to him for very kindly comparing the cultures and confirming the identifications.

REFERENCES.

(1) ASHBY, S. F. Oospores in cultures of *Phytophthora Faberi* Maubl. Kew Bull. IX (1922), 257–262.
(2) —— The Oospores of *Phytophthora Nicotianae* Br. de Haan, with Notes on the Taxonomy of *P. parasitica* Dast. Trans. Brit. Myc. Soc. XIII (1928), 86–95.
(3) DASTUR, J. F. On *Phytophthora parasitica* Nov. Spec. A new disease of the Castor Oil Plant. Mem. Dept. Agric. India, Bot. Ser. V (1913), 177–231.
(4) HOPKINS, J. C. Notes on Soft-rot of cotton bolls in the West Indies caused by *Phytophthora*. Ann. Bot. XXXIX (1925), 267–280.
(5) NOWELL, W. Report of Mycologist. St Vincent Official Gazette, Oct.-Dec., 1917.
(6) —— Diseases of crop plants in the Lesser Antilles. West Indian Committee, (1917), 274.
(7) ROBSON, W. Report on the Botanic Station, Montserrat. Imp. Dept. of Agric. for West Indies (1911–12), 17.

FURTHER NOTE ON THE PRODUCTION OF SEXUAL ORGANS IN PAIRED CULTURES OF SPECIES AND STRAINS OF PHYTOPHTHORA.

By S. F. Ashby, B.Sc., Mycologist, Imperial Bureau of Mycology.

(With two Text-figures.)

IN two recent papers (1, 2) I have recorded the ready development of sexual organs when certain strains of individual species of the genus *Phytophthora*, and also when certain distinct species were grown together in paired cultures. The species concerned were *P. parasitica* Dastur (emend.), *P. palmivora* Butler, *P. cryptogea* Pethyb. and Lafferty, and *P. Cinnamomi* Rands. With the exception of the last-named, oospores have been found, although sometimes after long delay, in some pure single strain cultures of all the species; so that the individual mycelia would appear to be homothallic, and there seems to be no need for assuming that those strains are heterothallic in which oospores have been formed in paired cultures but not, as yet, in pure cultures.

Opportunity has been afforded also of examining isolations of *P. Arecae* (Colem.) Pethyb. and *P. Meadii* McRae. Coleman (3) obtained oospores of *P. Arecae* when pure single strain cultures were inoculated on shelled areca nuts, and Rosenbaum (4) and Leonian (5) have recorded them in pure cultures on agar media.

McRae (6) recorded oospores in pure single strain cultures of *P. Meadii* on agar media. At the instance of Dr McRae, an isolation of *P. Meadii* from *Hevea brasiliensis* in India, made some three years ago, and another from the same host in India, made about one year ago, were sent to me. An isolation of the same species, made this year from Black Stripe of *Hevea* in Malaya by Mr A. Thompson, has also been available. The culture of *P. Arecae* was obtained from the American Type Culture Collection in Chicago (No. 1401). Oospores were detected in scanty numbers after several months in maize meal agar cultures of the older isolation of *P. Meadii*, soon after it was received, but they were not found in later cultures; they have not been detected in cultures of the later isolations. The

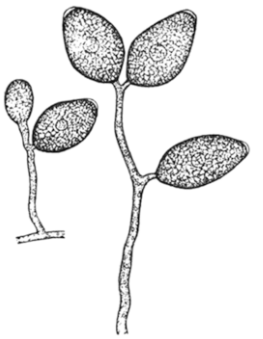

Fig. 1.

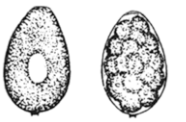

Fig. 2.

Fig. 1. Zoosporangia of *P. Cinnamomi*. × 345 (after Rands).
Fig. 2. Zoosporangia of *P. cryptogea*. × 510 (after Pethybridge and Lafferty).

isolation of *P. Arecae* has not developed oospores on any medium including maize, bean and quaker oats agars. When the strains of *P. Meadii* were grown together in pairs on maize-meal agar at 23°, sexual organs were not detected, but when each of the three isolations was grown with the strain of *P. Arecae* on the same medium at 23° C., sexual organs developed freely in four to five days. They were formed at first in the zone of contact of the two mycelia and later throughout most of the growth zone of the strain of *P. Meadii*, but were not present in the *P. Arecae* zone. The antheridia were amphigynous and after a few days the oogonia became thick-walled and golden-yellow; oospores developed but not in abundance, since the contents of the oogonia in many instances underwent an oily degeneration.

In Table I the dimensions in microns of the oogonia and oospores in the paired cultures of the strains of *P. Meadii* with

P. Arecae are shown, together with the data recorded by other observers on the two species in pure culture.

Table I.

Strains	Oospores Mean size	Oospores Range	Oogonia Mean size	Oogonia Range
P. Meadii (older isolation in India) + *P. Arecae*	24·0	20–28	28·5	24–34
P. Meadii (later isolation from, in India) + *P. Arecae*	25·8	18–29	—	—
P. Meadii (Malaya) + *P. Arecae*	25·3	20–28	29·0	24–35
P. Meadii (McRae's data)	25·0	16–32·8	32·0 (approx.)	22–45 (approx.)
P. Arecae (Coleman's data)	—	23–36	—	—
P. Arecae (Rosenbaum's data)	32·4	23–44	—	—

The size and distribution of the sexual organs in the paired cultures indicated, therefore, that they were actually those of *P. Meadii*. A comparison of the culture of *P. Arecae* with those of *P. Meadii* brought out no criteria sufficient to separate them as independent species.

The sporangia which are formed in sympodia freely in water cultures, are similar in size and shape with a papilla rather less prominent than in most strains of *P. palmivora*; a slender hollow pedicel is present several times longer than in *P. palmivora*. The mean size of the sporangia of *P. Arecae* on oat agar, according to Rosenbaum (*l.c.*) is 48 × 30 μ, and McRae (*l.c.*) records the same mean size for those of *P. Meadii* on french bean agar. I have not found chlamydospores in young cultures of either, but in old cultures resting spores may be present as terminal and mostly thick-walled papillate conidia. The larger recorded size of the oospores in *P. Arecae* does not in itself suffice to establish a difference of species. It seems highly probable that both are one species, separable by their behaviour in paired culture into two groups of strains—the "Arecae" and "Meadii" groups, comparable with the "cacao" and "rubber" groups of *P. palmivora* (*l.c.*). I hope soon, however, to be able to examine further cultures of *P. Arecae* both from the areca palm and from other hosts.

In an earlier paper(2) a brief reference was made to the development of sexual organs and oospores in paired cultures of *P. cryptogea* Pethyb. and Lafferty with *P. Cinnamomi* Rands. It may be of interest to record the data obtained in more detail.

One of the strains of *P. cryptogea* was sent by Dr G. H. Pethybridge, who had recently isolated it from a tulip; he sent also a piece of the flower stalk from which hyphae had grown out into water and had formed sporangia freely. The other strain was obtained from the Centraal-bureau at Baarn, Holland,

together with a culture of *P. Richardiae* Buis. isolated by Buisman (7) from the Calla lily. The culture of *P. Cinnamomi* was obtained from the American Type Culture Collection, Chicago (No. 1407).

The asexual spores of the strain from tulip, both the non-papillate zoosporangia and the flask-shaped conidia formed on the tulip stem in water, were quite similar to those described and figured by Pethybridge and Lafferty (8), and those obtained when mycelium from pure cultures of the tulip strain and that from Baarn were allowed to grow on sterilised centipedes in water were also quite alike.

When the two isolations were grown in paired culture with *P. Cinnamomi* on maize-meal agar at 23° C., sexual organs were found to be forming freely in the "cryptogea" zone after nine or ten days, and oospores matured in abundance a few days later; no sexual organs developed in the zone of growth of *P. Cinnamomi*, the mycelium of which can be readily distinguished by the abundant development of stalked, large, thin-walled vesicles occupied by one or more oil globules and produced terminally either singly or in bunches.

The tulip strain has not developed sexual organs in pure culture after six months, either in a cool cellar or in the laboratory on any medium, but they were present in rather scanty numbers in a pure culture of the strain from Baarn on maize-meal agar (but not on quaker oats or french bean agar) after six months in a culture kept in the cellar. The mature sexual organs were alike in the paired cultures and the pure culture, the antheridia being apparently always amphigynous, the oogonia with yellow walls, and the oospores with thickened colourless walls. The strain of *P. Richardiae* developed substantially larger sexual organs with amphigynous antheridia and mature oospores abundantly after a week or two in pure culture on quaker oats, bean and maize media, and, as was to be expected, formed them freely and quickly in the paired cultures with *P. Cinnamomi* but only in its own zone. Conidia of *P. Richardiae* were obtained also on sterilised centipedes in water cultures. They were non-papillate with a broad flattened apex and somewhat thick-walled, and there was no evidence of the production of zoospores. Those of *P. cryptogea* developed under the same conditions were also non-papillate, often with somewhat thickened walls and a broad flattened apex, but some were thin-walled and able to form and discharge zoospores; they were smaller and relatively broader than those of *P. Richardiae* but quite of the same type. In the water cultures of both species similar more or less spherical, thin-walled enlargements of the hyphae occurred separated by short hyphal bridges;

frequently the enlargements produced short branches which again formed subterminal enlargements. They were similar to the formations described and figured (Pl. XLVII, fig. 9) by Pethybridge and Lafferty (*l.c.*) but often of a more definite shape. Measurements of the oospores and mature oogonia in the paired and pure cultures are recorded in Table II and the dimensions of the conidia in Table III. It will be noticed that the conidia of both species developed from pure cultures in water on sterilised centipedes are smaller than those formed in water on hyphae growing out from lesions on the host. *P. Richardiae* differs from *P. cryptogea* in developing sexual organs and oospores more readily in pure culture, which although similar in development and structure, are substantially larger; the conidia of *P. Richardiae* are also larger and have a higher ratio than those of *P. cryptogea*; the greenish purple colour of the oospores of *P. Richardiae* recorded by Buisman (*l.c.*) was not seen in my cultures.

Table II.

Strains	Number measured	Oospores Mean size	Oospores Range	Oogonia Mean size	Oogonia Range
P. cryptogea (tulip) + *P. Cinnamomi*	50	24·6	20·5–29	29·2	24·8–34
P. cryptogea (Baarn)	(*a*) 75	23·6	19–29	28·2	24–34
+ *P. Cinnamomi*	(*b*) 24	23·1	19–29	—	—
P. cryptogea (Baarn) pure culture	33	23·0	19–28	27·5	24–32
P. Richardiae + *P. Cinnamomi*	55	31·0	24–36	38·5	32–43
P. Richardiae (pure culture)	100	30·7	19–41	39·0	29–48
P. cryptogea (Pethybridge and Lafferty)	—	25·0	—	30·0	—
P. Richardiae (Buisman)	—	± 29·0	—	—	± 34–38

Table III.

Strains	Conidia (Sporangia) Mean size	Ratio	Range
P. cryptogea (tulip) (flower stalk in water)	43·9 × 29·8	1·47	25–54 × 18–37
P. cryptogea (tulip) (pure culture in water)	32·3 × 22·8	1·42	20–42 × 16–29
P. cryptogea (Pethybridge and Lafferty)	40 × 27	1·48	24–50 × 17–30
P. Richardiae (pure culture in water)	41·7 × 26·2	1·59	24–66 × 18–33
P. Richardiae (Buisman)	—	—	—
P. Richardiae (Salmon and Ware) (11)	Commonly 52 × 33	1·58	—
P. Cinnamomi (Rands)	57 × 33	1·7	Commonly 38–84 × 27–39

The author's studies on the strains of *P. parasitica* Dast. (emend.) and *P. palmivora* Butler (*l.c.*) have led him to the conclusion that the essential similarities, apart from mere differences in the size of the conidia (sporangia) and sexual organs of *P. cryptogea* and *P. Richardiae*, do not justify a distinction of species, but that the differences are sufficiently recognised by recording it as a variety, namely *P. cryptogea* Pethyb. and Lafferty var. *Richardiae* (Buis.).

Zoosporangia of *P. Cinnamomi* have been developed also when mycelium from maize-meal and quaker oats agar cultures has been transferred to water which was changed a few times. They are non-papillate and similar to, but substantially larger than, those of *P. cryptogea* (see Table III), and discharge zoospores of the same size. Sexual organs have not been found in pure cultures, but those developed in a paired culture with a strain of *P. parasitica* (*l.c.*) showed amphigynous antheridia and oospores of similar size to those of *P. Richardiae*. The vesicles formed so abundantly on solid media are peculiar (Rands (9) calls them chlamydospores) and closely resemble the similar bodies developed on the same media by *Blepharospora cambivora* Petri, a culture of which was kindly sent by Prof. L. Petri. Sexual organs have not been detected in paired cultures of *B. cambivora* and *P. Cinnamomi* and the first has also not formed them in pure cultures. Sporangia of *B. cambivora* have developed in water cultures on sterilised centipedes; they show a more marked narrowing towards the broad tip approaching the papillate type, but the hyaline apical layer is relatively shallow. As Buisman has pointed out (*l.c.*), the proliferation of the pedicel with the production of new sporangia within those which have discharged zoospores and other characters indicate a close relationship of the four forms, but *P. Cinnamomi* appears to be nearer to *B. cambivora* than to *P. cryptogea*.

Pythiomorpha gonapodioides Peters (10) (for a culture of which thanks are due to Dr Westerdijk) has developed coloured thick-walled chlamydospores in culture; in paired cultures it grows vigorously, soon over-runs and suppresses any strain of *Phytophthora*, and no sexual organs have been detected in such cultures.

REFERENCES.

(1) Ashby, S. F. The oospores of *Phytophthora Nicotianae* Br. de Haan, with notes on the taxonomy of *P. parasitica* Dastur. Trans. Brit. Myc. Soc. XIII (1928), 86.

(2) —— Strains and taxonomy of *Phytophthora palmivora* Butler (*P. Faberi* Maubl.). Trans. Brit. Myc. Soc. XIV (1929), 18.

(3) Coleman, L. C. Diseases of the *Areca* Plam. I. Koleroga. Dept. Agric. Mysore Bull. II (1910).

(4) Rosenbaum, J. Studies of the genus *Phytophthora*. Journ. Agric. Res. VIII (1917), 233.

(5) LEONIAN, L. H. Amer. Journ. Bot. XII (1925), 444.
(6) MCRAE, W *Phytophthora Meadii* n.sp. on *Hevea brasiliensis*. Mem. Dept. Agric. India. Bot. Ser. IX (1918), 119.
(7) BUISMAN, C. J. Root Rots caused by Phycomycetes. Meded. Phytopath. Willie Comm. Scholt. XI (1927), 13.
(8) PETHYBRIDGE, G. H. and LAFFERTY, H. A. Sci. Proc. Roy. Dublin Soc. XV (1919), 487.
(9) RANDS, R. D. Streepkanker van Kaneel etc. Meded. Inst. Plantenziekt. (1922), 54.
(10) KANOUSE, B. Bot. Gazette, LXXIX (1925), 196.
(11) SALMON, E. S. and WARE, W. M. Root Rot of *Richardia*. Gard. Chron. LXXXI (1927), 234.

THE PRODUCTION OF SEXUAL ORGANS IN PURE CULTURES OF PHYTOPHTHORA CINNAMOMI RANDS AND BLEPHAROSPORA CAMBIVORA PETRI.

By S. F. Ashby, B.Sc., Mycologist, Imperial Bureau of Mycology.

(With two Text-figures.)

IN the preceding paper* on the production of sexual organs in paired cultures of strains and species of *Phytophthora* the statement was made that they had not been detected in pure cultures of *P. Cinnamomi* and *B. cambivora*. They have now been found in a pure culture of *P. Cinnamomi* (American Type Culture Collection, No. 1407) in two tubes of sloped maize-meal agar after six months at the temperature of the laboratory; and also in the "Cinnamomi" zone of a paired culture of that species with *B. cambivora* on the same medium and of the same age.

In one of the tubes of *P. Cinnamomi* alone, a yellowish brown patch, present on the glass at the base of the **V**, was found to be due to many mature sexual organs with large amphigynous antheridia which were mostly longer than they were broad. The oogonia, which showed more or less thickened yellow walls, although in some instances the coloured walls were quite thin, were for the most part broadly clavate with the wide funnel-shaped base within the antheridium. The oospores with hyaline thickened walls usually filled the upper main part of the oogonial cavity. The sexual organs appeared to be borne on separate hyphae. The measurements which follow, record in microns the

* See p. 252.