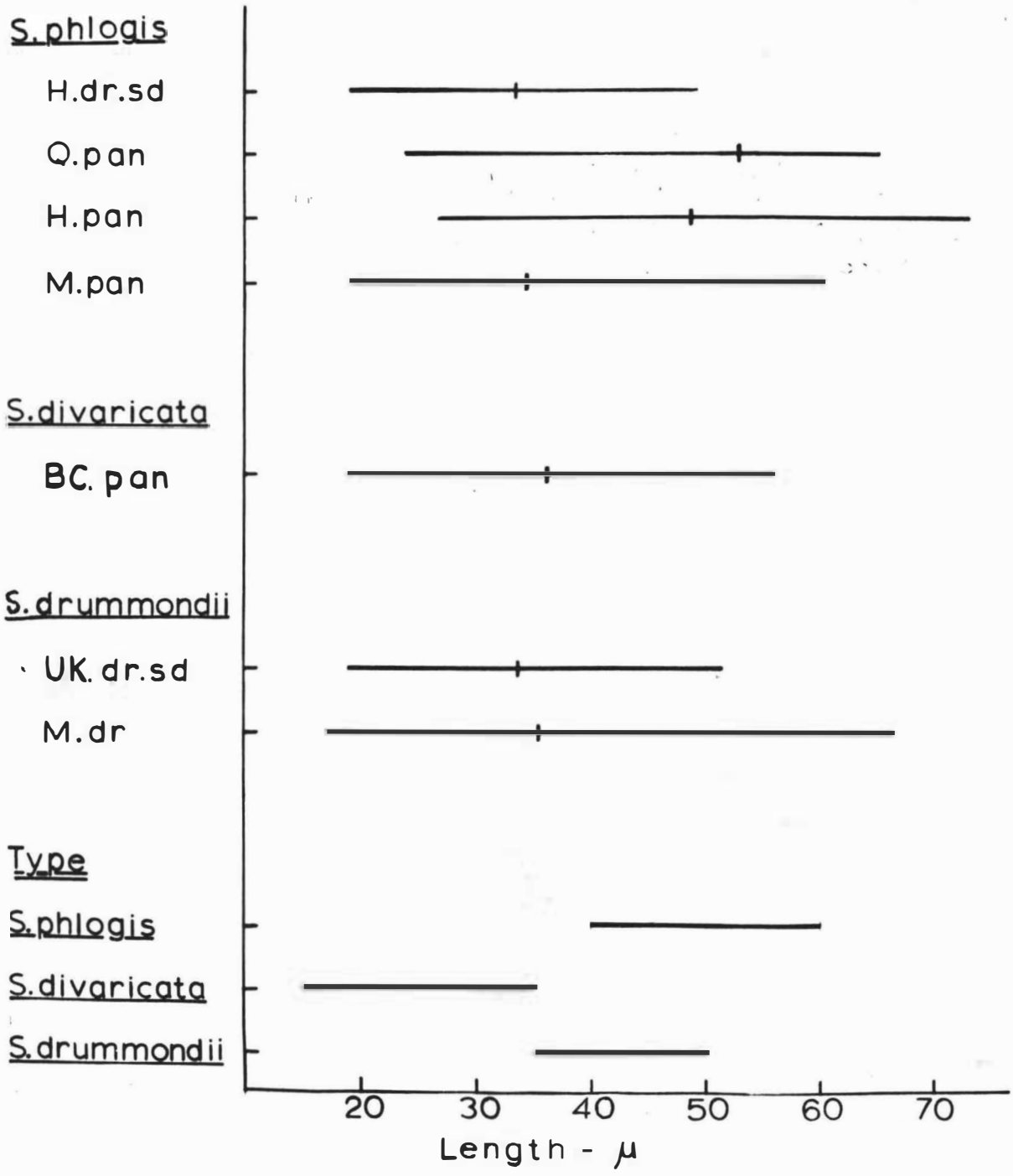


Figure 1 Range and mean of the length of pycnidiospores from P. drummondii and P. paniculata collections grouped according to species of pathogen and compared with data in the original descriptions of type material.

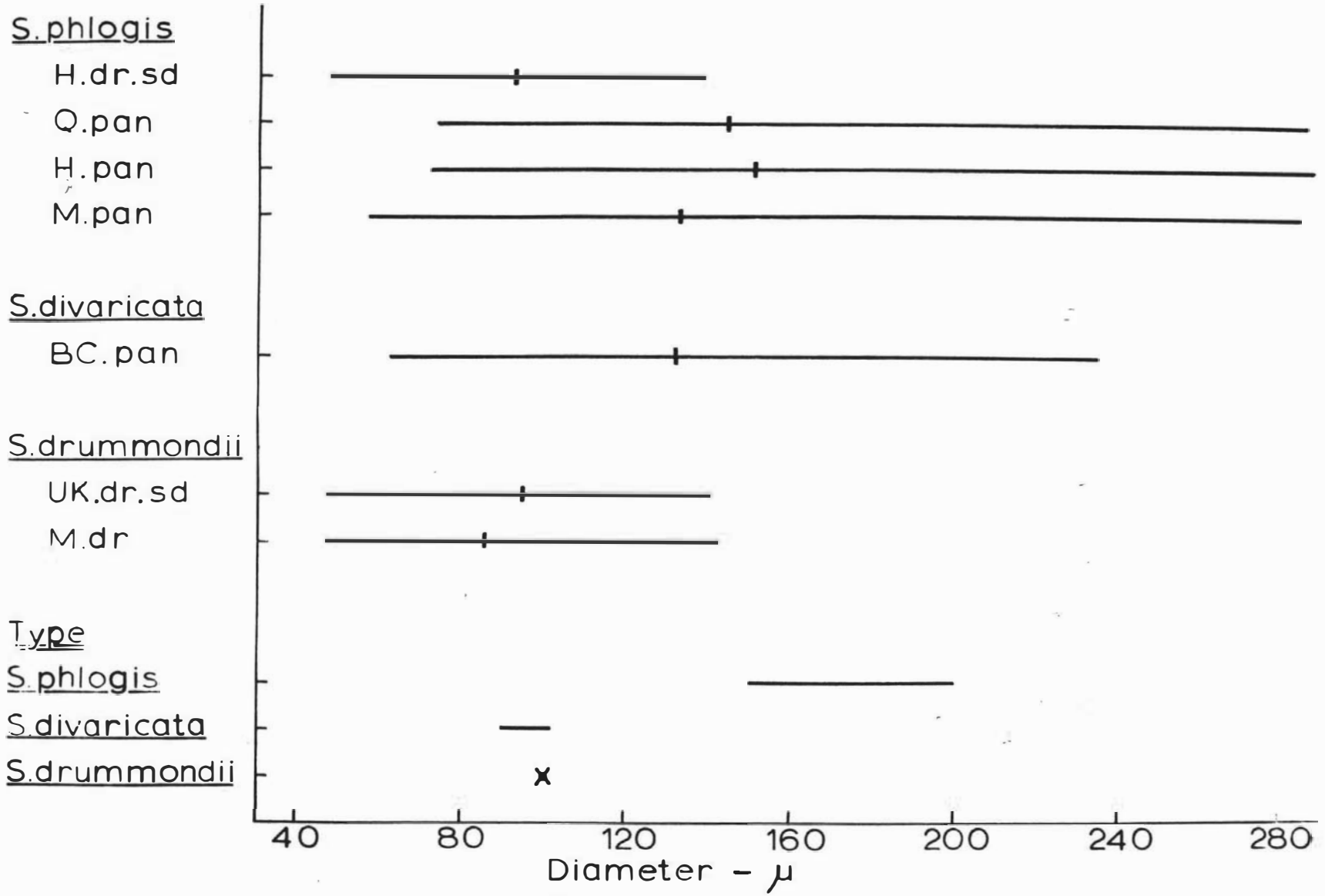


Figure 2 Range and mean of the diameter of pycnidia from P. drummondii and P. paniculata collections grouped according to species of pathogen and compared with data in the original descriptions of type material.

from all collections are grouped according to species of pathogen and compared with similar data from the three type descriptions.

3. Discussion

The above results show that collections presumed to be representative of the three species of Septoria could not be identified on the basis of their gross morphology in host tissues. Pycnidiospores were similar throughout in shape and septation, the little variation observed between collections being no greater than could be found within any one collection. Likewise the morphology of pycnidia, as revealed by study of microtome sections, proved to be very similar for all collections.

It was also shown that separation of the three species was not possible on the basis of pycnidiospore and pycnidium dimensions. As regards pycnidium diameter, the statistical comparisons of collections from both host species and representing the three species of fungus, showed the material could be arranged in two groups corresponding to the two host species (Table 16). That is, there was a host effect, the pycnidia from P. drummondii collections in all cases being significantly smaller on average than those from P. paniculata. In contrast, within each host species there were no clear differences between collections, even though different species of Septoria are alleged to be involved. In the case of spore length, the statistical analysis showed that collections could likewise be arranged in two groups, there being no differences within the two groups but a significant difference between groups. Thus pycnidiospore length from the P. paniculata material received from Holland and Quebec and both identified as S. phlogis was significantly greater than those from all other collections. However these latter represent collections from both host species and include all three

species of pathogen. That is, in respect of both pycnidia diameter and pycnidiospore length, consistent identification of all collections on the basis of these criteria was not possible.

Results of the experiment where repeated collections were made from plants raised from infected Dutch and English P. drummondii seed are of particular interest as they clearly demonstrate the dangers inherent in the practice of basing diagnoses upon a single collection. Although in both series the range of spore length and pycnidium diameter at different collection dates was so great as to warrant identification as all three species, statistical treatment of the data revealed no significant differences to exist (Appendices 5 and 6). Since the two series of plants were raised in complete isolation the variation could possibly be attributed to environmental influences. However as there were wide fluctuations between collection dates within any one series without a corresponding trend in the other series, this was probably not the cause. It is more likely that the variation is simply an expression of the genetic variability inherent in each collection.

The conclusion to be drawn from the above studies is that there was no convincing evidence of more than one species of Septoria causing the disease on the two species of Phlox. On the other hand, the detailed studies of variation in spore length and pycnidia diameter do indicate how infrequent collections of field material in a limited environment has encouraged the continued recognition of three species of Septoria pathogenic on Phlox spp.

D. Cultural Studies

As far as the author is aware there are no reports of studies on the cultural features of Septoria isolates from the genus Phlox. In the present work comparative studies were made of agar isolates from New Zealand and overseas collections of P. drummondii and P. paniculata and representing the three erected species of pathogen. The objectives were to characterise the causal organism on culture media, and to determine whether species separation was possible on the basis of cultural characteristics. The main features considered were gross colony characteristics such as shape, size and color, and growth rate, and the morphology of vegetative mycelium, pycnidia, pycnidiospores, and secondary conidia. The pattern of spore germination was also investigated since various workers have followed Drechsler (1923) in recognising two major groups of species within the genus Helminthosporium distinguished on the basis of the cells from which the germ tube arises.

1. Methods and Materials

Isolations from the field collections listed on pages 24 and 25 were made to agar by two methods. In the first, lesioned leaves with pycnidia were held in a sterile petri-dish lined with moistened filter paper for 24-36 hours, by which time spores had usually escaped to form cirrhi. Longer times were usually necessary for leaves of P. paniculata. The leaves were examined with a stereoscopic microscope and single cirrhi were transferred with a sterile needle to drops of sterile water in petri-dishes. Plates of agar inoculated with the resultant spore suspensions by streaking with a platinum loop were incubated at 24°C.

The above method was followed for all P. drummondii specimens, and on

those occasions where pycnidia were located on P. paniculata leaves. With this latter host, tissue plating had to be resorted to in those instances where pycnidia were not observed. Small pieces of leaf approximately 3 mm square from the edge of young lesions were immersed for one minute in 70% alcohol, transferred to 1:1000 mercuric chloride solution for 30 seconds, washed by five successive transfers through sterile water and plated to prune agar. This method was only moderately successful because of the slow growth of the pathogen and because of the large number of contaminants frequently present.

Single spore cultures were prepared by streaking a weak spore suspension over a limited area of potato dextrose agar (PDA) plates. After incubation for 24 hours well-isolated germinating spores were transferred to either agar plates or test-tube slopes.

In preliminary cultural studies the relative merits for growth and sporulation of the following 9 agar media were determined: PDA, cornmeal, potato, carrot, water, V8 juice (all laboratory prepared), and tomato juice, PDA, malt and prune (all Oxoid, Oxo Ltd., London). Of these laboratory-prepared PDA proved most satisfactory in every respect, being easy to prepare inexpensive and consistently supporting good growth and sporulation. Unless otherwise stated, all cultural studies were made on this medium.

Pycnidiospores produced on agar were collected for measuring by transferring to slides the spore mass which collected over the ostiole area of pycnidia on cultures incubated at 24°C. Lactophenol cotton blue was used throughout as a stain.

The production of secondary conidia, the manner of pycnidium formation and the pattern of spore germination was followed on PDA slide cultures incubated at 24°C. These were prepared by pouring a few drops of agar onto single slides in sterile petri-dishes lined with moistened filter paper.

The slides were inoculated by streaking with a dilute spore suspension, in each case prepared from a single cirrhus from naturally diseased leaves. By microscopically following selected spores at intervals of 3 - 6 hours the general pattern of germination was determined. The details of hyphal septation were observed after the application of a drop of lactophenol cotton blue to the agar surface of some slides at each inspection.

Brancato and Golding (1953) established that in cultural studies colony diameter is a valid measure of the effect of environmental factors. This criterion was used in the present study when determining the effect of temperature on growth of isolates from the various field collections. Plates were inoculated either with single spores, as previously described, or by mass transfer from established colonies. In this latter method equal sections of a 10 day old monosporous colony were cut with a cork borer (3 mm diameter) and transferred upper surface down to the centre of agar plates.

2. Results

As isolation of the pathogen from field collections progressed it was evident that no correlation existed between cultural features and the collection source or species of pathogen purported to be involved. In general, all first generation monosporous isolates, irrespective of origin, were sufficiently similar in appearance to give the impression of a single, cultural type. Differences in colony shape, colony colour and growth rate were frequently observed between collections, but these were no greater than could be found within any one collection, particularly when the inoculum was spores derived from cirrhi from different lesions.

The term "wild type" is henceforth used to designate the characteristic, naturally-occurring cultural type which appeared in first generation isolations,

the expression being used in the same sense as proposed by Miller (1945) for the Fusarium of muskmelon wilt. Wild type isolates are to be distinguished from cultural variants which subsequently developed from the initial field monosporous cultures.

(i) Colony Characters of the Wild Type

On plates of PDA incubated at 24°C colonies were first macroscopically discernible at three days. They were mucose and dull white at first with a smooth, moist surface and an entire edge. Over the next four days colonies gradually darkened, increased in diameter to approximately 8 mm and the centres became slightly raised (Plate 17). The presence of pycnidia was indicated within 10 days from inoculation by many cream colored, shining, spherical masses of escaped pycnidiospores located more particularly towards the centre of colonies. It was at this stage that colonies from P. drummondii collections least resembled those from the perennial host species; in general, P. paniculata isolates were lighter in colour because of a fine tomentum of short aerial hyphae which gave a distinctive mouse grey appearance and tended to mask the presence of the pycnidiospore masses (Plate 18). From the tenth day there was considerable variation in the development of colonies from all collections. Some became very irregular in outline while others remained symmetrical. Many developed a distinctive white peripheral border whereas others remained uniform in color. In all cases the colony centres finally became raised, uneven, carbonaceous, frequently with patches of course, white aerial hyphae (Plate 19).

(ii) Cultural Variants

Wild type isolates from all collections seldom remained constant in culture. With time there was cultural degradation in the sense that

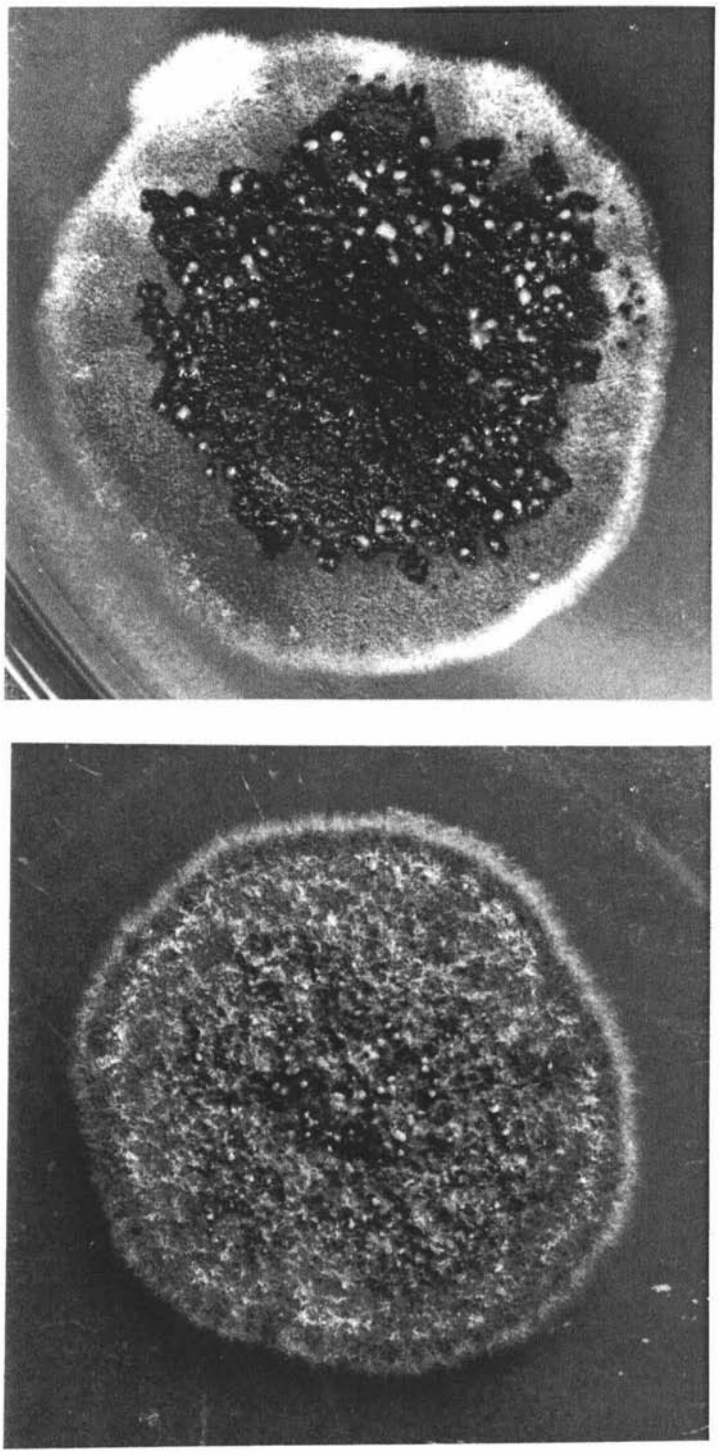


Plate 18 Wild type monosporous isolates from naturally infected P. drummondii (top) and P. paniculata plants (bottom) on P.D.A. plates incubated at 24°C. for 22 days.

Plate 19 Surface topography of aged P. drummondii isolate on P.D.A. and incubated at 24°C.

numerous variant strains appeared, which in many cases overgrew and replaced the parent wild type colony. Cultural variation was not studied as a specific topic but the following observations are relevant in indicating the limited value of cultural features in taxonomic considerations.

Sectors or patches differing from the parent colony appeared at any stage of growth and in positions varying from near the centre to the outer border (Plate 20). They were observed as early as five days in monosporous isolates of the wild type and in some instances crowded out the parent colony. In a few cases wild type isolates were stable through several transfers; more usually there was a gradual change in colony features to a stage where the isolate bore little resemblance to the original thallus. Cultural variants retained in pure culture and perpetuated by further subculturing showed far greater stability than parent wild type isolates (Plates 21 & 22). Many variant strains were sterile, and in all instances where pycnidia were produced the intensity of sporulation was considerably less than wild type isolates grown under identical conditions. In a limited series of inoculation tests selected cultural variants were without exception markedly less pathogenic than wild type isolates.

(iii) Morphology of the Wild Type

The features exhibited by colonies which developed on PDA plates incubated at 24°C are described below:

(a) Mycelium

The hyphae of young actively growing colonies was slender, 1 - 3 µ in diameter, septate, free-branching, hyaline at first but quickly acquiring pigmentation (Plate 29). With age hyphae became coarser, up to 6 µ in diameter, more closely septate, dark in color

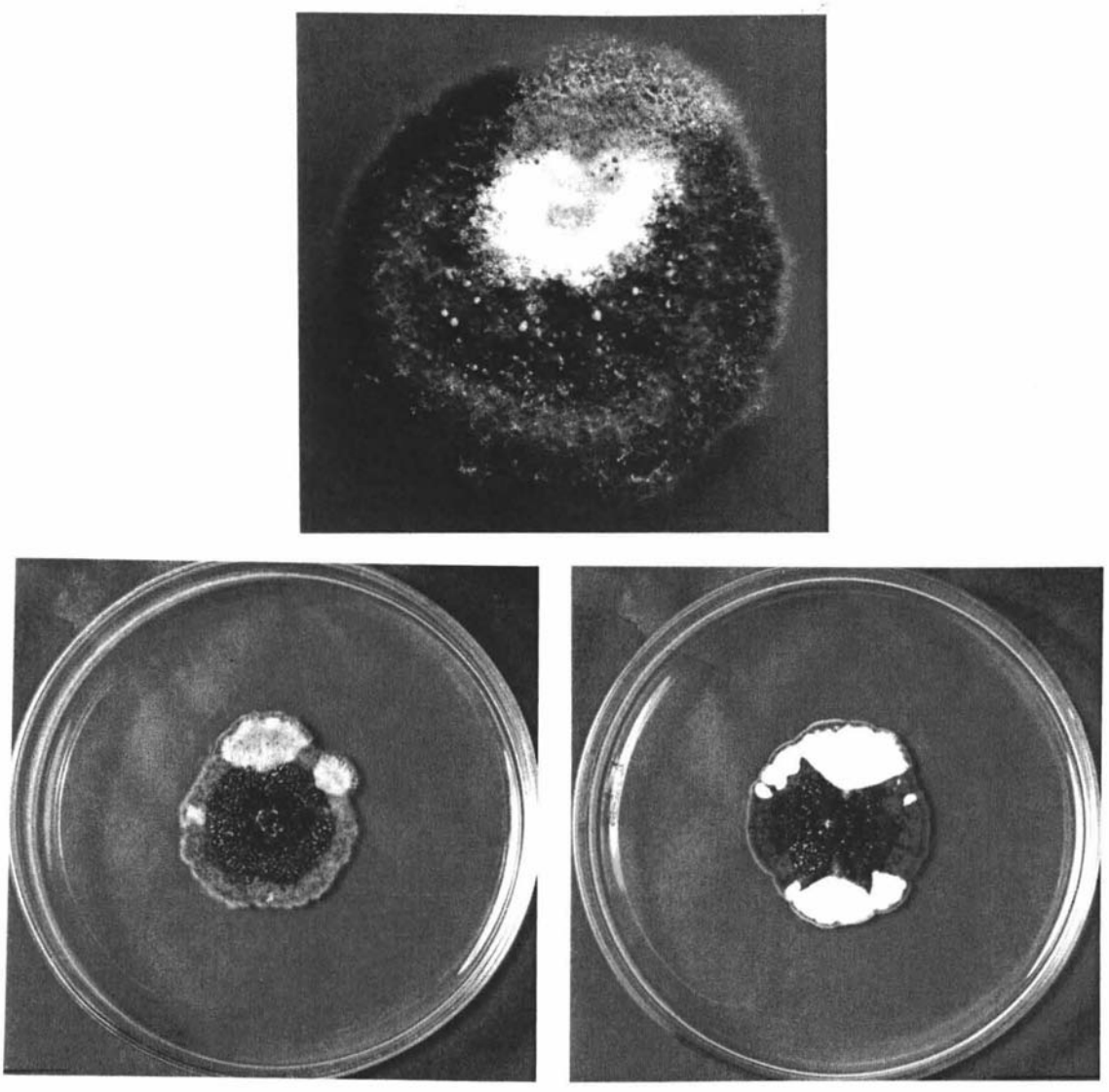


Plate 20 Cultural degradation in monosporous wild type isolates derived from infected P. drummondii seedlings.

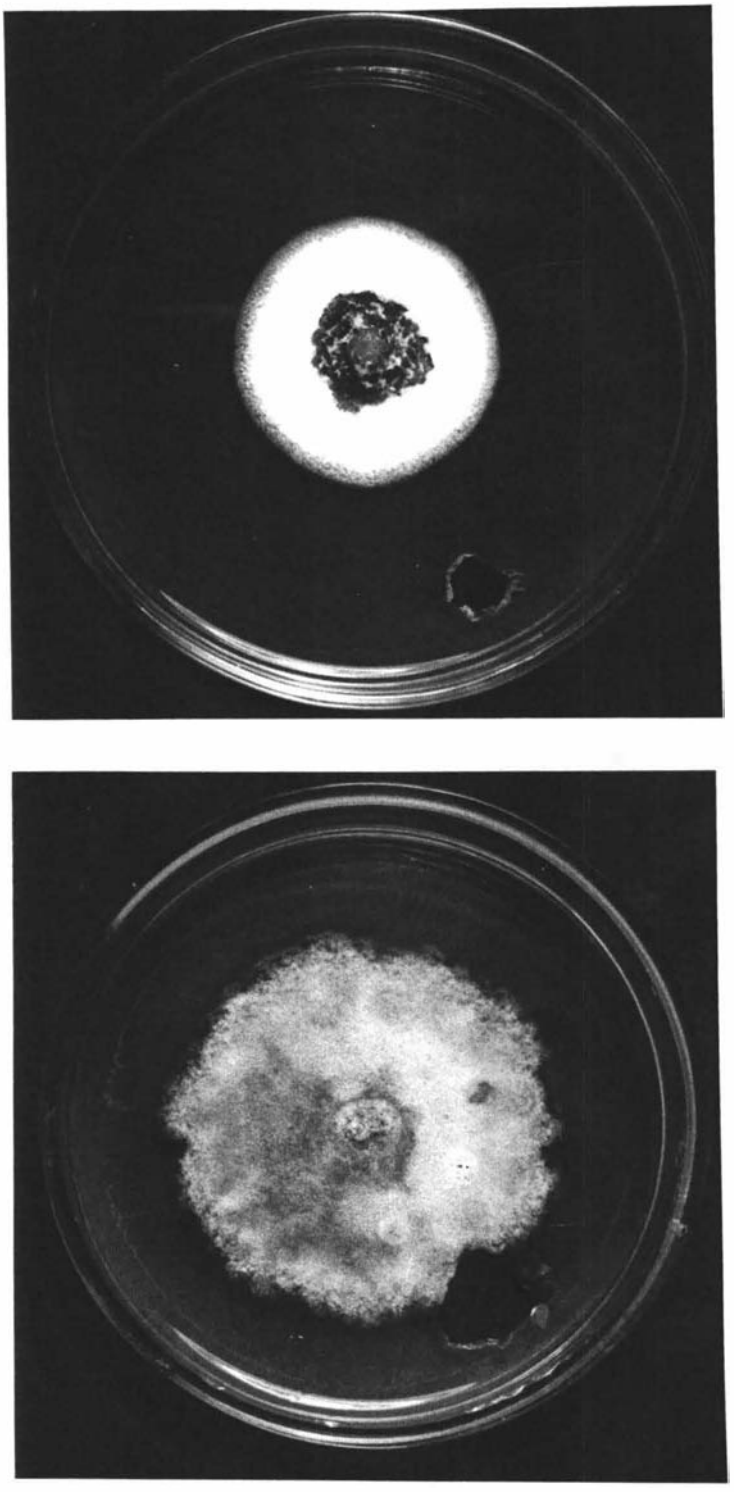


Plate 21 Cultural variants subcultured from monosporous wild type isolates derived from infected P. drummondii seedlings.

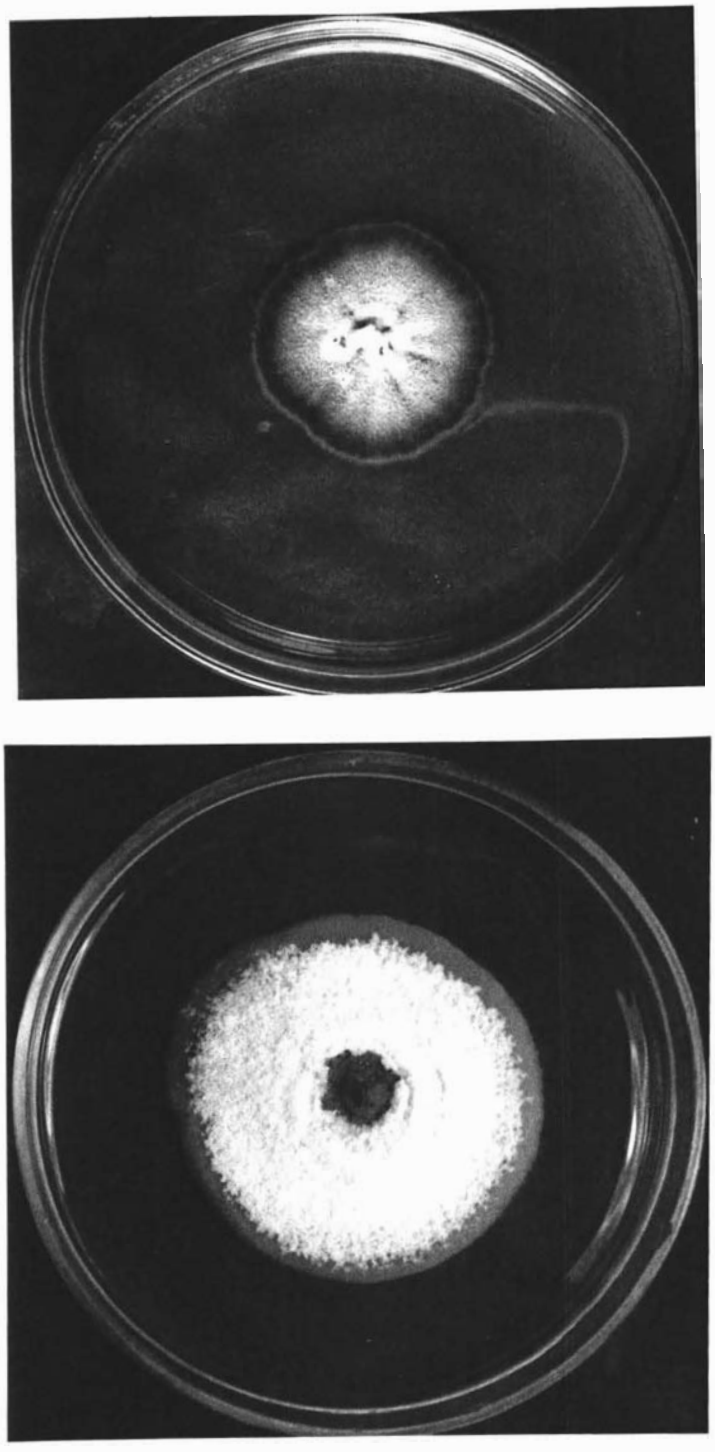


Plate 22 Cultural variants subcultured from monosporous wild type isolates derived from infected P. paniculata plants (Manawatu collections).

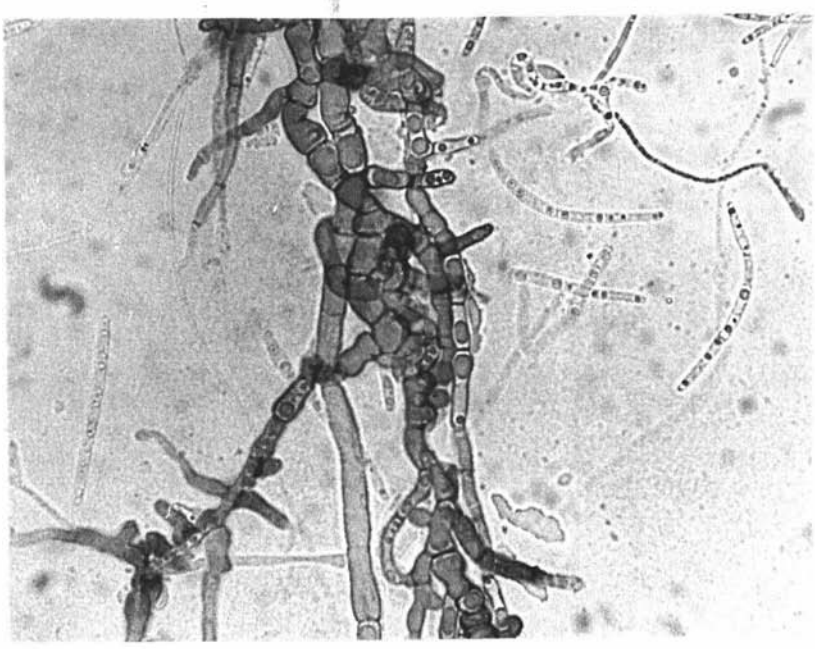
and often densely filled with oil globules. The hard, crust-like crown of mature colonies consisted of closely packed hyphae with short, thick walled, rounded cells reminiscent of chlamydospores. Similar dark, intercalary, chlamydospore-like bodies were present along submerged hyphae.

(b) Pycnidia

Pycnidia were usually produced by isolates within six days. Those from P. drummondii collections were black, crowded, spherical and either superficial or embedded in the raised crown area of colonies. The wall surface was loosely pseudoparenchymatous and often covered with short, dark grey, closely septate hyphae. In some cultures the presence of pycnidia was masked by a loose mat of light grey aerial hyphae. Pycnidiospores were released as early as five days in P. drummondii isolates to form dense, sticky, pale cream masses over the ostiole. Cirrhi were never observed in culture.

P. paniculata isolates differed in that pycnidial production was frequently delayed, and not as intense. They were consistent in shape and structure being crowded and spherical, with a firm pseudoparenchymatous wall. Pycnidia transferred to slides were more difficult to rupture than those from P. drummondii isolates. Spore masses released from pycnidia were only rarely present within two weeks of isolation.

The method of pycnidium formation by isolates from both host species was of the type described by Kemptom (1919) as meristogenous and was the same for all isolates examined. Stages in the formation of pycnidia on PDA slide cultures of a P. drummondii isolate are depicted in Plates 23 and 24. Primordia develop from adjacent cells of a single hypha, or more commonly from adjacent cells of two or more neighbouring



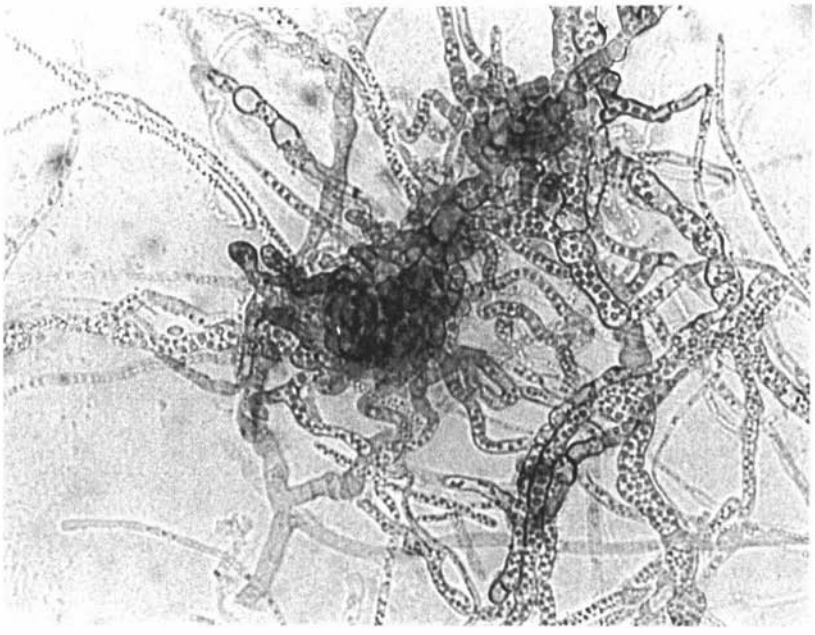
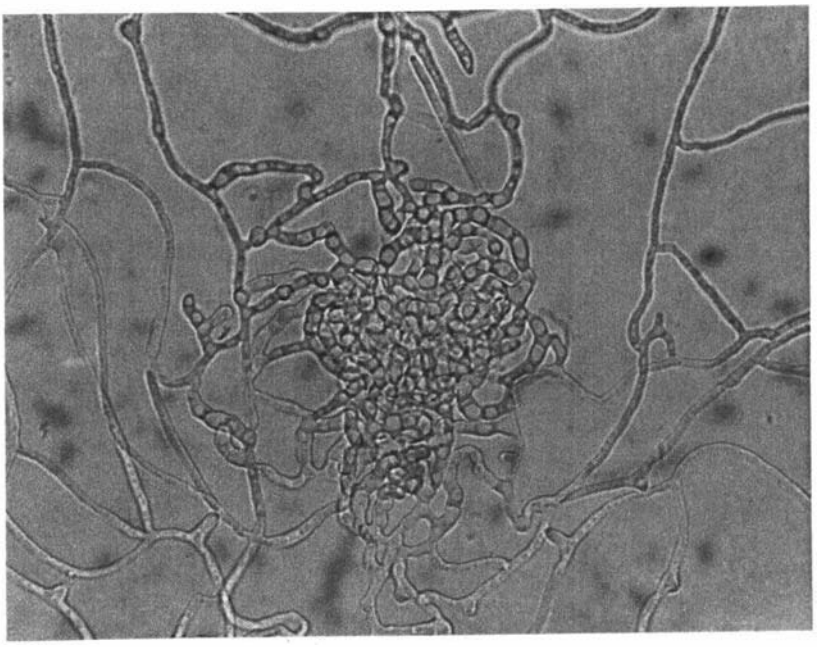


Plate 23 Stages in the formation of pycnidia by a *P. drummondii* isolate, on P.D.A. slide cultures (see next Plate).



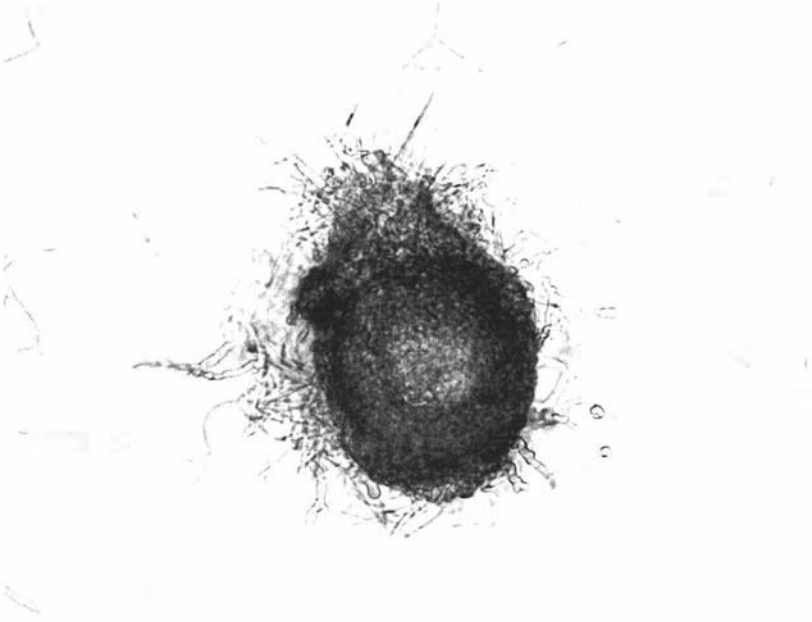


Plate 24 Stages in the formation of pycnidia by a P. drummondii isolate, on P.D.A. slide cultures (continued).

hyphae. These cells first darken and enlarge, become thick walled, divide in several planes and gradually merge to form a small knot of tightly intervoven hyphae. Free anastomosis between neighbouring cells follows, the effect then being a compact, spherical, black, pseudo-parenchymatous mass. Subsequent development leading to the formation of pycnidiophores and spores could not be followed on agar media.

(c) Pycnidiospores

There were no distinctive spore features to typify any one collection. Shape was similar for all isolates and ranged from straight to slightly curved. Septation was a very variable feature ranging from 0 - 7 with the 3-septate state most common.

The length of pycnidiospores produced by wild type isolates from P. drummondii and P. paniculata field collections following growth on PDA at 24°C for 12 days are presented in Tables 17 and 18. An analysis of variance showed there was no significant difference in the mean length of spores from the four series of P. paniculata isolates (Appendix 11). The data from all P. drummondii and P. paniculata isolates were grouped according to origin and the groups compared by an analysis of variance; there was no significant difference (Appendix 12).

(d) Secondary Conidia

The production on agar of asexual spores on free vegetative hyphae has been reported for several species of Septoria (Weber, 1922; Sprague, 1944; Hughes, 1949; Bond, 1952; Blair, 1962). These are similar in appearance and structure to pycnidiospores and are variously referred to as macroconidia or secondary conidia.

A capacity for intense production of secondary conidia was a feature common to all isolates, more particularly those from P. paniculata

TABLE 17 Length of pycnidiospores produced by monosporous wild type isolates from P. drummondii field collections at 12 days on PDA and incubated at 24°C; sample size of 50 spores in each instance

Source of Collection	Collection Number	Range (in microns)	Mean
H.dr.sd.	1	26.6–66.5	37.4 ± 0.47
	2	22.8–51.3	34.2 ± 0.34
	Overall	22.8–66.5	35.8
U.K.dr.sd.	1	24.7–55.1	33.8 ± 0.36
	2	19.0–62.7	39.8 ± 0.19
	Overall	19.0–62.7	36.8 ± 2.7

TABLE 18 Length of pycnidiospores produced by monosporous wild type isolates from 14 P. paniculata field collections at 12 days on PDA and incubated at 24°C; sample size of 50 spores in each instance

Collection	Collection Number	Range (in microns)	Mean
M.pan	1	22.3–49.4	31.2 ± 0.34
	2	22.8–55.1	36.0 ± 0.26
	3	19.0–36.1	23.6 ± 0.29
	4	20.9–55.1	40.7 ± 0.34
	Overall	19.0–55.1	32.9 ± 3.3
H.pan	1	26.6–49.4	37.1 ± 0.48
	2	22.8–62.7	46.6 ± 0.23
	Overall	22.8–62.7	41.9 ± 4.5
B.C.pan	1	22.8–41.8	33.3 ± 0.45
	2	19.0–43.7	28.3 ± 0.31
	3	22.8–47.5	37.6 ± 0.36
	Overall	19.0–47.5	33.1 ± 3.7
O.pan	1	28.5–45.6	36.1 ± 0.27
	2	26.6–57.0	47.7 ± 0.18
	3	26.6–49.4	41.4 ± 0.25
	4	24.7–39.9	32.6 ± 0.34
	5	24.7–55.1	33.8 ± 0.33
	Overall	24.7–57.0	38.3 ± 2.8

collections. On PDA slide cultures incubated at 24°C conidiophores appeared singly within four days as short, obclavate projections arising at right angles from parent vegetative hyphae. They were usually aseptate, rarely one or two septate, straight, non-branched, rounded at the tip 1.6 - 3.5 µ in diameter at the base and up to 18 µ long. The secondary conidia resembled pycnidiospores being filiform, 0-5 septate, slightly acicular, mostly straight, and borne singly and terminally on the conidiophores. Dimensions varied greatly in any one isolate, and ranged from 31 to 107.5 µ by 1.6 to 3.5 µ.

Plates 25 and 26 depict stages in the production of secondary conidia on PDA slide cultures by P. drummondii and P. paniculata isolates.

(e) Spore Germination

The general pattern and rate of germination was identical for all collections and is illustrated on Plates 27,28,29,30 and 31. In no instance were terminal or lateral germ tubes discernible during the first 10 hours. However during that time the overall body length at least doubled and this was assumed to be the result of cell division and growth from end cells. Slides stained at this stage revealed most germinated spores to be 5- or 7-septate whereas the majority of ungerminated spores were 3-septate. First lateral hyphae were observed at 15 hours and invariably were formed from the larger middle cells of the original spore. From here on close septation occurred, older cells enlarged in diameter, and first formed lateral branches sometimes developed secondary hyphae. Pycnidial primordia were formed at about 64 hours by P. drummondii isolates and immature secondary conidia were in evidence at 72 hours.

The absence of any readily recognisable structural change in spores

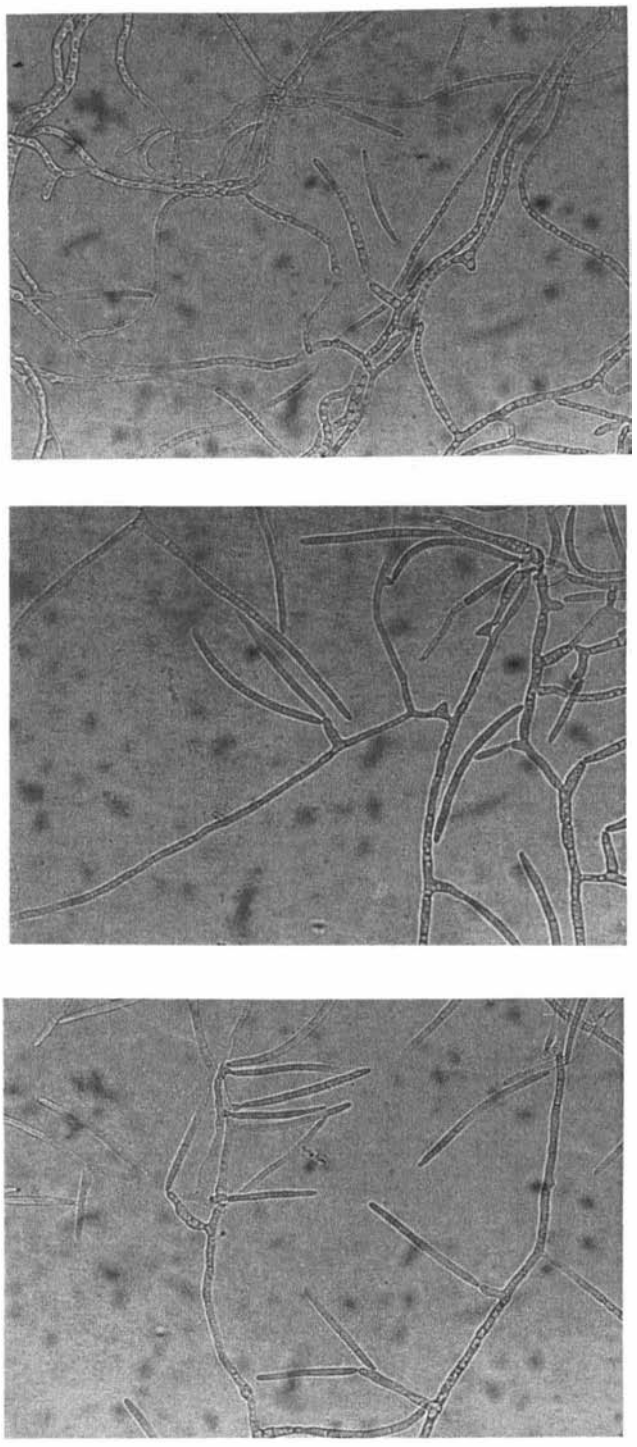


Plate 25 Secondary conidia production on P.D.A. slide cultures by *P. drummondii* isolates.

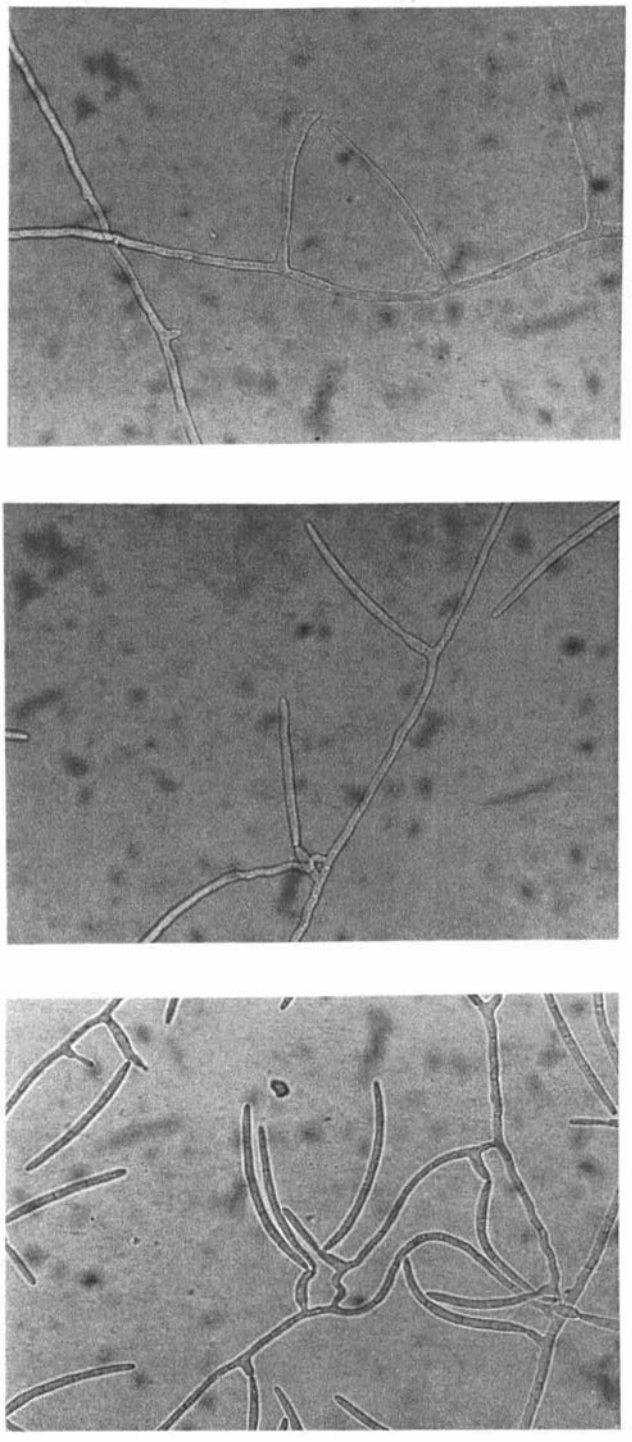
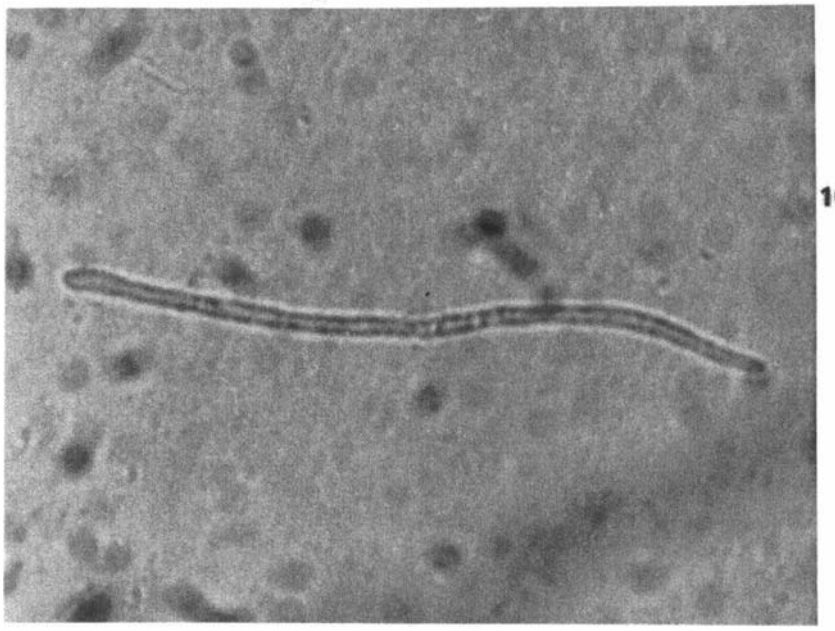
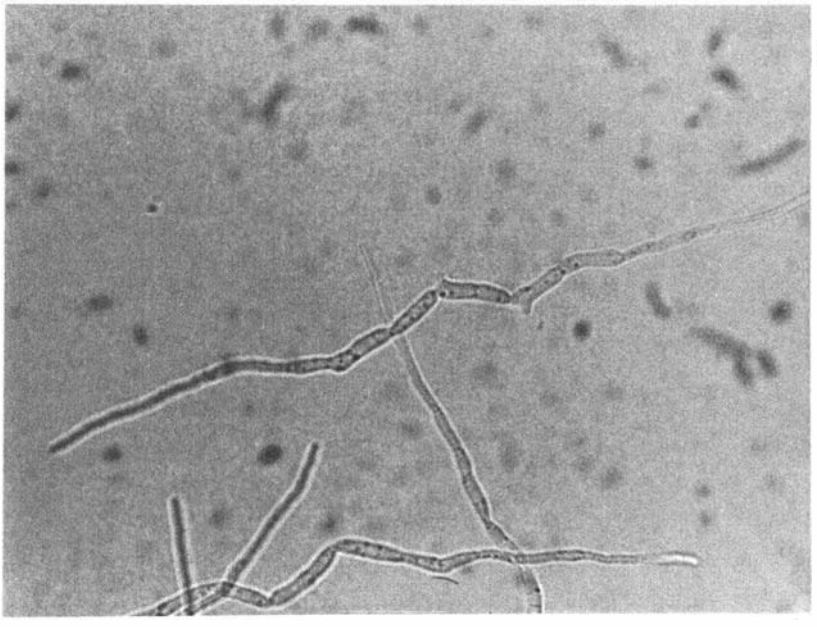


Plate 26 Secondary conidia production on P.D.A. slide cultures by *P. paniculata* isolates.

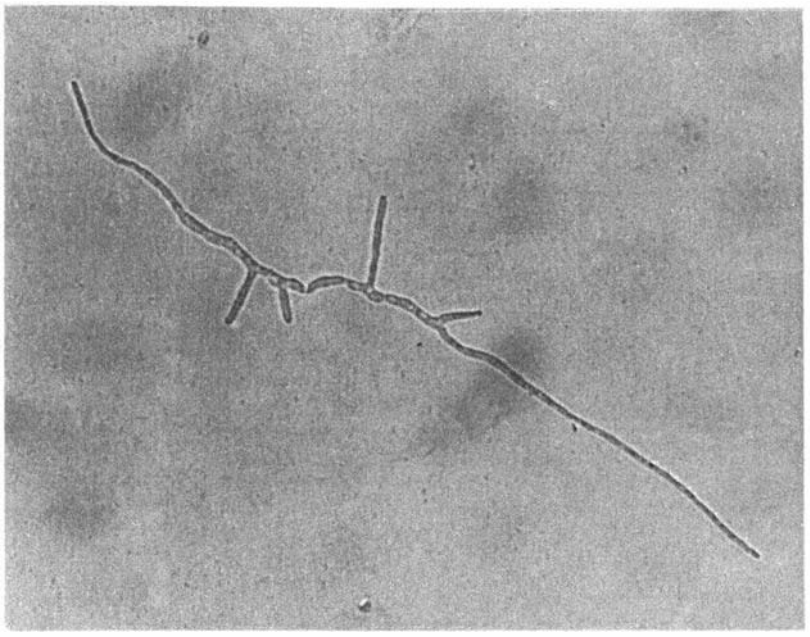


10½ hours

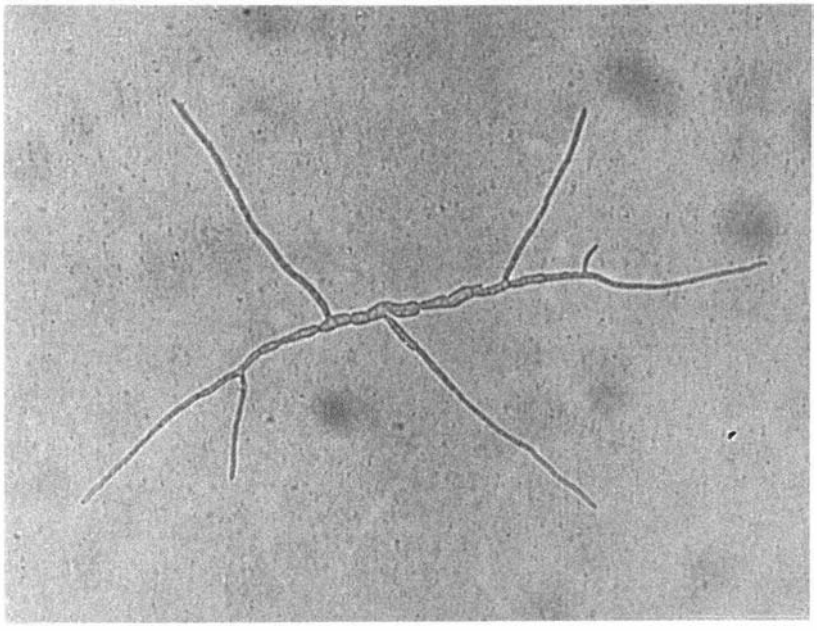


26 hours

Plate 27 Stages in the germination of pycnidiospores on P.D.A. slide cultures incubated at 24°C. Inoculum from an infected *P. drummondii* seedling.



38½ hrs



43 hrs

Plate 28 **Stages in the germination of pycnidiospores on P.D.A. slide cultures incubated at 24°C. Inoculum from an infected P. drummondii seedling.**

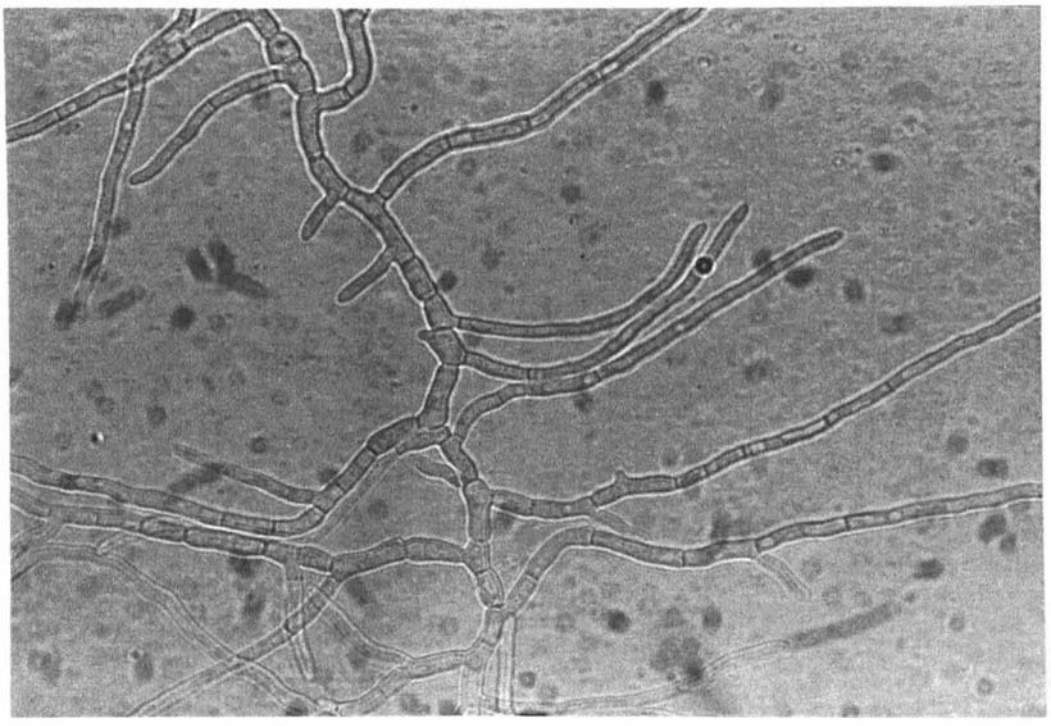
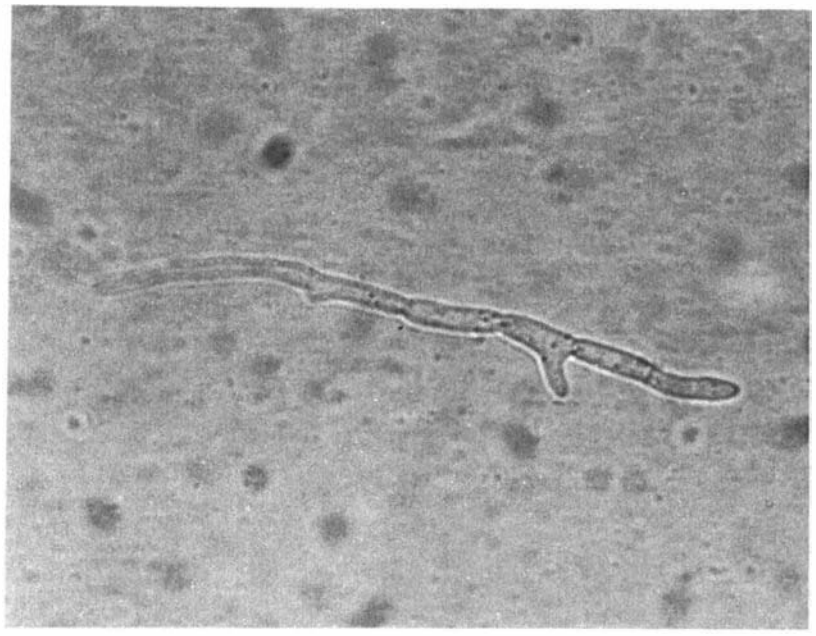
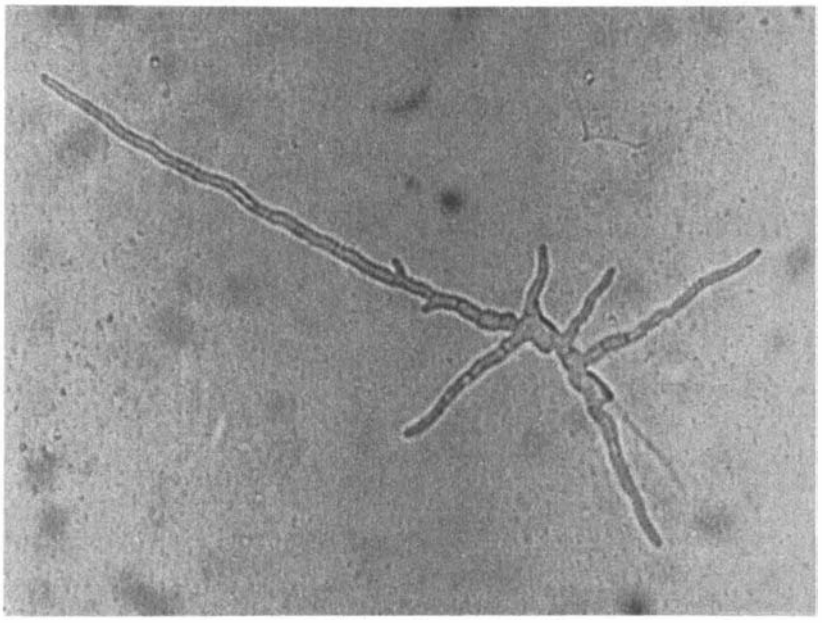


Plate 29 Vegetative mycelium of a monosporous P. drummondii isolate on a P.D.A. slide culture incubated at 24°C. for 68 hours.

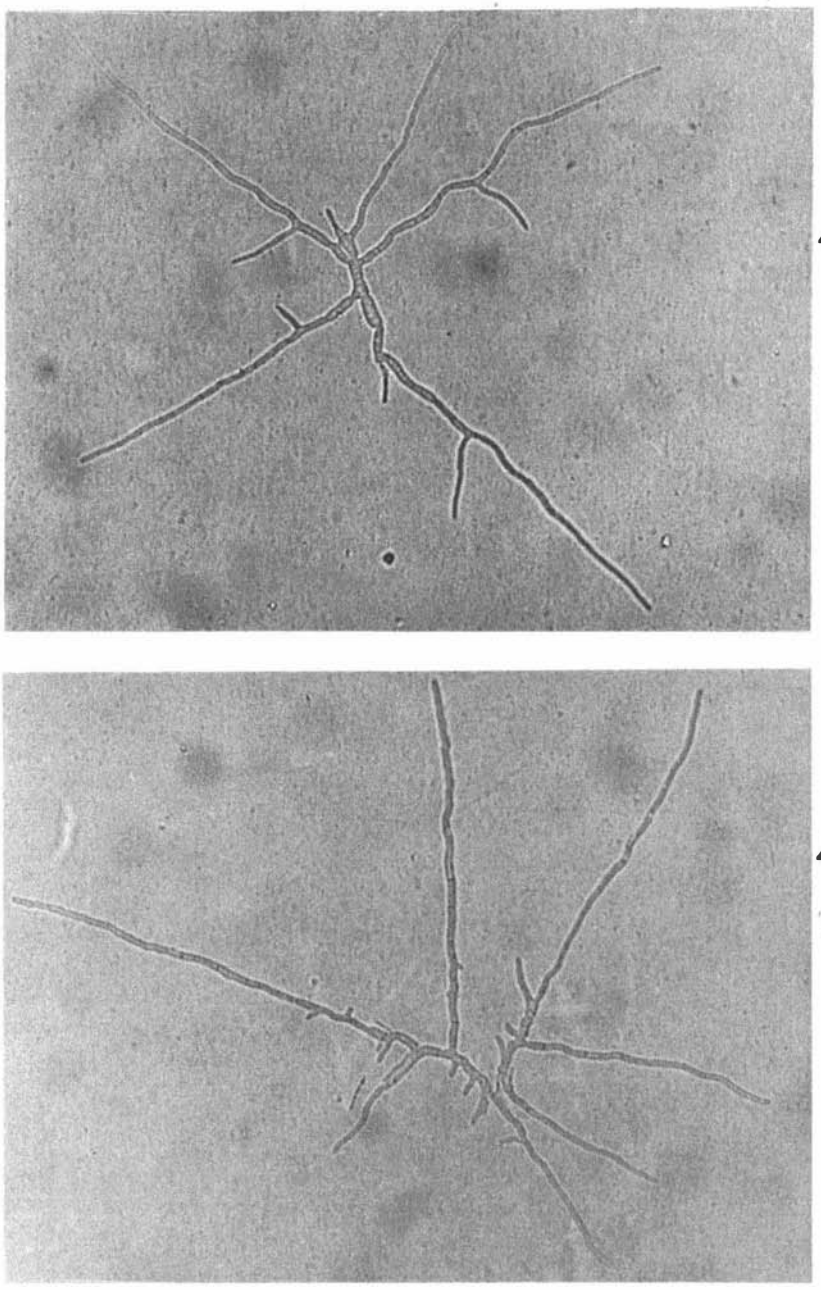


15 hours



36 hours

Plate 30 Stages in the germination of pycnidiospores on P.D.A. slide cultures incubated at 24°C. Inoculum from a field infected P. paniculata plant (Manawatu collection).



43 hours

48 hours

Plate 31 Stages in the germination of pycnidiospores on P.D.A. slide cultures incubated at 24°C. Inoculum from a field infected P. paniculata plant (Manawatu collection).

during the early stage of germination prevented studies on the effect of temperature on percentage spore germination, and the time requirement for the onset of germination at different temperatures. Invariably germination began with a considerable increase in overall body length and this was revealed only by following growth of selected spores against the graduations of the eyepiece micrometer.

(iv) Growth in Relation to Temperature

The effect of temperature on growth was determined for isolates from all collections in three experiments using single spores and mycelial discs (3 mm diam.) as inoculum (Plate 32). There was a similar response at any one temperature by all collections but some variation between collections in the extent of growth at some temperatures (Table 19). Thus for all collections the minimum and maximum temperatures for growth were approximately 6° and 30°C respectively, with maximum growth at 27°C.

For all collections pycnidiospore production occurred over the temperature range 9°-27°C but was most intense at 24°C. Pycnidia were never observed at 30°C. Although greatest mycelial growth occurred at 27°C relatively few pycnidia were produced at this temperature.

Temperature greatly influenced the macroscopic features of wild type colonies, even when the inoculum was single spores from the one pycnidium (Plate 33).

3. Discussion

Cultural studies are of value in clarifying the taxonomy of related groups of fungi where one or more features associated with growth on agar are so distinct and stable as to clearly characterise a particular species. However, there are dangers inherent in the use of cultural features in this

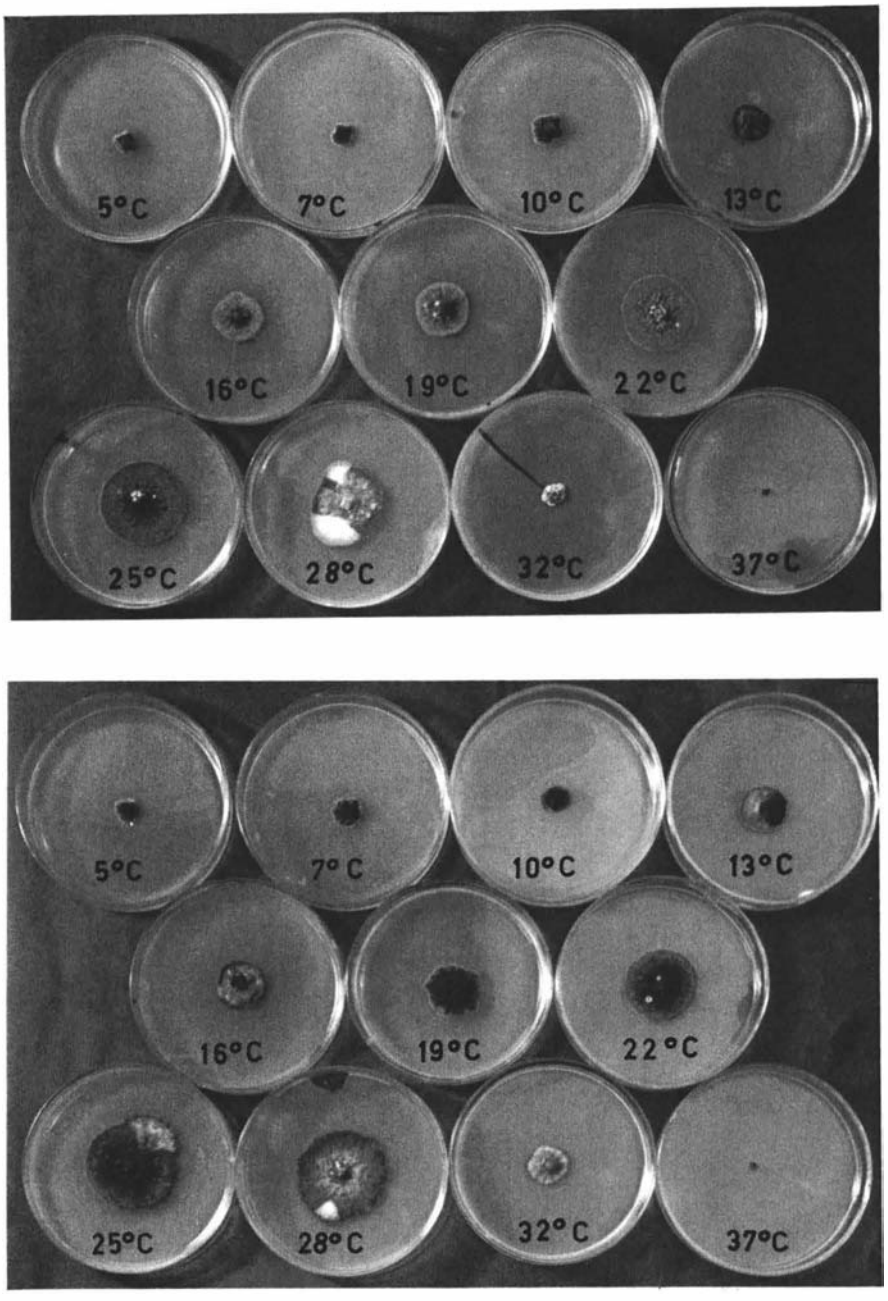


Plate 32 Effect of temperature on growth rate of Septoria isolates from P. drummondii (above) and P. paniculata plants (below). In each instance inoculum was a mycelial disc (3 mm diam.) cut from the periphery of 7-day-old monosporous wild type isolates grown on P.D.A. at 24°C. Note the development of cultural variants at several temperatures in both series.

TABLE 19 Effect of temperature on colony diameter (mm) of monosporous wild type isolates from P. drummondii (2) and P. paniculata (4) collections at 24 days on PDA plates. (Mean of 3 replications at each temperature for each collection)

Collection Source	TEMPERATURE (°C)										
	3	6	9	12	15	18	21	24	27	30	33
U.K.dr.sd.	-	2	8	9	9	10	14	24	27	20	-
H.dr.sd.	-	1½	3	7	9	10	17	22	28	22	2
M.pan	-	1½	6	11	13	16	24	25	34	26	-
H.pan	-	2	5	9	11	13	17	23	30	25	-
B.C.pan	-	1	3	7	12	15	20	28	35	21	-
Q.pan	-	2	6	8	13	17	22	24	31	20	-

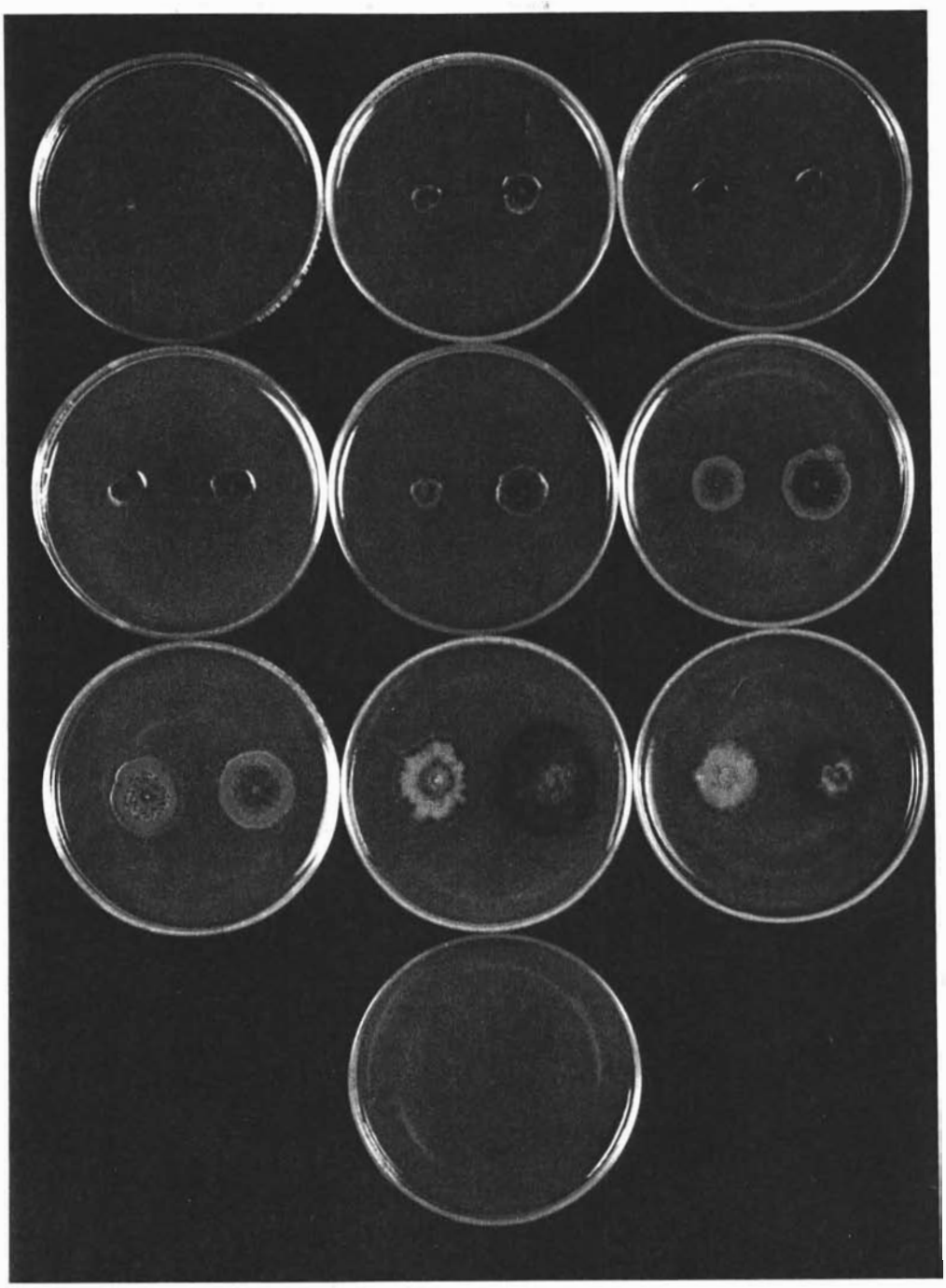


Plate 33 The influence of temperature on macroscopic features of monosporous wild type isolates from a P. drummondii (left) and P. paniculata plant (right). Inoculum in each instance derived from a single pycnidium. Temperature range from 6° to 33°C. in 3° intervals.

way. There is first the difficulty of assembling a population sufficiently representative to cover the range of genetic variability existing in the field. Only with a truly representative collection is it possible to define the species limits within which isolates can be placed with any degree of certainty. When cultural degradation is a feature of collections then comparisons must be made as soon as possible after isolation to ensure employment of characters which are exclusively those of the wild type. Further, comparisons must be made under uniform conditions since even minor changes in the external environment may significantly influence both gross colony features and morphology. For example, Williams (1959) has shown that the spore dimensions of four fungal species representing a wide range of morphological types were significantly altered by slight changes in temperature, p/H and light. More recently Rangaswami & Sambandam (1960) demonstrated that the nutritional content of the substrate significantly reduced the length of conidia of Alternaria melongenae. Obviously the findings from cultural studies must be used with great caution, and at best can serve only as supporting evidence for any taxonomic decision. In the first instance taxonomy must be concerned with and be based on stable morphological criteria of the fungi as they exist in the field.

In the present study, collections could not be distinguished on the basis of cultural features. In general, the gross colony appearance of monosporous isolates from P. drummondii collections were initially more alike than those from the various P. paniculata collections and as a group were more distinctive during the first 20 days of growth. However, the degree of variation in P. paniculata collections was such that isolates were often indistinguishable from those from P. drummondii collections. Distinguishing collections on the basis of the alleged species of pathogen involved and

using gross colony features as the criterion was likewise impossible. In particular the range of variation among the so-called S. divaricata isolates was so great as to provide cultures macroscopically identical with some from each of the other collections.

All isolates were morphologically uniform. A microscopic comparison of monosporous isolates of the same age and grown under identical conditions did not reveal consistent differences in the structure and dimensions of pycnidiospores, the method of pycnidium production or the pattern of spore germination. Distinguishing collections on the basis of these features was also impossible. That is, results from the cultural studies in no way provided support for the contention that more than one good species of fungus was involved.

The ready production of secondary conidia on agar was an outstanding feature of isolates from all collections. It is postulated that secondary conidia are produced in the field functioning there as a further form of inoculum for secondary dispersal of the pathogen. As stated earlier, pycnidia could be located on infected P. paniculata plants only with difficulty yet vast numbers of discrete lesions on leaves indicated an abundance of inoculum in the immediate environment. There is also the possibility that perithecia are produced, but a sexual stage of the pathogen was never located. In an attempt to demonstrate secondary conidia production on host tissues, infected leaves of both species were subjected to varying periods of high humidity in petri-dishes incubated at temperatures ranging from 15° to 30°C. On two occasions severely infected P. paniculata leaves (Manawatu collections) were located with a fine tomentum of short erect conidiophores bearing conidia in a manner identical to that observed on agar slide cultures. In both instances the fungus was isolated to agar

and used as inoculum to induce infections typical of the disease on this host. This provided proof that the fungus has the genetic capacity to produce secondary conidia on host tissues but it would be unwise to assume this occurs to any great extent in the field. However, there remains the distinct possibility that under the right environmental conditions pycnidiospore inoculum may be supplemented by asexual spores produced directly from vegetative mycelium.

E. Pathogenicity Studies

There are no records in the literature of pathogenicity tests within the genus Phlox using Septoria isolates. Pathogenicity studies were therefore conducted first to meet the requirements of Koch's Postulates. Cross inoculations were also made with isolates from both P. drummondii and P. paniculata and representing the three species of pathogen, to determine whether collections could be differentiated on the basis of restricted pathogenicity. It was the practice of earlier mycologists to split clearly defined morphological species into a multiplicity of "species" merely on the basis of host specificity. Cross inoculations were made with what are purported to be representatives of the three species of Septoria to determine whether any of the present isolates were host specific and whether they could be differentiated on this basis.

Materials and Methods

Plants of P. divaricata were not available as seed could not be procured of this weed species, which is confined to North America. Inoculation trials were thus restricted to use of the species P. drummondii and P. paniculata. Plants of the former were raised from pathogen-free seed. The latter were

propagated by root cuttings from established disease-free plants.

The inoculum in all cases was a spore suspension prepared from wild-type cultures grown at 24°C on PDA. The strength of the inoculum varied between experiments but was identical for all isolates used in any one trial. Plants were inoculated by painting both leaf surfaces or by spraying with the spore suspension. Following inoculation, plants were subjected to high humidity in a glass humidity cabinet, or by covering with cellophane bags.

In all experiments precautions were taken to prevent unintended infections, and adequate controls were always provided.

Results

1. Proof of Pathogenicity

The requirements of Koch's Postulates were successfully fulfilled on numerous occasions with isolates from Manawatu field collections of both P. drummondii and P. paniculata (Plates 34 & 35). In the case of the former series symptoms usually first appeared within seven days, and were followed by abundant production of pycnidia on lesions within a further 56 hours. Symptoms were slower to develop in P. paniculata inoculations, usually taking at least 10 days, and as in field infected plants, relatively few pycnidia were formed. In both inoculation series re-isolation to agar was achieved by streaking spores from a suspension prepared from cirrhi. Colonies which developed were essentially similar to those from which the parent inoculum was prepared.

2. Cross Inoculations

(a) Susceptibility of P. drummondii to isolates from P. paniculata

In preliminary experiments the susceptibility of the host species P. drummondii to Septoria isolates from Manawatu collections of P. paniculata was demonstrated on several occasions. On receipt of collections of in-

Plate 34 Proof of pathogenicity: artificially inoculated P. drummondii plant at 13 days.

Plate 35 Proof of pathogenicity: P. paniculata plant (raised from seed) at 21 days following inoculation.

fected P. paniculata from Holland, British Columbia and Quebec, the relative pathogenicity to P. drummondii was determined on four potted plants with isolates from this material and an isolate from a Manawatu collection of P. paniculata. In each instance the inoculum was a spore suspension prepared from 20 day old PDA cultures incubated at 24°C and adjusted to approximately 28,000 spores/ml. Controls were provided by two plants inoculated with an isolate from P. drummondii (approx. 28,000 spores/ml), and two plants sprayed with sterile water. All plants were subjected to high humidity for 56 hours following inoculation and then returned to glasshouse benches. Results recorded at 25 days are summarised in Table 20.

All isolates proved pathogenic to P. drummondii but there was considerable variation between isolates in symptom expression and the rate and extent of disease development. Whereas within six days well developed lesions typical of field infections were present on both plants inoculated with the P. drummondii isolate, symptoms were not present until 11 days in the case of Manawatu and Dutch P. paniculata isolates (Plate 36), and 13 days in the case of the British Columbia and Quebec isolates (Plate 37), and in each instance bore little similarity to field infections of either P. drummondii or P. paniculata. First evidence of infection was the appearance on the lower, older leaves of minute, circular, dark brown lesions which, during the following three days, became more distinct and enlarged to approximately 5 mm in diameter. In the New Zealand and Dutch series some few lesions reached 8–10 mm diameter. The lesion centres lightened very little in colour and only when the dead areas were very close together was there any coalescence. Severest infection in all cases was on the older leaves, particularly towards the leaf tip, and associated with this there was usually a gradual yellowing of tissues between lesions. At 25 days a few pycnidia were present but only on the larger

TABLE 20 Infection of P. drummondii plants inoculated with Septoria isolates from P. paniculata collections; results taken at 25 days

Source of Inoculum	Disease Rating a.	Pycnidial Production b.
M.pan	3	+
H.pan	2	+
B.C.pan	1	0
Q.pan	1	0
Controls		
M.dr.	5	+++
Sterile water	0	0

a. Subjective scale of 0-5, with 5 being severe infection.

b. + = very few pycnidia present.

++ = moderate numbers of pycnidia present.

+++ = large numbers of pycnidia present.

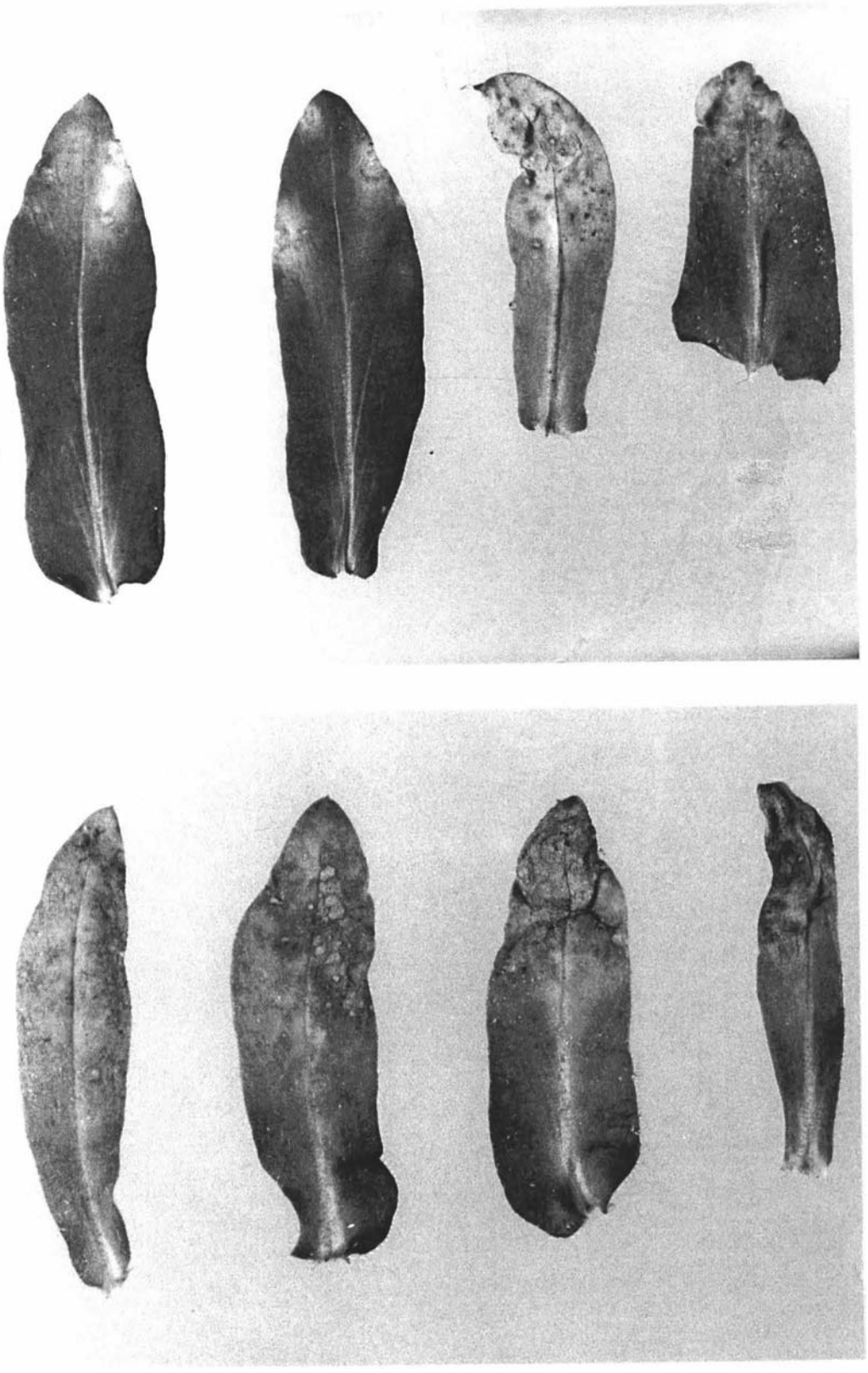


Plate 36 Infected leaves from P. drummondii plants artificially inoculated with a Manawatu P. paniculata isolate (top), and an isolate from a P. paniculata collection received from Holland (bottom).

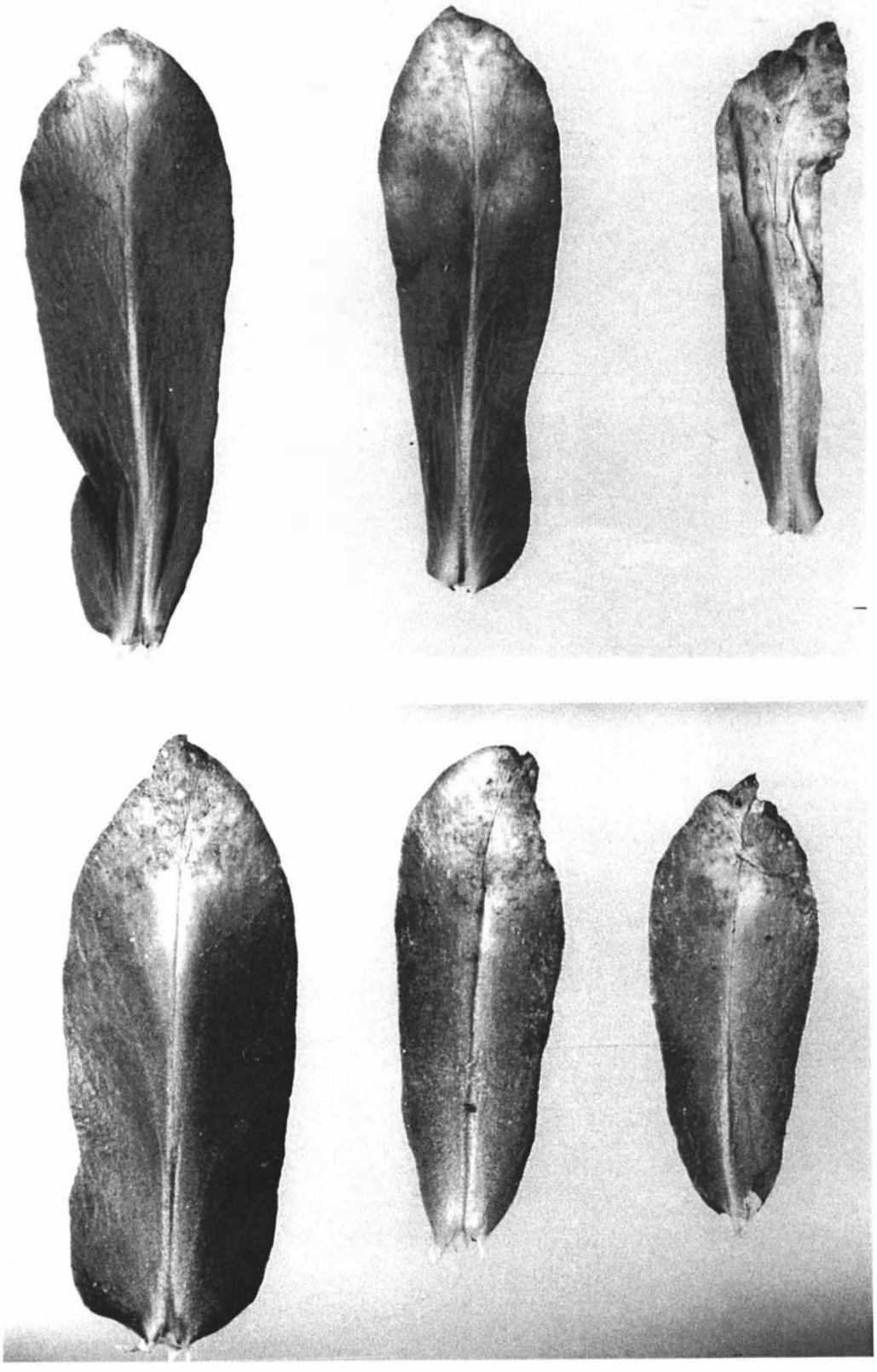


Plate 37 Infected leaves from P. drummondii plants artificially inoculated using isolates from P. paniculata collections received from Quebec (top), and British Columbia (bottom).

lesions on the lower leaves of plants inoculated with the Manawatu and Dutch isolates. However, when the more severely infected leaves of each series were subjected to high humidity in petri-dishes for three days pycnidia developed in all cases.

All control plants sprayed with sterile water remained disease free. The fact that plants inoculated with an isolate from P. drummondii became severely infected indicated that the environmental conditions associated with the experiment were favourable for infection.

(b) Susceptibility of P. paniculata to isolates from P. drummondii

Septoria isolates from P. drummondii seedlings raised from infected seed of Dutch and English origin proved weakly pathogenic to potted P. paniculata plants. Inoculum was a spore suspension prepared from ten day old PDA cultures, and inoculation was by spraying to run-off point. Controls were provided by two groups of four potted P. drummondii plants inoculated with the two spore suspensions, and a further four plants sprayed with sterile distilled water. All plants were subjected to high humidity for 56 hours following inoculation and then set out well-spaced on glasshouse benches.

There was very poor development of symptoms on P. paniculata plants inoculated with the two P. drummondii isolates. In both series scattered black lesions less than one millimetre in diameter and of irregular outline were first observed at 11 days on the upper surface of many leaves. By the fifteenth day a small percentage of lesions had enlarged to 3-5 mm diameter and in some few instances had coalesced. With age there was some lightening in color of the larger lesions but pycnidia were not observed. Pycnidia were also not produced when the older larger lesions were subjected to prolonged high humidity in petri-dishes. The spotting of the leaves was shown to result from Septoria infection by plating leaf tissues to PDA

following surface sterilisation with 0.1% mercuric chloride for 30 seconds.

Control plants sprayed with sterile water remained disease-free, whereas the two series of P. drummondii plants inoculated with the two spore suspensions became severely infected.

The susceptibility of P. paniculata to Septoria isolates from P. drummondii plants was further investigated in a series of experiments using shoots excised from healthy plants and held in bottles of tap water. P. paniculata leaves are decussately arranged. In each inoculation experiment all except the top four or five pairs of leaves were stripped from each shoot and both surfaces of one leaf of each pair inoculated by wiping with a spore suspension, the other leaf serving as a control. Further controls were provided by shoots similarly inoculated with a spore suspension from a P. paniculata isolate. Following inoculation all shoots were covered with cellophane bags for varying periods.

Results confirmed that Septoria isolates from P. drummondii are only weakly pathogenic to P. paniculata. Severest infection was induced when the inoculum potential was increased to 220,000 spores/ml., the period of humidity following inoculation extended to five days, and shoots were again covered with cellophane bags when first symptoms became apparent (Plate 38). Following such treatment larger lesions developed which frequently coalesced, and groups of up to 13 pycnidia were located from which the pathogen was readily re-isolated to agar.

Discussion

In the Saccardian system of Deuteromycete classification the two main criteria for species delimitation are pathogen morphology, and host specificity. It appears that earlier mycologists viewed host specificity within the genus Septoria as of considerable importance since well over 1,000 species have been

described, primarily on the basis of the host on which they were found. The fact that the spore length of 700 of the Septoria "species" in Saccardo's "Sylloge Fungorum" fall within the limits of 20-50 µ (Beach, 1919) further emphasises the importance previously attached to host specificity in this genus. The three species of Septoria at present under consideration were originally described on P. paniculata, P. divaricata and P. drummondii respectively, with overall spore length falling within the limits of 15-60 µ. One may conclude that here also, host specificity played some part in the consideration that three species of fungus were involved.

The above pathogenicity studies have shown that isolates from P. paniculata, regarded as S. phlogis and S. divaricata were pathogenic to P. drummondii. Similarly, isolates from P. drummondii, regarded as S. phlogis and S. drummondii were pathogenic to P. paniculata. Although in all cases collections were more pathogenic to the host from which the isolate was obtained it was clear that from the standpoint of association with host species there were no essential differences of taxonomic value.

Plate 38 Excised P. paniculata shoot artificially inoculated with an isolate from a P. drummondii seedling (see text).

II SEED TRANSMISSION STUDIES

A. Introduction

Seed-borne diseases are those which may result from the use of seed carrying viable inoculum of a pathogenic micro-organism; that is, is such instances the seed functions as a source of primary inoculum. Depending on the nature of the association between seed and inoculum so two broad situations are recognised:

(i) Seed Contamination: where free propogules of the pathogen are present as superficial adherents, or where minute fragments of crop detritus bearing viable inoculum accompanies the seed and are sown with it.

(ii) Seed Infection: where the pathogen is established as vegetative mycelium in the testa and/or embryo of the seed.

Knowledge of the manner of seed dissemination of pathogens is of importance since treatments adopted for control of specific seed-borne diseases are based on predetermination of whether seed contamination and/or seed infection is involved.

The recently published annotated list of known seed-borne diseases compiled by Noble, de Tempe and Neergaard (1959) emphasises the extent to which seed pathology has developed in recent years. Included are fifteen seed-borne diseases caused by species of Septoria, of which eight are considered to be of economic importance. In each of these diseases both seed contamination and seed infection are involved, with the former of minor significance because the free conidia associated with seed are relatively short-lived. Where seed infection is involved, vegetative mycelium, and in some few cases pycnidia (Gloyer 1931, Maude 1964), are present on and beneath the seed surface. Spores within pycnidia and sub-surface mycelium in the testa are of considerable

importance in initiating infection because both may remain viable for some months.

Field observations in the present study suggested that both seed contamination and seed infection occur in diseased P. drummondii seed crops. Disease development had been followed in several Manawatu field plantings and in late autumn lesions with abundant pycnidia were frequently observed on leaves, stems, petioles, pedicels and sepals in the immediate vicinity of maturing ovaries (Plate 39). Because of the proximity of pycnidia to the flower head there is ample opportunity for infected tissues to be included with the seed during harvesting. During threshing, pycnidiospores, cirrhi and pycnidia would be liberated from this material to contaminate the seed. The close relation of pycnidia to inflorescences would also facilitate spore dispersal by splashing during wet weather. Germinating spores could then infect developing seed within ovaries. It was postulated, therefore, that both seed contamination and seed infection could be involved in dissemination of the Septoria leaf blotch disease of P. drummondii.

B. Initial Evidence of Seed Transmission in Commercial Seed Lines

Evidence of seed transmission was first obtained in October 1953, using three United Kingdom lines of P. drummondii each of which had produced infected seedlings in two local commercial nurseries. Four new boxes of sterilised soil were sown with each seed line at the rate of 1.5 grams (approx. 800 seeds) per box and set out well-spaced in a glasshouse which had not previously held P. drummondii plants. As controls, four boxes were similarly sown with seed of two further lines which in both nurseries had produced only disease-free seedlings.

At 45 days from sowing a count was made of the number of seedlings in

Plate 39 Lesions with pycnidia on stems and leaves in the vicinity of maturing ovaries of P. drummondii.

each box with lesions and pycnidia on one or both cotyledons. Several diseased seedlings were removed from each box and the pathogen isolated to agar. The results are given in Table 21.

TABLE 21 Seedling infection resultant on use of commercial seed.

Seed Line	Percentage Germination[a]	No. of Emerged Seedlings	No. of Infected Seedlings				% Infected Emerged Seedlings			
			Box A	Box B	Box C	Box D	Box A	Box B	Box C	Box D
1	82	577	5	2	2	3	0.85	0.34	0.34	0.51
2	91	612	-	3	1	1	-	0.48	0.16	0.16
3	87	558	7	3	5	2	1.26	0.54	0.9	0.36
Control 1	95	701	-	-	-	-	-	-	-	-
Control 2	83	547	-	-	-	-	-	-	-	-

[a] Determined by the Government Seed Testing Station, Department of Agriculture, Palmerston North.

As seedlings in the control boxes were disease free, infection clearly resulted from viable inoculum associated with the seed at the time of sowing. However, these results provided no evidence of whether seed contamination or seed infection was involved.

C. Studies Relating to Seed Contamination

1. Detection of Surface-Borne Inoculum

The technique for detecting surface contamination of P. drummondii seed was based on that developed by Bolley (1902) for detecting spores of Tilletia foetida (Wallr.) Liro and Fusarium oxysporum f. lini (Bolley) Sny. and Hans.

on seed of wheat and linseed respectively.

Three grams of the three United Kingdom seed lines which previously had given rise to infected seedlings were added to 15 ml. of distilled water in an Erlenmeyer flask and vigorously shaken. The wash water from each flask was centrifuged for 10 minutes at 3,000 r.p.m. and the supernatant discarded. Slides were prepared by adding some of the deposit in each tube to drops of lactophenol cotton blue.

Pycnidiospores typical of the causal fungus were observed on several slides from each of the three series. In preparations from two of the seed lines small tissue pieces with pycnidia were also observed. In both cases pressure applied to the cover slip induced rupture of the pycnidia and release of Septoria type conidia.

It was thus established that commercial seed of P. drummondii may include conidia of the causal organism either in free form, or in pycnidia associated with crop debris.

2. Viability of Inoculum Contaminating Seed

The following experiments were conducted using the three commercial seed lines referred to above, which at the time were not more than eleven months old.

Cruikshank (1954) demonstrated the viability of conidia of Septoria linicola (Speg.) G. Roda associated with seed of linseed by observing spore germination in Van Tieghem cells. His method involved preparing a concentrate of conidia from seed using Bolley's centrifuge technique, transferring spores to Van Tieghem cells and incubating at 23°C for 24 hours. Preparations were then stained with lactophenol cotton blue and examined for germ tubes. This method was tried in the present study but found to be unsatisfactory as heavy

bacterial growth made examination of the conidia extremely difficult and those few observed showed no evidence of germination although observations continued until 56 hours. This lack of evidence of germination did not necessarily imply non-viability of the observed spores as germination could well have been inhibited by the high concentration of associated saprophytic bacteria and fungi.

In a further attempt to observe germination, plates of prune agar and malt agar were streaked with the centrifuged deposit from the three seed lines, and incubated at 24°C. Within three days however all plates were covered with rapidly growing saprophytes which effectively masked any development of the slower growing Septoria.

The viability of free inoculum associated with seed was finally demonstrated by use of a plant inoculation method. A 10 gram sample of each of the three seed lines was shaken in 50 ml. of sterile water and the wash water centrifuged at 3,000 r.p.m. for 10 minutes. The supernatant was discarded and the sediment re-suspended in 30 ml. of sterile water and painted over eight leaves of four potted, disease-free seedling plants using a camel hair brush. As controls, another four plants were similarly inoculated with a spore suspension (113,000 spores/ml.) prepared from a stock culture, and four plants were inoculated with distilled water. All plants were subjected to high humidity for 72 hours to facilitate infection. Subsequent watering was by steeping in trays. Where lesions and pycnidia developed identification of the pathogen was confirmed by isolating to agar.

The results, recorded at 28 days following inoculation, are presented in Table 22.

TABLE 22 Viability of inoculum in free association with commercial seed.

Source of Inoculum	No. of Leaves Infected			
	Plant A	Plant B	Plant C	Plant D
Seed Line 1	2	1	0	1
Seed Line 2	0	0	0	0
Seed Line 3	0	0	0	0
Stock Culture	8	8	8	8
Sterile Water	0	0	0	0

The above results show that viable inoculum is carried on the surface of P. drummondii seed imported to New Zealand, and thus allows the hypothesis that seedling infections in nurseries may originate from this inoculum. As the seed at the time of testing was eleven months old the results imply the ability of pycnidiospores to remain viable for at least that length of time, more probably when associated with cirrhi or pycnidia. The ability of spores to remain viable in cirrhi and pycnidia for long periods was investigated as follows:

(a) Viability of Spores in Cirrhi

Naturally infected leaves bearing abundant pycnidia were subjected to high humidity at 24°C in petri-dishes lined with moistened filter paper to induce the production of cirrhi. After 24 hours the lids were removed to allow leaves and filter paper to dry, and the leaves were then transferred to sterile petri-dishes for storage at room temperature. To determine survival of pycnidiospores with time, sample leaves were removed at 3 day intervals for 69 days and not less than ten cirrhi transferred to a drop of sterile water to

produce a dense spore suspension. Twelve plates of PDA were then streaked with this inoculum and incubated at 24°C for 10 days. The production of colonies of the fungus was the criterion indicating spore viability.

Up until 27 days little loss in viability occurred, as evidenced by large numbers of colonies which appeared on all streaked plates. Thereafter a gradual reduction in colony numbers occurred until the fifty-seventh day when only three colonies developed on the twelve plates. All plates streaked using inoculum prepared at 60, 66 and 69 days failed to produce colonies. Seven colonies grew from the 63 day inoculum.

In a second experiment colonies failed to appear after 51 days.

Spores in acervuli and pycnidia are held together by a gelatinous matrix which is believed to protect and prolong their viability (Lilly & Barnett, 1951). In the above experiment, the outer spores of each cirrhus probably died gradually and after 50 days only a few more deeply located spores remained viable. This suggests that spores freed from pycnidia cannot remain viable for periods in excess of three months. As imported European seed spring sown in New Zealand is at least 10 months old it follows that disease outbreaks in this country do not arise from conidia in cirrhi present as superficial contaminants on seed.

(b) Viability of Spores in Pycnidia

Spores in pycnidia of some sphaeropsidaceous pathogens may remain viable for periods in excess of one year (Machacek 1945, McKay 1946, Gabrielson 1962). Microscopic examination of the detritus concentrate from imported P. drummondii seed had revealed not only Septoria spores but also leaf fragments bearing pycnidia of the pathogen. There was thus the possibility of spores within such pycnidia functioning as primary inoculum in New Zealand.

Naturally infected leaves bearing pycnidia were stored in petri-dishes at room temperature. At monthly intervals a spore suspension was prepared from at least twelve pycnidia and streaked over not less than six plates of PDA.

Until the eighth month large numbers of colonies developed on all plates but thereafter there was an obvious decline in numbers until growth ceased at fourteen months. The results thus indicate the possibility of European seed being contaminated with viable inoculum in the form of spores within pycnidia at the time of spring sowing in New Zealand. In the experiment above (Section C (2)) where infection resulted from inoculating healthy plants with the centrifuged concentrate from year-old seed the inoculum was probably spores released at that time from pycnidia associated with minute pieces of host tissue included with the seed.

3. Seedling Infection from Contaminated Seed

The above trials had done no more than to demonstrate the fact of imported seed being contaminated with viable inoculum, and to reveal the probable form in which that inoculum occurred. The following two experiments were conducted to investigate whether seedling infection could in fact result from use of seed so contaminated.

1. Six grams of pathogen-free seed (83% germination) was added to a suspension of spores (110,000/ml.) prepared from a ten day old PDA culture. After thorough agitation the seed was removed and dried between blotting paper for 24 hours at room temperature and sown as follows:

(a) 400 seeds were set out in a Copenhagen germinator. As a control, 400 untreated seeds of the same line were similarly sown.

(b) Two new boxes of sterilized soil were each sown with two grams (approx. 1,100 seeds) of the artificially contaminated seed. A similar box sown with the untreated seed served as a control.

Watering of seed boxes was by steeping in trays, thereby reducing the possibility of secondary spread from the first exposed infected cotyledons. In both series infected seedlings were removed as they appeared, and the experiment concluded at 50 days from sowing. Confirmation of infection was by microscopic examination of cirrhi from pycnidia developed on cotyledons.

In the Copenhagen germinator test first infection was apparent at 21 days, and the last positive record was at 36 days, at which stage 116 seedlings (29% of sown seed) were infected. In all cases infection was severe, there being abundant pycnidial production on one or both cotyledons, frequently followed by death of the seedling. All seedlings from untreated seed were disease free.

Results of the seed box sowings are presented in Table 23.

TABLE 23 Seedling infection from box sown artificially contaminated seed

Seed Source	No. of Emerged Seedlings	No. of Infected Seedlings	% of Emerged Seedlings Infected
Box 1	768	51	6.6
Box 2	814	36	4.4
Control	841	-	-

2. The circumstances under which commercial seed may become contaminated with the pathogen at harvest was simulated by crushing naturally infected leaves bearing many pycnidia and adding them to six 500 ml. flasks

each with six grams of pathogen-free seed. Following vigorous shaking and removal of the larger leaf fragments the seed was bulked. Each month this seed was used to sow one box at the rate of two grams per box. Watering throughout was by steeping in trays, and all infected seedlings were removed as they appeared during the first 50 days from sowing. Confirmation of infection was by microscopic examination of cirrhi from lesioned cotyledons.

Infection was recorded only in boxes sown at one, two and five months with four, six and two seedlings respectively being found. The experiment was repeated on two subsequent occasions with essentially similar results. Infected seedlings failed to appear in the majority of boxes and in no instance later than four months from commencement of the experiment.

From these experiments it is concluded that seedling infection can result from viable inoculum in loose form associated with seed at the time of sowing.

4. Discussion

Results from the above experiments probably overstate the case for seed contamination as a source of primary inoculum in New Zealand sown seed. The contamination of imported seed with viable surface inoculum is revealed, and the possibility of seedling infection arising from use of artificially contaminated seed demonstrated, but it does not necessarily follow that initiation of the disease in commercial nurseries is from viable inoculum loosely associated with seed.

The concept of an 'inoculum potential' has been discussed at length by Dimond and Horsfall (1959), and Garrett (1960) and its significance demonstrated by several workers (Cole and Couch 1958, Garrett 1959, and Gooding & Lucas 1959). The concept is that under favourable field conditions there is a minimum spore density or numerical spore threshold which is necessary to establish infection. Although the concept has been developed only as it relates to air-borne and soil-borne pathogens, support for its application to the situation of seed-borne contamination is provided by Heald (1921). In studying the relation between the numbers of seed-borne chlamydospores and field establishment of covered smut of wheat caused by Tilletia caries (D.C.) Tul. and T. foetida (Wallr.) Liro, Heald found that for even low percentages of infection high numbers of viable spores must be present on seed. In the above experiment where seedling infection resulted from sowing P. drummondii seed artificially contaminated by dipping in a strong spore suspension, an inoculum potential was provided far in excess of that likely to occur in the field. And in the case of seed line No.1, it may be assumed that by centrifuging the wash water the viable spore load from all seed was concentrated to a level sufficient to ensure infection when leaves were painted with this preparation. For seedling infection to be a consequence of field sowing of

that particular seed requires that in the soil, in the immediate vicinity of at least one seed, sufficient viable spores must escape from pycnidia to reach the minimum density required for infection. Since imported seed is at least 10 months old at the time of sowing, and since the viability of spores in pycnidia decreases rapidly from the eighth month, it is unlikely that the minimum inoculum potential required for infection would be reached. Accordingly it is unlikely that seed contamination is of significance in accounting for initiation of infection in seedlings in New Zealand.

D. Studies Relating to Seed Infection

1. Detection of Infected Seed

Mature ovaries with the associated sepals densely covered with pycnidia may be readily located in diseased garden plantings of P. drummondii throughout the Manawatu during the months of March and April (Plate 40). Seed harvested from such ovaries was screened for infection in the following manner.

Individual ovaries were broken open under aseptic conditions and the three seeds they each contained bulked in a sterile test-tube. After washing in four changes of sterile water to remove possible surface contaminants the seed was dried between sterile blotting paper and transferred, one each, to prune agar slopes. Checking seed individually in this way was a precaution intended to prevent contaminant saprophytes from growing over neighbouring seeds and masking the presence of the slower growing Septoria colonies. The slopes were incubated at 24°C and those colonies considered typical of the pathogen on this medium were microscopically examined. This experiment was repeated on two subsequent occasions with seed harvested from similarly diseased crops. Results are presented in Table 24.

Plate 40 Mature ovaries of P. drummondii with sepals bearing pycnidia of the causal organism.

TABLE 24 Percent of infected seed from mature ovaries with the associated sepals and pedicels infected

Trial No.	No. of Seeds	No. of Seeds Yielding Septoria	Percent of Seed Infected
1	128	16	12.4
2	208	21	10.1
3	75	5	6.6

In those cases where the pathogen was the only fungus present, mycelium could be observed within 48 hours and by four days covered the seed and adjacent agar to form a dense, dark-grey colony. Pycnidia were usually apparent within six days and by eight days shiny, moist, cream-colored masses of released pycnidiospores were clearly visible (Plate 41).

The above figures for number of infected seed may be regarded as minimal as dense mycelium of contaminant fungi grew out from many seeds, effectively preventing detection of the pathogen.

2. Seedling Infection from Infected Seed

Evidence indicating that seedling infection may arise from use of field-infected seed was provided by two trials in which seed harvested by hand from ovaries with severe infection of the sepals were set out in a Copenhagen germinator, and box-sown in sterilised soil. Results are recorded in Table 25. In each case the trial was continued for 50 days, during which time infected seedlings were removed as they appeared.

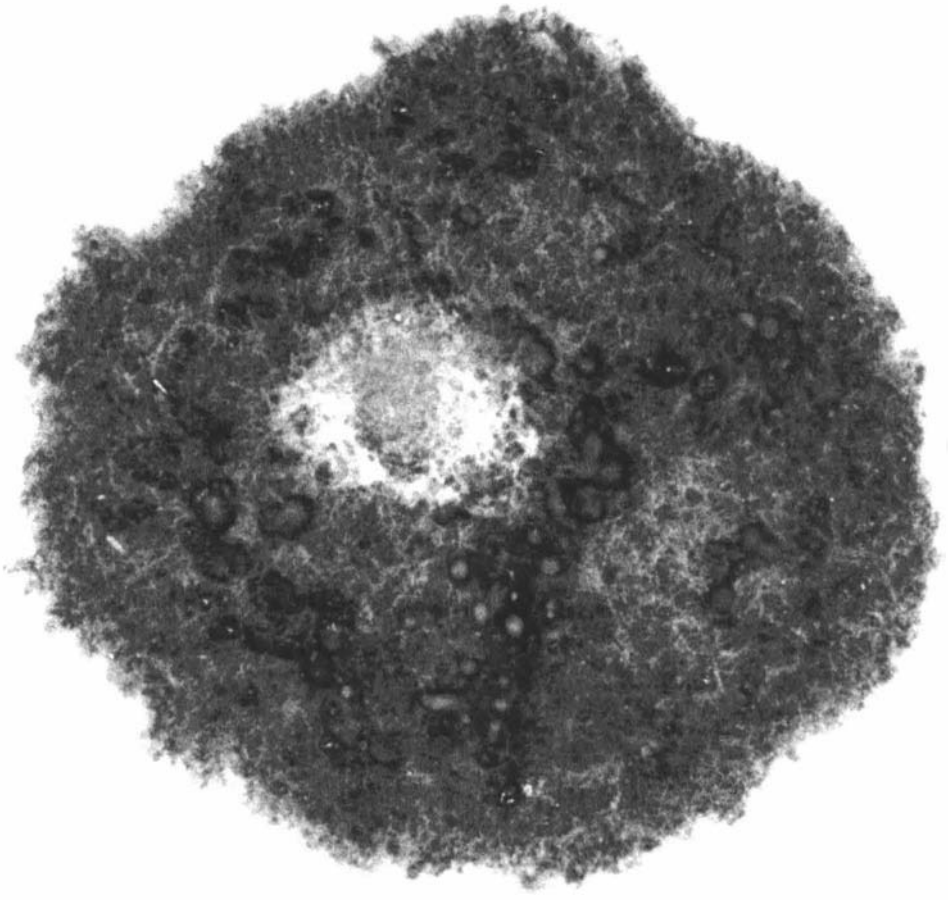


Plate 41 Colony of the pathogen arising from infected *P. drummondii* seed plated to P.D.A. Note pycnidia and released spores.

TABLE 25 Seedling infection resulting from sowing seed from ovaries with the associated sepals and pedicels infected

Test Method	No. of Seeds Sown	Percentage Seed Germination	No. of Infected Seedlings	Appearance of First Infected Seedlings (days)	Appearance of Last Infected Seedlings (days)
Copenh. Test	121	39	5	26	33
Soil Test	214	31	3	31	39

In both trials first evidence of infection was the lightening in color of the upper surface of the cotyledon to which the testa was still affixed, followed within 36 hours by the appearance of light brown pycnidia. Usually a lesion was produced on only one cotyledon of a seedling (Plate 42) but in a few instances symptoms had developed on the other cotyledon within a further two or three days.

Microscopic examination of the 74 ungerminated seeds in the Copenhagen trial revealed seven to have the pathogen present. In each case the seeds were small, shrivelled and partly covered by associated saprophytic fungi. Positive identification of the pathogen was by pycnidia on the seed and by masses of released cream-colored pycnidiospores. Dense mycelium of contaminant fungi masked the possible presence of the pathogen on other non-germinated seed.

3. Pathogen Survival in Naturally Infected Seed

Seed harvested by hand from ovaries with infected sepals and free of surface contaminants was held at room temperature in a sterile Erlenmeyer flask. At intervals of three calendar months for 15 months seed was removed,

Plate 42 Infected seedling showing cotyledonary lesion and pycnidia.

washed in 4 changes of sterile water and plated to prune agar at the rate of 10 seeds per petri-dish. Results are presented in Table 26.

TABLE 26 Survival of the pathogen in naturally infected seed

Seed Age from Harvest (months)	No. of Seeds Plated	No. of Seeds Yielding the Pathogen	Percent of Seed Infected
3	75	10	13.3
6	84	11	11.9
9	102	6	5.9
12	74	7	9.4
15	63	4	6.3

These results clearly indicate that the pathogen may be viable in imported European seed at the time of sowing in New Zealand. Since at the end of the experiment some of the seeds yielding the pathogen were observed to be germinating it may be concluded that seedling infection in commercial nurseries may arise from use of imported, infected seed sown within 15 months of harvest.

4. Method of Seed Infection

Although lesions and pycnidia may be located on leaves, stems, petioles, pedicels, and sepals they were never observed on the walls of ovaries. Even in cases where all sepals of a calyx were liberally covered with pycnidia and the seed on plating yielded the pathogen, no symptoms were observed that suggested direct penetration of the ovary wall and seed infection by that path. Further, plating of ovary walls to a range of media after various

surface sterilization treatments in no case yielded Septoria colonies. In a few instances ovaries were located with infected sepals closely adhering to the ovary surface, giving the impression that at least some of the pycnidia present had arisen from ovary tissues (Plate 43). In these cases infection of the contained seed could conceivably have occurred directly through the ovary wall. However, close study of each example showed pycnidia to be present only on sepal tissues. Infection of the ovary wall is thus not a feature of this disease and seed infection must occur in some other manner.

Loughnane, McKay and Lafferty (1946) provided proof that infection of flax seed with Septoria linicola was achieved by mycelium advancing from infected sepals to the pedicel at its junction with the base of the ovary, thence up through placental tissues to the immature seed. They found that when bolls borne on diseased pedicels were broken open many of them had pycnidia developed in profusion along the placenta. When seed from such bolls was microscopically examined, incipient pycnidia were observed on the testa in the region of the hilum.

In the present investigation it was noted that where sepal infection occurred pycnidia were almost invariably present on the pedicel as well (Plate 44). This fact, plus the lack of evidence of penetration through the ovary wall suggested the possibility of seed infection in P. drummondii occurring in a manner similar to that of flax seed by S. linicola. Experiments designed to substantiate this hypothesis were conducted as follows. In each case mature ovaries with the associated calyx and pedicel severely lesioned and covered with pycnidia were used.

(a) Seed was harvested by hand and the placenta* removed from the crushed ovary and examined for pycnidia under a stereoscopic

---

*See footnote p.125.

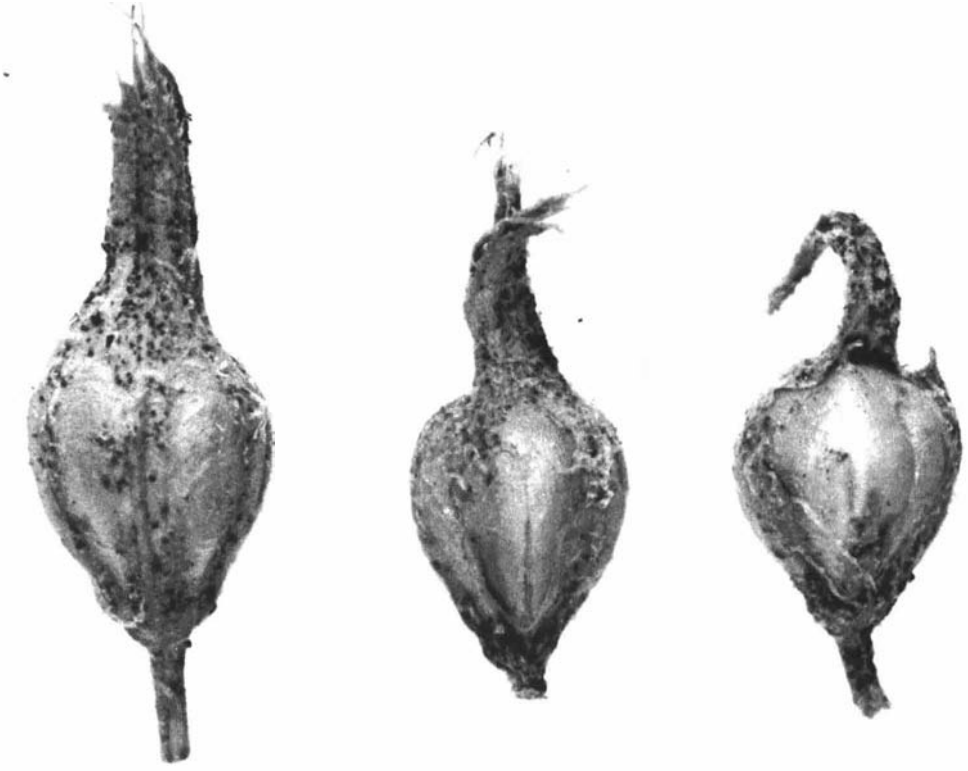


Plate 43 Sepals bearing pycnidia adhering to the surface of matured ovaries from adult P. drummondii plants.

Plate 44 Continuous production of pycnidia on sepals and pedicels from P. drummondii inflorescences.

microscope. In some instances pycnidia were clearly observed (Plate 45). When subjected to high humidity in petri-dishes these pycnidia readily produced cirrhi. Isolates resulting from transfer of such cirrhi to agar were shown to be pathogenic in inoculation trials.

(b) The placentas and seed were removed from 74 ovaries and bulked separately in two sterilised test tubes. Following washing in three changes of sterile water and drying between sterile blotting paper the placentas and seed were plated to prune agar, and incubated at 24°C. Septoria colonies were positively identified from 18 (24.3%) of the 74 plated placentas, and from 21 (9.4%) of the 222 plated seeds.

(c) In a subsequent experiment the placenta and three seeds from each of 41 ovaries were washed separately in two changes of sterilised water and plated to prune agar (Plate 46) with the following results:

Plates free of Septoria colonies	- 19
Septoria from the placenta only	- 12
Septoria from the placenta and one seed	- 6
Septoria from the placenta and two seeds	- 3
Septoria from one seed only	- 1

The above results substantiate the hypothesis that in P. drummondii the path of seed infection is by way of the pedicel to the placenta within the ovary, and thence to the developing seed.

---

*In P. drummondii the ovaries are distinctly triloculate, and placentation is exile with one seed developing in each locule. During maturation of the seed the three interlocular septa remain joined at the centre but individually come free from the outer wall of the ovary. In strict definition the term placenta applies to the area where the funicles are attached but hereafter is used in the expanded sense to denote the total structure consisting of the joined remains of the three interlocular septa and that area where the developing seeds are attached (Fig. 3).

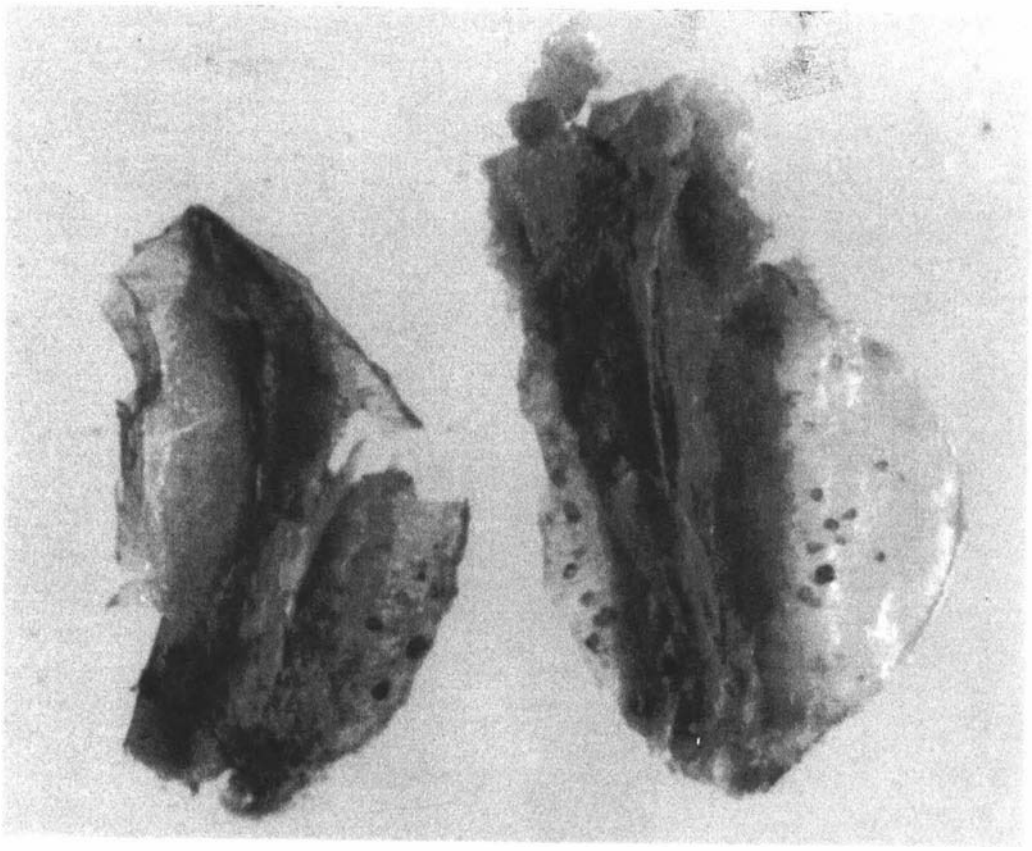


Plate 45 Placentas from two matured ovaries showing presence of pycnidia of the pathogen.

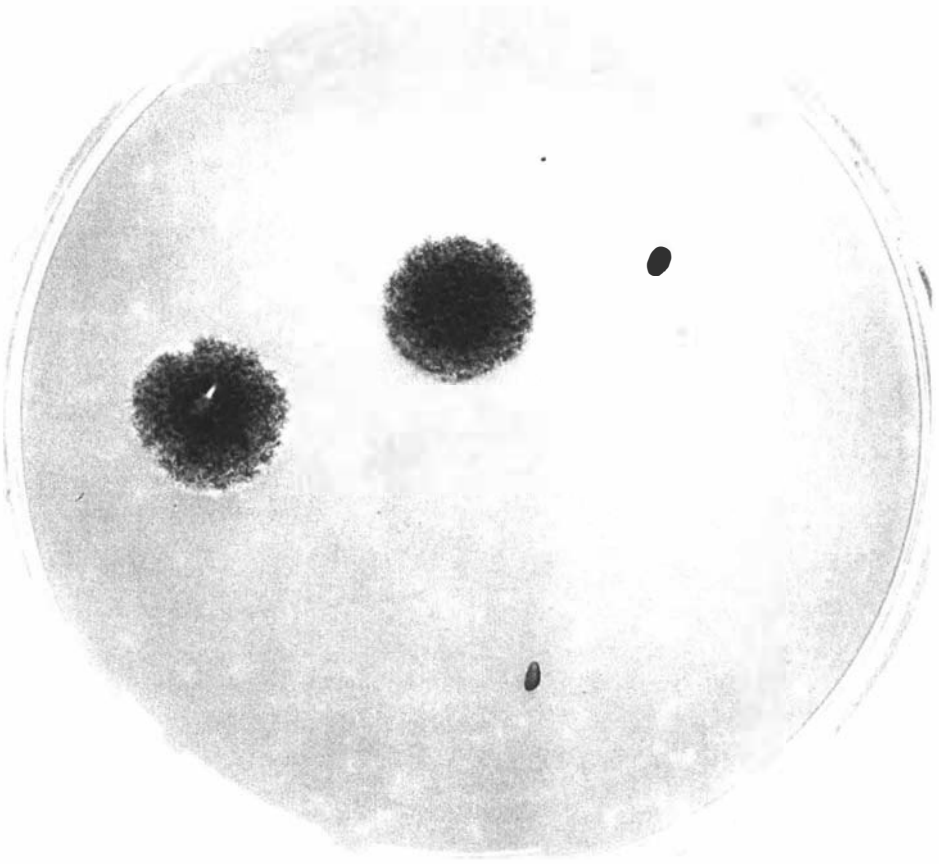


Plate 46 Colonies of the pathogen developing on P.D.A. from the placenta and one of the three seeds from a matured ovary.

FIGURE 3 Diagrams of a mature ovary of *P. drummondii* showing location of the seed in relation to the interlocular septa.

Top - longitudinal section

Bottom - transverse section

KEY

A - remains of stigma and style

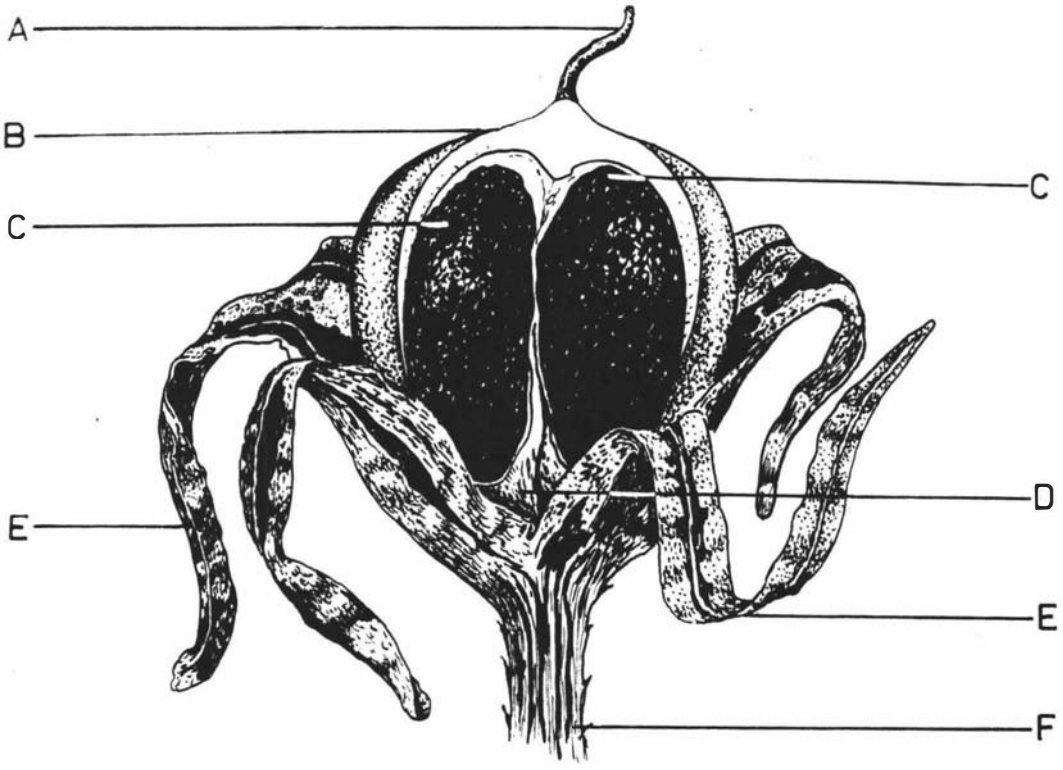
B - ovary wall

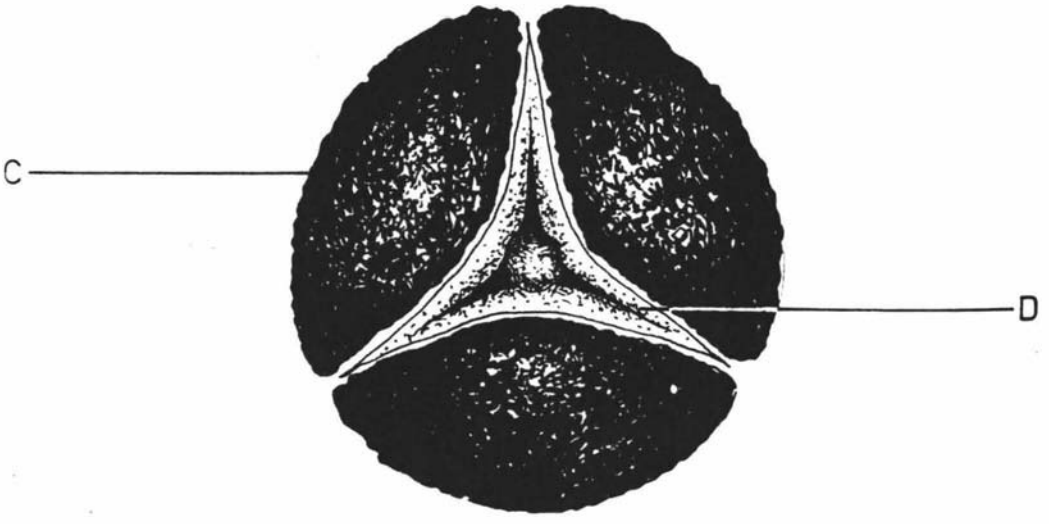
C - seed

D - interlocular septa

E - sepals

F - pedicel


A
B
C
C
D
E
E
F


C
D

5. Artificial Infection of Seed

Seed infection as it is believed to occur in the field was experimentally achieved in the following manner. A series of potted, disease-free plants in full flower were selected after fertilisation had occurred and when seed within the ovaries were at various stages of development. Using a fine camel hair brush the lower surface of the sepals of 37 calyxes were painted with a spore suspension (385,000 spores/ml.) prepared from a ten day old PDA culture. The plants were then held in a humidity cabinet for three days before setting out on a glasshouse bench. Care was taken to apply the spore suspension only to the sepals.

Infection was apparent at twelve days with the appearance of lesions and pycnidia on the great majority of sepals, which soon died. In no instance was there evidence of infection of ovary walls. Forty-seven days following inoculation seventeen ovaries which had matured were removed. The placenta and three seeds from each was washed in three changes of sterile water and plated to prune agar.

On thirteen of the petri-dishes profuse growth of saprophytic fungi arising from the placenta and seeds was so great as to mask the growth of any Septoria which may have been present. However, the remaining four plates gave positive evidence of seed and placental infection, as follows:

Plate 1 - Septoria from the placenta and two seeds.

Plate 2 - Septoria from the placenta only.

Plate 3 - Septoria from the placenta and one seed.

Plate 4 - Septoria from the placenta and one seed.

These results further support the contention that seed infection in the field is by the way of the pedicel to the placenta within the ovary and thence

to the developing seed.

6. Discussion

The above studies have established the fact that seed infection may occur in the field in crops infected with the Septoria leaf blotch disease. Further, it has been shown that when sown, such infected seed may give rise to diseased seedlings. Observations indicate that the transference of infection from seed to seedling is associated with the epigeal manner of germination. During emergence, as the testa is borne aloft the mycelium in the testa resumes growth and invades tissues of the cotyledons. Evidence that infection has occurred is provided by the appearance of lesions bearing pycnidia on one or both of the expanded cotyledons.

The studies on the decline of fungal viability with time in naturally infected seed indicate the pathogen may survive in seed for at least 15 months following harvest, and further, that seed of this age may germinate to produce infected seedlings. That is, the fact that European seed is approximately 10 months old at the time of sowing in New Zealand cannot be used as an argument against the contention that primary infections in New Zealand nurseries may arise from use of infected imported European seed.

The way in which seed comes to be infected in the field has also been established. Observations and experimentation have shown that the pathogen penetrates the ovary via the pedicel, infecting first the placenta and then the developing seed. While this phase of the disease cycle is of considerable importance in that it ensures perpetuation of the disease and its likely establishment in new areas, observations also suggest that the pathogen at this stage may have a considerable adverse effect on seed yield. In experiments where seed harvested from ovaries with sepal lesions and pycnidia were

either sown in Copenhagen germinators or plated to agar the percent of seed germinating was consistently very low, with most of the non-germinating seed being shrivelled and of small size. Further, in agar plating tests it was noted that colonies developed from these non-germinating seeds rather than from the larger, fully developed seeds. Assuming that invasion of an ovary may occur in the field any time between anthesis and seed maturity then there must be considerable variation in the stage of seed development at the time of infection by the pathogen. In the case of immature seed, penetration of the testa and invasion of the embryo would seem likely, resulting in death of the embryo at this stage. In more mature seed only testa invasion would occur with seed development in no way impaired. During seed-cleaning operations the light infected seed would be dressed out. The remaining seed would be fully developed and of high germination capacity, but would include a few seeds with the pathogen established in the testa. It is this latter seed which is believed to initiate epidemic outbreaks of the disease in New Zealand nurseries each spring.

E. Extent of Disease Transmission in Commercial Seed Lines

Appreciation of the significance of seed-borne inoculum in initiating primary infections has in recent years stimulated interest in methods for evaluating seed health. A considerable number of techniques have been devised, the usefulness of each being dependent on the specific objectives of the study and the peculiar circumstances associated with the pathogen/host seed relationship. The suitability of the following methods for screening P. drummondii seed was investigated.

1. Visual Recognition of Infected Seeds

Celery seed infected with Septoria apiicola Speg. may be visually detected

by the presence of pycnidia on the seed surface. Subjecting infected seed to high humidity in petri-dishes lined with damp filter paper causes pycnidia to swell and thereby allows ready recognition under a stereoscopic microscope (Marshall, 1960). Phlox drummondii seed considered likely to be infected was carefully scrutinised on many occasions, but pycnidia were never observed. Seed of this species is dark grey to black and of relatively small size (2 mm. diam. approx.) and pycnidia would be difficult to locate, particularly if immersed or poorly erumpent.

Seed harvested by hand from ovaries where pycnidia were present on the placenta were subjected to high humidity for periods up to five days to induce production of the more readily discernible cirrhi. However, on no occasion were cirrhi observed. Seed from the same source was bleached in a solution of sodium hypochlorite (10.2% available chlorine) for five minutes, soaked overnight in lactophenol cotton blue and microscopically examined for pycnidia. None were observed. In hand-prepared sections of these seeds only free-branching, septate hyphae were located. The testas retrieved from seedlings with lesioned cotyledons have never been observed to have pycnidia present. During Copenhagen germinator tests pycnidia have been observed on seeds, but in all instances this was following at least 20 days incubation and then only on poorly developed shrivelled seed. These seeds showed no evidence of germination and would not normally be present in machine dressed commercial seed lines.

Production of pycnidia in the testa does not appear to be a normal consequence of seed infection, and therefore, recognition of infected seed lines by this means is not possible.

2. Bolley's Centrifuge Technique

This method previously mentioned on p.106 provides a means of removing and identifying surface-borne elements of pathogens and therefore has application only in the study of seed contamination. The method has the advantage of speed but does not reveal the viability or pathogenicity of the fungal elements. This latter restriction may be overcome by supplementing with a pathogenicity test in which the spore concentrate is applied to healthy plants, but even then the results are of limited value. Successful infection merely indicates that loose, viable inoculum was associated with the seed and in no way implies that seedling infection would inevitably result from use of this seed.

3. Agar Test

The first extensive use of an agar plate method to detect seed-borne pathogens was by Christensen and Stakman (1935). Seeds are set out on agar and the pathogens recognised by their cultural characteristics during colonization of the media. In cases where the pathogen is slow growing, as in the present instance, seed is surface-sterilised prior to plating to remove the more rapidly growing associated saprophytes. Thus the method is useful for testing for seed infection, rather than seed contamination. The advantages of plating are that test conditions can be controlled, results are quickly obtained, and relatively little space is utilised. The method has limited value in that it is not a pathogenicity test; the total number of infected seeds in a seed sample, whether alive or dead, is alone indicated. Thus the actual pathogenic value of the seed-borne inoculum is not revealed and a forecast of the field performance of the seed is therefore not possible. The method was not used in the present study because of the time required to plate out sufficient seeds to detect the low percentage infection in many

seed lines under test.

4. Blotter Test

This method involves spacing seeds on moist blotters in an environment of high humidity following which seed and seedlings are inspected for infection symptoms, and for the pathogen itself. Greatest value is where the pathogen induces characteristic disease symptoms on the seedling, the technique then becoming a pathogenic method. In such cases, depending on whether or not the seed is first surface sterilised so the method provides information on total inoculum, or seed infection only.

In the present study Copenhagen germinators proved suitable in blotter tests for seed infection. Seed was either first washed in running water for some hours or surface sterilised in sodium hypochlorite (1% available chlorine) for 30 seconds. Infected seedlings were readily identified by cotyledonary lesions and pycnidia which usually developed within 30 days from sowing. The method is not used in routine screening of commercial seed lines because of the large numbers of seeds which require to be set out to ensure the detection of trace infections.

5. Seed Germination in Sterilised Soil

As a method for routine seed health surveys this approach is impractical because of space requirements and the length of time before results are available. It is, however, extremely valuable in research studies where the number of seed lines to be indexed is limited. It has the considerable advantage of also being a pathogenicity test and therefore reflects field performance of the seed, and allows a quantitative evaluation of seed infection.

Health tests were conducted on 38 imported European seed lines of P. drummondii commercially available for spring sowing in the years 1958, 1959 and 1961, using the following methods:

(a) Three grams of each line was vigorously shaken in 15 ml. sterile water and the wash water centrifuged at 3,000 r.p.m. for ten minutes. Several slide preparations of the concentrated sediment were microscopically examined for spores and pycnidia of the pathogen.

(b) The sediment from each of the above preparations was re-suspended in 20 ml. sterile water and painted over eight leaves of four potted, disease-free plants. Controls were provided by plants similarly inoculated with sterile water. All plants were subjected to high humidity for 56 hours to encourage infection. Subsequent watering of plants was by steeping in trays. Results were recorded 20 days following inoculation.

(c) 4.5 grams (approx. 2,400 seeds) of each seed line was continuously washed in tap water for at least four hours, air dried and sown in a box of sterilised soil. Boxes were set out well spaced on benches, and infected seedlings removed as they appeared until the fiftieth day from sowing.

The results of these three experiments are presented in Table 27, and the data are summarised in Table 28.

TABLE 27 Extent of seed transmission in commercial seed lines of P. drummondii

Seed Sample		Variety	Germn. Capacity 1/.	Experimental Method (a) Centrif. Test 2/.	(b) Pathogenicity Test	(c) Soil Sowing Test No. Infected Seedlings	(c) Soil Sowing Test % Infected Seedlings 3/.
1958	1	Derwent strain, mixed	76	+	-	-	-
	2	Nana compacta, mixed	87	+	-	-	-
	3	Alba oculata	63	-	-	-	-
	4	Grandiflora, mixed	91	++	-	-	-
	5	Nana compacta, mixed	86	-	-	3	0.14
	6	Cuspidata	75	-	-	-	-
	7	Nana compacta, beauty	53	+	-	-	-
	8	Grandiflora, mixed	94	+++	-	4	0.18
	9	Vermillion, red	63	-	-	-	-
	10	Atropurpurea	58	-	-	-	-
	11	Cinnabar	67	+	-	3	0.18
	12	Nana compacta, mixed	89	-	-	-	-
	13	Coccinea	73	-	-	-	-
	14	Scarlet beauty	79	+	+	1	0.05
1959	1	Cinnabar	89	-	-	3	0.14
	2	Grandiflora, mixed	93	-	-	1	0.04
	3	Atropurpurea	95	++	-	-	-
	4	Grandiflora, mixed	96	+	-	1	0.04
	5	Nana compacta, mixed	82	-	-	4	0.20
	6	Derwent strain, mixed	89	+	-	7	0.32
	7	Grandiflora, mixed	74	+	-	-	-
	8	Chamois rose	63	-	-	1	0.06
	9	Scarlet beauty	92	-	-	-	-
	10	Dwarf globe	86	++	+	1	0.05
	11	Nana compacta, mixed	79	-	-	3	0.16
	12	Nana compacta, mixed	73	+	-	6	0.35
	13	Coccinea	64	+	-	-	-
	14	Derwent strain, mixed	92	-	-	2	0.09
	15	Cuspidata	87	++	-	2	0.09
	16	Twinkle stellata	71	-	-	-	-
1961	1	Grandiflora, mixed	92	-	-	-	-
	2	Dwarf globe, mixed	63	-	-	-	-
	3	Twinkle stellata	51	-	-	-	-
	4	Vermillion, red	88	+	-	3	0.14
	5	Grandiflora, mixed	74	-	-	-	-
	6	Nana compacta, mixed	83	-	-	-	-
	7	Nana compacta, mixed	93	+	-	5	0.22
	8	Scarlet beauty	91	+	-	-	-

1/. Determined by the Seed Testing Station, Department of Agriculture, Palm. Nth.
2/. Subjective evaluation of spore numbers observed.
3/. This figure is approximate only, being based on predicted emergence as suggested by the germination capacity test of the seed.

TABLE 28 Summarised data from Table 27

Year	No. of Seed Lines Tested	Number of Lines with Free Conidia	Pathogenicity Test Positive	Soil Sowing Test Positive
1958	14	7	2	4
1959	16	8	1	11
1961	8	3	-	2
Totals	38	18 (47.4%)	3 (0.08%)	17 (44.7%)

These results provide further evidence of the Septoria leaf blotch disease of P. drummondii being transmitted by imported seed, to the extent of 44% of the 38 seed lines tested for the years 1958, 1959 and 1961. As the sterilised-soil method for screening was essentially similar to the manner in which seedlings are produced commercially the results may be considered to accurately reflect likely performance of the seed in nurseries. Seed infection levels were low, in all cases less than 1%. However, because seedlings are densely crowded prior to pricking-out and because the practice of daily overhead watering ensures effective secondary spread of inoculum, one established infection centre in a seed box at this stage is of great significance.

Seed carrying only surface contaminants may be regarded as playing little if any part in transmitting the disease to New Zealand. Although three seed lines were shown to have viable inoculum present in loose form, seedling infection from this source is not likely, for reasons discussed earlier (p.114).

A particularly significant point revealed by the above results is the fact that 26 (68.4%) of the 38 seed lines were harvested from infected parent crops,

as evidenced either by spores present in the wash water or by the development of infected seedlings. This figure is minimal as in some lines spores were probably overlooked because they were present in very low numbers. Since harvesting seed from infected crops seems to be an accepted practice in Europe, seed transmission of the disease may be viewed as a continuing problem in commercial nurseries in New Zealand so long as seed from this source is used.

III EPIDEMIOLOGY

The present study was primarily concerned with taxonomy of the causal organism of the Septoria leaf blotch disease of P. drummondii, and the question of its transmission by means of seed. However the following studies on aspects of the epidemiology of the disease were necessary as a prerequisite for pathogenicity experiments, and to further explain the appearance and development of the disease under New Zealand conditions.

A. Humidity and Establishment of Infection

Early observations indicated that a period of high humidity following inoculation was essential for establishment of infection. The following experiment was designed to determine the length of time plants need be exposed to a saturated atmosphere following inoculation for infection to result.

Sixteen 5 inch pots each containing three P. drummondii plants were sprayed to run-off point with a spore suspension of strength 57,000 spores/ml., the inoculum being prepared from PDA first generation cultures. Following inoculation all except two pots were placed in a humidity cabinet. Thereafter at 12 hour intervals and until 84 hours the high humidity treatment of two further pots was terminated by removing them to the glasshouse bench. For each series a record was taken of when symptoms first appeared, and on the twentieth day following inoculation all plants were indexed for infection. Results are presented in Table 29.

The results indicate that plants exposed to high humidity following inoculation for less than 36 hours failed to develop symptoms, and that there was a gradual increase in the severity of infection as the exposure time was increased from 36 to 60 hours. Exposure to high humidity also markedly

TABLE 29 Effect of humidity following inoculation on infection and disease severity

Exposure Time (- in hours)	Disease Severity Rating [a]	No. of Days Following Inoculation for First Symptoms
0	0	-
12	0	-
24	0	-
36	+	17
48	++	14
60	+++	10
72	+++	10
84	+++	7

[a] Severity ratings taken at 20 days after inoculation, and based on the following subjective scale:

0 = no infection
+ = trace infection
++ = moderate infection
+++ = severe infection

influenced the length of the incubation period, longer exposures having the effect of shortening the time interval between inoculation and disease expression.

In subsequent glasshouse pathogenicity trials plants were subjected to continuous high humidity for 2½ - 3 days following inoculation.

B. Inoculum Potential

The concept of an inoculum potential has been fully reviewed in recent years (Dimond & Horsfall 1959, Garrett, 1960). The basic thought is that there is a minimum spore density, the so-called numerical threshold for infection which is necessary for disease establishment under favourable conditions. Since environmental conditions in the field are rarely stable this threshold level will vary considerably. Ideally, glasshouse experiments on this subject should be conducted with strict control of humidity and temperature, with the inoculum density the only variable. As controlled climate facilities were not available precise exporimentation was not possible. However, one trial was conducted with the objective of identifying the minimum spore strength necessary for routine inoculation experiments. Observations were also made on the influence of inoculum strength on disease severity and symptom expression.

A bulk spore suspension of strength 111,000 spores/ml was prepared from first generation PDA isolates from field diseased P. drummondii plants. Ten suspensions of varying strengths were prepared from this parent suspension, the lowest concentration being 120 spores/ml. Using each concentration nine, five week old potted plants were sprayed to run-off point, and all pots placed in a humidity cabinet for 3 days before returning to the glasshouse bench. At 20 days following inoculation each series was indexed for infection. The experiment was conducted during the month of July in a heated glasshouse where temperatures ranged from 52° to 73°F. Results are presented in Table 30.

Although a concentration of 1,000 spores/ml was sufficient to establish infection, at this strength less than 10 lesions developed on any of the nine inoculated plants. As the inoculum concentration increased so the number of

TABLE 30 Influence of spore concentration on infection

Inoculum Density (spores/ml)	Severity of Infection
111,000	Very severe
57,000	Very severe
33,000	Very severe
17,000	Severe
13,000	Severe
6,000	Moderate
3,000	Moderate
1,000	Trace
500	-
120	-

lesions per plant increased until at 33,000 spores/ml spots readily coalesced, followed by death of many of the leaves. Symptoms were slower appearing at the lower concentrations but lesions were larger in size than those at the higher concentrations. Other than this influence on lesion size the inoculum strength did not affect the nature of symptoms.

In subsequent work involving plant inoculations a minimum inoculum density of 20,000 spores/ml was used.

C. Host Penetration

The manner of leaf penetration in *P. drummondii* was investigated by the excised leaf method. Young leaves from disease-free plants were inoculated by painting limited areas of both the lower and upper surface with a concentrated spore suspension prepared from field diseased plants. A saturated atmosphere was maintained by placing the leaves on slides in petri-dishes lined with moistened filter paper. At intervals leaves were removed, cleared and stained. Of the several methods and modification tried for clearing and staining the following proved highly satisfactory, the spores, germ tubes and young hyphae being well stained and readily observable against the cleared-leaf background. Leaves were first gently boiled in a 1:1 solution of glacial acetic acid and 95% alcohol (Latch & Hanson, 1962) until the chlorophyll was lost and they became semi-opaque. The leaves were then rinsed by gently agitating in a beaker of water to which a few drops of alcohol had been added to encourage release of trapped air bubbles and ensure complete wetting of the leaf surface. Finally the cleared leaves were transferred to slides and stained for approximately five minutes by flooding with lactophenol acid fuchsin.

Leaf penetration occurred only by way of stomata, and without the formation of appressoria (Plate 47). In no instance was there evidence of direct penetration of the epidermis. During the initial stages the pattern of spore germination was similar to that observed on agar slides. Germ tubes invariably developed from one or both end cells of spores, were of the same diameter as the spores and did not branch until several times the length of the parent spore. Growth seemed to be indiscriminate in that germ tubes appeared not to be attracted to the stomata. Frequently hyphae grew alongside or over

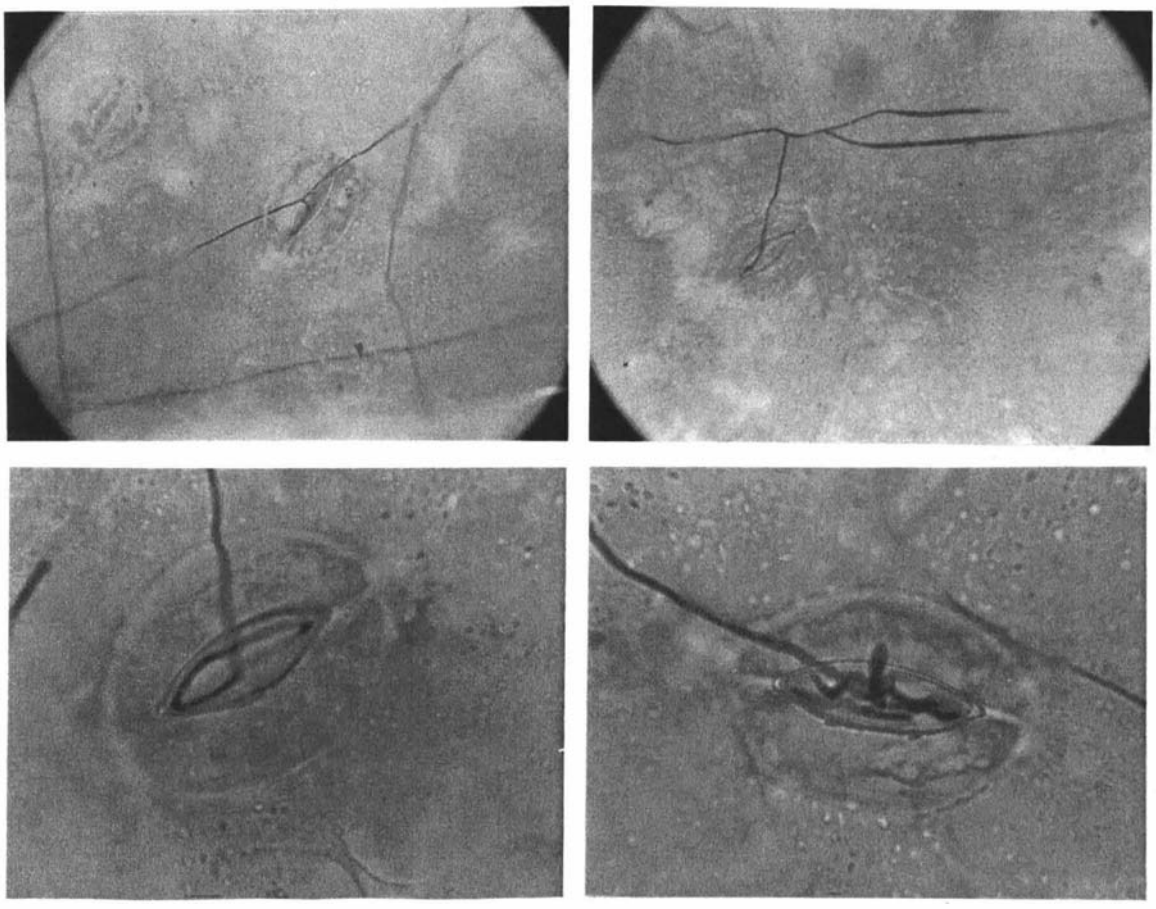


Plate 47 Stomatal penetration of a P. drummondii leaf by hyphae from germinating pycnidiospores

stomata without entering. Only rarely was growth of germ tubes or young hyphae terminated by entering a stoma. More usually entry was by a penetration hyphae developed from a main hypha which continued growth on the leaf surface.

D. Spread of Infection

Wide dispersal of species of Septoria by air-borne asexual spores does not occur because released pycnidiospores are held together in a gelatinous, water-soluble matrix. However, dissemination to distant localities has been shown to occur by wind blown infected debris bearing pycnidia (Weber 1922, Lutey & Fezar 1960). Once infection is established leaf-wetting or persistent high humidity induces the production of cirrhi and thereafter localised spread is by pycnidiospores dispersed by any mechanical means. Christensen, et al. (1953) for example, demonstrated in Minnesota that at least 20 species of insects were important agents in the dissemination of Septoria linicola and concluded that - "any creature that walks, runs, jumps or crawls through infected fields when plants are wet is an agent in the spread of the fungus, because animals such as spiders, mice, frogs, birds and dogs have been shown to disseminate spores". The effectiveness of rain in localised spread of Septoria chrysanthemi has been demonstrated by Dimock (1942) who found that direct splashing of pycnidiospores from soil may reach a height of 18 inches. Dimock (1951) also found that pycnidiospores of this species were readily dispersed by insecticidal sprays, most of which had little or no effect on the viability of the spores.

Observations on the commercial production of bedding plants in the Manawatu pointed to the probable importance of water splashing in local spread of Septoria infections of P. drummondii in nurseries. Seedlings are in close

proximity to each other and the practice of daily overhead watering seemed likely to ensure free movement of pycnidiospores to new infection courts. This subject was investigated as follows in a glasshouse which had not previously held P. drummondii plants.

Sixteen boxes were each pricked-out with 72 healthy seedlings from a sowing of seed previously shown to be pathogen-free. One seedling towards the centre of 12 boxes was then replaced by an infected seedling with one or both cotyledons lesioned and bearing pycnidia. The remaining four boxes were used as controls. All boxes were set out on glasshouse benches so that at least 10 yards separated any two boxes, and thereafter were watered daily. In the case of the four control boxes and six of the remaining boxes watering was by overhead application using a fine spray hose, following common nursery practice. The remaining six boxes were watered by steeping in trays. At 15, 30 and 45 days a count was made of the number of infected plants in each box. The presence of one lesion bearing pycnidia was sufficient to rate a plant as being infected. Results are presented in Table 31.

The importance of overhead watering in local dispersal of inoculum is well demonstrated in the above experiment. In the six boxes watered in this way all plants were infected within 45 days. Growth was noticeably arrested and at the conclusion of the experiment all plants were quite unfit for sale. On the other hand there was relatively little development of infection in those boxes watered by steeping in trays, and most of this occurred when plants were well advanced. At that stage the crowding of the plants ensured high localised humidity which in turn favoured infection from the relatively few spores which may have been mechanically dispersed.

It is not known by what means inoculum reached the one control box in which symptoms appeared.

TABLE 31 The effect of watering method on spread of infection among seedlings in seed boxes

Box No.	Watering Method	No. of Infected Plants		
		15 days	30 days	45 days
1	overhead hosing	18	63	72
2		13	71	72
3		17	66	72
4		31	76	72
5		21	72	72
6		13	58	72
7	steeping in trays	4	10	31
8		6	17	26
9		3	12	19
10		8	21	33
11		4	14	25
12		2	20	53
Control Box				
A	steeping in trays	0	0	0
B		0	0	0
C		0	0	12
D		0	0	0

E. Field Survival Between Seasons

Infected volunteer seedlings were frequently observed in the early spring in flower beds which had supported infected plants during the previous autumn. The fact that first symptoms appeared on cotyledons strongly suggested such seedlings had arisen from fallen infected seed. There was also the possibility however, of survival as vegetative mycelium in soil, or as viable pycnidiospores in pycnidia associated with crop debris, and that primary infections among volunteer seedlings was from this source.

The question of field survival of the fungus and possible sources of primary inoculum was investigated as follows:

(a) Survival as Seed-borne Inoculum

Ten boxes of infected pricked-out seedlings were permitted to flower and set seed during the summer and the disease encouraged by regular overhead watering. In all boxes lesions with pycnidia developed on leaves in the immediate vicinity of the flower heads and matured ovaries were observed to shatter, with the seed falling to the soil. In March all plants were removed and the boxes of soil stacked in a glasshouse. Five months later (August) and thereafter at intervals of one month one box was steeped in a tray of water. Subsequent watering was always by steeping in trays. For each box a count was taken of healthy and infected P. drummondii seedlings that emerged during the first sixty days following initial watering. Once positively identified infected seedlings were carefully removed. Results are summarised in Table 32.

The results show that following removal of an infected crop the pathogen can persist in soil and in the absence of growing plants for at least 14 months. The fact that in all cases infection was first apparent on cotyledons

TABLE 32 Seedling infection resulting from infected seed held over in soil

Seed Box No.	Time in Storage (months)	Total Emerged Seedlings	No. of Infected Seedlings
1	5	64	9
2	6	11	2
3	7	56	11
4	8	49	5
5	9	73	5
6	10	66	7
7	11	121	18
8	12	52	7
9	13	60	3
10	14	87	5

again strongly suggests survival during this period was by means of infected seed which fell towards the end of the previous season. The circumstances of this experiment do not reflect field conditions in that boxes were permitted to dry out and soil temperatures in the glasshouse were higher than outside. However, the observations mentioned earlier of infected seedlings appearing in the spring following a diseased crop indicate the glasshouse conditions of the present experiment did not necessarily enhance the changes of carry-over between seasons in this way.

(b) Survival as Free Living Mycelium in Soil

During periods of rain large numbers of spores released from pycnidia on infected plants are washed to the soil. As spores no longer associated with pycnidia are short lived such spores may not function as primary inoculum the following spring. Conceivably however, pycnidiospores reaching the soil in the autumn could germinate, the fungus then becoming established as a free-living soil saprophyte not in association with host tissues. In the event of this occurring primary infections the following spring could well originate from rain splashed inoculum in the soil reaching the lower leaves. This possibility was investigated in the following manner.

Six new seed boxes of field soil were each inoculated by adding spores produced on eight PDA cultures incubated at 24°C for 15 days. The inoculum was prepared by flooding the plates with sterile distilled water, gently disturbing the surface with a glass rod to encourage spore dispersal, and passing the liquid through three layers of muslin. The soil was garden loam collected from an area which had not previously supported ornamental plants. Three boxes similarly prepared were inoculated with sterile water. All boxes were set out well spaced in a farm area and allowed to weather through the winter. Six months later (September) each box was thickly sown with seed previously shown to be pathogen-free. Throughout the winter and subsequent to seed sowing seedlings other than P. drummondii were removed.

In all boxes there was good establishment of P. drummondii plants all of which remained disease free until termination of the experiment. A similar experiment the following winter using three seed boxes and inoculum from double the number of plates gave identical results.

From the above results it is concluded that free pycnidiospores reaching the soil are of no significance in survival of the pathogen between seasons.

(c) Survival in Plant Debris in Soil

A previous experiment had shown that some spores in pycnidia on leaves could remain viable for at least 15 months when stored in the laboratory. The following experiment was designed to reveal not only whether similar survival could occur in the field but also whether seedling infection could arise from such inoculum.

The dry lower leaves of heavily infected P. drummondii plants were collected in March, crumbled, and mixed with field soil. This mixture was then used to form the upper inch of soil in six new seed boxes previously filled with field soil from another location. Three boxes of the same field soil but without the addition of infected material were used as controls. All boxes were then set out at least 50 yards apart in an area remote from ornamental plantings and allowed to weather through the winter. The following August each was thickly sown with P. drummondii seed previously shown to be pathogen-free.

Thirty-two days from sowing the disease was positively identified by the appearance of lesions and pycnidia on one plant in each of four of the inoculated boxes. Seven days later the disease was identified in the remaining two inoculated boxes. In no instance were cotyledonary lesions observed. Within three months from sowing all plants in all six inoculated boxes were heavily infected. Plants in the control boxes remained disease free.

These results indicate that viable inoculum may persist through the winter as spores in pycnidia associated with crop debris. The following spring, with production of cirrhi during periods of high humidity, primary infections could result from splashed spores reaching the lower leaves. However, debris from an infected crop will be wetted many times through the

winter, possibly exhausting the inoculum supply by the spring. There is thus the question of whether pycnidia in infected debris can in fact liberate spores after repeated wetting over a period of several months. This point was investigated as follows.

(d) Release of Spores from Pycnidia Wintered in Soil

Severely infected P. drummondii leaves collected in March were subjected to high humidity for 36 hours, agitated in running water to remove cirrhi, air dried and stored in envelopes in the laboratory. One calendar month later the leaves were again subjected to high humidity for 24 hours, and a spore suspension prepared from cirrhi which developed was used to inoculate healthy glasshouse plants. The stock leaves were washed with water again to remove remaining spore horns, air dried and stored as before. This procedure was continued at monthly intervals through until November, using the original infected leaves throughout.

Some cirrhi were produced following each high-humidity treatment; and symptoms typical of the disease developed on plants following each inoculation, whereas control plants remained disease free. It seems clear therefore that under field conditions the pathogen may persist through the winter in crop debris even after repeated wetting over a period of seven months, and that released pycnidiospores may function as primary inoculum to initiate infections in the following growing season.

A recent report by Scharen (1966) discusses the ability of Septoria modorum Berk. to produce pycnidia and spores in dead tissue. Scharen found that following repeated wetting and drying of naturally infected wheat leaves collected several months earlier there was replenishing of spores in the older pycnidia so that additional spores were produced each day. Further wetting of the leaves at intervals induced the formation of new pycnidia. Thus it

was shown that plant debris left in the field after harvest not only harboured the fungus but also served as a medium for growth with a resulting increase in the inoculum potential. A similar situation could well exist in the case of infected P. drummondii plants. That is, not only may the pathogen survive between seasons as pycnidiospores in pycnidia associated with crop debris, but there is also the possibility of an increase in the inoculum just so long as the debris remains intact. However for new season infection to result from this source requires that plants be available in the immediate vicinity, since spore dispersal is mainly by splashing during wet weather.

IV DISCUSSION AND CONCLUSIONS

In attempting to identify the species of *Septoria* causing the leaf blotch disease of *P. drummondii* it became necessary to consider the taxonomic status of all described *Septoria* species pathogenic to the genus *Phlox*, regardless of the host species involved. The problem was approached by critically examining the type collections and other herbarium material, and studying New Zealand and overseas collections of both *P. drummondii* and *P. paniculata* purported to be infected by one or other of the three erected species of *Septoria*. The possibility of separating species on the basis of cultural characters was also investigated by a series of comparative studies of isolates from the above mentioned field collections. Morphology of the pathogen in host tissues and dimensions of pycnidia and pycnidiospores were the features particularly considered.

The findings have already been discussed, and in effect provide no evidence of the existence of more than one species of *Septoria*. It is considered that collectively the material examined represents a completely integrating series which cannot be morphologically separated. Accordingly, it is recommended that a single species be recognized pathogenic to the genus *Phlox* and that on the basis of priority this be

Septoria phlogis Sacc. & Speg., Michelia 1 : 184 (1879),
with synonymy as follows:

Septoria divaricata Ell. & Ev., Jour. Mycology 5 : 151 (1889)

Septoria drummondii Ell. & Ev., Jour. Mycology 7 : 133 (1892)

Septoria longispora Vogl., Atti. R. Acad. Sc. Torino XLIII (1907-08)

According to Bisby (1945) the task of the taxonomist is to -

(a) arrange categories of species, genera and families so as to facilitate identification; and

(b) present a 'natural' arrangement on the basis of phylogenetic relationships.

Septoria is a Deuteromycete genus and as such constitutes a convenient grouping of organisms showing morphological similarity of their conidial stages, but which are unrelated. That is, classification within the Deuteromycetes is wholly artificial and the sole objective in any taxonomic treatment of a member genus must therefore be that of providing a practical, convenient system of species delimitation so as to enable ready identification. The present arrangement of Septoria species falls far short of this ideal. More than 1,000 species have been erected and in most cases the original descriptions are of single field collections with no allowance made for either inherent or environmentally induced variability. Separations have been made primarily on the basis of slight differences in the dimensions and septations of spores, with host association also extensively used. In the earlier days of descriptive mycology a widely held concept was that fungi belonging to the same genus but parasitising different host genera or species were of necessity distinct species of pathogen. As a consequence a multiplicity of "species" were erected, separated on a host basis and correlated with only minor morphological differences. Thus species of Septoria as presently defined so overlap one another in morphological features as to prohibit determination from the descriptions available. This becomes evident when one inspects Saccardo's "Sylloge Fungorum", where the spore length of nearly 700 of the Septoria species described fall within the limits of 20 to 50 u (Beach, 1919).

Although there is frequent reference to the chaotic state of species differentiation in Deuteromycete genera it is only the genus Fusarium which

has been critically revised in the light of twentieth century knowledge of fungal genetics and variability. In a series of three papers Snyder and Hansen (1940, 1941, 1945) forcefully advocated acceptance of a nine species scheme based strictly on morphological features to replace that of Wollenweber and Reinking (1935) which involved 65 species and 55 varieties and which had proved to be largely unworkable. By use of the single spore technique Snyder and Hansen determined the extent of variability in Fusarium populations and concluded the only characters sufficiently stable to serve as taxonomic criteria were shape of macroconidia, presence of microconidia, and presence of chlamydospores. On such morphological criteria they differentiate their nine species. Physiological characters were recognised also, but used only to indicate intra-specific categories. Thus formae speciales are diagnosed solely by pathogenicity tests, and physiological races within formae are identified on the basis of their relative pathogenic capabilities to a series of differential varieties of the host species. The wide acceptance of this scheme over the past 20 years without significant changes is evidence of the simplicity and convenience of the scheme. The only refinement has been the employment of "cultivar" names at the sub-specific level to accommodate clonal groups that show some distinctive features that are of importance to the plant pathologist (Snyder, Hansen and Oswald, 1957).

The Snyder and Hansen classification of the fusaria has relevance to the present study as it provides a working example of the current trend towards consolidation of species and suggests the pattern that could well be followed in revision of the whole genus Septoria. The first step would be an experimental analysis of variability in field collections, and the identification of those morphological characters least prone to genetic variation

and most stable under changing environmental conditions. Species differentiation would be strictly on the basis of such morphological criteria, and this would inevitably lead to consolidation of many previously erected "species" and necessitate changes in taxonomy and nomenclature. As regards categories below the species level, these could be differentiated on the basis of their restricted pathogenicity and be designated as trinomials, as Snyder and Hansen have done for the fusaria. There would appear to be no difficulty in this since in his studies on biological specialization in 15 species of Septoria Beach (1919) found that the host range did not usually extend beyond the limits of a genus, or in some cases included only two or three genera.

The studies on seed transmission have shown that the Septoria leaf blotch disease is transmitted in commercial lines of P. drummondii seed imported from Europe. Further, it has been established that epidemic outbreaks regularly experienced in local nurseries are a direct consequence of sowing such infected seed. These studies have also provided other essential information on the disease cycle, which is now considered to be as follows.

Transmission of the disease by seed may occur when seed is harvested from an infected crop. Inoculum may be associated with the seed either in free form (seed contamination) or as vegetative mycelium established within seed tissues (seed infection). Seed contamination occurs during harvesting and threshing in the course of which both spores and pycnidia are released from infected tissues and become effectively distributed among the seed. Although inoculum of this type is frequently present in commercial seed lines it is of no significance in accounting for seedling infections in New Zealand. The time lapse of approximately 10 months between harvesting in Europe and

sowing in New Zealand is in excess of the maximum time that spores freed from pycnidia can remain viable. Some spores within pycnidia in crop detritus associated with seed may remain viable for 10 months following harvest but the decline in viability is so rapid after eight months as to make it extremely unlikely that an inoculum load necessary for infection will be present.

Seed infection may occur at any stage between anthesis and seed maturity, with entry of the ovary being achieved by mycelial growth from the infected pedicel to the placenta, and thence to the developing seeds. As a consequence there may be considerable seed loss at this time, the loss depending upon the extent to which the disease is established throughout the seed crop and the stage reached in seed development at the time contact is made with the fungus. If the pathogen reaches the seed early in its development the embryo is invaded and the seed killed before attaining normal size. Such seed is light and is removed during seed-cleaning processes. On the other hand, fully developed seed with the pathogen established in the testa is not dressed out. It is such infected seed that provides primary infection foci in nurseries.

Appreciation of the significance of even very low levels of seed infection requires an understanding of the procedures and circumstances associated with the commercial production of seedlings. P. drummondii is used as a spring bedding species in the Manawatu, first sowings usually being made in early September. Seed is sown in boxes (20" x 15") and the seedlings pricked-out approximately 30 days later to boxes of the same size at the rate of 56–80 plants per box. Within two weeks the boxes are transferred to cold frames for hardening-off and at nine weeks from sowing are normally ready for sale. Seed sowing rates are excessively high. In tests it was determined that approximately 3,000 seeds are sown per box, and assuming a 75% emergence, a dense

stand of some 2,000 seedlings occupies a soil surface area of only 300 square inches. This intense crowding inevitably causes seedlings to be drawn and soft, and the practise of daily overhead watering ensures high localised humidity among seedlings at all times.

First infections are in evidence at about 30 days from sowing, which coincides with the time seedlings are pricked-out. Symptoms are lesions with pycnidia on one or both cotyledons and are the result of mycelial infection from the testa during epigeal germination. Since over 70 pycnidia are frequently present on one infected cotyledon at this stage, each producing many hundreds of spores, a high inoculum potential is immediately available. The persistent high humidity causes spores to be released in cirrhi and overhead watering ensures local dispersal of the inoculum by splashing. The close proximity of seedlings results in spores reaching many plants and the establishment of numerous secondary infections. That is, at the time of pricking-out many of the young plants are infected, although as yet there is no expression of symptoms. However, lesions and pycnidia soon develop and with further disease cycles the stage is reached where all plants of a box are severely infected.

It must be stressed that the actual level of infection in commercial seed is relatively unimportant in that the presence of one infected seedling immediately prior to pricking-out is sufficient to enable epiphytotic development of the disease within a nursery. Further it should be noted that the presence of inoculum with seed is of significance only as a means of providing initial infection foci for secondary spread. That is, seed-borne inoculum does not affect stand establishment by inducing either pre-emergence or post-emergence damping-off.

In the Manawatu the disease is of only minor importance in established

garden plantings, primarily because of the restrictive influence of the summer climate. Following prolonged wet weather there may be a temporary build-up of infection but only rarely is the damage sufficient to warrant the application of protective fungicides. This is in direct contrast to the situation in nurseries where losses are such that control of the disease is a recurring problem each year.

A first principle of plant pathology is that only pathogen-free seed be used in establishing crops. While this statement has general application it is particularly true in those cases where other sources of primary inoculum do not exist or are extremely uncommon, and where the pathogen has only a limited capacity for secondary spread between crops. In such instances breaking the disease cycle by the simple expedient of using only healthy seed is likely to provide the most practical means of control. Septoria leaf blotch of P. drummondii is a disease in this latter category. Other than by means of infected seed, survival between seasons is known to occur only in intact crop debris. However this is regarded as of minor importance as a source of inoculum in the following season because of the limited distance over which rain-splashed spores can be dispersed. Unless new season plantings are in the immediate vicinity of infected crop debris such inoculum will not be effective.

Theoretically, air-borne sexual spores may be produced and serve as an alternate form of primary inoculum, but no evidence has been found to suggest that this fungus reproduces in this way. In spite of a thorough search extending over several years a perfect stage was not found. Thus it may be safely assumed that either a sexual stage is not produced, or is so rare as to be of no significance in the disease cycle. That is, the pathogen is adapted to survival by way of seed, and any consideration of disease prevention and control

must therefore initially centre on the question of pathogen-free seed.

Seed will in fact be disease-free if harvested from healthy crops, or if treated in some way to inactivate the associated pathogens. Various seed treatments have been devised for specific diseases, of which the hot water method is the most commonly used in cases of seed infection. A temperature/time combination could probably be determined for treating P. drummondii seed, but there is still the problem of identifying infected seed lines requiring treatment. Visual examination of seed lots is of no help, and routine germination tests give no indication of the health condition of a seed line. A laboratory method for seed indexing could possibly be devised but to be reliable the sample size would need to be large since the levels of infection are invariably low.

Control by seed treatment is only a means to an end however, and avoids the real issue, namely, the production and use of seed harvested only from disease-free crops. This of course is the ideal and in effect requires that seed crops be raised in areas where the climate is such that the disease cannot flourish. In this regard it is perhaps significant that 18 P. drummondii seed lines of North American origin have been tested over several years by the soil sowing method and found to be free from infection. Further, during September and October 1964 the writer inspected seed crops in Santa Barbara County, California of several major American seed houses and found no evidence of Septoria leaf blotch. It is not suggested that immediate control of the disease will result from use of only American seed, but since 26 (68.4%) of 38 seed lines of European origin screened over a three year period were shown to be harvested from infected crops it would seem a logical precaution in the meantime for New Zealand seed firms to import P. drummondii seed from America, rather than Europe.

SUMMARY

1. A *Septoria* leaf blotch disease of *P. drummondii* is prevalent in the Manawatu but economically important only in commercial nurseries.

2. Experimental evidence has established that the causal fungus is seed-borne in *P. drummondii* seed lines imported from Europe, and that seedling infections in commercial nurseries are consequent on the use of such seed.

3. Although world-wide in distribution the disease has not previously been reported as seed-borne.

4. Both seed infection and seed contamination occurs but only the former is of significance in New Zealand.

5. In infected seed the pathogen is established as vegetative mycelium; pycnidia are not produced in seed tissues.

6. In seed crops the path of seed infection is by way of the pedicel to the placenta within the ovary and thence to the developing seed.

7. Cotyledonary lesions are the result of mycelial invasion from the testa of infected seed during epigeal germination.

8. Infected seed was present in 17 (44.7%) of 38 European seed lines of *P. drummondii* indexed over a three year period. Twenty-six (68.4%) of the seed lines were harvested from infected crops.

9. In the Manawatu the fungus can persist through the winter in intact debris and this constitutes a possible source of primary inoculum for field infections.

10. The question of controlling seed-borne inoculum is discussed.

11. The morphology of the fungus in culture and in tissues of New Zealand and overseas P. drummondii and P. paniculata collections is described in detail. Pycnidia are dark-brown to black, globose to pyriform, clearly ostiolate, 47-288 /u in diameter; pycnidiospores are hyaline, filiform, usually guttulate, 0-5 septate (mostly 3), 17-73 x 1-3 /u.

12. On PDA secondary conidia (macroconidia) are produced in profusion. The possible role of secondary conidia in the disease cycle is discussed.

13. The taxonomic status of Septoria species pathogenic to the genus Phlox was investigated. A comparative study of type collections, herbarium collections and field material indicated that on the basis of morphological criteria only one species should be recognised.

14. Septoria phlogis Sacc. & Speg., published in 1879 has priority over other species epithets and is proposed as the correct name.

BIBLIOGRAPHY

AINSWORTH, G.C. and G.R. BISBY, 1961. A Dictionary of the Fungi. Comm. Mycol. Instit., Kew, Surrey. 547 pp. (5th ed.)

ALEXOPOULUS, C.J., 1962. Introductory Mycology. John Wiley & Sons, Inc. New York. 401 pp. (2nd ed.)

BEACH, W.S., 1919. Biological specialization in the genus Septoria. Amer. Jour. Bot. 6 : 1-34.

BISBY, G.R., 1945. An Introduction to the Nomenclature of Fungi. Comm. Mycol. Instit., Kew, Surrey. 143 pp. (2nd ed.)

BLAIR, J.D., 1962. Studies on some diseases of plants indigenous to New Zealand. II Septoria myopori Cooke & Massee. Trans. Roy. Soc. N.Z. 1 : 231-233.

BOLLEY, H.L., 1902. The use of the centrifuge in diagnosing plant diseases. Soc. Prom. Agr. Sci. Proc. 23 : 82-85.

BOND, T.E.T., 1941. A leaf spot disease of annual Phlox. Trop. Agriculturist 46 : 142-146.

_____ 1952. Septoria ameriae Allesch., the cause of a leafspot disease of 'Bees Ruby' Ameria. Trans. Brit. Myc. Soc. 35 : 81-90.

BRANCATO, F.F. and N.S. GOLDING, 1953. The diameter of the mould colony as a reliable measure of growth. Mycologia 45 : 848-864.

BRIEJER, C.J., 1965. Pers. comm.

BRIEN, R.M., 1939. A list of plant diseases recorded in New Zealand. Bulletin No. 67. Dept. Sci. Industr. Res. 39 pp.

BRIEN, R.M. and Joan M. DINGLEY, 1955. Second Supplement to "A revised list of plant diseases recorded in New Zealand". 1952-1955. N.Z. J. Sci. Tech. 37A : 1.

CHRISTENSEN, J.J. and E.C. STAKMAN, 1935. Relation of Fusarium and Helminthosporium in barley seed to seedling blight and yield. Phytopath. 25 : 309-327.

COLE, H. and H.B. COUCH, 1958. The etiology and epiphytology of northern anthracnose of red clover. Phytopath. 48 : 326-331.

CRUICKSHANK, J.A.M., 1954. Thermo-chemical seed treatment. Nature 173 : 217-218.

DIMOCK, A.W., 1942. Controlling Septoria leaf spot of chrysanthemum. Bull. Chrysanth. Soc. Amer. 10 : 6-11.

_____ 1951. The dispersal of viable fungus spores by insecticides. Phytopath. 41 : 152-156.

DIMOND, A.E. and J.G. HORSFALL, 1959. Plant Chemotherapy. Ann. Rev. Plant Physiol. 10 : 257-276.

DRECHSLER, C., 1923. Some graminicolous species of Helminthosporium. J. Agr. Res. 24 : 641-739.

ELLIOTT, J.A., 1917. Taxonomic characters of the genera Alternaria and Stemphylium. Amer. Jour. Bot. 4 : 439-476.

ELLIS, J.B. and B.M. EVERHART, 1889. New and rare species of North American fungi (Sphaeropsideae). Jour. Mycol. 5 : 145-157.

_____ 1892. New species of fungi. Jour. Mycol. 7 : 130-135.

FRIES, E., 1828. Elenchus fungorum, sistens commentarium in systema mycologicum. Vol. 2, 154 pp. (Gryphisewald).

_____ 1832. Systema mycologicum. Vol. 3, 480 pp. (Gryphisewald).

GARRETT, S.D., 1960. 'Inoculum Potential' Ch. 2 in Plant Pathology Vol. III (ed. by Horsfall & Dimond). Academic Press, N.Y. 715 pp.

GABRIELSON, R.L. and R.G. GROGAN, 1962. Survival of the celery late blight organism. Phytopath. 52 : 361.

_____ 1964. The celery late blight organism Septoria apiicola. Phytopath. 54 : 1251-1257.

GLOYER, W.O., 1931. China aster seed treatment and storage. N.Y. State Ag. Exp. Sta. Tech. Bull. 177, 41 pp.

GOODING, G.V. and G.B. LUCAS, 1959. The effect of inoculum level in the severity of tobacco black shank. Phytopath. 49 : 277-281.

GREENE, H.C., 1951. Host Index of Parasitic Fungi Collected on Plants in Wisconsin, 1880-1950. Edward Bros., Ann Arbor, Mich. 99 pp.

GROVE, W.B., 1935. British Stem and Leaf Fungi (Coelomycetes). 1. Sphaeropsidales. Cambridge Univ. Press. 488 pp.

HAY, Roy, 1937. Annuals. Martin Hopkinson Ltd., London. 126 pp.

HEALD, F.D., 1921. The relation of spore load to the percent of stinking smut appearing in the crop. Phytopath. 11 : 269-278.

HUGHES, S.J., 1949. Studies on some diseases of sainfoin (Onobrychis sativa), II. The life history of Ramularia onobrychidis Allescher. Trans. Brit. Myc. Soc. 32 : 34-59.

_____ 1953. Conidiophores, conidia and classification. Canad. Jour. Bot. 31 : 577-659.

_____ 1965. Pers. comm.

KEMPTON, F.E., 1919. Origin and development of the pycnidium. Bot. Gaz. 68 : 233-261.

LACEY, M.L. and C.E. HORNER, 1965. Verticillium wilt of mint : interaction of inoculum density and host resistance. Phytopath. 55 : 1176-1178.

LANJOUN, J. et al., 1961. International code of botanical nomenclature. Utricht. 372 pp.

LARGE, E.C., 1957. Pers. comm.

LATCH, G.C.M. and E.W. HANSON, 1962. Comparison of three stem diseases of Melilotus and their causal agents. Phytopath. 52 : 300-315.

LILLY, V.G. and H.L. BARNETT, 1951. Physiology of the Fungi. McGraw-Hill, New York. 464 pp.

LOUGHNANE, J.B., R. McKAY and H.A. LAFFERTY, 1946. Observations on the pasmo disease of flax and on the causal fungus Sphaerella linorum Wall. Sci. Proc. R. Dublin Soc. 24 (N.S.) No.10, 89-98.

MacMILLAN, H.G. and O.A. PLUNKETT, 1942. Structure and germination of Septoria spores. Jour. Ag. Res. 64 : 547-559.

MACHACEK, J.E., 1945. The prevalence of Septoria on celery seed in Canada. Phytopath. 35 : 51-53.

MARSHALL, G.M., 1960. The incidence of certain seed-borne diseases in commercial seed samples. III. Septoria leaf spot or blight of celery. Ann. Appl. Biol. 48 : 27.

MAUDE, R.B., 1964. Studies on Septoria on celery seed. Ann. Appl. Biol. 54 : 313-326.

MARTIN, G., 1887. Enumeration and description of the Septorias of North America. Jour. Mycol. 3 : 85.

MILLER, J.J., 1945. Studies on the Fusarium of Muskmelon wilt. Canad. Jour. Res., C. 23 : 16-43.

McKAY, R., 1946. A study of Septoria oxyspora Penz and Sacc. isolated from diseased barley. Sci. Proc. R. Dublin Soc. 24 (N.S.) No.11, 99-110.

NOBLE, M., J. de TEMPE, and P. NEERGAARD, 1958. An annotated list of seed-borne diseases. Comm. Mycol. Instit. (in collaboration with International Seed Testing Association). 159 pp.

OUDEMANS, C.A.J.A., 1901. Contributions to the knowledge of some undescribed or imperfectly known fungi. Proc. Sect. Sci. 3 : 140-156.

_____ 1923. Enumeratio Systematica Fungorum. Vol. 4, p. 530.

PAPE, H., 1935. A common leaf spot disease of Phlox. Blumen-u Pfl Bau der Gartenwelt 39 : 426-427. Abstract in Rev. App. Myc. 15 : 25, 1936 (original not seen).

RANGASWAMI, G. and C.N. SAMBANDAM, 1960. Influence of substrate on spore size of Alternaria melongenae. Phytopath. 50 : 486-488.

SACCARDO, P.A., 1879. Michelia 1 : p. 184.

_____ 1884. Sylloge fungorum. Vol. 3. p. 474.

_____ 1913. Sylloge fungorum. Vol.22. p. 1102.

SAVILLE, D.B.O., 1952. 'Diseases of Ornamental Plants'. - in 32nd. Ann. Rep. Canadian Pl. Dis. Survey, compiled by I.L. Connors and D.B.O. Saville, 123 pp.

SCHAREN, A.L., 1966. Cyclic production of pycnidia and spores in dead wheat tissue by Septoria nodorum. Phytopath. 56 : 580.

SEYMOUR, A.B., 1929. Host Index of the Fungi in North America. Harrard University Press, Cambridge, Mass., 732 pp.

SNEDECOR, G.W., 1956. Statistical Methods. Iowa State Coll. Press. (5th ed.)

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

_____ 1941. The species concept in Fusarium, with reference to section Martiella. Amer. J. Bot. 28 : 738-742.

_____ 1945. The species concept in Fusarium, with reference to Discolor and other sections. Amer. J. Bot. 32 : 657-666.

SPRAGUE, R., 1944. Septoria disease of Graminaeae in Western United States. Oregon State Momographs No. 6, Oregon State College, Corvallis.

THOMPSON, H.S., 1964. Pers. comm.

TOMS, H.N.W., 1949. 'Diseases of Ornamental Plants'. - in 29th Ann. Rep. Canadian Pl. Dis. Survey, compiled by I.L. Connors and D.B.O. Saville, 114 pp.

TRELEASE, S.D., 1885. Preliminary list of Wisconsin parasitic fungi. Trans. Wisc. Acad. Sc. Arts and Letters 6 : 123.

VOGLINO, P., 1907. Some new fungi from the Piedmont. Proc. Roy. Acad. Sc. Turino (1907-1908) 43 : 96.

WAKEFIELD, E.M., 1940. Nomina generica conservanda. Contributions from the Nomenclature Committee of the British Mycological Society. Trans. Brit. Myc. Soc. 24 : 282-293.

WEBER, G.F., 1922. *Septoria* disease of cereals. 2. *Septoria* diseases of wheat. Phytopath. 12 : 537-585.

WILLIAMS, C.N., 1959. Spore size in relation to cultural conditions. Trans. Brit. Myc. Soc. 42 : 213-322.

APPENDIX 1

Analysis of variance of the mean length of pycnidiospores from type material of S. divaricata Ell. and Ev. and S. drummondii Ell. and Ev. (see Tables 2 and 3)

Source	d.f.	S.S.	M.S.	F.	Result
Collection	1	17.14	17.14	< 1	N.S.
Error	11	223.55	20.32		
TOTAL	7	314.56			

APPENDIX 2

Analysis of variance of the length of pycnidiospores from herbarium collections (see Table 4)

Source	d.f.	S.S.	M.S.	F.	Result
Collection	2	112.83	56.42	1.40	N.S.
Error	5	201.73	40.35		
TOTAL	7	314.56			

APPENDIX 3

Analysis of variance of the length of pycnidiospores from twelve Manawatu collections of P. drummondii (see Table 5)

Source	d.f.	S.S.	M.S.	F.	Result
Collection Dates	3	456.55	152.18	2.55	N.S.
Error	8	476.55	59.57		
TOTAL	11	933.10			

APPENDIX 4

Analysis of variance of the diameter of pycnidia from 12 Manawatu collections of P. drummondii (see Table 5)

Source	d.f.	S.S.	M.S.	F.	Result
Collection Dates	3	346.40	115.47	< 1	N.S.
Error	8	1155.09	144.39		
TOTAL	11	1501.49			

APPENDIX 5

Analysis of variance of the length of pycnidiospores from Manawatu *P. drummondii* collections raised from infected Dutch and English seed (see Table 6)

Source	d.f.	S.S.	M.S.	F.	Result
Collection	1	0.06	0.06	< 1	N.S.
Time	7	188.99	26.99	1.071	N.S.
Error	7	176.43	25.20		
TOTAL	15	265.48			

APPENDIX 6

Analysis of variance of the diameter of pycnidia from Manawatu *P. drummondii* collections raised from infected Dutch and English seed (see Table 6)

Source	d.f.	S.S.	M.S.	F.	Result
Collection	1	21.16	21.16	< 1	N.S.
Time	7	1416.30	202.33	2.527	N.S.
Error	7	560.40	80.06		
TOTAL	15	1997.86			

APPENDIX 7

Analysis of variance of pycnidiospore length from 14 Manawatu collections of *P. paniculata* (see Table 10)

Source	d.f.	S.S.	M.S.	F.	Result
Collection Date	3	82.69	27.56	1.65	N.S.
Error	10	166.66	16.67		
TOTAL	13	249.35			

APPENDIX 8

Analysis of variance of pycnidium diameter from 14 Manawatu collections of *P. paniculata* (see Table 10)

Source	d.f.	S.S.	M.S.	F.	Result
Collection Date	3	1842.84	614.28	1.64	N.S.
Error	10	3734.32	373.43		
TOTAL	13	5577.16			

APPENDIX 9

Analysis of variance of the length of pycnidiospores from seven group collections of *P. drummondii* and *P. paniculata* (see Table 16)

Source	d.f.	S.S.	M.S.	F.	F. Rqd.
Collections	6	1854.05	309.01	8.61	2.31(3.24)
Error	45	1614.94	35.89		
TOTAL	51	3468.99			

APPENDIX 10

Analysis of variance of the diameter of pycnidia from seven group collections of *P. drummondii* and *P. paniculata* (see Table 16)

Source	d.f.	S.S.	M.S.	F.	F. Rqd.
Collections	6	29674.96	4945.83	21.37	2.31(3.24)
Error	45	10415.76	231.46		
TOTAL	51	40090.72			

NOTE: With regard to Appendices 9 and 10, those means differing from each other were located by the "t" test where

$$d_{0.05} = t_{0.05}\ (45\ \text{d.f.}) \sqrt{\text{E.M.S.}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)};$$

$d_{0.05}$ = difference required for significance at the 5% level of probability

E.M.S. = error mean square

$n_1 + n_2$ = number in the two groups being compared

APPENDIX 11

Analysis of variance of the length of pycnidiospores produced on agar by isolates from Manawatu and overseas collections of *P. paniculata* (see Table 18)

Source	d.f.	S.S.	M.S.	F.	Result
Collection	3	159.94	53.31	1.32	N.S.
Error	10	403.83	40.38		
TOTAL	13	563.77			

APPENDIX 12

Analysis of variance of the length of pycnidiospores produced on agar by isolates from *P. drummondii* and *P. paniculata* collections (see Tables 17 and 18)

Source	d.f.	S.S.	M.S.	F.
Collection	1	0.08	0.08	< 1
Error	16	587.89	36.74	
TOTAL	17	587.97		