STUDIES IN THE GENUS PHYTOPHTHORA

I. Oospore Formation and Taxonomy of *Phytophthora palmivora* Butler

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The development of the sexual bodies in *Phytophthora palmivora*, Butl. has been investigated by a number of workers from time to time. Aided by these and other physiological reactions the status of this species has been revised every now and then and modified to take in more than one species created by later workers in its fold. The arguments put forward by the revisionists have been varied.

Coleman (1907) was the first to report on oospore formation in cultures of *P. omnivora* var. *arecæ* Colem. [(later named *P. arecæ* (Coleman) Pethy]. Rosenbaum (1917) obtained oospores of *P. arecæ* in cultures received from Coleman. But other workers have not been able to obtain these sexual bodies in single strain cultures. Narasimhan (1930) has put forward the explanation that the cultures sent to Rosenbaum were probably not a single strain but a mixed one and that this might be responsible for the formation of oospores. The same explanation is applicable for the development of oospores in Coleman's cultures also. McRae (1917) has described the production of oospores in single strain cultures of *P. meadii* McRae. But this ability is shortlived and other workers to whom cultures of this fungus had been supplied found that oospores did not develop in the cultures. The isolate now studied also does not produce oospores in single strain cultures. Ashby (1922) noted that in paired cultures of *P. palmivora* and *P. faberi* Maub. (coconut and cacao strains) oospores developed. Later (1929) he continued the studies on the development of sexual bodies in paired cultures of *Phytophthora* and has recorded their formation in paired cultures of isolates from coconut and cotton; citrus and coconut; rubber and cacao; and coconut (India) and coconut (Jamaica). As a result of these studies and with isolates from other sources he recognised that the isolates he had, could be placed into two groups, the members of one group forming oospores when mixed with members of the other. Adopting Gadd's (1925) group nomenclature, he arranged the isolates into the 'cacao' and 'rubber'

groups. In the 'cacao' group were isolates from cacao, coconut (India), papaya, *Vanda* and *Cattleya* and in the 'rubber' group isolates from coconut (Jamaica and Philippines), Citrus, *Hevea*, *Dendrobium* and *Odontodenia*. Lester-Smith (1927) grew three isolates of *P. faberi* Maubl. in paired cultures and obtained oospores. In combination with *P. parasitica* Dast. also *P. faberi* formed oospores Paired cultures of *P. parasitica* strains and *P. nicotianæ* Br. de Haan, also produced oospores. Gadd (1925) made a comparative study of the strains of *Phytophthora* isolated from cacao, papaya, *Hevea*, *Dendrobium*, *Odontodenia* and breadfruit in Ceylon. He found that these strains produced oospores in paired cultures; the isolates from cacao and papaya behaved as 'plus' strains and others as 'minus' strains. His later studies (1927) have confirmed his earlier conclusions but he found that the isolates from areca in Ceylon did not form oospores with isolates of *P. faberi* strains. Thompson (1929) obtained eight isolates of *Phytophthora* from *Hevea brasiliensis* which he classified into *P. palmivora*, *P. meadii*, and *P. heveæ* Thompson. These formed oospores in paired cultures with other strains (coconut and roselle) of *P. palmivora* and *P. parasitica*. Narasimhan (1930) studied oospore formation in paired cultures of isolates from *Areca*, *Santalum album* L., *Loranthus longiflorus* Desv., *Jatropha curcas* L., *Bryophyllum calycium* Salisb., *Artocarpus integrifolia* L., *Colocasia antiquorum* Schott and *Ficus hispida* L. He found that in paired cultures the isolates from *Loranthus* and *Areca* developed oospores with the isolates from *Santalum* and *Jatropha*. Leonian (1931) studied the behaviour of 85 cultures of *Phytophthora*. He found that 48 of them were heterothallic equally divided into males and females while the remainder were classified into inconstant forms exhibiting heterothallism, and neutral behaviour. The cultures he tested included *P. palmivora*, *P. faberi*, *P. parasitica*, *P. terrestris* Sherb., *P. manoana* Sid. and *P. nicotianæ*. Venkatarayan (1932) was able to obtain oospores in paired culture of two isolates of *P. arecæ* and *P. palmivora*. Uppal and Desai (1939) obtained oospores in paired cultures of two isolates of *P. arecæ* from Bombay Province. Marudarajan (1941) investigated the formation of oospores in six isolates from *Areca*, *Hevea*, coconut, palmyra and *Cacao* and agreed with Gadd in the existence of two groups. He continued his investigations with two isolates from *Clerodendron infortunatum* L. and *Spondias mangifera* Willd., each belonging to one of the above groups.

Materials and Methods

The availability in the stock cultures of the Government Mycologist, Coimbatore, of a large number of isolates of this genus and of *P. palmivora* as it is now understood was taken advantage of to study their sexual behaviour

under controlled cultural conditions. The list of isolates used in these studies with the accepted identifications as far as they were known at the commencement of these studies is given in Table I.

TABLE I

List of the isolates of Phytophthora used in the study of paired cultures

S. No.	Host	Part affected	Pathogen	Locality	Source from which the isolate was obtained
1	*Agave wightii*, Dr. and Pr.	Leaf	*Phytophthora parasitica* Dast.	Coimbatore	Local isolation from Madras Province
2–6	*Areca catechu* L. (Four isolates)	Fruit	*P. arecæ* (Colem.) Pethy.	South Kanara	do
	(One isolate)	do	do	Mysore	Mr. M. J. Narasimhan, Bangalore
	do	do	*P. arecae* (Strain Tyagli.	North Kanara, Bombay	Dr B. N. Uppal, Poona
	do	do	*P. arecae* (Strain Nilekani)	do	do
7	*Artocarpus incisa* L.	do	*P. palmivora* Butl.	South Kanara	Local isolation from Madras Province
8	*A. integrifolia* L.	do	*P. arecae* (Colem.) Pethy.	do	do
9	*Borassus flabellifer* L.	Bud	*P. palmivora* Butl.	Malabar	do
10	*Citrus nobilis* Lou. (I)	Leaf and fruit	do	do	do
11	*Citrus sinensis* Osbeck (11)	Base of stem	*P. palmivora* Butl.	Kistna	Local isolation from Madras Province
12	*Clerodendron infortunatum* L.	Leaf	P. sp	South Kanara	do
13	*Cocos nucifera* L.	Bud	*P. palmivora* Butl.	Malabar	do
14	*Colocasia antiquorum* Schott.	Leaf	P. sp.	South Kanara	do
15	*Hevea brasiliensis* M. Agg.	Leaf and fruit	*P. meadii* McRae	Cochin State	do
16	*Lycopersicum esculentum* Mill.	Fruit	*P. arecae* (Colem) Pethy.	Coimbatore	do
17	*Nicotiana tabacum* L.	Stem	*P. parasitica* var. *nicotianae*	Salem	do
18	*Piper betle* L. (3 isolates)	do	*P. palmivora* Butl.	Chingleput and Tanjore	do
19	*Spondias mangifera* Willd.	Fruit	do	South Kanara	do
20	*Theobroma cacao* L.	do	*P. faberi* Maubl.	Ceylon	Government Mycologist, Ceylon
21	*Jatropha curcas* L.	do	P. sp.	South Kanara	Local isolation from Madras Province

All the isolates were pure strains and non-oospore forming at the time when the study was commenced. Some of them have been reported to have produced oospores in single strain culture but at the time of the studies no

oospores could be detected in any of the cultures. The isolates from agave, breadfruit and *Hevea* come under this class. Paired cultures were grown in petri-dishes or agar slants. In petri-dishes, quadrants were marked on the dishes by cutting out furrows 2–3 mm. wide in the media through the centre of the dish at right angles. The two strains used in paired cultures were inoculated on adjacent quadrants so that oospore formation if any could be detected easily in the clear furrows where the two growths meet when examining the undersurface of the dish under the low power of the microscope. On agar slants in tubes, the two strains were placed side by side on one edge of the slant half way down its length so that the periodic examination of the tube under the low power of the microscope was facilitated. Except when otherwise mentioned, the cultures were grown on oat agar media at laboratory temperature.

Results of Experiments

Experiment I.—At the outset the two isolates from *Areca* which were obtained from Dr. Uppal were grown together; oospore development was observed on the fifth day in the furrows between adjacent quadrants. In the course of ten days numerous oospores had developed in the zones of both the strains besides those formed in the furrow. Dark lines or zones representing the areas of oospore formation described by Narasimhan (1930) were, however, absent.

Experiment II.—The next step was to find out the sexual behaviour of the different isolates from *Areca* available at Coimbatore. Each of the five isolates, four from South Kanara and one from Mysore, was grown together with each of the two Bombay strains. It was found that all these five isolates formed oospores with the Tyagli strain but not with the Nilekani strain. This explains why Marudarajan (1941) failed to get oospores in mixed cultures of the areca strains from this province. Obviously all of them happened to belong to the same group and further studies revealed that these isolates corresponded with the Nilekani strain from Bombay.

Experiment III.—In a third series of experiments a large number of the isolates of *Phytophthora* available at Coimbatore and originally isolated from a variety of hosts were grown in paired cultures with the two Bombay strains of *P. arecæ* with the following results (Table II).

It is clear from Table II that all the isolates used in this experiment fall into two distinct groups one forming oospores with the Tyagli strain and the other with the Nilekani strain.

Experiment IV.—Paired cultures were then made of various permutations and combinations of all isolates available at Coimbatore other than

TABLE II

Results of paired culture studies made with two Bombay isolates of P. arecæ

Isolate	Result of pairing with	
	Tyagli strain	Nilekani strain
Agave ..	No oospores	Oospores formed
Breadfruit ..	do	do
Citrus II ..	do	do
Coconut ..	do	do
Hevea ..	do	do
Jatropha ..	do	do
Palmyra ..	do	do
Cocoa ..	do	do
Spondias ..	do	do
Areca ..	Oospores formed	No oospores
Betel vine (3 isolates)	do	do
Citrus I ..	do	do
Clerodendron ..	do	do
Jak ..	do	do
Tomato ..	do	do

the two Bombay isolates of *P. arecæ* supplied by Dr. Uppal and observations on the formation of oospores were recorded.

The results are given below:—

TABLE III

Results of paired culture studies among original collections of Phytophthora available at Coimbatore

Isolates	Areca	Betel Vine	Cit. I	*Clerodendron*	Jak	Tomato	Agave	Breadfruit	Cocoa	Cit. II	Coconut	*Colocasia*	*Hevea*	*Jatropha*	*Spondias*	Palmyra	Tobacco
Areca ..	0	0	0	0	0	0	×	..	×	×	×	0	×	×	×	×	×
Betel vine ..	0	0	0	0	0	0	..	..	..	..	×	..	×	..	×	..	..
Citrus I ..	0	0	0	..	..	0	..	..	×	×	×	..	×	×	×	×	..
Clerodendron ..	0	0	..	0	0	0	×	..	×	..	×	0	×	..	×	..	..
Jak ..	0	0	..	0	0	0	×	..	..	×	×	..	×	×	×	×	..
Tomato ..	0	0	0	0	0	..	×	×	×	..	×	..	×	×	×	×	×
Agave ..	×	..	..	×	×	×	0	0	0	..	0	..	0	0	0	0	..
Breadfruit ..	×	×	×	×	×	×	0	0	..	..	0	×	..	..	0	..	..
Cocoa .	×	..	×	×	×	×	0	..	..	0	0	..	0	0	..	0	..
Citrus II ..	×	..	×	..	×	..	..	..	0	..	0	..	0	0	..	0	..
Coconut ..	×	×	×	×	×	×	0	0	0	0	0	..	0	0	0	0	0
Colocasia ..	0	..	..	0	..	..	..	..	..	..	..	..	..	..	×	..	..
Hevea ..	×	×	×	×	×	×	0	..	0	0	0	..	0	0	0	0	..
Jatropha ..	×	..	×	..	×	×	0	..	0	0	0	..	0	0	0	0	..
Palmyra ..	×	..	×	..	×	×	0	..	0	0	0	..	0	0	0	0	0
Tobacco ..	×	..	..	..	..	×	..	..	..	..	0	..	..	..	..	0	0
Spondias ..	×	×	×	×	×	×	0	0	..	..	0	×	0	0	0	0	..

Remarks.—The isolate from breadfruit died soon after the commencement of this experiment; it could not be utilised for further study of paired cultures.

0 = No Oospores. × = Oospores formed. .. = Combinations not tried.

The behaviour of these isolates has been consistent and in conformity with the results obtained in previous experiments. The isolates fall into two sexually distinct types. The members of one group invariably form oospores in paired cultures with members of the other group but not amongst themselves.

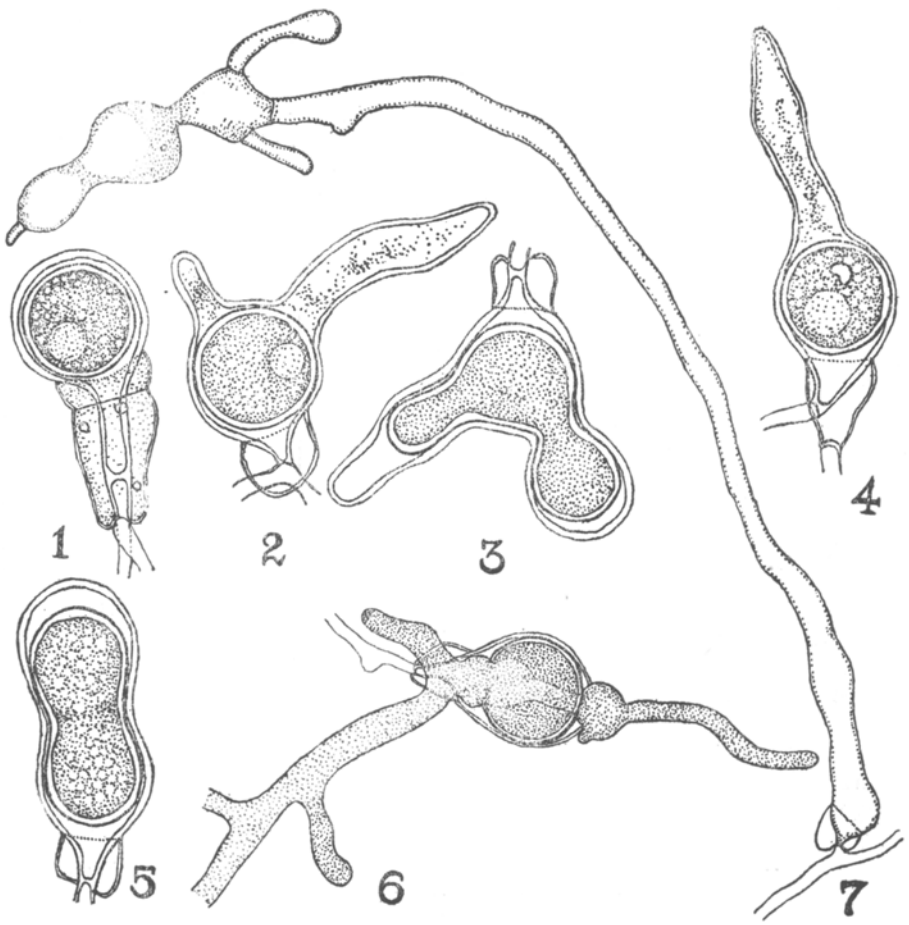
Morphology of the Sexual Organs

The oogonia are spherical, thick-walled, light yellow to yellowish brown in colour, persistent and always with one amphigynous antheridium at the base. Sometimes double antheridia may be seen one above the other (Text-Fig. 1). The antheridia and oogonia always developed on different hyphæ. No instance of the two organs developing on the same hypha was seen. The oogonial wall exhibits variations. In some combinations all the oogonia were smooth, but in others like *Citrus* I × *Jatropha*, *Citrus* I × *Citrus* II, *Citrus* I × palmyra and *Citrus* I × coconut some of the oogonia had thicker walls which were not as clear cut as in others but possessed a rough outline due to uneven thickening of the outer surface (Figs. O, P, Q). This feature has been noted by Gadd (1924) in some of the paired cultures of isolates from Ceylon. Tompkins and Tucker (1937) observed a thick brown crystalline encrustation on the wall of the oogonium produced in paired cultures of *P. capsici* Leon. Consequently the exact limits of the oospore and oogonia could not be determined while taking measurements. With the addition of concentrated solution of potassium hydroxide the encrustation disappeared and the walls of the oogonia and oospores became clear. The thickening of the oogonial wall noticed in some of the cultures under study however did not react in a similar manner when potassium hydroxide solution was added.

The oospores are spherical, thickwalled and light yellow to yellowish-brown or reddish-brown in colour. They may either completely fill the oogonial cavity or there may be some space between the two walls. In all paired cultures varying proportions of the two kinds can be observed.

In some of the cultures (*e.g.*, *Citrus* I × *Jatropha*) some of the later formed oogonia and oospores were peculiar. The oogonium was elongated, irregularly swollen and sometimes developing one or more branches (Text-Figs. 2–5, 7). Usually only one oospore was found in such oogonia but this occupied only a portion of the oogonium while the rest of the cavity

was either empty or filled with vacuolate hyaline or yellowish protoplasmic contents. Oospores also exhibited lobulation in some cases. Some of the oogonia were very much elongated and branched and usually these did not contain oospores. They resembled empty, swollen, elongated and branched hyphæ with scanty or disintegrated contents. The presence of the amphigynous empty antheridium at the base distinguished these structures from vegetative hyphæ and showed them as malformed oogonia. Leonian (1931) has observed similar structures in a culture of *P. palmivora*. But he describes them as germinating oospores and the round bodies inside some of them as secondary oospores. But the writers do not agree with this. These structures represent abnormal proliferating oogonia. The



TEXT-FIGS. 1–6.—Sexual bodies from Citrus I X *Jatropha*. 1. Double antheridia. 2–5. Abnormal oogonia with differently shaped oospores. 6. Germinating oospore. 7. An abnormal oogonium showing peculiar growth and branching (× 680).

germination of the oospore can be easily distinguished by the presence of the germ tube which bursts through the wall of the oospore and later through the wall of the oogonium and grows out (Text-Fig. 6). But in most of the cases this was not evident and the oogonium alone had elongated and developed branches. When the oospore germinates the oogonium does not elongate. The sketches given by Leonian (1931) also do not bear out his statement. They can be regarded only as abnormal oogonia. Those oogonia which have not been fertilised probably resume vegetative activity and develop into branched structures of limited growth.

In tubes and petri-dishes the sexual bodies develop on the medium or are submerged; they are also formed on the sides of the glass in tubes and in the clear space between the quadrants in petri-dishes. The first formed oospores are usually at the junction of the growths from the two isolates.

TABLE IV

Measurements of oogonia and oospores as recorded by various workers

Author	Species or pair of isolates	Oogonia		Oospores	
		Range μ	Mean μ	Range μ	Mean μ
Rosenbaum ..	*P. arecae* ..			23–44	32·4
Coleman ..	do ..			23–36	
Ashby ..	Cacao+Coconut 3 ..	22–35	28·6	17·8–27·8	23·1
	Cacao+Cotton boll ..	do	29·0	17·8–28·6	23·1
	Cacao+*P. parasitica* (castor) ..	25–33	28·8	20·0–25·3	23·6
	Coconut (Jamaica)+Coconut (India)			26–39	31·8
	Hevea+Cacao (Hevea Zone) ..			22–33	27·6
	(Cacao Zone) ..			20–30	25·0
	P. meadii+*P. arecae* ..	24–34	28·5	20–28	24·0
	P. meadii (Malaya)+*P. arecae* ..	24–35	29	23–28	25·3
McRae ..	*P. meadii* ..	24–35	32	16–32·8	25·0
Gadd ..	Cacao+*Odontodenia* ..			19–25	22·1
				21–28	23·8
	Cacao+rubber ..			20–28	23·7
	Cacao+Breadfruit ..			22–27	24·0
	Papaw fruit+*Odontodenia* ..			17–28	23·4
Narasimhan ..	Areca+*Santalum* ..			30–31	
	Areca+*Jatropha* ..			26–27	
	Santalum+*Loranthus* ..			30–31	
	Loranthus+*Jatropha* ..			29–30	
Venkatarayan ..	*Aleurites*+areca (fruit) ..		27·8		23·04
	do + do (top rot) ..		25·8		22·2
	Areca (top rot)+*Santalum* ..		28·9		25·5
	Areca (fruit) + do ..		32·6		28·1
Marudarajan ..	*P. arecae*+*P. meadii* ..	28–42	35·7	24·5–38·5	31·5
	do +Palmyra ..	28–40	33·6	do	29·5
	do +Coconut ..	28–42	36·0	do	30·8
	do +Citrus ..	24·5–33·3	29·4	22·8–31·5	27·3
	do +Cacao ..	18·3–33·3	28·0	17·5–29·8	24·5

Later they may be observed in other portions also. In some combinations oospores are formed in plenty while in others they are few. This difference in the intensity of formation of sexual bodies may be due to the fact that the isolates were originally brought into culture at different periods and consequently varied in the number of generations they had passed through in subcultures on agar media.

The size of the oogonium and the oospore exhibited wide variations. The measurements of the oogonia and oospores obtained by previous workers are given in Table IV.

These measurements were compared with those of the sexual bodies produced in the paired cultures under study. One hundred measurements were made in each case. The sexual bodies were taken from paired cultures within ten to fifteen days after inoculation (Table V).

TABLE V

Measurements of sexual bodies obtained from different combinations

Serial No.	Isolates grown in paired cultures	Diameter			
		Oogonia		Oospores	
		Range in μ	Mean μ	Range in μ	Mean μ
1	*Areca* (Nilekani)+Palmyra	23–41	30·0	18·6–30·0	24·4
2	*Areca* (Nilekani)+*Jatropha*	21·7–40·3	28·6	14·0–31·0	21·8
3	*Areca* (Nilekani)+*Spondias*	14·0–33·3	23·9	10·5–28·0	17·5
4	Jak+Palmyra	20·2–27·9	25·1	15·5–21·7	19·5
5	Jak+*Jatropha*	21·7–34·1	27·1	15·5–26·5	20·9
6	do+*Citrus* II	21·7–34·1	29·5	14·0–27·9	23·0
7	do+*Hevea*	20·2–34·1	27·2	15·5–27·9	20·8
8	do+*Agave*	21·7–31·0	25·8	15·5–24·8	19·9
9	do+*Spondias*	23·3–34·0	28·4	18·6–27·9	22·5
10	do+*Areca* (Tyagali)	24·8–34·4	29·8	20·2–27·9	12·3
11	*Citrus* I +*Spondias*	21·7–34·1	27·1	15·5–27·9	21·6
12	do +*Citrus* II	24·8–37·2	30·6	18·6–31·0	24·7
13	do +Palmyra	21·7–34·1	28·1	17·1–26·4	21·6
14	do +*Jatropha*	24·8–37·2	29·1	18·6–31·0	23·0
15	*Clerodendron*+*Hevea*	21·7–32·6	27·5	17·1–24·8	21·1
16	*Clerodendron*+*Spondias*	23·3–34·1	29·4	18·6–31·0	24·0
17	Betel vine I+*Spondias*	21·7–32·6	27·5	18·6–27·9	21·7
18	Betal vine II+*Spondias*	24·8–37·2	30·0	18·6–31·0	23·8
19	Betel vine+*Areca* (Tyagali)	24·8–36·5	28·4	15·5–31·0	21·9
20	Citrus I+Tyagali	21·7–30·0	27·5	15·5–24·8	21·0
21	*Cleiodendron*+*Areca* (Tyagali)	24·8–35·7	30·5	17·1–27·9	23·4
22	*Areca* (Kanara)+*Spondias*	21·7–34·1	28·4	18·6–27·9	22·4
23	*Colocasia*+*Spondias*	21·7–34·1	27·5	15·5–24·8	21·9
24	Tomato+Tobacco	20·2–27·9	24·1	15·5–21·7	18·0
25	Tomato+*Jatropha*	21·7–31·0	25·1	18·6–27·9	21·9
26	Tomato+*Citrus* II	21·7–34·1	29·1	18·6–27·9	24·8
27	Tomato+Coconut	23·3–34·1	27·7	20·2–29·6	23·4
28	Breadfruit (alone) (suspected to be mixed)	20·0–28·5	25·0	16·5–24·5	20·6
29	Tomato+*Hevea*	24·8–34·1	28·1	20·2–29·6	24·1

The mean diameter of the oospores varies from 17·5 to 24·4μ and lies within the range obtained by other workers. The wide variation in size of the sexual bodies observed in these studies and by other workers goes to show that this character is highly variable and plastic and that no reliance can be placed on this for taxonomic purposes. However the ability to form the oospores in paired culture brings out the specific relationship of the complementary isolates.

Some Physiological Studies

The influence of medium on oospore formation.—It has been stated by previous workers that certain media favoured the formation of oospores in mixed cultures while others did not. This factor differs with isolates. Thus Tucker (1931) found that certain isolates of *P. parasitica* produced large numbers of oospores in lima bean and oat-meal agars and few or none on cornmeal agar, while still others developed more oospores on cornmeal agar and a smaller number on oat-meal agar. The investigations on paired cultures recorded here were carried out on oat agar which was found to be quite satisfactory. Leonian (1931) also found that oat agar was the most suitable. Two complementary strains, *viz.*, *Citrus* I and *Citrus* II were grown on oat, frenchbean and maize agars. The reaction of the media was adjusted to pH 5·6 in each case. The growth of the fungi was very luxuriant on oat and frenchbean agars and less profuse on maize agar. Oospores were formed in all cases but were more numerous in french-bean and oat agars than in maize agar. Tucker's observations only show the possibility that different races have preference to particular media for growth and reproduction.

Liquid oat-extract was prepared by boiling 50 gm. of powdered oat grains in a litre of water for one hour and then filtered through cottonwool. After filtering, the extract was autoclaved for 20 minutes at 15 lbs. pressure. In this medium two strains were grown for 15 days after which the medium was filtered through Chamberland filters under aseptic conditions. Five and ten cubic centimetres of the filtrates were mixed with 10 c.c. of melted oat agar medium which was then poured into plates. After the agar had set, the plates were inoculated with the complementary strain. Even after 30 days' growth oospores were not formed. This indicates that a strain does not secrete any extra-cellular substance into the medium to stimulate oospore formation in its complementary strain. Further work on these lines is in progress.

Temperature and oospore formation.—Ashby (1929) has recorded that if paired cultures are maintained at 23° C. (or 20°–25° C.) prompt development

of oospores takes place. Other workers also have experienced that exposure to lower temperatures or maintenance of cultures in ice-chests is conducive to oospore formation. Marudarajan (1941) observed oospore formation to be good at 20° C. In the course of the present investigation it was observed that oospores did not develop in the paired cultures started in the months of March, April and May when the laboratory temperatures varied between 28° C. and 31° C. But in July and August and from October to January the paired cultures readily produced oospores when the laboratory temperature was below 26° C. Paired cultures kept inside a controlled temperature cabinet in which the temperature varied from 8°–10° C. failed to develop oospores. Very low temperatures evidently do not favour the development of oospores in this tropical species.

Age of isolates and oospore formation.—In fungi, it is common experience that the intensity of sporulation gradually diminishes as the isolate is kept on for a large number of generations on agar media and may even disappear eventually. This behaviour is often seen in *Phytophthora* especially with regard to oospore formation. Paired cultures of fresh isolates of complementary strains produce oospores quite readily and in large numbers in 3–8 days depending on the distance separating the inocula of the two strains. But after several generations of sub-cultures the capacity to form oospores decreases in some strains until it is finally lost. For instance the Nilekani strain on *Areca* obtained from Bombay used to form large numbers of oospores with its complementary strains as mentioned earlier. But at the time of writing, *i.e.*, two years after its arrival, it does not form oospores with the isolates with which it was forming oospores before. It has become neutral.

The isolate from *Spondias* is another good example of the waning of the capacity to form oospores with ageing. A fresh isolate formed oospores with all the complementary isolates in four days. Another which had been isolated two years ago produced sexual bodies in combination with the same complementary strain. But the development was incomplete. Antheridia and oogonia were formed but mature oospores did not develop. The oogonia had grown through the antheridia and assumed the normal size and shape after emergence but later the contents disintegrated. Six months later, even this phenomenon did not occur in the combinations. Thus there has been a gradual decline of the sexual capacity of the isolate. This phenomenon is attributable either to the senescence of the isolate through successive subculturing on media for a long period or to formation of indistinguishable dissociants which were neutral or had lost their sexuality. These dissociants

have possibly been carried over in the transfers and thus the change might have occurred. Further experiments are necessary to decide the correctness of this view.

Discussion

The study of the formation of oospores in *Phytophthora* has been an interesting subject for investigation and several workers have been on this problem, though the last word has not yet been written. Oospore formation is influenced by various factors such as the temperature at which the organism is grown, the medium on which it is grown, the age of the isolates and lastly the innate character of the isolate itself. There are some species in which the sexual bodies have not been recorded yet. The development of the sexual bodies in the *Phytophthora palmivora* group has been investigated by different authors and divergent views have been expressed about the causes leading to this phenomenon. One school represented by Ashby (1928-29) and Lester-Smith (1927) is of the opinion that the oospore formation is brought about by some sort of biochemical stimulation of one strain by the other. Lester-Smith states that the production of oospores " in mixed cultures is due to the influence of one vegetation on the other acting through its effect on the medium or on certain constituents of the medium ". Gadd (1924), Thompson (1929), Narasimhan (1930), Leonian (1931). Venkatarayan (1932) and Uppal and Desai (1939) on the other hand believe in the heterothallic nature of the isolates of this species. Some prefer to call the isolates ' Plus ' and ' Minus ' strains while Narasimhan and Leonian who have traced the origin of the hyphæ producing the antheridia and oogonia, call the isolates male and female. Tucker (1931) is not convinced of the heterothallic nature of the isolates.

The present investigations carried out with 25 isolates of *Phytophthora*, the bulk of which were obtained from this province and a few from outside the province, have shown that all of them fall into two distinct groups based on their capacity to produce oospores in paired cultures. The isolates of one group form oospores when mixed with isolates from the other. Different combinations of the members of the two groups have been made and the results have been consistent throughout. In the light of present knowledge this behaviour can only be attributed to heterothallism within the same species.

It has moreover been found that in all cases the oogonia and antheridia are borne on different hyphæ and never on the same hypha. This again is an indirect evidence of heterothallism. Narasimhan (1930) and Leonian (1931) have claimed to have traced the antheridia and oogonia to different thalli, which is a direct proof of heterothallism of the isolates studied.

It has also been found that some isolates have gradually lost their sexuality in course of time when grown on media. The deterioration of the capacity for oospore-formation seems to be attributable to gradual loss of the sexual vigour of the strains through continued growth on agar media.

The behaviour of some of the isolates which formed oospores in single strain cultures originally but later failed to produce them, is intriguing. This can be explained away in two ways. It is possible that the original culture was itself a mixed one as might be expected when the fungus is isolated by tissue cultures and the original host had been infected by both the strains. An observation made by the writers in 1946, favours this view. In 1946 a fresh isolate was obtained from breadfruit by tissue culture. In the first generation abundant oospores were formed. From this culture single hyphal tips were transferred to agar slants. Oospores failed to form in these secondary cultures indicating that the original isolate was mixed. But McRae (1917) recorded oospores in single sporangial isolates of *P. meadii*. It has been noticed by several investigators that the cultures supplied to them from Coimbatore did not develop oospores. Even in India the same phenomenon was experienced. McRae had observed oospores on *Hevea* fruits also. This could be explained on the assumption that the strain was originally homothallic but during the growth of the cultures on agar media for a number of generations dissociation took place and the loss of one sexual factor resulted therefrom. It is, however, interesting to note that the isolates from *Agave*, *Hevea* and *breadfruit*, which were originally reported to be forming oospores in single strain cultures and have now become non-oospore-forming, fall into the same sexual thallus group which produces oospores in paired cultures with individuals of the same complementary group. No isolate belonging to the opposite group isolated in this province has ever been known to form oospores in single strain cultures. Leonian (1931) obtained seven dissociants from a culture of *P. parasitica*. Of these six behaved as females and one as a male. He has also obtained other dissociants which could be termed neutral since they failed to form oospores with either of the male or female isolates. Even in the heterothallic strains under study the sex vigour has been lost owing to long culturing on agar media or formation of neutral dissociants. Thus members of the *P. palmivora* group behave as homothallic, heterothallic or neutral strains though with continued growth on agar media many of the strains may become neutral. This change is observable in both groups of complementary strains. Therefore, for the correct identification of the isolates fresh cultures are essential.

These investigations have been helpful in deciding the taxonomic relationships of the isolates. The isolates studied have been variously classified at present. Tucker (1933) and Leonian (1934) have suggested certain revisions of the classification of *Phytophthora* species. Tucker has merged together *P. palmivora*, *P. arecæ*, *P. faberi* and *P. meadii* into one species under *P. palmivora*. Leonian believes that *P. mexicana* Hots. and Hart., *P. parasitica*, *P. parasitica* var. *rhei* God., *P. parasitica* var. *nicotianæ* Tucker, *P. terrestris* Sherb., *P. melongenæ* Saw. and *P. symmetrica* Sid. also should be brought under *P. palmivora*. The isolates under investigation are usually classified as follows: *P. palmivora* on coconut, palmyra, citrus and betel vine; *P. arecæ* on arecanut and tomato; *P. faberi* on cocoa; *P. meadii* on *Hevea*; *P. parasitica* var. *nicotianæ* on tobacco; *P. sp.* (not determined) on *Clerodendron*, breadfruit, *Spondias*, *Jatropha*, Jak and *Colocasia* and *P. parasitica* on Agave.

The basis for specific differentiation has been morphological features of the hyphæ, sporangia, chlamydospores and oospores, when formed. Pathogenicity has also been utilized for separating species. Studies on this genus have shown (Tucker, 1931 and Leonian, 1934) that the morphological characters of the mycelium, sporangia and chlamydospores are so plastic as to be of little use in specific differentiation. Leonian (1934) says that "pathogenicity is of still less value, the shape and size of the chlamydospores altogether useless and that of the sporangia not much better in the taxonomy of species of *Phytophthora*".

The work now recorded has shown that all the isolates under study produce oospores when grown mixed with complementary isolates. All the oospores formed in the various combinations are of the same type and the measurements fall within the range recorded for oospores produced from complementary strains occurring on the same host, *e.g.*, *Areca* (Tyagali) $\times$ *Areca* (Nilekani) 14·0–31·0 μ. This feature coupled with the readiness with which oospores are formed in the paired cultures of these isolates brings out the close specific relationship of these isolates. There can be no question of regarding these oospores as of hybrid origin between different species because no constant differences can be made out between these either in the size of the oospores, the nature of the antheridia or any other important character. All these isolates, therefore, fall under one species, *viz.*, *P. palmivora* Butler.

This species has a wide host range. Not less than sixteen species of host plants have so far been recorded from S. India. It is heterothallic but the present state of our knowledge suggests the possibility of some isolations

being homothallic. Two distinct sexual strains—the plus and the minus—are seen and the collections in our possession are classified under the two heads as follows:

Plus	Minus
Areca (Nilekani)	*Areca* (Tyagali)
Betel vine	Palmyra
Citrus I	Coconut
Clerodendron	Cacao
Colocasia	Rubber
Jak	Breadfruit
Tomato	*Spondias*
	Agave
	Tobacco
	Citrus II
	Jatropha

The grouping of the isolates into the " Cacao " and " Rubber " groups adopted by Gadd and followed by Ashby is rather confusing. The same host has been found to be affected by both the strains. For example, the " Cacao " isolate from Ceylon really behaves like an isolate belonging to the " Rubber " group. Ashby has also found that different isolates from cacao and coconut may fall into different groups. Therefore, the naming of the groups according to the host is misleading.

Uppal and Desai (1939) have obtained two complementary isolates Tyagali and Nilekani—form the same host, *viz.*, *Areca*. Tyagali behaves like the isolates from palmyra and coconut in Madras; Butler (1910) has recorded that *P. palmivora* affects arecanuts causing bud-rot. It is possible that the Tyagali strain represents the coconut strain (minus) which has become parasitic on *Areca* in that locality. Coconut is also infected by both the strains. *Citrus* in India is also parasitised by both. When such mixed or combined infections occur in nature on the same host plants, there is every possibility of oospores developing as has been recorded in breadfruit and *Hevea* rubber. This represents one of the methods of ' over-summering ' of the fungus under tropical conditions obtaining in South India. Whether sexual reproduction gives rise to new races is a matter for further investigation.

The plant pathologist has to consider the significance of these results. These facts bring out the necessity for vigilance on his part concerning the occurrence of *Phytophthora palmivora* on a variety of hosts some economically important and others of no importance. Inasmuch as this species

has a wide host range, the passage from one host to another is easy under favourable conditions. Further, the part played by the non-crop-hosts in the survival of the pathogen, the formation of sexual bodies when the same host becomes infected by the two sexual strains and the possible production of new strains as a result of sexual reproduction cannot be overruled. The parasitism of *P. palmivora* being by no means specialised, every record of this species on any host has to be considered as a source of potential danger to the crop plants known to serve as hosts of this species in the locality.

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SUMMARY

The formation of oospores in paired cultures of twenty-five isolates of *Phytophthora* was studied. These isolates fall into two main groups—the " plus " and the " minus " and the members of one group form oospores when paired with members of the other group. Some of the isolates were found to lose their sexual capacity with continued cultivation on agar media. Fresh isolates form oospores quickly with complementary strains.

All these isolates belong to *P. palmivora*, Butl. The other species of *Phytophthora*, *viz.*, *P. arecæ*, *P. meadii*, *P. faberi* and *P. parasitica* var. *nicotianæ*—are to be merged in *P. palmivora* as they are found to be morphologically similar and do not exhibit any constant and reliable differences from *P. palmivora* and readily form oospores when paired with it. This species is heterothallic; but homothallism has been reported to have been noticed in some isolates.

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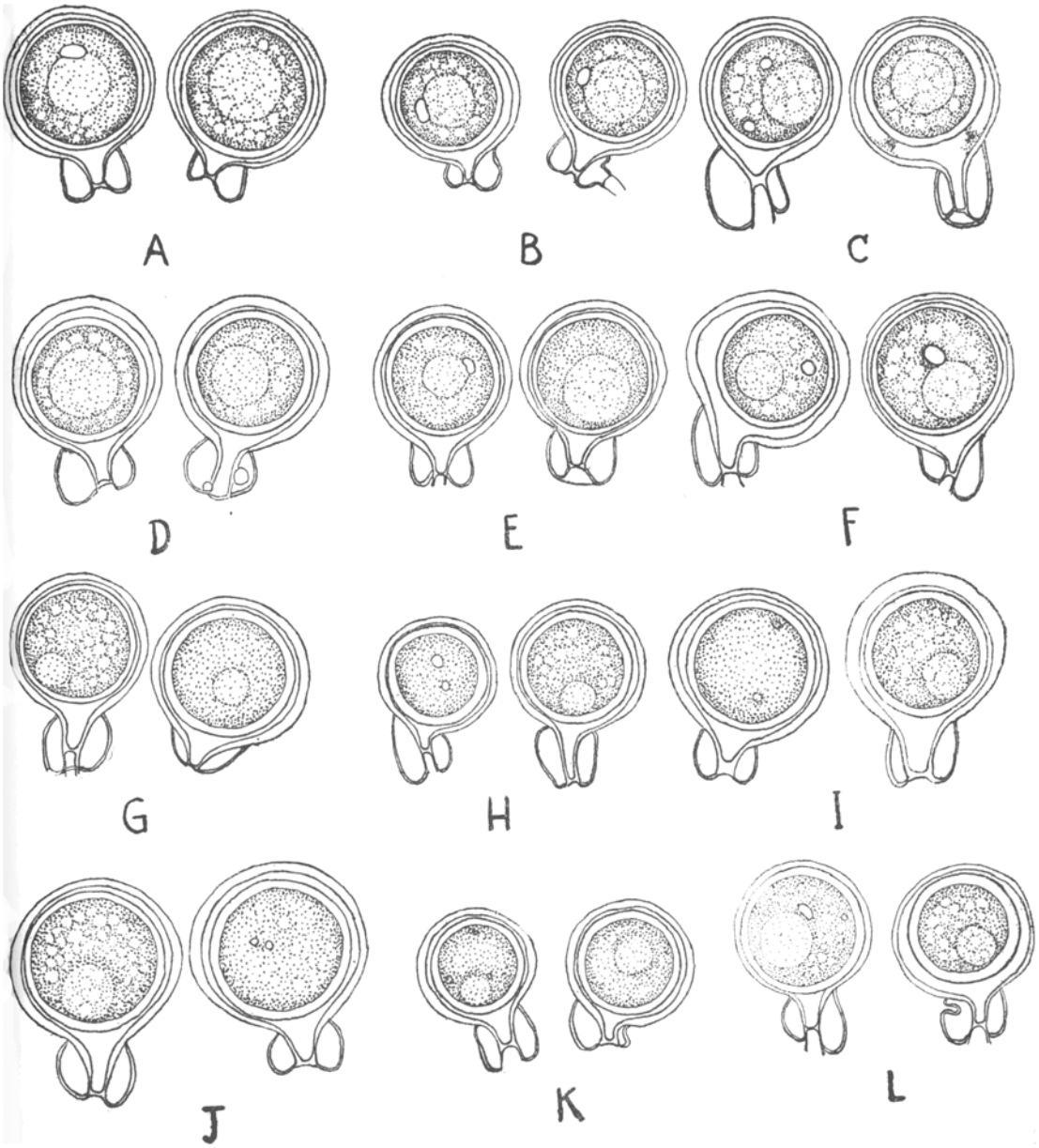
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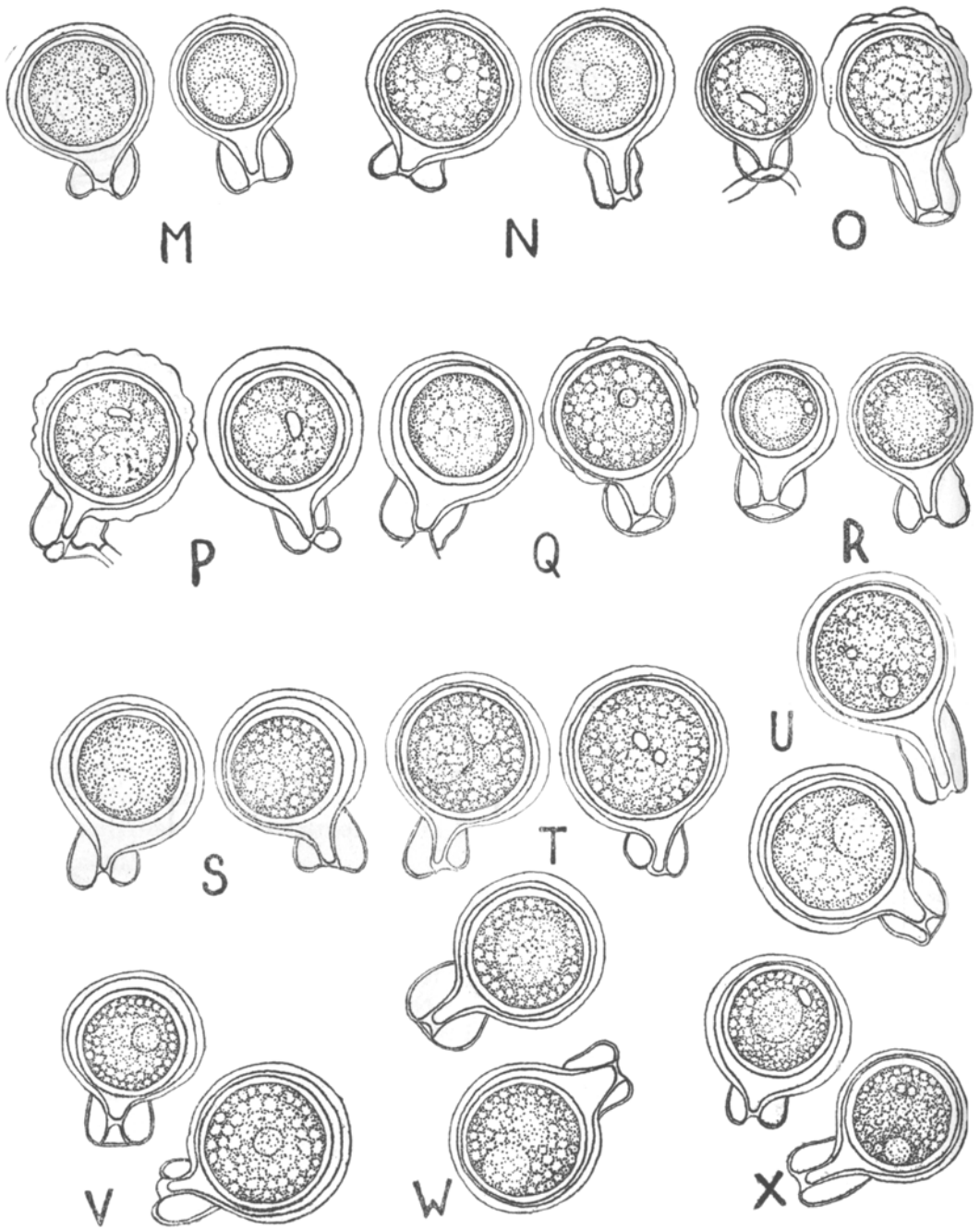
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Explanations of Plates

All figures were drawn with the aid of an Abbe camera lucida at a uniform magnification of × 680.

Plates VIII and IX : Sexual bodies produced in paired cultures of 'plus' and 'minus' strains of *Phytophthora palmivora* Butler.

Plate VIII

A. Jak × Jatropha
B. Jak × Palmyra
C. Jak × Hevea
D. Jak × Citrus II
E. Jak × Agave
F. Jak × Spondias
G. Tomato × Citrus II
H. Tomato × Jatropha
I. Tomato × Cocoa
J. Tomato × Hevea
K. Tomato × Tobacco
L. Tomato × Agave

Plate IX

M. Tomato × Coconut
N. Citrus I × Spondias
O. Citrus I × Jatropha
P. Citrus I × Palmyra
Q. Citrus I × Citrus II
R. Breadfruit (alone)
S. Clerodendron × Hevea
T. Clerodendron × Spondias
U. Betel vine I × Spondias
V. Betel vine II × Spondias
W. Colocasia × Spondias
X. Areca × Spondias