A SEPTORIA DISEASE OF *EUPHORBIA PEPLUS* L.

By Dorothy E. Shaw,
Faculty of Agriculture, University of Sydney.

(Plate i; one Text-figure.)

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Synopsis.

A *Septoria* disease of Petty Spurge is described, and the Australian distribution is given. The cultural characteristics and morphology of the causal organism are described. The results of investigations concerning the host-parasite relations, the longevity of the spores, the search for the perfect stage, and pathogenicity tests with other plants are given. The literature describing species of *Septoria* parasitizing species of *Euphorbia* is examined, and the name *S. pepli*, n. sp., is proposed for the causal organism.

Introduction.

A leaf and stem spot disease of *Euphorbia peplus* L., caused by a species of *Septoria*, was pointed out to the writer by Professor W. L. Waterhouse in 1947. He had had it under observation for some time, and noted that infection on Petty Spurge was quite widespread around Sydney. At his suggestion an investigation of the disease was carried out, with particular reference to the host range, as some serious diseases of economic plants are caused by species of *Septoria*.

Economic Importance.

Euphorbia peplus L., Petty Spurge, native to Europe and Asia, occurs on the New South Wales coast and tablelands, and in Queensland, Victoria, South Australia and Western Australia, as recorded by Hurst (1942). She listed its reputed medicinal and photographic properties, but to the writer's knowledge it is not used commercially in Australia. Hurst also recorded it as containing a poisonous principle, euphorbin. It is a weed of gardens and waste places, but is easily eradicated. The disease is not of economic importance.

Review of Literature.

Australian Records.

No mention of a leaf and stem spot disease of *E. peplus* was made by Cooke (1892) or by McAlpine (1895). The disease was noted by Waterhouse (unpublished data) in June, 1921, in the Sydney area. Pieces of material collected by him and embedded in wax in 1921 are filed at Sydney University. Also filed are pieces of diseased material in wax from a collection made in July, 1932, again from the Sydney area. A record of a *Septoria* leaf spot disease of *E. peplus* was made by Noble *et al.* (1934), without either date or locality of occurrence. The herbarium specimen lodged at the Department of Agriculture, Sydney, is also without date of occurrence, locality or collector's name. A *Septoria* species is recorded occurring on *E. peplus* in Brittlebank's catalogue of Australian Fungi (unpublished), compiled between 10th May, 1937, and 2nd March, 1940, but no indication of the date or locality is given, and no specimen is filed in the herbarium.*

Overseas Records.

Diseases caused by six species of *Septoria* and two species of *Rhabdospora* were recorded by Saccardo (1884, 1892, 1913)), on eight species of *Euphorbia*, but none on *E. peplus*. Oudemans (1921) listed species of *Septoria*, *Phleospora* and *Rhabdospora* on various *Euphorbia* species in Europe, but none on *E. peplus*. No *Septoria* was recorded for any *Euphorbia* by Grove (1935), although a *Rhabdospora* was noted on one species of *Euphorbia* in Britain.

* Personal communication from Mr. S. Fish, Government Biologist, Department of Agriculture, Victoria.

In a personal communication, Dr. G. R. Bisby, of the Commonwealth Mycological Institute, reported that he could find only one record of a species of *Septoria* on *E. peplus*, and that was of *Septoria euphorbiae* Guep. in the Russian book "Key to Fungi, Vol. 2. Fungi Imperfecti", by A. A. Jaczewski (1917). I am indebted to Dr. Bisby for his translation of the significant paragraph: "p. 102. *S. euphorbiae* Guep. on *Euphorbia amygdaloides*, *E. peplus*. Round, olive-coloured spots. Stylospores 40 to 45μ by 2 to 2·5μ with 3 to 4 indistinct septa." Dr. Bisby also reported that no *Septoria* was recorded on *Euphorbia* spp. for North America by Seymour (Host Index of the Fungi of North America. Cambridge, Mass., 1929), this volume being unavailable to the writer.

No *Septoria* on any species of *Euphorbia* was recorded for South Africa by Doidge and Bottomley (1931), by Brien (1939) for New Zealand, or by Shigekatsu Hirayama (1931) or Nakato Naito (1940) for Japan.

Australian Occurrence.

The disease occurs on Petty Spurge over a wide area around Sydney, and collections were made throughout the area extending from Mangrove Mountain, near Gosford, in the north, Windsor in the west, and Mt. Keira, near Wollongong, in the south, in 1948 and 1949. In 1950, two further collections were made at Canberra, A.C.T., and Wagga, N.S.W.

The disease has not been recorded for Western Australia, South Australia, or for Queensland.* It is not known whether Brittlelank's record was for Victorian occurrence. In Tasmania in 1948 the writer collected diseased Petty Spurge plants at Launceston, Hobart, and Port Arthur.

From the information available at present, the disease is known to occur around Sydney, at Wagga, Canberra and throughout Tasmania.

Appearance of the Disease.

Lesions first appear on the leaves as small, mostly circular areas, pale green in colour, later spreading and turning pale yellow, then light brown, becoming papery and covered with scattered black pycnidia. Many lesions per leaf were recorded (in one case 14 apparently separate ones), but the majority of field specimens examined showed that infection was from one centre only, although sometimes two were observed. The pycnidia occur on both sides of the leaf, but mostly on the undersurface. A few lesions examined had a total lack of pycnidia on the upper surface, although numerous ones were found on the lower. In some cases a slight zonation of pycnidia occurs radially from the centre of infection. The pycnidia are produced singly, only rarely being grouped in twos or threes. As the necrotic tissue enlarges, slight puckering often occurs between this area and the rest of the leaf. The edge of the necrotic area is generally quite regular and definite, but without cicatrix formation. Pycnidial production is usually confined to the necrotic area, but occasionally lesions were found where pycnidia occurred at the edge of the lesions in advance of necrosis. Where several lesions occur per leaf, the diseased areas later coalesce. In the advanced stages of the disease premature leaf fall occurs.

Lesions on the stem occur less frequently than on the leaves. The leaf infection, in many cases, extends to the petiole and thence to the stem. Pycnidia occur on both petioles and stems. Stem infections were produced in the glasshouse, which were so severe as to cause death to the uninfected upper part of the plants.

Pycnidia were recorded on the seed capsules on material from the field, as well as on plants inoculated in the glasshouse. No pycnidia were noted on the seeds.

Cultural Characteristics.

Isolation Methods.

Isolations of the causal organism were first made by the usual method of tissue transplants, pieces of diseased material of about $\frac{1}{4}'' \times \frac{1}{4}''$ being cut from the edge of

* Personal communications from Mr. W. P. Cass Smith, Government Plant Pathologist, Western Australia, Mr. D. B. Adam, Department of Plant Pathology, University of Adelaide, and Professor D. A. Herbert, University of Queensland.

the lesions, or just in front, and taken through 95% alcohol for 5 seconds, mercuric chloride 1 in 1000 for 20 seconds, and three washings with sterile water, then transferred with sterile forceps to freshly poured and cooled plates of potato dextrose agar. It was found, however, that if a contaminant present had escaped the surface sterilization, it usually grew at a rate much in excess of the *Septoria* mycelium, which was subsequently difficult to obtain in pure culture. The method later adopted was to allow the spores to exude from the pycnidia in the leaf, into a drop of distilled water, and to streak a loopful of the spore suspension over the surface of the P.D.A. plates. Bacterial and fungal contaminants were sometimes present, but because of the scattered nature of the *Septoria* spores isolations were not difficult. Transfers were then made to P.D.A. slopes, of single spores or hyphal tips, as well as mass transfer of mycelium. Nine isolations were made from collections, five from within the Sydney area, three from Tasmania, and one from Wagga. Nineteen collections were made from many diseased plants observed in the field, three of these being from Tasmania, one each from Wagga and Canberra, and the rest from a wide area around Sydney.

Media.

The fungus grew on P.D.A., water agar, standard agar, maize meal agar, maize husks and maize cobs, potato cubes, lucerne shoots, lima bean agar, dried pea agar, lentil agar, soaked rye and peanut husks, also on Czapeck-Dox with marmite, and wort agar. It also grew on a water extract of *E. peplus* leaves in agar, and on sterile shoots and stems of *E. peplus*. Growth on water agar was very slow and meagre. Pycnidia, mostly well formed, were produced on all the media, often with abundant pinkish exudate of spores, especially on the sterile stems of Petty Spurge, and on the rye-peanut husk media. P.D.A. proved the most satisfactory media for mycelium and spore production and for maintaining cultures.

Appearance of Cultures.

Spores germinate on P.D.A. usually within twenty-four hours. After three to four days the colonies are visible, being whitish in colour, and distinctly mucose, with a smooth surface and an entire edge to the naked eye. As the colonies develop, the edge becomes slightly irregular and the surface slightly ridged. At seven days most colonies have become black in the centre, and at 17 days are all black, sometimes with a tinge of greeny-grey, with a minute border of white at the edge.

On P.D.A., cultures later produce small greyish patches of very short, pile-like aerial mycelium. Black carbonaceous pycnidia are embedded over the surface, and these, in most cultures, give pink exudates of masses of spores. Where pycnidia occur near the side of a tube, cirri can be easily distinguished under the low power of the microscope. These spore masses are yeast-like in appearance to the naked eye. The mycelial mass later becomes carbonaceous, piled and convoluted in the centre, and with sub-surface hyphae at the edge. The hyphal mass remains very compact and difficult to separate, with growth upwards nearly as great as lateral growth. Seldom is the whole surface of the slope covered with mycelium, and often cracks occur in the agar. After about three months small tufts of white cottony mycelium appear in patches over the surface in some slopes. Colonies are usually non-sporulating about this time. In some cultures more than four months old, hard carbonaceous bodies appeared on the surface and along the cracks in the agar. The structures, when sectioned after fixing in chrom-acetic and stained with gentian violet-orange G, were roughly circular to oval, but measured anything from 200 to 500μ wide (measuring from the innermost walls). The interior had no definite structure, but consisted of wispy strands of fungal material, which could not be distinguished as hyphae. The walls consisted of 6–9 layers of very dark brown, thick-walled, pseudoparenchymatous cells, which pass into a region of brownish, twisted, strongly septate, clearly distinguished hyphae. No development further than this sclerotial-like stage was observed.

Optimum Temperature.

Optimum temperature was determined by growing the fungus in small (2¼″) Petri dishes on P.D.A., and incubating over a range of temperatures. Three tests were

carried out at different times. In the first two tests duplicate plates were used, in the third only one plate was inoculated for each temperature, except for 20°C., when duplicate plates were used. All the tests gave approximately the same results for the temperatures available. Inoculum was of the Pennant Hills isolate, approximately 2 mm. square, and readings were taken at four weeks. The readings for the third test are given, as the range available at that time was a little more comprehensive.

TABLE 1.
Growth of the Causal Organism at Various Temperatures.

Temperature. °C.	Colony Diameter. mm.	Spore Production and Character of Growth.
3	2× 2	No growth discernible.
5	2× 4	Very slight growth.
10	7× 9	Colony black with small white margin; no spores.
15	13×14	Colony similar to above; no spores.
20	16×17 (mean of 2 plates)	Colony black with greenish tinge; on the reverse side, clumps of pycnidia in concentric circles; abundant spores.
25	10×12	Colony black; no spores.
30	2× 2	No growth discernible.
Room temp. (light)	13×16	Colony as at 20° C.; abundant spores.
Room temp. (dark) (June-July)	12×13	Colony as at 20° C.; very abundant spores.

Optimum temperature for mycelial growth and spore production is around 20°C. Colonies at the other temperatures might have produced spores when older. No measurement was made of the height of the colonies, but this increased with increase in diameter.

Optimum pH.

Optimum pH was determined by growing the fungus on plates of P.D.A. adjusted with N/5 NaOH or N/5 HCl to give a series varying in hydrogen-ion concentration. Two tests were conducted, and the pH was read on a Vane Electronic pH Meter. Duplicate plates were used in the first test, triplicate plates for the second. Readings of pH were taken before inoculation. Inoculum was of the Sandy Bay (Tasmania) isolate, approximately 2 mm. square. Plates were kept at room temperature during May and June, and measured after seven weeks.

TABLE 2.
Growth of the Causal Organism at Various Hydrogen-ion Concentrations.

pH.	Colony Diameter. mm. (Mean of 3.)
3·6	6·0× 9·3
4·8	23·6×25·3
5·6	26·0×29·5
6·8	24·3×26·3
7·7	22·0×24·0
8·6	16·0×18·6

Optimum pH is about 5·6 or a little more alkaline. Specificity is not very marked over a wide central range, the greatest effect being shown on the very acid side at the concentrations taken.

Test for Pathogenicity.

Isolations of the organism were made as outlined previously, and the fungus maintained on P.D.A. slopes. Spores from pycnidia produced in culture were used to inoculate leaves of Petty Spurge seedlings in the glasshouse as outlined under "Host-parasite Relations, Method". Typical symptoms of the disease appeared on the leaves, with production of pycnidia. Isolations made from these lesions yielded the organism which was similar in detail to that isolated initially.

Morphology of the Causal Organism.

Mycelium.

The mycelium in young colonies consists of stellately radiating hyphae, which are minutely guttulate, hyaline, septate, and branch profusely. Pigmentation occurs in hyphae about six days old. Old hyphae (i.e., after a period of months), become very nobbly in outline, olive in colour, often with large refractive globules, but usually without any apparent contents. Young hyphae at the edge of colonies are very fine. Mycelium in colonies 13 days old measured up to 3μ in diameter, and at four months measured about 4μ in diameter. Hyphae in the plant tissues measure 2–3μ in diameter.

Pycnidia.

Pycnidia occur on both sides of the leaf, but mostly on the under-surface, scattered over the centre and sometimes on the marginal green areas of the necrotic spots; singly, only rarely in twos or threes; visible to the naked eye, black in colour, but reddish-brown by transmitted light; globose, immersed but later erumpent; ostiole about one-quarter the diameter of the pycnidium; pycnidial wall smooth, composed of 2–3 layers of pseudoparenchymatous cells; 85–135μ (65–160μ), mean 110·19μ $\pm$ 18·98μ.

Pycnidiospores.

Pycnidiospores hyaline, straight to very slightly curved, attenuated at one end, with a varying number of guttulae; usually three septa, often two, sometimes one, rarely aseptate or four-septate; 25–44μ (17–51μ), mean 35·77μ $\pm$ 5·94μ.

Cirri.

Cirri were often found exuded from the ostioles in material examined straight from the field, and practically always from material in the glasshouse, owing to the high humidity. They are colourless under reflected light, and in strong sunlight can be seen with the naked eye as a glistening whitish spot on the pycnidium. The horns vary in length, nearly always curl over, and often adhere to the neighbouring horns. On diseased material from the glasshouse, cirri were noted from quite small pycnidia. Cirri were observed microscopically by placing diseased stems from the glasshouse (with cirri still attached) into lacto-phenol cotton-blue, and by allowing pycnidia produced in culture to exude spores into dilute lacto-phenol. The pycnidiospores are oriented with their long axes parallel to the horn. When material with cirri already exuded is placed into water the spores immediately separate from one another. When pycnidia with unreleased spores are placed in water, the spores are ejected separately, not exuded in cirri.

Observations on Pycnidiospores.

Spore Structure.

Pycnidiospores of all isolates examined were hyaline, the septa being indistinguishable unless stained. Stains used included cotton-blue lacto-phenol, aqueous gentian violet, nigrosin, aceto-carmine, and gentian violet, orcein and carbol fuchsin in lacto-phenol. Cotton-blue lacto-phenol proved to be the best stain to show cell contents. This stain acts quickly, the protoplasm staining blue of varying intensity. Guttulae were of various sizes, sometimes two large ones appearing in each cell, one at each end near the septa, sometimes one large guttula only, as well as numerous small ones. The number of large guttulae was not constant per cell or per spore. Septa were unstained with gentian violet, the spore contents appearing granular with large refractive drops in most cells. In material fixed, sectioned and stained with gentian violet-orange G,

nuclei were clearly distinguishable in the spores and conidiophores. Spores mounted in water and examined with dark field illumination, showed the cell contents as circular areas of light of varying size and intensity, as shown in Plate 1, B, by MacMillan and Plunkett (1942).

Septation of Spores.

Because of the confusion in the literature regarding the number of septations in most species of *Septoria*, this aspect of the causal organism was particularly observed. MacMillan and Plunkett (1942), following on the work of Garman and Stevens* (cited by the afore-mentioned but unavailable to the writer), have shown that there is a great inadequacy on this point for most published descriptions of *Septoria* species. Sprague (1944) and MacMillan and Plunkett consider that the number of septations of the spores is usually 1, 3 or 7, depending on whether there are 1, 2, or 3 nuclear divisions in the spores, and that there is simultaneous division of the end cells, after formation of the primary septum. MacMillan and Plunkett concluded that the spores become mature on attaining the 3-septate condition, but not before, and that an even number of septa in the spores is anomalous. These authors found it difficult to account for the very large number of observations in the literature for even-numbered septate spores. Sprague (1944) found that some species infecting grasses are characterized by 2-septate spores, while five septa are common in others. He concluded that in some species the number of nuclear divisions evidently is dependent on the available cell nutrients; that large spores produced in humid winter weather may have two or even three nuclear divisions, while later in the season, when the weather is warmer and drier, the same species may produce aseptate or 1-septate spores.

Septa are indistinguishable in unstained spores. Stained with cotton-blue lactophenol, the septations are visible, but the granular nature of the cells does not make for easy observation. The best method found was as follows: diseased material with pycnidia was placed in a drop of water on a slide, and the spores allowed to exude. After a minute or so, the tissue was lifted from the slide, and a drop of iodine in potassium iodide in 80% alcohol was added, this being a modification of the method used by Brodie and Neufeld (1942). The cover slip was lowered, and the septation counts made immediately. The spores, with this method, appear a homogeneous golden-brown, with the septa, under slightly reduced light, clearly distinguished.

TABLE 3.

Septation of Spores from Different Collections, at Various Intervals from Time of Collection, and from Culture.

Collection.	No. of Spores Examined.	Time from Collection.	Percentages of Spores in the Various Septate Classes.					
			0	1	2	3	4	5
Mangrove Mtn.	200	2 weeks.	—	10·0	28·5	60·5	1·0	—
Mt. Keira	200	2 months.	0·5	17·5	37·0	44·5	0·5	—
P. Arthur, Tas.	200	4 months.	—	19·0	46·5	33·0	1·0	0·5
Sandy Bay, Tas.	200	4 months.	—	10·5	36·0	53·5	—	—
Sandy Bay, Tas.	100	6 months.	—	6·0	15·0	79·0	—	—
*Sandy Bay, Tas.	142	—	—	1·9	46·0	52·1	—	—
Penshurst	100	11 months.	1·0	21·0	38·0	40·0	—	—

* Spores from culture.

The results shown in Table 3 indicate that the maximum number of septa in mature spores is three, practically no further division taking place until germination. The first cell-division in the spore would appear to take place early, because of the very small number of aseptate spores which appear in the counts. Spores in which

* Garman, P., and F. L. Stevens, 1920.—"The genus *Septoria*." *Ill. State Acad. Sci. Trans.*, 13 : 176-219.

two cell-divisions have taken place (giving four-celled spores) would most probably be spores produced under optimum conditions. The large percentage of 2-septate spores could only be explained where only one cell divided after the formation of the primary septum. It is to be noted that a large number of 2-septate spores was also produced in culture. Counts from all the above collections are of the same general type, i.e., 3>2>1-septate spores (except the Port Arthur isolate, where the 2-septate spores predominate), with 0-, 4-, and 5-septate spores occurring only very rarely. The relative percentage of 3-, 2-, and 1-septate spores is most likely conditioned by nutrition. Age, under the conditions of storage of the material, did not appear to affect the relative percentages greatly.

Germination of Spores.

1. *Method and manner of germination.*

Germinations were studied on plates of P.D.A., the media having been strained, and only that amount which would just cover the bottom poured into Petri dishes. Spores from a water suspension were streaked over the surface with a loop. With this method, each streak of spores could be followed under the microscope with ease. Germination conformed in general to that described by MacMillan and Plunkett (1942) for 20 representative spores of *S. apii-graveolentis*. These workers noted that, at the end of four hours, the two end-cells had divided, and the tapered end of the spore had increased in length more than the other cells. At twelve hours the end-cells had divided, and at 24 hours the two original centre-cells had divided, and the outer cells of this division had sent out tubes. The spores were originally all 3-septate, and the germination was quite symmetrical.

In the present study, it was noted that germination was not always symmetrical, and counts were made of the positions of emergence of germ tubes. Readings were taken on 200 spores from the Penshurst collection, taken at random for both readings, on P.D.A.

TABLE 4.
Germination of Spores at 24 and 50 Hours.

Type of Germination.	24 Hours. %	50 Hours. %
Not germinated	4	3
Germ tube at 1 end	38	1
1 side	6·5	1
both ends	33	11
both sides	1·5	2
1 end, 1 side	12·5	1
2 ends, 1 side	4	17
1 end, 2 sides	0·5	9
both ends and more than one side	—	55

As indicated in Table 4, emergence of germ tubes was asymmetrical, although by the second reading at 50 hours, emergence had proceeded towards the "normal" type expected from a 3-septate spore, as shown by MacMillan and Plunkett. Spores from the Penshurst collection included many with one and two septations, and the asymmetrical emergences were most likely a reflection of this condition. The above workers considered that spores with less than three septations were immature, but it is to be noted that spores in this study which would be classed as "immature" were capable of germination, as shown in Table 4, where 96% had germinated in 24 hours.

Germinations on water agar were slower than on P.D.A. (e.g., in one test, 94% had germinated on P.D.A. in 48 hours, against 74·5% on water agar), and hyphae were shorter, with fewer branches. For this reason, strained P.D.A. was used in preference to water agar, although the latter is a little clearer. Germinations in water were comparable with those on P.D.A. Spores retained their identity in most cases, up to approximately 48 hours on P.D.A., but for a shorter time in water.

No secondary spores borne directly on the mycelium were observed, as noted by Weber (1922) and Sprague (1944) for several species of *Septoria*. Anastomoses between germinating spores occurred in water, but none were observed on P.D.A. or water agar.

2. *Effect of Temperature on Germination.*

Several loopfuls of a spore suspension in water were placed on coverslips, which were then inverted over van Tiegham cells containing several drops of water, and sealed with vaseline. After seven days cotton-blue lacto-phenol was run in, to fix and stain the hyphae, the coverslips were removed and placed on clean slides for observation.

TABLE 5.
Germination of Spores and Degree of Development at Different Temperatures.

4° C.	8° C.	13° C.	Room Temp. July.	25° C.	30° C.	37° C.
+	+	++	+++	+	–	–

The results of the temperature test for germination agree fairly well with that for vegetative growth, as in Table 1.

Spore Size.

1. *Length.*

Great discrepancy exists in the literature regarding methods employed for mounting and measuring, and methods of recording measurements, and the position has been reviewed by Bisby (1945) and Ramsbottom (1948). There are also wide differences in opinion as to the number of items, e.g., spores, which are required to be measured. Many workers have not recorded the number measured. Bisby (1945) suggested 20 or so, to include spores at both ends of the range. Cochrane (1932) measured 1,000 spores of each of the two species studied. Beach (1919) measured 200 spores for each test when checking the effect of various microclimates on the length of *Septoria* spores.

In this study, spores were allowed to exude from pycnidia into a drop of water on a slide, for not longer than two minutes, when lactophenol cotton-blue was added, to prevent swelling and to stain the hyaline spores. One thousand spores were measured, from different isolates and environments, and the results examined to see if there was any difference between the spores of seven collections, between the Tasmanian and Sydney area collections, and between the spores from diseased material and those from culture. In some of the collections, material was limited.

Examination of the results in Tables 6 and 7 shows that the means range from 32μ to 41μ, and variation between groups is no greater than variation within groups. The greatest number of long spores was produced in culture. Other workers have found that even slightly different environments affect the length of *Septoria* spores, e.g., MacMillan and Plunkett (1942), found that spores of *S. apii-graveolentis* from pycnidia measured 36·8μ (average of 50 spores), while spores from cirri measured 44·5μ (average of 20 spores). Moore, cited by Hughes (1949), found that the average length of spores of *S. lactucae* increased from 27·5μ to 35μ after a week of dull or wet weather. Beach (1919), measuring 200 spores for each test, with *S. tritici* and *S. verbascicola*, found that size was affected by environmental conditions such as bright and dull light, moist and dry culture conditions, and summer and winter development. While the readings shown in Table 6 are not sufficiently comprehensive for a detailed comparison, it is considered that the differences in length of spores of the various isolates can be accounted for by differences in environment at time of spore production.

TABLE 6.
Length of Spores from Different Isolates.

Isolate.	Month of Collection.	Source.*	No. Measured.	Mean. μ.	Standard Deviation. μ.
Sandy Bay, Tas.	January.	N.	200	32·38	5·100
Sandy Bay, Tas.	January.	C.	100	33·46	4·676
Port Arthur, Tas.	January.	N.	200	35·60	5·821
Launceston, Tas.	January.	N.	200	37·37	4·901
Pennant Hills (7)	May.	C.	100	41·11	5·091
Rydalmere	May.	C.	50	39·71	4·525
Penshurst	July.	N.	100	33·52	6·225
Pennant Hills (9)	August.	N.	50	38·08	3·994

* N. = nature, C. = culture.

TABLE 7.
Comparison between Groups.

	Tasmania μ.		Sydney Area μ.		Bulked Mean μ.	Bulked Stan. Dev. μ.
Nature	32·38 35·60		33·52 38·08		35·01	5·778
	37·37		41·11			
Culture	33·46		39·71		37·77	5·775
Bulked	34·88 Mean	5·564 Stan. Dev.	37·84 Mean	6·184 Stan. Dev.	35·77 Mean	5·939 Stan. Dev.

2. *Width.*

Spores were prepared for measuring as described under the previous section. Three hundred spores were measured, at the widest part, using a 20× eyepiece with a calibrated ocular micrometer, and fifty were measured using a filar micrometer.

TABLE 8.
Width of Spores.

Method.	Isolate.	Source.	Number Measured.	Mean. μ.	Standard Deviation. μ.
Filar micrometer	Penshurst.	N.	50	1·8975	0·2204
Ocular micrometer	Sandy Bay.	C.	100		
	Sandy Bay.	N.	100	1·9384	0·1430
	Launceston.	N.	50		
	Pennant Hills.	C.	50		

No difference between isolates was detected. The use of the calibrated ocular entailed making estimates for those spores whose widths fell between graduations. Although greater accuracy is obtained by using the filar micrometer, whose graduations are only 0·178μ apart, the former method is quicker, and is sufficiently accurate to

warrant its use under most circumstances, if a large number of spores are to be measured.

Size of Pycnidia.

Diseased material was mounted in lactophenol to prevent shrinking and swelling, and a coverslip gently lowered on top to avoid squashing. Measurements were made from the outside of the pycnidial wall on those pycnidia whose ostiole was uppermost, i.e., if the adaxial surface of the leaf was uppermost, only adaxial pycnidia were measured. Readings for each collection, however, were made on pycnidia on both sides of the leaf. After a preliminary examination of the pycnidia, it was decided that their practically spherical formation required only one measurement. Where length and width differed, the diameter measured was the one which happened to lie parallel with the graduated scale of the eyepiece.

Table 9.
Size of Pycnidia from Different Isolates.

Isolate.	No. of Pycnidia Measured.	Mean. μ.	Standard Deviation. μ.
Sandy Bay, Tas.	200	117·55	18·694
Port Arthur, Tas.	100	107·44	18·712
Pennant Hills (7)	50	102·34	15·760
Rydalmere	100	110·33	17·847
Penshurst	50	104·04	18·241
Pennant Hills (9), ...	100	105·06	18·093
Bulked	600	110·19	18·982

As shown in Table 9, the mean of the bulked readings was $110{\cdot}19\mu \pm 18{\cdot}982\mu$. Measurements of pycnidia grouped in twos and sometimes in threes fell within the range of solitary pycnidia, and are not included in the above figures.

The mean of 50 measurements of ostioles of pycnidia on diseased leaves was $29{\cdot}24\mu$, ranging from 17μ to $47{\cdot}6\mu$.

Host-Parasite Relations.

Method.

Seedlings of *E. peplus*, raised from seed, or transplanted from the field, were grown in pots in the glasshouse. Leaf surfaces were difficult to wet, and the best method proved to be moistening with the fingers, followed by spraying from an atomizer. Several loopfuls of inoculum were placed on both surfaces of the leaves, the inoculum consisting of a water suspension of spores, either from pycnidia on leaves, or from pycnidia in culture. Seedlings were incubated from 48 to 72 hours, then placed on benches in the glasshouse.

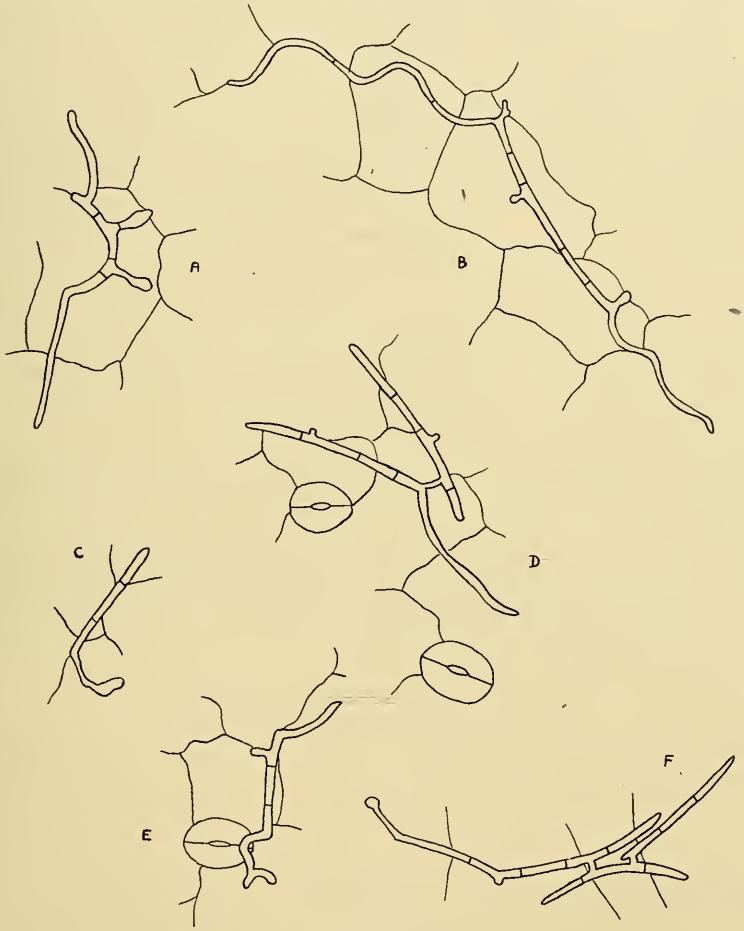
Leaves were picked and fixed after 24 and 48 hours, and thereafter every two days until pycnidia were abundant. Several methods of fixing and staining were tried, including staining with Pianese 111*b* as recommended by Weber (1922), but the method finally followed was as follows: leaves were fixed in Farmers (absolute alcohol and glacial acetic acid 3:1) for 20 minutes, washed several times in 95% alcohol, decolourized in 95% alcohol for 18–24 hours, and either mounted in lactophenol cotton-blue permanently, or taken from it to lactophenol after ten minutes. This method was found to be very satisfactory. The leaf tissue after treatment was quite colourless, and the spores and hyphae on the leaf surface stained blue, standing out vividly. Spores in pycnidia stained in deepening amounts of blue, depending on the maturity. Hyphae in the tissues were quite colourless when first mounted, but after several months appeared faintly blue. Material mounted in this way and examined after fourteen months was in good condition. The hyphae were clearly visible below the epidermis,

and although the chloroplasts by this time were stained blue, this assisted observations by making cell boundaries easily discernible.

Stem tissue was stripped, fixed, stained and mounted as above.

Observations.

Spores had germinated on the leaf surface after 24 hours, usually from one end, sometimes from both, more rarely from a side branch. The infection hyphae were usually not much thinner than the width of the spore. Many germ tubes terminated in small, appressoria-like bodies, usually at the junction of two epidermal cells. In many cases hyphae were observed to pass alongside or over stomates. No entry was



Text-figure 1.

Camera-lucida drawings of spores germinating on the leaf surfaces. × 300. A, B and C, spores germinating on the upper surface. D, fusion of spores germinating on the under surface, with germ tube passing by stomate. E, spore germinating on the under surface, with germ tube passing over stomate. F, multiple fusion of spores germinating on the uppper leaf surface.

observed through stomates, and there appeared to be no attraction for the hyphae to do so. One case only was observed where the hypha ended at a stomate, and in that case there appeared to be further development.

Germination was similar on both leaf surfaces. Branching was observed only rarely, and spores retained their identity longer than on P.D.A. The number of septa

did not increase in most spores during germination. Anastomoses were frequently observed between two spores, sometimes between three spores.

At a later stage, hyphae were observed in the leaf tissue, spreading out radially from the infection centre, branching and passing beneath the epidermis, and around the cells, mainly in the mesophyll region. Later, knots of hyphae were noted below the stomates, sometimes not directly beneath, but always in the sub-stomatal cavity. On no occasion did a knot develop on one hypha only: pycnidial formation therefore is probably symphogenous.

On a few leaves, notably in one collection kept wrapped in moist paper for 24 hours, hyphae were observed issuing from the stomates above immature pycnidia. These "aerial" hyphae measured approximately 3μ wide, and were from several to 70μ long. In many cases not just one hypha projected from the stomate, but two or three, and some cases were observed where bunches of five to eight short hyphal tips projected through the stomates.

Pycnidia were apparent to the naked eye after about 14 days. The most mature pycnidia occurred at the centre of the lesions, and radially from these were immature pycnidia, aggregations of hyphae under stomates, and then hyphae ramifying through the tissue. Hyphal tips were quite distinct, and measured about 3μ across, being just slightly wider than the older hyphae. Tips were located up to 500μ in advance of pycnidia formation. In the undiseased tissue, the chloroplasts appeared distributed evenly around the cell walls, and remained so even with the advance hyphae passing around them. In the region between the advance hyphae tips and the pycnidia, the chloroplasts lose their discreteness, and the cells appear collapsed. The boundary between the normal-appearing cells and the collapsed cells was usually quite distinct. Not all the stomates had pycnidia forming under them, but where a stomate nad been missed in the first place, it often showed aggregations at a later stage, i.e., young pycnidia could form in the zone of older mature pycnidia.

Strippings from stems showed a similar condition, with hyphae mostly running up and down the stem, and pycnidia being more linearly placed, instead of zoned. Pycnidial formation was symphogenous.

Examination of healthy Petty Spurge leaves showed that stomates occur on the abaxial surface on the average of eight to a field (magnification ×840), while very few occur on the adaxial surface, most fields having none, or two or three, at certain parts, e.g., along the sides of the main vein. This would account for the predominance of pycnidia on the lower surface of the leaves.

TABLE 10.
Viability of Spores at Various Periods After Collection.

Collection.	Age at Test.	No. of Spores Counted.	Germinated. %
Mt. Keira	2 months.	170	94
	3½ ,,	200	87
	5 ,,	200	71
	7 ,,	100	0
Mangrove Mtn.	7 ,,	200	12
Pennant Hills (9)	10 ,,	200	0
Penshurst	11 ,,	300	0

Diseased tissue was also fixed in Flemming's weaker solution and Farmers Fluid, the former being stained with gentian violet-orange G, the latter with carbol fuchsin-light green. Sections were cut at 8μ and 10μ. No cicatrix was ever observed at the edge of the lesion. Hyphae ramified throughout the tissue, and the stages in pycnidial formation were noted: hyphae loosely woven below the stomates, later developing into a knot, followed by the development of the pycnidial wall. The pycnidia were subepi-

dermal, and later erumpent with a widened ostiole. Spores were produced on one-celled pycnidiophores. Nuclei were clearly visible in sections stained with gentian violet-orange G.

Longevity of Spores.

Germination tests were made with spores from diseased material which, after the initial test, was kept between paper at room temperature. Several loopfuls of the spore suspensions were streaked across strained P.D.A. plates, and germination counts were made at 48 hours. Spores from all isolates gave more than 95% germination at the initial tests.

Spores retained within the pycnidium under the above conditions of storage were viable for about six months.

Source of Inoculum and Transmission of the Causal Organism.

Diseased plants of Petty Spurge were found in the field throughout the year, with the exception of late spring, and further search might have revealed infected plants even during this period. These diseased plants, therefore, provide an immediate source of inoculum. Pycnidia on fallen leaf and stem fragments could also liberate spores, given favourable conditions of moisture, and provide an additional source of inoculum in the absence of growing infected plants.

To determine whether the disease is seed-borne or not, tests were carried out as follows:

A collection was made of the ripest capsules on heavily diseased plants at Penshurst. In the laboratory, seeds (most of which had left the capsule cases) were picked out with forceps. The cases were floated and turned over in distilled water in watch glasses and examined under low power. Many pycnidia were noted on them. The cases were drawn up onto the sides of the watch glass with forceps, and the residue water examined with reduced light. Many spores were noted. The seed was turned over in distilled water in watch glasses and examined under low power. No pycnidia were noted on the seed, but spores were found in the water residue. The seed was then sown in pots containing sterilized soil, and the residue water with the spores sprayed over the seed. The seedlings were examined every day after emergence, but no sign of disease was apparent on the stems or leaves. Random seedlings were selected at four weeks, washed in fast running tap water, and several times in distilled water, and plated on P.D.A. No *Septoria* mycelium grew from the tissue. Capsules and seeds from diseased plants at Allawah were examined as above. Pycnidia were found on the cases, and spores in the washing, but no pycnidia were detected on the seeds.

Ripe seed capsules were collected from Petty Spurge plants in an isolated patch at the University, the plants being apparently quite free from disease. The capsules and seeds were examined as above, and as no pycnidia or spores were observed, the seed was presumed to be clean. The seed was divided into four groups of about 40 seeds each, and treated as follows:

(*a*) Seed sown in pots in sterilized soil (control).
(*b*) Seed sown in pots in sterilized soil, and the surface of the soil sprayed with a suspension of spores and mycelium of the Pennant Hills isolate.
(*c*) As (*b*), but from the Sandy Bay isolate.
(*d*) Seed soaked for 24 hours in a spore and mycelium suspension, then sown in pots in sterilized soil.

The seedlings were examined after emergence, up to a period of eight weeks, but no sign of disease was observed on the stems or leaves. Random seedlings were washed as above, and plated on P.D.A., but no *Septoria* mycelium grew from the tissue.

Seeds from heavily diseased plants were surface sterilized and plated on P.D.A., but no *Septoria* mycelium developed.

Although the above tests are not conclusive, it seems unlikely that mycelium is carried in the seed, and although spores are carried on the surface, no infection was established from these. It must not be overlooked that spores in pycnidia on shed

capsule cases could constitute a source of inoculum, for example, if splashed up by the rain on to the leaves of young seedlings.

Search for the Perfect Stage.

The perfect stage of some species of *Septoria* has been recorded, and the literature was examined to note the environment favourable for perithecial production. Stevens (1925) cited Klebahn, who recorded *Mycosphaerella sentina* as the perfect stage of *S. piricola*, occurring on over-wintered leaves of apple and pear. Stevens also recorded *Leptosphaeria phlogis* as the perfect stage of *S. phlogis*. Roark (1921) showed that a *Mycosphaerella*, occurring on over-wintered leaves and in pure culture (media not given), was the perfect stage of *S. rubri*. Weber (1922) recorded *Leptosphaeria avenaria* as the perfect stage of *Septoria avenae* Frank, perithecia occurring in oatmeal agar and potato dextrose agar cultures. Stone (1916) showed that *Mycosphaerella grossulariae*, occurring on dead over-wintered leaves of currant and gooseberry, was the perfect stage of *S. ribis*, and that *Mycosphaerella aurea*, found on old leaves of *Ribis aureum*, was the perfect stage of *S. aurea*. Thompson (1941) recorded *Mycosphaerella populorum* as the perfect stage of *S. musiva* on poplars, and *Mycosphaerella populicola* as the perfect stage of *S. populicola*, the ascigerous stages occurring on over-wintered leaves. Ruggieri (1936) reported a *Mycosphaerella* as the perfect stage of *S. aurantiorum*, having obtained perithecia in pure culture. Klebahn (1934) found that a *Mycosphaerella* on overwintered leaves of chestnut was the perfect stage of *S. castanicola*. Wollenweber (1938) found perithecia of *Sphaerella linorum*, the perfect stage of *S. linicola*, on flax straw in the Argentine. Johnson (1947) found a *Leptosphaeria* on wheat, and rarely, barley leaves in Canada, and also obtained perithecia on corn meal agar, this being the perfect stage of *S. avenae* Frank F. sp. *triticea*. Cochrane (1932) could not find the perfect stages of *S. apii* or *S. apii-graveolentis*, either in culture, on artificially wintered leaves, or in response to treatment with ultra-violet light.

During the present study, all cultures on P.D.A. were periodically examined for any evidence of the perfect stage, some slope cultures being kept for more than a year, Cultures on the various types of media used, on P.D.A. in various environments, and cultures used for temperature tests were examined, in case a necessary factor or combination of factors giving optimum conditions for perithecial growth had been supplied.

Cultures of the various isolates were opposed in all combinations on P.D.A., in nearly all combinations on corn meal agar and on sterile lucerne shoots. These cultures were kept for more than five months.

Heavily diseased leaves were placed on and just below the surface of soil sterilized for four hours, in Petri dishes, and were (*a*) held at approximately 2°C. for four months, then at room temperature, or (*b*) moistened periodically at room temperature.

Diseased leaves on plants infected in the glasshouse were allowed to fall onto the surface of the soil in the pots; some pots were kept in the glasshouse for six months, others were put into the open, and the decomposing leaves examined from time to time. A patch of heavily diseased plants was marked off in a garden at Hurstville, and allowed to remain undisturbed. The diseased leaves shed on to the soil surface, and later the stems, were examined. The Petty Spurge leaves are so delicate that they do not retain their identity for long on the soil surface.

No evidence of a perfect stage was found under any of the above conditions.

Pathogenicity Tests with Other Plants.

In order to determine the host range of the fungus, plants of the family Euphorbiaceae, being species of *Euphorbia* and *Ricinus*, were inoculated in the glasshouse. The writer particularly desired to determine whether the species of *Euphorbia* reported as hosts of species of *Septoria* were susceptible or not to the fungus under study, but difficulty was experienced in obtaining seed, as most of the species mentioned are of European-Asian habitat, and are not present in Australia. Black

(1922) recorded *E. exigua* as present in South Australia, and the C.S.I.R. Bulletin No. 156 (1942) listed *E. exigua* and *E. Esula* as present in Australia, but seed of these species was unobtainable at Sydney Botanic Gardens, Adelaide University (Waite Institute), Melbourne Botanic Gardens and the Tasmanian University. Seed was also requested through the medium of the Australian Plant Disease Recorder, circulating through all the States. Seed of *E. Esula*, *E. exigua*, *E. serrata*, *E. silvatica*, *E. palustris*, *E. aspera*, *E. angulata* and *E. amygdaloides* was requested from overseas institutions by the Plant Introduction Office, C.S.I.R.O., and viable seed of *E. Esula* and *E. exigua* was eventually obtained.

Plants not of the family Euphorbiaceae, but known to be hosts of other species of *Septoria*, were also inoculated, together with other grasses, weeds and ornamentals.

The inoculum consisted of a suspension of spores, either from pycnidia on fresh leaves, or from cultures of the various isolates. The plants were either transplanted from the field, raised from seeds, or grown from cuttings. Seedlings of *E. peplus* were inoculated at each test.

Plants inoculated were as follows:

Euphorbia helioscopia L., "Sun Spurge".
E. Drummondii Boiss, "Caustic Weed".
E. Lathyris L., "Caper Spurge".
E. terracina L., "False Caper".
E. splendens Bojer (*E. Millii* Desm., *Sterigmanthe splendens* Kl. et Garcke), "Crown of Thorns".
E. neriifolia L.
E. Bojeri Hook (*Sterigmanthe Bojeri* Kl. et Garcke).
E. exigua L.
E. Esula L.
E. pulcherrima Willd. ex Klotzsch, "Poinsettia".
Ricinus communis L., "Castor Oil".
Lycopersicon esculentum Mill., "Tomato".
Linum usitatissimum L., "Flax".
Pastinaca sativa L., "Parsnip".
Apium graveolens L., "Celery".
Apium leptophyllum (DC) F. Muell.
Triticum vulgare Host. "Federation" wheat.
Triticum monococcum L., "Einkorn" wheat.
Avena sativa L., "Richland" oats.
Hordeum vulgare L., "Kinver" barley.
Secale cereale L., "Open-pollinated rye".
Zea Mays L. (var. *indentata*).
Poa annua L., "Winter grass".
Poa pratensis L., "Kentucky Blue grass".
Bromus unioloides H.B.K., "Prairie grass".
Lolium multiflorum Lam., "Italian rye grass".
Hordeum bulbosum L.
Agrostis alba L.
Digitaria adscendens (H.B.K.) Henrard.
Dianthus spp., "Carnation".
Malva parviflora L., "Small-flowered Mallow".
Sonchus oleraceus L., "Sow Thistle".
Geranium sp., "Geranium".
Geranium dissectum L.
Erodium cygnorum Nees, "Blue-flowered Crowsfoot".
Erodium moschatum (L.) L'Hér., "Crowsfoot".
Oxalis corniculata L.
Stellaria media (L.) Vill.

No infection was obtained in any of the above species, while the *E. peplus* controls gave lesions and pycnidia. The plants were kept under observation for weeks after inoculation, in case development of the fungus was slower than in "Petty Spurge".

No *Septoria* disease was detected in the field on plants of *E. helioscopia* at Hobart, Castle Hill and Hurstville, or on *E. Drummondii* at Allawah and Sydney University, or on plants of the latter species sent from Toowoomba, Queensland. At Hurstville, several plants of *E. helioscopia*, growing in a patch of severely diseased *E. peplus*, showed complete immunity.

Judged by the species tested, it appears as if the causal organism has a limited host range. Specificity by *Septoria* species for one or a few hosts has been noted by other workers, e.g., Beach (1919) working with 15 species, Weber (1922*a*, 1922*b*, 1923) with eight species, Cochrane (1932) with two species, Thompson (1941) with two species, and Sprague (1944) working with many species on grasses.

Name of the Causal Organism.

The history of the genus *Septoria* has been reviewed by Wakefield (1940) and Sprague (1944). The former's recommendation that *Septoria* Sacc. (1884) described from type species *S. Cystisi* Dem. be conserved against *Septoria* Fr. (1828), which was based on non-pycnidial species, was upheld by Sprague. *Phleospora* Wallr. (1833), based on non-pycnidial species, is an exact synonym of *Septoria* Fr. (1828). Grove (1935), Clements and Shear (1944) and Ainsworth and Bisby (1945) followed Saccardo's use, employing *Phleospora* for the forms with incomplete pycnidia. The writer, on the above authorities, retains the genus as described by Saccardo (1884) on page 474.

From examination of fresh diseased material and microtome sections, it is evident that the causal organism of the disease of "Petty Spurge" conforms to *Septoria* Sacc. The filiform nature of the spores, their length in relation to their width (ratio approximately 15-20:1), the yeasty, later carbonaceous, scanty mycelium and distinct black pycnidia (to the naked eye), distinguish it from *Stagnospora*, whose spores are typically cylindrical (although grading into more filiform types), with a length:width ratio of less than 10:1, with a cottony appearance on P.D.A., and with pale brown, less prominently distinguished pycnidia to the naked eye (Sprague, 1944; Clements and Shear, 1941). The pycnidia are too well-formed for *Phleospora*, and lesions occur too often on the leaves to consider *Rhabdospora*.

The only species of *Septoria* recorded on *Euphorbia* spp. are as follows:

1. *S. bractearum* Mont., 1849. The spore length is given as 50μ. It was described on *E. serrata*. (Saccardo, 1884.)
2. *S. Kalchbrenneri* Sacc. The type of this species is Rabenhorst *Fungi europaei* 1854, issued as *S. euphorbiae* Kalchbrenner in *Hedwigia*, 1865, p. 158, *nec* Guep. No spore measurements are given. It was described on *E. silvatica*, *E. palustris* and *E. aspera*. (Saccardo, 1884.)
3. *S. Euphorbiae* Guep., 1879. The spore measurements are given as "40–45μ × 2–2½μ, with 3–4 indistinct septa. It was described on *E. Esula* and *E. angulata*. (Saccardo, 1884.)

The rest of the very brief descriptions of the above three species is more or less the same.

Oudemans (1902), after examining the Exsiccati of Desmazieres, recommended that *S. bractearum* Mont. should become *S. Euphorbiae* Desm., and that *S. Euphorbiae* Guep. should yield place to *S. Guepini* Oud. *S. Kalchbrenneri* Sacc. remained unchanged.

4. *S. media* Sacc. et Brun. A fairly full description is given. The spots are described as having a dark reddish margin, with spores 50–55μ × 1μ. It was described on *E. palustris*. (Saccardo, 1892.)
5. *S. euphorbicola* Hollós, 1910. The description of this species is fuller, and the spots are given as 1 mm. in diameter, pycnidia 140–160μ in diameter, and spores 16–20μ × 2–2·5μ. It was recorded on *E. procera*. (Saccardo, 1913.)
6. *S. Hariotiana* Sacc., 1906. A full description is given: the spots 1 mm. in diameter, with a dark purple margin, pycnidia 120–125μ in diameter, spores 3–4 septa, 30–32μ × 3μ. It was recorded on *E. palustris*. (Saccardo, 1913.)

Because of the inadequacy of the description of *S. Kalchbrenneri* Sacc., a request was made to the Commonwealth Mycological Institute for further information, if it was available, and Dr. G. R. Bisby kindly supplied the following notes: "The Kew specimen of this No. 584 consists of three leaves bearing spots 1–3 mm. in diameter, roundish, visible on both sides of the leaf, at first brownish, then ashen, particularly on the upper surface of the leaf, at all times surrounded by a slightly raised, distinct, reddish brown margin; pycnidia circa 100–200μ wide, brown, with an ostiole which becomes 35μ or more wide; spores (25) 30–35μ × 2–2·5μ, somewhat elevate and tapering

to 1–1·5μ at one end, hyaline, 0–3 septate, straight or somewhat curved. Most of the pycnidia appear to open on the upper surface of the leaf."

The only reference in the literature to a *Septoria* disease of *E. peplus* is in Jaczewski's "Key to Fungi", Vol. 2, p. 107, 1917: "*S. euphorbiae* Guep. on *E. amygdaloides*, *E. peplus*. Round, olive-coloured spots. Stylospores 40 to 45μ by 2 to 2·5μ with 3 to 4 indistinct septa." (Jaczewski was apparently unaware of the entry in *Revue Mycologique*, 1902, whereby *S. euphorbiae* Guep. became *S. Guepini* Oud.). It is not known whether Jaczewski was referring to *E. amygdaloides* Lam. or *E. amygdaloides* L. Hooker and Jackson (1895) listed *E. amygdaloides* L. as being synonymous with *E. sylvatica* L., on which a *Septoria* disease had already been described, namely, *S. Kalchbrenneri* Sacc. Hooker and Jackson also listed *E. amygdaloides* Lam. as a synonym of *E. nicaecnsis* All., and *E. nicaeensis* (St. Am. Fl. Agen., 192) as a synonym of *E. Esula* L. It was on *E. Esula* L. that *S. euphorbiae* Guep. (now *S. Guepini* Oud.) was originally described. It is not known whether the Russian organism was identified on the grounds of morphological similarity. The spore sizes given in the Russian text are the same as those given by Saccardo (1884).

The fungus under study, with spores 35·8μ ± 5·9μ long by 1·9 ± 0·17μ wide and pycnidia 110·2 ± 19·0μ and with unrestricted spots, differs in spore size from *S. Euphorbiae* Desm. (once *S. bractearum* Mont.) (spores 50μ), and from *S. media* Sacc. et Brun (spores 50–55μ by 1μ); and in pycnidia and spore size from *S. euphorbicola* Hollos (spores 16–20μ, pycnidia 140–160μ). *S. Euphorbiae* Desm. was also recorded by Oudemans (1921) on *E. exigua*, and no infection was obtained on this plant with the organism from *E. peplus*.

The fungus more closely resembles *S. Hariotiana* Sacc., *S. Guepini* Oud. and *S. Kalchbrenneri* Sacc. The spores of *S. Hariotiana*, however, are given as 3μ wide, and the length of the spores of *S. Guepini* as 40–45μ, and these are respectively wider and longer than the spores under study. The pycnidia of *S. Kalchbrenneri* (100–200μ) are larger than those on Petty Spurge. *S. Guepini* was recorded on *E. Esula*, but no infection was obtained on this plant with the organism under study. The appearance of the lesions caused by *S. Hariotiana* and *S. Kalchbrenneri* on their hosts, differs markedly from those caused by the *Septoria* on *E. peplus*.

It is realized that the same organism can sometimes produce quite dissimilar lesions on even closely related hosts, and that the difference in spore sizes of some of the above species could, perhaps, be accounted for by natural variation or environment. However, it is considered that the fungus is sufficiently different morphologically (as far as can be determined from the brief description of some of the other species), and with regard to host range, to be described as a new species. In correspondence with the C.M.I., Dr. Bisby was of the opinion that this would be the best course to follow. It is therefore proposed to name the fungus *Septoria pepli*, n. sp.

Septoria pepli, n. sp.

Pycnidiis amphigenis, sed vulgo hypogenis, in orbicularibus non limitatis maculis; sparsis aut raro aggregatis, atris, globosis, innatis vel dein erumptibus, ostiolatis, 85–135μ (65–165μ); sporulis hyalinis, rectus vel leniter curvatis, sursum attenuatis, guttulatis, 3-septatis, saepe 2-, raro 0- vel 4-septatis, 25–44μ (17–51μ) × 1·5–2·0μ (1·3–2·5μ).

Hab. in foliis et in cauli *E. peplus* L. in N.S.W., A.C.T. et Tasmania.

Pycnidia on both sides of the leaves, but mostly on the undersurface, in circular, unrestricted spots, singly or rarely aggregated in twos or threes, visible to the naked eye, black, but reddish-brown by transmitted light, globose or slightly elongate, immersed but later erumpent; ostiole about one-quarter the diameter of the pycnidium; pycnidial wall smooth, composed of 2–3 layers of pseudoparenchymatous cells; 85–135μ (65–165μ); spores hyaline, straight or slightly curved, tapering at one end, with a varying number of guttulae, 3 septate, often 2-, rarely 0- or 4-septate; 25–44μ (17–51μ) × 1·5–2·0μ (1·3–2·5μ).

Hab. on leaves and stems of *E. peplus* L., around Sydney, and at Wagga, N.S.W., in the A.C.T. and Tasmania.

Type specimen collected at Pennant Hills, December, 1949.

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DESCRIPTION OF PLATE I.

Fig. 1.—Leaves of *Euphorbia peplus* L. showing lesions caused by *Septoria pepli* n. sp. × 2.

Fig. 2.—Six months old culture of the fungus on P.D.A., showing "staling". Note the convoluted centre and the sub-surface hyphae at the edge. × 2.

Fig. 3.—Pycnidium produced in culture, on rye and peanut husks, with spores exuded in a cirrus. Mounted in cotton-blue lactophenol. × 100.

Fig. 4.—Section through pycnidium showing filiform spores. Cut at 8μ and stained with gentian violet-orange G. × 400.