I hereby declare that the work presented in this thesis:

"Studies on the Development of Secondary sporangia of *Phytophthora palmivora* (Butl.) Butl."

was done entirely by me in the Department of Botany, University of Ghana, from August 1975 to August 1977. This work has never been presented either in whole or in part for any other degree of this University or elsewhere.

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STUDIES ON THE DEVELOPMENT OF SECONDARY SPORANGIA OF *PHYTOPHTHORA PALMIVORA* (BUTL) BUTL.

A thesis presented by

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CONTENTS

A B S T R A C T

A small proportion of sporangia of P. palmivora, less than 1.0 per cent, formed by hyphae growing on cocoa pods germinated in humid air at 25°C whilst still attached to the hypha to produce germ tubes terminated by secondary sporangia. Sporangia in suspension in distilled water formed the greatest number of secondary sporangia at 30°C. Germination in samples of sporangia of the same age and formed under the same conditions varied, and, the percentage of directly germinated sporangia bearing secondary sporangia at 30°C ranged from 1.5 to 17.1 in the various samples.

The majority of sporangia germinating in Distilled water formed a single germ tube. The maximum number of germ tubes observed on a sporangium was three. Directly germinating sporangia consequently generally produced a single secondary sporangium. The length of germ tubes bearing the secondary sporangia varied. Secondary sporangium could germinate directly on maturation to produce another secondary sporangium and secondary sporangia to as far as the fourth order were formed in certain instances.

Secondary sporangia produced by sporangia germinating in distilled water and in solutions of amino acids, carbohydrates, mineral salts, proteins and vitamins were markedly smaller than the primary sporangium. The average lengths of the primary and secondary sporangia ranged from 38.1 to 42.4 µm, and 26.9 to 32.7 µm respectively. Secondary sporangia of 2nd, 3rd and 4th order were closely similar to the first secondary sporangium in size.

Secondary sporangium formation was limited in nutrient solutions prepared with carbohydrates at concentration of 0.1 to 0.8 per cent used as both germination and growth media. More secondary sporangia were formed in distilled water than in solutions of Fructose, Glucose, Sucrose, Mannose, Xylose, in the mixtures containing fructose, glucose and sucrose and in solutions of alanine, arginine, asparagine, glycine, histidine, leucine, lysine; mixtures of fructose, glucose and sucrose, amino acids at 100 to 400 ppm and Peptone and Yeast-extract at a concentration of 1.0 per cent.

Withdrawal of nutrients by transfer of the germinated sporangia to distilled water or severe reduction of nutrient concentration induced extensive secondary sporangium formation in 2 - 3 hour old germ tubes of sporangia germinated in nutrient solutions of either proteins or carbohydrates. Growing germ tubes transferred from nutrient solutions to solutions of low concentrations of Aneurine hydrochloride, D-Biotin Cyanocobalamin, Nicotinamide, Pantothenic acid calcium salt, Pyridoxine hydrochloride and Riboflavin and into solutions of 5mM KH_2PO_4 and $MgSO_4.7H_2O$ or into solutions of mixtures of these two inorganic salts and either 0.1 per cent protein (peptone or yeast-extract) or 0.05 per cent carbohydrates (fructose and glucose), profusely formed secondary sporangia. Induction of secondary sporangium by this method was possible at 30°C and lower temperatures of 20° and 25°C.

Secondary sporangia were produced by 92.6 - 100 per cent of directly germinated sporangia at pH 5.0 to 9.0. Only 1.0 - 9.7 per cent of the directly germinated sporangia formed secondary sporangia at pH 3.0.

Lipids, proteins and RNA were detected, by staining, in both primary and secondary sporangia. The concentration and distribution of each were the same in the two types of sporangia. Indirect germination was, however, greater and proceeded at a faster rate in the primary than in the secondary sporangia.

I. INTRODUCTION

Two types of direct germination are distinguishable among spores of fungi : 'tube-germination' and 'sprout-germination'. The less common type is sprout germination in which the germinating spore under certain circumstances gives rise to a mass of yeast-like cells. The ascospores of Bulgaria inquinans Fries are a commonly cited example. In the majority, tube germination is the rule. The germinating spore produces a germ tube which grows into a mycelium.

Growth of the germ tube, in certain instances, during tube germination may, however, be very limited and a mycelium is never produced. The germ tube directly gives rise to a secondary spore. The germ tubes of teliospores of Uredinales and chlamydospores of Ustilaginales are converted into basidia and promycelia which bear basidiospores and sporidia, respectively, as a regular definite stage in the germination. Similarly, germ tubes of germinating oospores of some members of the Peronosporales, and, zygospores of members of the Mucorales stop growth when they are only a few microns long and then form sporangia at their apices.

In other fungal species, secondary spores are not a regular feature and their formation is dependent on certain environmental conditions. Secondary spore formation among species of this category has been found to be associated with all sorts of spores. De Bary (1869) found that when primary conidia from cultures of Beauveria species were germinated in a thin film of water, the germ tubes, after reaching a length of 6 to 10 times the diameter of the conidia, produced oblong - cylindric secondary conidia. He named these "cylinder conidia". Secondary conidia were also reported among Beauveria species by Clerk (1963), Macleod (1954), Petch (1926), Pettit (1875) and Siemaszko (1937).

Clerk o.p. cit observed that secondary conidia were produced by germ tubes of primary conidia of Beauveria bassiana (Bals.) Vuill. sown in distilled water and also incubated at 100% R.H. He showed that whereas the primary conidia consisted of a mixture of globose (2.1 - 2.8 µm in diameter) and oval (2.8 - 4.7 x 2.1 - 2.4 µm) spores, the secondary conidia were uniformly oval in shape and measured 3.5 - 4.6 x 1.5 - 2.5 µm.

Hawn and Vauterpool (1953) reported the occurrence of

secondary basidiospores in Rhizoctonia solani (Kuhn). They described the germination of the basidiospore as a 'repetition'. A sterigma appeared at the apex of the spore, its length being about twice that of the primary basidiospore and at the apex of the sterigma developed a secondary basidiospore. The sterigma formed by the basidiospore of Ceratobasidium cornigerum (Bourd.) Rogers, in a similar repetitive germination, on the other hand, emerges from the side of the basidiospore (Martin, 1944). Germination by repetition is a common phenomenon in the Ascomycete, Sporobolomyces roseus (Kl. and van N). The ballitospores form sterigmata upon which smaller ballitospores are produced (Bessey, 1950).

Secondary spore production is unusually common in certain groups of fungi, notably in the Order Entomophthorales and in the Genus Phytophthora.

The greatest contribution on the Entomophthorales has come from the observations of Drechsler. Secondary conidium formation has been described by Drechsler in Conidiobolus firmipilleus Drechsler (Drechsler 1953), Conidiobolus osmodes Drechsler (Drechsler, 1954), Conidiobolus nigosus Drechsler, Conidiobolus nanodes Drechsler and Conidiobolus lachnodes Drechsler (Drechsler, 1955a), Conidiobolus polytocus Drechsler and Conidiobolus chlamydosporus Drechsler (Drechsler, 1955b),

Conidiobolus incongruus sp. n. and in Conidiobolus multivagus sp. n. (Drechsler, 1960).

Drechsler (1955b) gave the specific epithet polytocus to C. polytocus which means "bringing forth many young ones" to signify its unusual prolificness. In an early stage of miltiplicative sporulation the primary conidium shows several warty protuberances. Each protuberance, after elongating a few microns, forms a terminal swelling that grows into a globose microconidium.

Srinivasan and Thirumalachar (1962) studied the morphology of several Conidiobolus species from India. Many of these species readily formed secondary sporangia. Conidiobolus globuliferus Drechsler conidia showed repetitional development by giving rise to a secondary globose conidium on a short tubular secondary conidiophore. In rare cases, ellipsoid secondary conidia were formed. Discharged conidia of Conidiobolus heterosporus Drechsl. germinated directly or gave rise to secondary globose conidia on long (50 - 60 µm long) tubular secondary conidiophore. Under certain conditions, the primary conidia formed secondary conidia of elongate - ellipsoid shape on slender conidiophores. Formation of the ellipsoid secondary conidia appeared to be more

frequent among conidia deposited on glass. Conidiobolus khandalensis Srinivasan and Thirumalachar possesses globose conidium with a characteristic conical papilla which germinated directly or gave rise to a secondary globose conidium on a short secondary conidiophore.

Usually the conidium of Conidiobolus coronatus (Cost) Batko produces one secondary conidium that is slightly smaller than its parent, but, occasionally, it may produce a number of small microconidia that are borne on radially oriented sterigmata (Page and Humber, 1973).

Callagham (1969) observed secondary spores on erect germ tubes of Basidiobolus ranarum Eidam. Dring (1958) similarly reported that discharged conidia of Conidiobolus rhysosporus Drechsler caught on 2 per cent malt agar plates formed secondary sporangia at the tips of short (about 50 µm) germ tubes of similar size and shape.

Hotson and Hartage (1923) reported one of the earliest observations in the Phytophthoras. They observed that, on some occasions, a sporangium of Phytophthora mexicana Hotson and Hartage germinated by sending out a single germ tube, often with a single sporangium forming at the apex.

The sporangium so formed could germinate by a germ tube, which in turn, formed another sporangium at the tip so that as many as four successive sporangia could be observed to form and germinate in series. The fungus also produced chlamydospores that germinated by germ tube and often carried a sporangium at the end of the germ tube.

Leonian (1922), a year earlier, observed secondary sporangium formation in Phytophthora capsici Leonian. He described the germinating sporangium as ovoid with prominent papilla , either with a single and apical germ tube or sometimes up to three germ tubes which would be variously dispersed on the sporangium and each carrying a sporangium.

Since then, secondary sporangia have been found in germinating sporangia of several species and the monograph, 'The Genus Phytophthora' by Waterhouse (1956) contains drawings of secondary sporangia at the apices of germ tubes of germinating sporangia of Phytophthora canavaliae Hara, Phytophthora cinchonae Sawada, Phytophthora cinnamomi Rands, Phytophthora citrophthora R.E. Smith, Phytophthora megasperma Drechsler, Phytophthora parasitica Dastur, Phytophthora phaseoli Thaxter, Phytophthora pini var. antirrhini Sundararaman and Ramakrishnan and Phytophthora tabaci Sawada.

In addition, repetitive development could be found in the zoospores. Zoospores of P. megasperma, whether properly liberated or retained within the sporangial envelope owing to frustrated dehiscence, often gave rise to a secondary zoospore, the repetitional development taking place either by direct discharge of contents through an evacuation tube 3.5 - 5.5 µm in diameter and 1.0 - 10 µm in length, or by the production of an elongated miniature sporangium, commonly 6.0 - 10.0 µm in diameter and 16.0 - 22.0 µm in length on a germ tube 10.0 - 60.0 µm long.

The production of secondary spores is seemingly dependent on environmental conditions which have been identified in only very few instances.

Light encouraged formation of secondary conidia in Conidiobolus rhyosporus (Dring, 1958). Most of the discharged conidia left in light germinated directly to produce secondary conidia. When, however, the spores caught on agar were allowed to germinate in the dark, mycelium production was promoted.

Zuckerman (1957) was able to induce production of secondary conidia from conidia of Endoconidiophora fagacearum (Bretz.) by means of X-ray irradiation. He reported that secondary spores were produced by germinated conidia after having been exposed to X-ray dosage up to 50kr.

From the results obtained, it was obvious that at dosages above 20kr, the higher the dosage, the longer was the delay in both the initiation of germination and in secondary conidium formation.

Mineral oil, osmotic pressure and pH have been identified as factors influencing secondary conidium production in Conidiobolus coronatus (Page and Humber, 1973). Many conidia formed microconidia (secondary spores) when they were germinated under mineral oil or on a medium with a higher osmotic pressure.

The effect of pH on the course of germination was tested with asparagine - glucose agar medium adjusted to different pH's. The proportion of germinated conidia which formed secondary conidia was greatest at the extremes of the pH range tested (pH 4 and pH 8-10), but especially so on alkaline media.

On malt agar medium, germinating conidia of Basidiobolus ranarum invariably developed a vegetative mycelium. In contrast, spores on the surface of the parent colony and, on water agar, nearly always germinated directly to give a conidiophore bearing a conidium. In this instance, nutrients (malt extract) suppressed secondary sporangium development on one hand, whilst on the other, secondary sporangia were formed

in the absence of any nutrients.

The habit of secondary spore production is invaluable to fungi. Dissemination of the fungus is enhanced by the rapid production of spores by germinating spores. In disease - species, infection units are multiplied. Development of secondary spores under conditions unsuitable to the hyphae ensures survival of the fungus.

The extensive occurrence of this phenomenon in the genus Phytophthora suggested an examination for secondary sporangium formation in Phytophthora palmivora (Butl.) Butl., an information of relevance to attempts at control of one of the most destructive Phytophthora species in the tropics.

P. palmivora is very widespread in the tropics and has a very wide host range. It is known to infect over 50 genera of flowering plants (Hickman, 1958). The most recent review of the host range of P. palmivora by Chee (1969) mentioned 138 host plants. The most important among these are cocoa (Theobroma cacao L.) and rubber (Hevea brasiliensis Muell-Arg.).

P. palmivora causes a variety of diseases in cocoa depending on the part of the plant it attacks. It causes a black-pod disease and a canker of the stem and floral cushions in all cocoa-growing countries in West Africa, the West Indies

and the Far East; brown pod-rot disease, cushion canker and a blight of terminal shoots in South America; mild bark and cushion canker, die-back of chupon and fan-shoots in Costa Rica; and a wilt of seedlings in Costa Rica and Nigeria (Gregory,; Wills 1962).

In Ghana, it is most serious as a pathogen of the pod, where it is responsible for an annual loss of about 33-35 per cent of the pods (Wharton, 1956), but it is difficult to obtain an accurate estimate of how much cocoa is lost since cultivation of the crop is done, principally, on peasant farms. The annual loss of yield of cocoa attributed to black-pod is conservatively estimated to average 10 per cent of the world's crop. This figure averages out a wide diversity of incidence, ranging from nil in Malaya, a few per cent in the drier areas of the Ivory Coast to a potential loss of 80-90 per cent in wetter parts of the Cameroun Republic.

The sporangia of P. palmivora germinate in distilled water and at moderately low temperatures to give motile zoospores, which together with the sporangia form the main agents of dissemination. At 30° - 35°C and, in the presence of nutrients the sporangia germinate to produce germ tubes (Clerk, 1972; 1974).

Numerous germ tubes are often formed by a sporangium turning it into a ' many - pronged' infection body. The production of secondary sporangia would further increase the potential of the sporangium as disease inoculum. This thesis reports on investigations made to study the formation of secondary sporangia in P. palmivora and to define the conditions that encourage production of the secondary sporangia.

II. LITERATURE REVIEW

There are very few references in the literature on the germination of sporangia of P. palmivora. These are reports on both indirect germination by production of zoospores and direct germination by formation of germ tubes.

Sporangia of P. palmivora formed zoospores in water (Bimpong and Clerk 1970; Clerk, 1972; Tarjot, 1967; Turner, 1963), in soil extract and, in root exudate of cocoa and 20 other flowering plants (Turner, 1960; 1963). Clerk op. cit. obtained the best indirect germination of more than 90 per cent, at 22°C.

The sporangia germinated in water by means of germ tubes only at 30°C and above. Germ tube formation was, however, encouraged at a lower temperature (22°C) by peptone and yeast-extract, cocoa pod extract and exudate, and very low concentrations of $CaCl_2$ and $MgSO_4.7H_2O$ in addition to thiamine and a number of sugars and amino acids (Clerk, 1972).

There is apparently no information on the development of secondary sporangia in P. palmivora nor on factors influencing the production of the secondary sporangia.

III. MATERIALS AND GENERAL METHODS

(i) MATERIALS:

The isolate of P. palmivora used in these studies was obtained from the stock culture collection of the Botany Department, University of Ghana, Legon.

Cocoa pods for raising sporangia were collected from the Botanical Garden of the University of Ghana, Legon.

(ii) GENERAL METHODS

(a) Maintenance of Stock Culture

Stock cultures of P. palmivora were maintained on slopes of Cassava Dextrose Agar medium (200g cassava; 20g dextrose; 30g agar; 1000ml water) in McCartney tubes. They were incubated at 25°C and subcultured at fortnightly intervals.

(b) Method of inoculation

Cocoa pods were wound-inoculated with 3 mm discs removed from the growing edge of 7 day old P. palmivora cultures on plates of Cassava Dextrose Agar growing at 25°C.

(c) Production of Sporangia

Surface sterilised cocoa pods were inoculated by making a triangular incision measuring 1 cm long each side, at the stylar end of the pod and placing mycelium-bearing disc upside down in the wound. The pods were then incubated in sterile rectangular perspex containers (22.5 cm long x 16.0 cm wide x 30.5 cm deep) which were open at one end. The pods were placed

on moist cotton wool pads and the perspex chambers inverted over them. The cultures were grown at 26 ± 2°C. The mycelium which grew mostly within the husk of the pod, sporulated on the surface on emergent hyphal branches. An average pod of 15 cm long was completely rotted by the fungus in 10 days.

The fungus on the inoculated pod was allowed to develop for six days. Sporangia were thereafter collected from the growing edge of the mycelium on each succeeding day, with flame-sterilised microspatula, until the entire pod was infected. Preliminary studies had earlier shown that such sporangia were less than 24 hours old and generally viable.

(d) Sporangial Germination Tests

Germination of the sporangia was studied by incubating sporangia harvested from the surface of inoculated cocoa pods in the various test solutions in Petri dishes (7 cm diameter). Each Petri dish contained 20 ml sporangial suspension. There were six replicates of each treatment.

(e) Sporangial Suspension for Germination Tests

The number of sporangia in suspension for every germination test was strictly standardised to 200×10^3 sporangia per millilitre of solution with the aid of a haemacytometer.

(f) Assessment of Effect of Treatment on Secondary Sporangium formation

The effect of the various treatments on secondary sporangium formation was assessed by noting the proportion of directly germinating sporangia bearing secondary sporangia. Data in the tables of results and values used for graphs and histograms were obtained from random readings from all replicates of each treatment.

Observations were made immediately at the end of the incubation period. Where this was not possible further development was stopped by adding a few drops of 1.0 per cent formalin solution to each Petri dish.

A secondary sporangium was regarded to have been formed if a germ tube from a sporangium carried at its apex a discernible sporangium of any size.

Record was generally made during each experiment of :

1. Number of sporangia out of the total number observed germinating indirectly by means of zoospores.
2. Number of sporangia out of the total number observed germinating directly by means of germ tubes.
3. Number of directly germinating sporangia bearing secondary sporangia

The total number of sporangia observed for each treatment has been indicated in the tables of results.

(g) Incubation

Incubation conditions varied with the experiment and are described at the appropriate places in the text.

(h) Staining Methods for Detection of Concentration of Organelles and Substances in the sporangia

Sporangia on albumen-coated microscope slides, except during examination of lipids, were variously stained to study the concentration of RNA, Glycogen, Lipids and Proteins in the sporangia.

RNA : The material was fixed in acetic alcohol (1 part glacial acetic acid, 3 parts of 95% ethyl alcohol) for approximately 30-60 minutes. The fixed sporangia were brought down to water through graded series of alcohol and water mixtures. The slides supporting the fixed sporangia were placed in Korson's solution A in a staining dish for 2 minutes (Korson's solution A : 4% Orange G in distilled water). The material was washed in distilled water to which a drop of Korson's solution A had been added. It was next placed in Korson's solution B for 15 minutes (Korson's solution B : 0.15 Methyl Green in distilled water).

The stain was drained off at the end of this period and the material stained in Korson's solution C for 5 minutes (1part 0.15% Methyl Green in distilled water plus 1 part 0.1% Toluidine Blue in distilled water). The sporangia were then rinsed in pure tertiary butyl alcohol and left overnight in fresh tertiary butyl alcohol to finish destaining. It was finally washed in xylol and mounted in Canada Balsam. R.N.A. stained blue.

LIPIDS: The presence of lipids was demonstrated using Sudan III. This stain dissolves in lipid material which then shows a red colour. The sporangia were mounted in water and allowed to dry, and then a drop of the stain (0.1 g Sudan III; 50.0 ml, 95% ethyl alcohol; 50 ml, glycerine) added. The stain was run off the material after 5 minutes, and the material mounted in glycerol for immediate examination under the microscope.

GLYCOGEN: The presence of glycogen was demonstrated using Best's carmine stain. The sporangia were fixed for 30 minutes in acetic alcohol; washed several times in 90% ethyl alcohol and dried on slides. The slides were placed in absolute alcohol, then in 80% ethyl alcohol for 5 minutes. The slide with the sporangia was put into Erlich's haematoxylin for 2 minutes; rinsed in water and transferred to 2 parts.

Best carmine stock solution (2 parts 880 ammonia, 3 parts methyl alcohol) for 5-10 minutes. The sporangia were next differentiated in Best's fluid (80 ml absolute alcohol, 40 ml methyl alcohol, 100 ml distilled water) until the stain stopped diffusing out. They were then cleared in xylene and mounted in Canada Balsam. Glycogen stains bright Red.

PROTEIN: The sporangia were fixed in acetic alcohol for 20-30 minutes and then left overnight in 10% perchloric acid at 4°C, so as to extract all R.N.A. The material was removed the next day, washed twice in distilled water and stained for 30-60 minutes at room temperature in a mixture of equal volumes of 0.1% safranin and Fast Green (mixed equal volumes of stains; allow to stand for 10 minutes with occasional shaking, filtered and the precipitate discarded). It was then washed twice in distilled water; followed by washing in two changes of absolute alcohol. The stained sporangia were finally cleared in xylene and mounted in Canada Balsam. Proteins were stained green.

(i) Method of Sterilisation

Nutrient media, distilled water, centrifuge tubes, test tubes and filter paper were sterilised by autoclaving for 15 minutes at 15 pounds per square inch pressure. Cotton wool plugs of Erlenmeyer flasks and test tubes were temporary covered with grease paper to prevent penetration of any

condensed water during autoclaving.

Petri dishes were sterilised by heating at 160°C for 6 hours in an electrically heated oven.

Cocoa pods were surface sterilised by washing them with 70% ethyl alcohol and then rinsing thoroughly with several changes of sterile distilled water.

The perspex humidity chambers which held the inoculated cocoa pods were sterilised by washing the inner surface with 70% ethyl alcohol and dried with sterile filter paper.

The inoculation room was sprayed with 5% dettol solution (5 ml Dettol : 95 ml water) 10 minutes before use.

(j) Adjustment of pH

Acidic pH, where required, was obtained by adding 0.5 N HCl to the medium. Alkaline pH was obtained by adding 0.5 N NaOH. The pH of the media was measured with a Zeromatic pH meter.

(k) Determination of pH

The pH of samples of all liquid media was determined by means of a Zeromatic pH meter before and after autoclaving.

(l) Chemicals

All chemicals used in the preparation of media were either of the Analar grade or of the B.D.H. (British Drug House) grade both obtained from British Drug House, Poole, England.

(m) Statistical Analysis

Experimental results were statistically analysed where necessary. The Least Significant Difference (L.S.D.) at 5% level has been quoted.

(n) Experimental Precaution

1. Glass-ware was kept scrupulously clean. Glass-ware which had been already cleaned with water and detergents was rinsed several times with tap water and three times with distilled water and allowed to drain before use.

2. The density of sporangial suspensions was kept fairly constant in all treatments.

3. The incubation chambers covering inoculated cocoa pods were removed for a few seconds every morning, to let off any gases accumulated in the closed chambers and, dripping condensed water on the inner surface of the chambers was wiped off with sterile filter paper.

4. In order to use viable sporangia for all the experiments, the viability of the sporangia for every test was determined prior to setting up the experiment. A quantity of the sporangia was put in distilled water and incubated at 22°C. Samples of sporangia accepted for any experiment should produce

at least 70 per cent indirect germination after one hour.

5. Some of the sporangia, on development of germ tubes, became buoyant and floated on the incubating medium. The microscope was, therefore, focused, during observations at the end of the incubation period, at all levels of the suspending medium to ensure that all sporangia were seen.

IV. RESULTS

A. GERMINATION OF SPORANGIA ATTACHED TO THE PARENT MYCELIUM

Waterhouse (1956) produced illustrations of germinating sporangia of numerous Phytophthora species bearing secondary sporangia. The germinating sporangia were generally detached. There was no information on any observations made of sporangia on the mycelium.

Cochrane (1958) noted that although germination of fungal spores in situ has occasionally been reported, it is certainly not a common phenomenon. P. palmivora seems to be one of the few species which produces spores capable of germinating whilst still attached to the hypha. For, sporangia attached to the parent mycelium have been shown to produce zoospores readily (Bimpong, 1969). P. palmivora culture transferred after 6 days of growth at 25°C in V-8/$CaCO_3$ broth into distilled water and maintained at the same temperature formed sporangia copiously within 5 days. The sporangia formed zoospores within one hour when the culture was then transferred to 20°C.

Clerk (1976) induced direct germination in P. palmivora sporangia attached to the parent mycelium when he incubated P. palmivora culture raised in V-8/$CaCO_3$ broth in yeast-extract solution at 30°C. He did not examine the germinating sporangia for secondary sporangia.

Several samples of sporangia removed from mycelium growing on cocoa pods at 26° - 27°C were examined immediately after removal under the microscope in drops of distilled water for the presence of secondary sporangia formed *in situ* on the infected pod. Examination of each sample was limited to only 10 minutes since the sporangia germinate rather quickly, particularly by means of zoospores, over a wide range of temperature (Clerk, 1972).

Samples examined on a particular day constituted one observation as recorded in Table 1.

Secondary sporangium formation *in situ* was rare. Less than 1.0 per cent of the total number of sporangia observed, ranging from 4,054 to 5,999, on each occasion germinated by means of germ tubes and formed secondary sporangia on the pod. Directly germinating sporangia without secondary sporangia were not detected.

TABLE 1

Formation of Secondary Sporangia by P. palmivora Sporangia still attached to mycelium growing on cocoa pod at 26-27°C

Experiment No.	Date August 1975	Total No. of Sporangia observed	% Sporangia with secondary Sporangia
1	8th	5704	0.9
2	9th	4636	0.7
3	10th	5592	0.5
4	11th	5999	0.3
5	12th	4054	0.4

B. SECONDARY SPORANGIUM FORMATION IN DISTILLED WATER

Evidence from the preceding study showed that the sporangia were capable of forming germ tubes and secondary sporangia, even though at a very low rate, in humid air. It is likely that the proportion of germinating sporangia incubated in distilled water that could form secondary sporangia would be greater. This supposition was verified in a subsequent experiment. It would be more rewarding to investigate this at temperatures where direct germination normally occurs in distilled water.

Clerk (1972) reported that higher temperatures encouraged direct germination of the sporangia of P. palmivora, in addition to supporting zoospore formation. He found that some of the sporangia (not more than 10 per cent) germinating in distilled water formed germ tubes at either 30° or 34°C., whilst others (approximately 50 per cent) produced zoospores. Generally, temperatures below 30°C encouraged zoospore formation only.

Clerk (1976) observed in subsequent studies that occasionally, germinating sporangia in distilled water at 22° - 26°C produced short germ tubes with secondary sporangia at their apices. The rate of formation of the secondary sporangia, he noted, was extremely low, usually less than 1.0 per cent.

Secondary sporangium formation in distilled water was studied by incubating aqueous sporangial suspension at 30° and 35°C. The proportion of directly germinating sporangia bearing secondary sporangia was assessed after 24 hours incubation. Table 2 contains data obtained during this investigation.

The higher temperature, 35°C, was clearly less suitable for sporangial germination. Only 1.6 to 3.8 per cent of the sporangia produced zoospores at this temperature in contrast to 10.7 to 16.2 per cent at 30°C.

Similarly, more sporangia, 15.5 per cent germinated by means of germ tubes at 30°C than at 35°C, which supported only 7.6 per cent direct germination.

Secondary sporangium formation was better at 30°C, and this temperature was adopted for subsequent experiments. The percentage of sporangia bearing secondary sporangia at 30°C and 35°C, were 13.5 and 1.7 per cent respectively.

TABLE 2

Germination of Sporangia of P. palmivora incubated in distilled water at 30° and 35°C for 24 hours

Temperature (°C)	Total No. of Sporangia observed (in four tests)	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
			Single Secondary Sporangium	more than one Secondary Sporangium	no Secondary Sporangium
30	1403	12.4	17.1	4.3	2.8
	1087	16.2	13.2	3.2	1.3
	1367	10.7	10.8	0.9	1.8
	2130	15.1	3.9	0.2	2.0
	Mean	13.6	11.3	2.2	2.0
35	1348	1.6	1.1	0.1	3.1
	1021	2.4	2.1	0.1	10.0
	1014	1.7	2.3	0.0	6.5
	1143	3.8	0.7	0.1	4.1
	Mean	2.4	1.6	0.1	5.9

C. Development of Secondary Sporangia

The earliest directly germinating sporangia incubated in distilled water at 30°C produced germ tubes in 1 to 2 hours. Maximum direct germination was achieved in 6 to 8 hours. A sporangium produced from one to three germ tubes which usually emerged close to the papilla (Plates 1 and 2).

In common with development in other Phytophthora species, the secondary sporangium was always borne at the tip of the germ tube. Germ tubes bearing secondary sporangia were of variable length. They could be as short as 32.0 um or as long as 242.0 um (Plates 3a, b and c).

Electron-micrographs of directly germinating sporangia of Phytophthora parasitica (Hemmes and Hohl, 1969) and P. palmivora (Clerk, 1974) showed very clearly that only a portion of the protoplasm of the sporangium participates in germ tube formation. A considerable proportion of the protoplast remains in the sporangium. Similar observations were made here. The protoplast remaining in the sporangium seemingly degenerated later. For, several of the germinating primary sporangia appeared empty when observed under the light microscope (Plates 2a and b).

This feature was again observed in secondary sporangia that germinated to produce, in turn, another secondary sporangium (Plates 4a, b). Such germinating secondary sporangia

were usually observed within 24 to 48 hours of incubation. Text - fig. 1 contains Camera lucida drawings of the numerous features described and supplements Plates 1 to 4.

The secondary sporangium never attained the size of the primary sporangium that produced it (Plates 1 to 4). Where there was a series of two or more sporangia in a chain (Plate 4) each succeeding sporangium was smaller than the preceding one. Table 4 shows that the primary sporangium with a mean length of 42.4 µm was far larger than the two secondary sporangia, 27.8 and 26.2 µm long, respectively, in the chain. The difference in size of the two secondary sporangia was not significant.

Only a fraction of the first secondary sporangia produced a subsequent sporangium. Approximately a quarter of the secondary sporangia produced formed another sporangium. Germination of this generation of secondary sporangia was rare and it was observed during this investigation in only 15 out of 568 secondary sporangia (Table 3).

TABLE 3

Ability of P. palmivora sporangia germinating in distilled water at 30°C to produce a chain of a series of secondary sporangia.

Period of Incubation (Hours)	Total No. of Sporangia observed	No. of Sporangia bearing Secondary Sporangia	No. of Sporangia bearing 1, 2 or 3 secondary sporangia in a chain		
			1	2	3
48	3222	291	205	72	14
72	2641	568	412	141	15

TABLE 4

Comparative size of Secondary Sporangia produced by *P. palmivora* sporangia germinating in distilled water at 30°C for 72 hours

Sporangium	Mean length of 60 sporangia (μm)
Primary	42.4 ± 1.5
1st Secondary	27.8 ± 0.9
2nd Secondary	26.2 ± 1.0

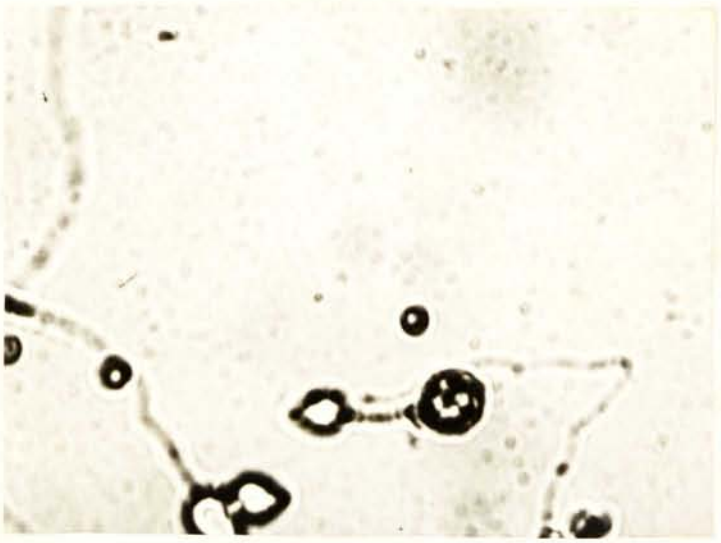

Plate 1. Unstained germinating sporangium of P. palmivora after 24 hours incubation in distilled water at 30°C bearing secondary sporangium on germ tube emerging through the papilla. x 300.

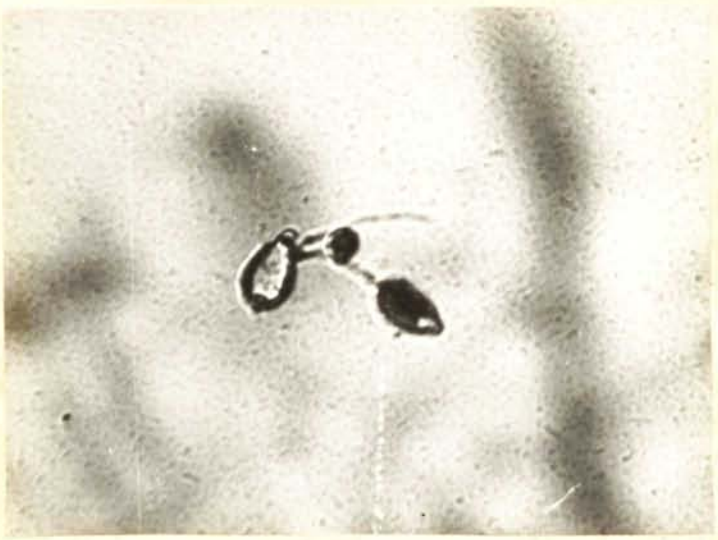

Plate 2a. Unstained germinating sporangium of P. palmivora after 24 hours incubation in distilled water at 30°C with two secondary sporangium - bearing germ tubes. x 250.

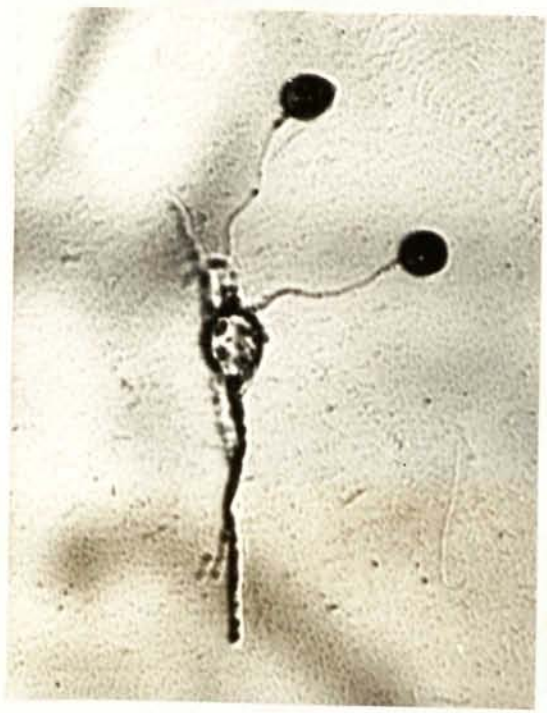

Plate 2b. Unstained germinating sporangium of P. palmivora after 24 hours incubation in distilled water at 30°C with two secondary sporangium-bearing germ tubes. x 300.

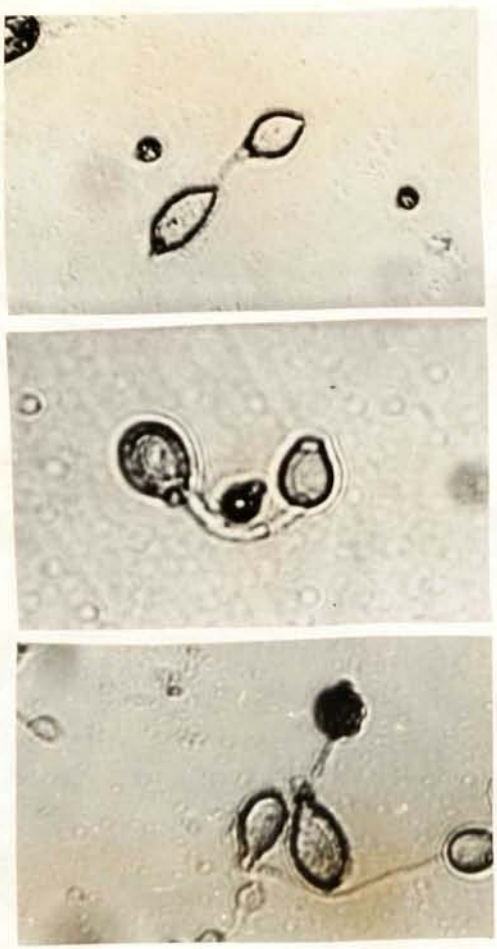

Plate 3a. Samples of unstained germinating sporangia of P. palmivora after 24 hours incubation in distilled water at 30°C showing secondary sporangia at apices of very short (averagely 32 µm) germ tubes x 300.

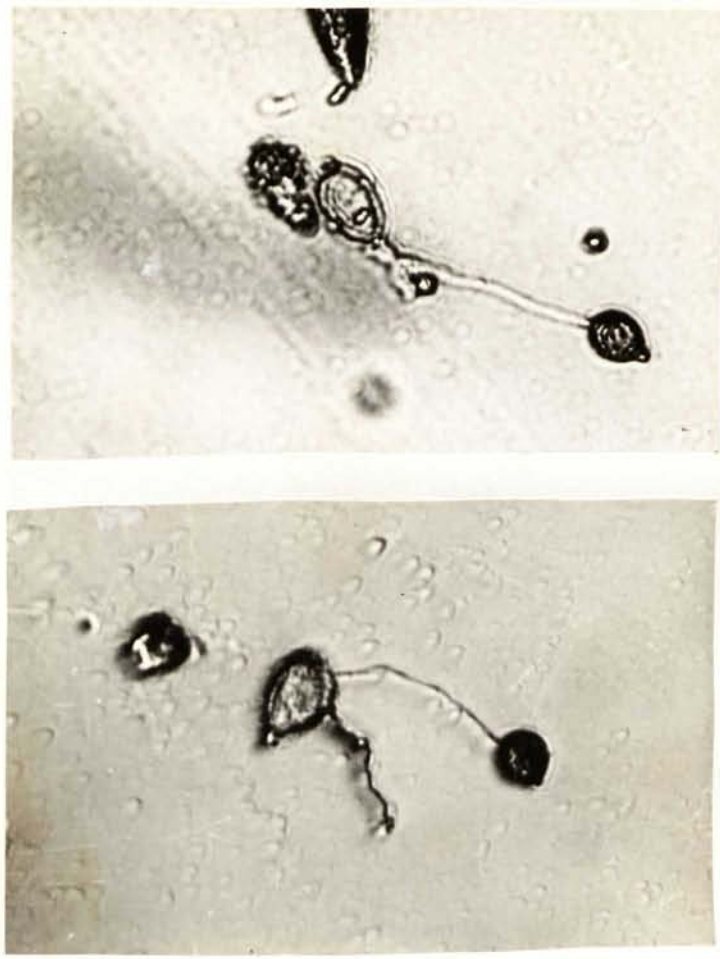

Plate 3b. Two unstained germinating sporangia of P. palmivora after 24 hours incubation in distilled water at 30°C showing secondary sporangia at apices of slightly longish (average 100 µm) germ tubes. x 300.

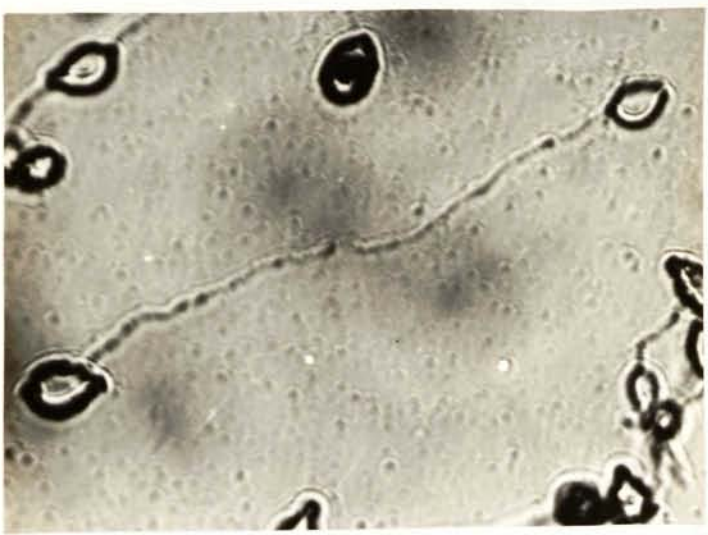

Plate 3c. Unstained germinating sporangium of P. palmivora after 24 hours incubation in distilled water at 30°C showing secondary sporangium at apex of long (240 µm) germ tube. x 300.

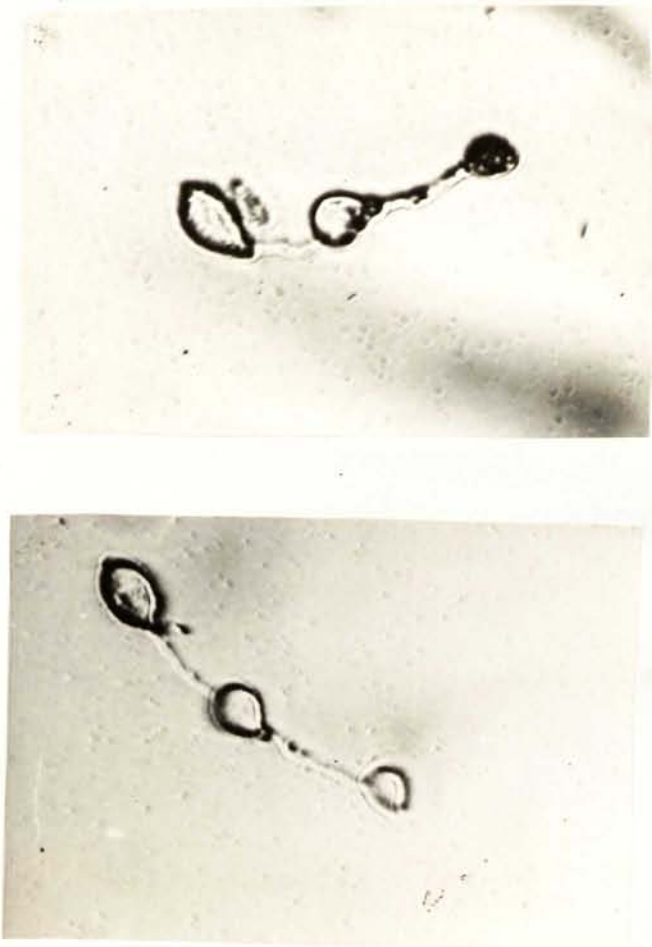

Plate 4. Two unstained germinating sporangia of P. palmivora after 48 hours incubation in distilled water at 30°C showing secondary and tertiary sporangia. x 300.

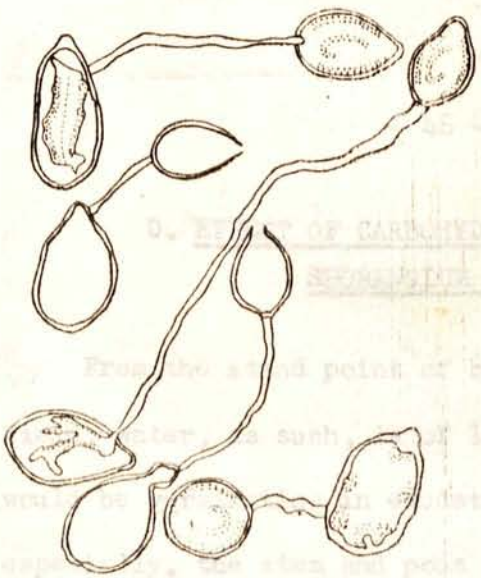

FIG.1 GERMINATING SPORANGIA BEARING SINGLE SECONDARY SPORANGIUM.

FIG.3 GERMINATING SPORANGIUM BEARING SUCCESSIVE SECONDARY SPORANGIA

FIG.2 GERMINATING SPORANGIA BEARING MORE THAN ONE SECONDARY SPORANGIUM

TEXT-FIG. 1 FORMATION OF SECONDARY SPORANGIA BY P. PALMIVORA SPORANGIA GERMINATING IN DISTILLED WATER AT 30°C.

D. EFFECT OF CARBOHYDRATES ON SECONDARY SPORANGIUM FORMATION

From the stand point of behaviour of the sporangia in the field, water, as such, is of less significance; the sporangia would be germinating in exudates and extracts of the host tissues, especially, the stem and pods of cocoa, and, in soil solution. These media would, naturally, contain nutrients and mineral salts in considerable quantities which would influence the germinating sporangia. This consideration prompted an investigation into the effects of selected carbohydrates and nitrogenous compounds on secondary sporangium formation.

Bimpong (1969) identified, by means of paper chromatography, the amino acids, Asparagine, Glutamic acid, Glycine and Tyrosine in cocoa pod extract. Any nutrients in the exudate occurred in too low concentrations to be detected by paper chromatography. Several amino acids have been detected with an Eel 199 Amino Acid Analyser in investigations in progress in the mycology laboratory of the Department of Botany, Legon (Asomaning, 1977).

Formation of secondary sporangia in P. palmivora in the presence of selected carbohydrates was first examined to find to what extent, if any, these nutrients influence sporangial behaviour.

Eleven carbohydrates at four concentrations, 0.1, 0.2, 0.4 and 0.8 per cent (weight/volume), were used (see Tables 5 to 10). Equal parts of sporangial suspension, at double the density used in other experiments, and solutions of appropriate carbohydrates, also at double the required concentration were mixed to obtain the desired concentration. Sporangial suspensions in Petri dishes were then incubated at 30°C and observed after 24 hours.

The results are tabulated in Tables 5 to 10 and illustrated in histograms in Fig. 4. The histograms were constructed to bring the various observations together for better comparison and to provide information on the lengths of germ tubes, bearing the secondary sporangia, information not provided in the tables of results. The lengths recorded in Fig. 4 were those of germ tubes bearing only a single secondary sporangium (see Text-fig. 1, Figs. 1 and 2).

Results in Tables 5 to 10 and in Fig. 4 showed that carbohydrates could affect secondary sporangium formation in several ways. The percentage of germinating sporangia bearing secondary sporangia and the length of germ tubes bearing them varied with concentration of the carbohydrate. The pattern of effect varied with the compound. Halm (1970) clearly demonstrated the erratic nature of germinating P. palmivora sporangia.

Samples of sporangia, of the same age, removed from cocoa pod surfaces showed differences in germination capacity. The effect of any treatment should, therefore, be judged by comparing observations with that in distilled water carried out at the same time.

In brief, the influence of these carbohydrates were :

Arabinose : Zoospore formation was better in distilled water than in solutions of Arabinose. Direct germination was, however, better in the Arabinose solutions with a peak at 0.2 per cent where the greatest number of secondary sporangia was also produced.

The germ tubes bearing secondary sporangia in the Arabinose solutions, particularly at 0.1, 0.2 and 0.4 per cent, were generally longer (148 - 226 µm) than germ tubes in distilled water (80 µm).

Cellobiose : Indirect germination was higher (24.5 - 34.3 per cent) at 0.1 to 0.4 per cent Cellobiose than in distilled water. Again distilled water supported lower direct germination rate of 1.0 per cent than any of the Cellobiose solutions, in which germination ranged from 2.9 to 9.2 per cent. Secondary sporangium formation was only slightly improved by Cellobiose at 0.2 to 0.8 per cent.

Germ tubes carrying the secondary sporangia were longer

in Cellobiose solutions than in distilled water. The mean length in distilled water was 105 µm in comparison to a mean of 391 µm at 0.8 per cent Cellobiose.

Cellulose : Cellulose at a concentration of 0.1 per cent supported almost twice (53.6 per cent) indirect germination of 28.3 per cent obtained in distilled water. Concentration of 0.8 per cent depressed zoospore formation.

At the best concentration of 0.4 per cent, Cellulose had almost the same effect on both secondary sporangium formation and germ tube growth as distilled water.

Fructose : Zoospore formation was severely suppressed by Fructose. Whilst 14.4 per cent of the sporangia produced zoospores in distilled water, the highest germination found at 0.1 per cent Fructose was 6.2 per cent. Concentrations of 0.4 per cent and above were totally inhibitory.

Although a very high percentage of direct germination, 56.2 per cent, was obtained at 0.2 per cent Fructose in contrast to a germination percentage of only 11.1 per cent in distilled water, greater number of secondary sporangia was formed in distilled water than in any of the test solutions.

Germ tubes bearing secondary sporangia were, however, longer, at certain concentrations of Fructose, 0.1, 0.2 and 0.4 per cent, 159 - 262 µm, than those in distilled water, 110 µm.

Galactose : Indirect germination decreased with increase in Galactose concentration. The lowest concentration of 0.1 per cent supported a lower germination of 13.5 per cent than distilled water, 17.9 per cent.

Secondary sporangium formation was greatest, 13.2 per cent at 0.2 per cent Galactose. Only 2.6 per cent formed secondary sporangia in distilled water and Galactose at 0.8 per cent was completely inhibitory.

Direct germination was higher at all concentrations than in distilled water. Concentration at 0.2 per cent promoted the highest percentage direct germination.

Germ tubes bearing the secondary sporangia were longer in 0.1, 0.2 and 0.4 per cent Galactose solutions (345-538 µm) than in distilled water (180 µm).

Glucose : Distilled water promoted the formation of the highest number of secondary sporangia of 2.5 per cent. The percentage of sporangia in Glucose solutions bearing secondary sporangia was low (0.3 to 0.5 per cent), although direct germination was greater, (5.8 to 11.4 per cent) in the Glucose solutions of concentrations of 0.2 to 0.8 per cent.

Approximately the same proportion of sporangia produced zoospores in distilled water and in 0.1 per cent Glucose solution. Percentage germination then fell with increase in

Glucose concentration.

Germ tube growth was encouraged by Glucose. Germ tubes bearing secondary sporangia were longest at 0.2 per cent Glucose. The mean length was 523 µm, more than three times the lengths in 0.1 per cent Glucose and distilled water, 169 µm and 150 µm, respectively.

Lactose : Lactose at 0.2 per cent was the optimum for secondary sporangium formation in the concentration range tested and it was also the best concentration for direct germination. In both instances the values obtained, 13.7 and 21.9 per cent, were far greater than the corresponding values of 3.7 and 3.9 per cent, respectively, in distilled water.

The pattern of indirect germination was closely similar to that in Glucose solutions.

Germ tubes bearing the secondary sporangia were generally longer in the Lactose solutions than in distilled water. The greatest effect was produced by 0.4 per cent Lactose which supported germ tubes more than twice (262 µm) the length of those in distilled water (109 µm).

Maltose : Concentrations of 0.2 and 0.4 per cent promoted higher secondary sporangium formation, 5.5 and 7.3 per cent respectively, than distilled water, 2.6 per cent.

Germ tubes carrying the secondary sporangia were longer in all the Maltose solutions than distilled water. The mean germ tube length increased with increase in Maltose concentrations

of 0.4 per cent and above.

Mannose : Mannose was not a suitable medium for secondary sporangium formation. Mannose, Sucrose and Xylose were the only carbohydrate compounds inferior to distilled water at all concentrations used. In the Mannose solutions, however, direct germination was better than in distilled water and the germ tubes bearing secondary sporangia were longer.

Approximately one-fifth of the sporangia germinated indirectly in distilled water. A slightly higher proportion, 25.1 and 31.0 per cent, did so in 0.1 and 0.2 per cent Mannose solutions, respectively. Percentage germination at higher concentrations of 0.4 and 0.8, on the other hand, decreased markedly (13.4 and 0.6 per cent, respectively).

Sucrose : Both secondary sporangium formation and direct germination were generally poor in the sucrose solutions.

Germ tubes bearing secondary sporangia in sucrose solutions were in contrast longer (168 - 290 µm) than those in distilled water (150 µm).

Zoospore formation was greatly suppressed in 0.2 to 0.8 per cent sucrose solutions.

Xylose : Although secondary sporangium formation was poor in the xylose solutions, greater percentage of the sporangia germinated directly than in distilled water.

Zoospore formation was also severely depressed. Percentage germination ranged from 1.8 to 3.3 in the xylose solutions but was as high as 24.0 per cent in distilled water.

Germ tube growth was only slightly improved by xylose at the concentrations used.

Multiple secondary sporangium formation was not common in the carbohydrate solutions, and only a few, 0.2 per cent Galactose (2.2 per cent), 0.2 per cent Lactose (0.7 per cent) and, 0.4 and 0.8 per cent Maltose (1.1 and 1.7 per cent respectively) provided values greater than those obtained in distilled water.

TABLE 5

Influence of Arabinose and Cellobiose on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Compound and Concentration % (w/v)	pH	Total No. of Sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
ARABINOSE						
0.0	7.6	2312	12.1	4.0	0.0	2.3
0.1	5.6	2097	2.6	8.3	0.0	8.1
0.2	5.5	2106	3.8	2.1	0.0	11.3
0.4	5.5	2112	0.3	2.1	0.0	2.6
0.8	5.5	2100	1.1	1.3	0.0	1.5
CELLOBIOSE						
0.0	7.6	2189	26.7	0.5	0.0	0.5
0.1	5.5	2083	34.3	0.0	0.0	2.9
0.2	5.5	2090	24.5	1.4	0.0	3.0
0.4	4.5	2081	31.4	2.3	0.0	2.8
0.8	5.5	2358	2.7	1.9	0.0	7.3

TABLE 6

Influence of Cellulose and Fructose on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Compound and Concentration % (w/v)	pH	Total No. of sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangia	more than one secondary sporangium	no secondary sporangium
CELLULOSE						
0.0	7.6	2201	28.3	0.8	0.1	0.2
0.1	7.7	2169	53.6	0.6	0.1	0.3
0.2	7.7	2289	33.9	0.5	0.0	0.3
0.4	7.7	2094	16.9	1.1	0.0	1.1
0.8	6.7	2114	12.2	0.4	0.0	0.9
FRUCTOSE						
0.0	7.6	2063	14.4	9.7	0.4	1.4
0.1	4.5	2118	6.2	6.4	0.1	12.2
0.2	4.5	2077	0.2	1.7	0.0	54.5
0.4	5.5	2107	0.0	1.4	0.0	23.8
0.8	5.5	2035	0.0	1.2	0.0	31.3

TABLE 7

Influence of Galactose and Glucose on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Compound and Concentration % (w/v)	pH	Total No. of sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
GALACTOSE						
0.0	7.6	2232	17.9	2.6	0.2	1.8
0.1	6.6	2258	13.5	4.3	0.3	3.5
0.2	5.5	2188	3.6	13.2	2.2	5.6
0.4	5.5	2218	0.0	0.9	0.0	17.1
0.8	4.8	2250	0.4	0.0	0.0	13.9
GLUCOSE						
0.0	7.6	2016	31.2	2.5	0.3	0.6
0.1	5.5	2207	29.6	0.5	0.0	1.1
0.2	5.5	2294	18.3	0.3	0.0	6.8
0.4	4.8	2294	5.7	0.3	0.0	5.5
0.8	5.6	2113	7.1	0.3	0.0	11.1

TABLE 8

Influence of Lactose and Maltose on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Compound and Concentration % (w/v)	pH	Total No. of sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
LACTOSE						
0.0	7.6	2213	19.0	3.7	0.2	0.0
0.1	5.6	2137	20.1	4.8	0.0	1.2
0.2	5.6	2184	11.6	13.7	0.7	7.5
0.4	5.6	2088	2.2	1.3	0.1	0.6
0.8	5.6	2168	5.0	1.2	0.1	0.8
MALTOSE						
0.0	7.6	2201	37.6	2.6	0.0	0.5
0.1	5.6	2189	23.1	2.6	0.0	1.5
0.2	5.6	2004	10.7	5.5	0.0	1.6
0.4	5.6	2103	1.0	7.3	1.1	2.4
0.8	5.6	2018	0.0	2.0	0.7	0.8

TABLE 9

Influence of Mannose and Sucrose on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Compound and Concentration % (w/v)	pH	Total No. of sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
MANNOSE:						
0.0	7.6	2260	21.1	2.0	0.3	0.4
0.1	5.6	2243	25.1	1.7	0.0	7.8
0.2	5.5	2165	31.0	1.3	0.2	4.2
0.4	5.5	2294	13.4	1.5	0.0	7.4
0.8	4.5	2130	0.6	0.2	0.0	4.7
SUCROSE						
0.0	7.6	2151	8.0	11.2	0.8	2.7
0.1	6.6	2255	9.6	2.1	0.1	12.8
0.2	6.6	2079	1.9	1.6	0.0	3.1
0.4	6.6	2081	0.5	1.5	0.0	2.2
0.8	5.6	2220	0.7	2.3	0.0	2.8

TABLE 10

Influence of Xylose on Germination of Sporangia of *P. palmivora* incubated at 30°C for 24 hours

Concentration % (w/v)	pH	Total No. of sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
0.0	7.6	2158	24.0	8.1	0.0	1.8
0.1	5.5	2154	1.8	3.9	0.0	9.0
0.2	5.5	2380	3.2	1.9	0.0	8.2
0.4	5.6	2297	3.3	1.7	0.0	9.1
0.8	4.5	2306	3.1	3.1	0.0	11.7

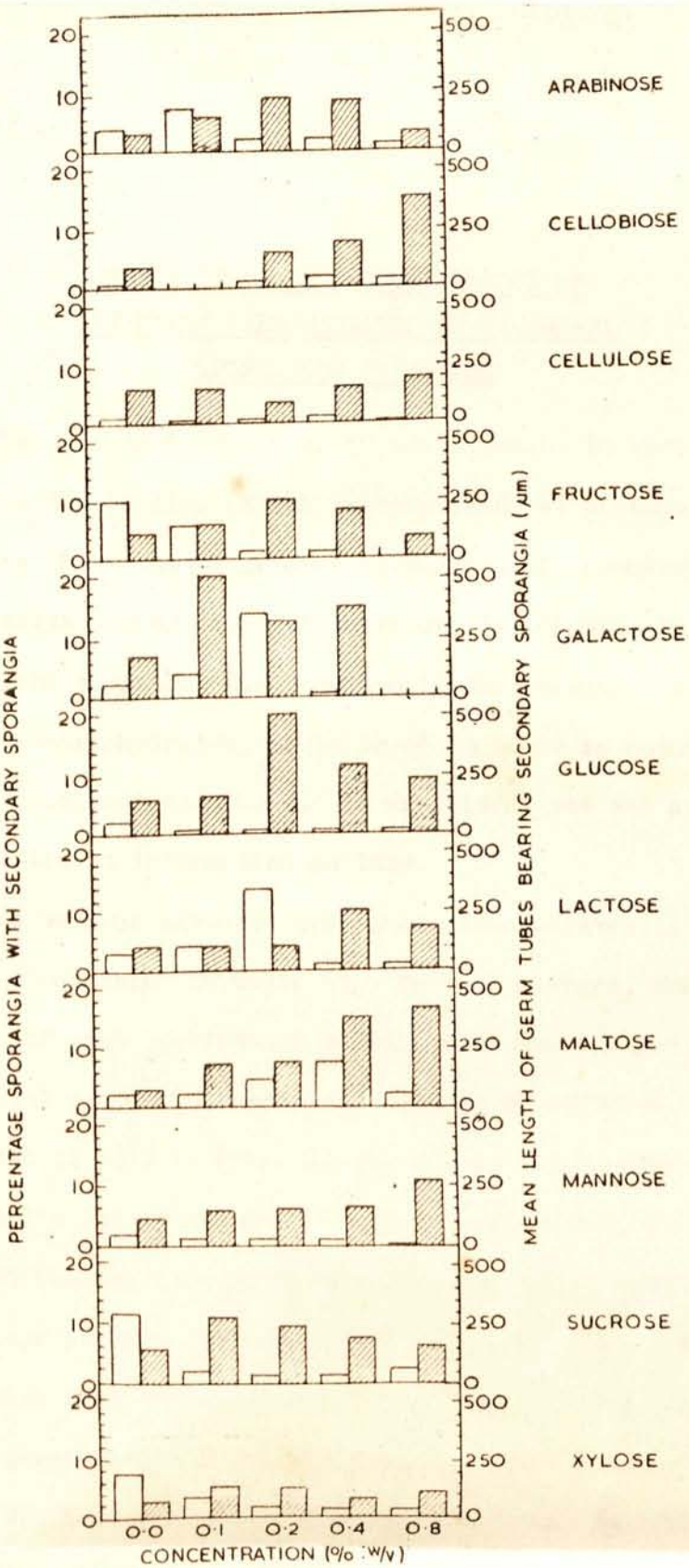

FIG. 4 EFFECT OF CARBOHYDRATES ON THE FORMATION OF SECONDARY SPORANGIA BY P.PALMIVORA AND ON THE GROWTH OF GERM TUBES BEARING THE SPORANGIA

PERCENTAGE SPORANGIA

MEAN LENGTH OF GERM TUBES (µm)

E. EFFECT OF VARIOUS COMBINATIONS OF DIFFERENT CARBOHYDRATES ON SECONDARY SPORANGIUM FORMATION

The data in Tables 5 to 10 are valuable in that they indicate the ability of the carbohydrates at certain concentrations to stimulate greater production of secondary sporangia. The results so far obtained, however, do not provide any clue to how the sporangia might behave in the presence of a mixture of these carbohydrates, as is bound to occur in nature in complex nutrient solutions. An experiment was set up to give some pertinent information on this.

The various mixtures contained carbohydrates in the combinations shown in Table 11. In each mixture, the concentration of each constituent carbohydrate was 0.1 per cent. Sporangial suspensions prepared with these mixtures, were incubated at 30°C in Petri dishes and examined after 24 hours. The results are presented in Table 11 and in Fig. 5.

The results (see Table 11) indicate that generally the percentage of secondary sporangia produced in the mixtures was lower than that in distilled water, although there was higher direct germination of 7.9, 8.1 and 14.3 per cent, respectively, in the Fructose - Glucose, Fructose - Sucrose and Glucose - Sucrose solutions in contrast to a percentage germination of 5.3 per cent in distilled water.

The mixtures encouraged greater germ tube growth before the onset of secondary sporangium formation. The Fructose - Glucose and Glucose - Sucrose mixtures supported the greatest mean lengths of 398 and 406 µm, respectively. The mean germ tube length in distilled water was 157 µm. Whilst the lengths of germ tubes produced in the Fructose - Glucose - Sucrose mixture were median between these two extremes. The lengths of germ tubes formed in the Fructose - Sucrose solution were only slightly superior to those in distilled water.

TABLE 11

Formation of Secondary Sporangia by P. palmivora sporangia incubated in mixtures of different Carbohydrates at 30°C for 24 hours

(Concentration of each component of mixture = 0.1% : w/v)

Components of Mixture	pH	Total No. of Sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium observed
Distilled water	7.6	2250	35.0	2.2	0.0	3.1
Fructose + Glucose	4.5	2220	30.1	1.5	0.0	0.4
Fructose + Sucrose	4.5	2139	23.9	1.4	0.0	6.7
Glucose + Sucrose	5.5	2146	26.0	0.5	0.0	13.8
Fructose + Glucose + Sucrose	4.5	2031	31.1	1.5	0.0	3.6

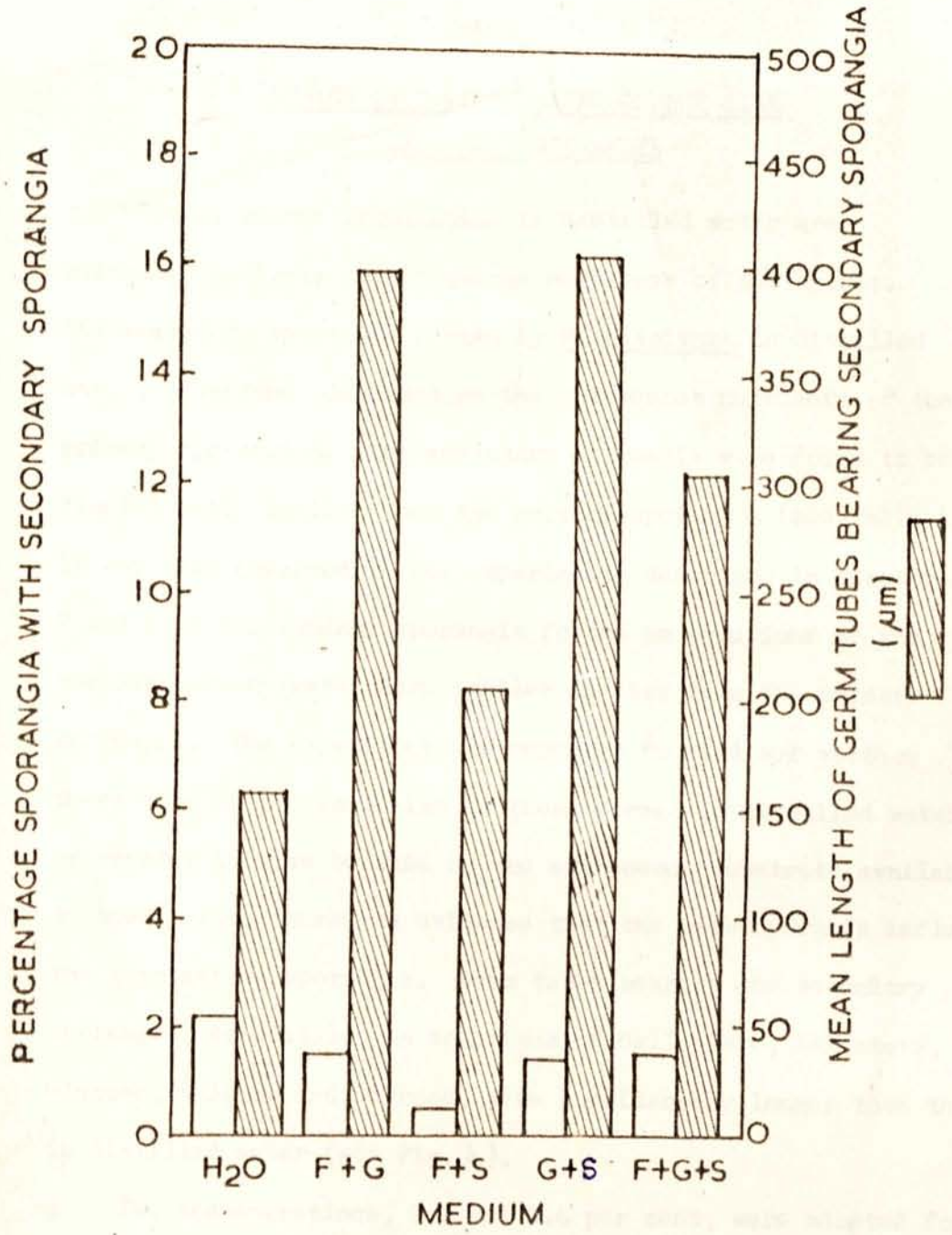

FIG. 5 EFFECT OF MIXTURES OF FRUCTOSE (F), GLUCOSE (G) AND SUCROSE (S) ON THE FORMATION OF SECONDARY SPORANGIA BY P. PALMIVORA AND ON GROWTH OF GERM TUBES BEARING THE SPORANGIA.
(CONCENTRATION OF EACH SUGAR IN THE MIXTURE WAS 0·1 PER CENT).

F. EFFECT OF CARBOHYDRATES ON THE SIZE OF SECONDARY SPORANGIA

Fungal spores germinating in distilled water are sustained entirely by endogenous nutrients of the spores. The secondary sporangia formed by P. palmivora in distilled water, therefore, depended on the endogenous nutrients of the primary sporangium. The secondary sporangia were found to be significantly smaller than the primary sporangia (see Table 4). It was also observed in the experiments described in chapters D and E that secondary sporangia formed in solutions of the various carbohydrates were smaller in size than the primary sporangia. The experiment was repeated to find out whether these were of the same size as those formed in distilled water or greater in size because of the extraneous substrate available to them. For, there was evidence that the carbohydrates influenced the germinating sporangia. Germ tubes bearing the secondary sporangia, especially, in solutions of Cellobiose, Galactose, Glucose, Maltose and Sucrose, were considerably longer than those in distilled water (see Fig. 4).

Two concentrations, 0.2 and 0.4 per cent, were adopted for this investigation using the eleven carbohydrates of the previous experiment and listed in Tables 12 and 13. The effect of the carbohydrates was assessed by comparing the lengths of secondary sporangia formed in distilled water with those formed

in solutions of the various carbohydrates. The mean sporangial lengths, estimated from 50 measurements in each case are presented in Tables 12 and 13.

Secondary sporangia formed in both distilled water and in the various nutrient solutions were clearly smaller than the primary sporangia. In the two tests, mean lengths of the secondary sporangia formed in distilled water were 29.9 µm (Table 12) and 29.5 um (Table 13). Values obtained for secondary sporangium length in the carbohydrate solutions of 0.2 per cent concentration also ranged from 26.9 µm, for sporangia produced in Cellulose solution, to 32.3 µm, for those in Arabinose solution. This range coincided with that for the 0.4 per cent solutions. The least value was 27.5 µm in Galactose solution and the greatest, 32.7 µm, in Mannose solution. The lengths of the primary sporangia, 38.1 and 39.3 µm, far exceeded those values. The curves in Fig.6 were drawn from the histograms of class - length distribution of the primary and secondary sporangia for each test. They demonstrate very well the greater size of the primary sporangia.

The carbohydrates tested did not increase the size of the secondary sporangia at both 0.2 and 0.4 per cent concentrations.

TABLE 12

Size of Secondary Sporangia formed in solutions of carbohydrates at a concentration of 0.2% (w/v) by *P. palmivora* sporangia germinating at 30°C for 24 hours (Each value is a mean of 50 measurements)

Compound	Mean Length of Secondary Sporangia (µm)
Arabinose	32.3 ± 1.0
Cellobiose	31.6 ± 1.2
Cellulose	26.9 ± 0.7
Fructose	30.6 ± 0.8
Galactose	29.8 ± 0.9
Glucose	28.7 ± 0.8
Lactose	27.0 ± 0.9
Maltose	30.3 ± 0.8
Mannose	27.9 ± 0.8
Sucrose	29.6 ± 0.8
Xylose	30.8 ± 1.0
Distilled water	29.9 ± 1.2

Mean length of Primary Sporangia : 39.3 ± 0.9 µm

LSD at 5 per cent level: 4.5

TABLE 13

Size of Secondary Sporangia formed in solutions of carbohydrates at a concentration of 0.4% (w/v) by *P. palmivora* sporangia germinating at 30°C for 24 hours

(Each value is a mean of 50 measurements)

Compound	Mean Length of Secondary Sporangia (µm)
Arabinose	28.3 ± 0.9
Cellobiose	27.8 ± 0.7
Cellulose	30.0 ± 0.8
Fructose	27.6 ± 0.9
Galactose	27.5 ± 0.7
Glucose	30.5 ± 1.1
Lactose	28.5 ± 0.7
Maltose	29.5 ± 0.8
Mannose	32.7 ± 1.0
Sucrose	27.6 ± 0.8
Xylose	29.0 ± 0.9
Distilled water	29.5 ± 0.6

Mean length of Primary Sporangia : 38.1 ± 0.8 µm

LSD at 5 per cent level: 5.5

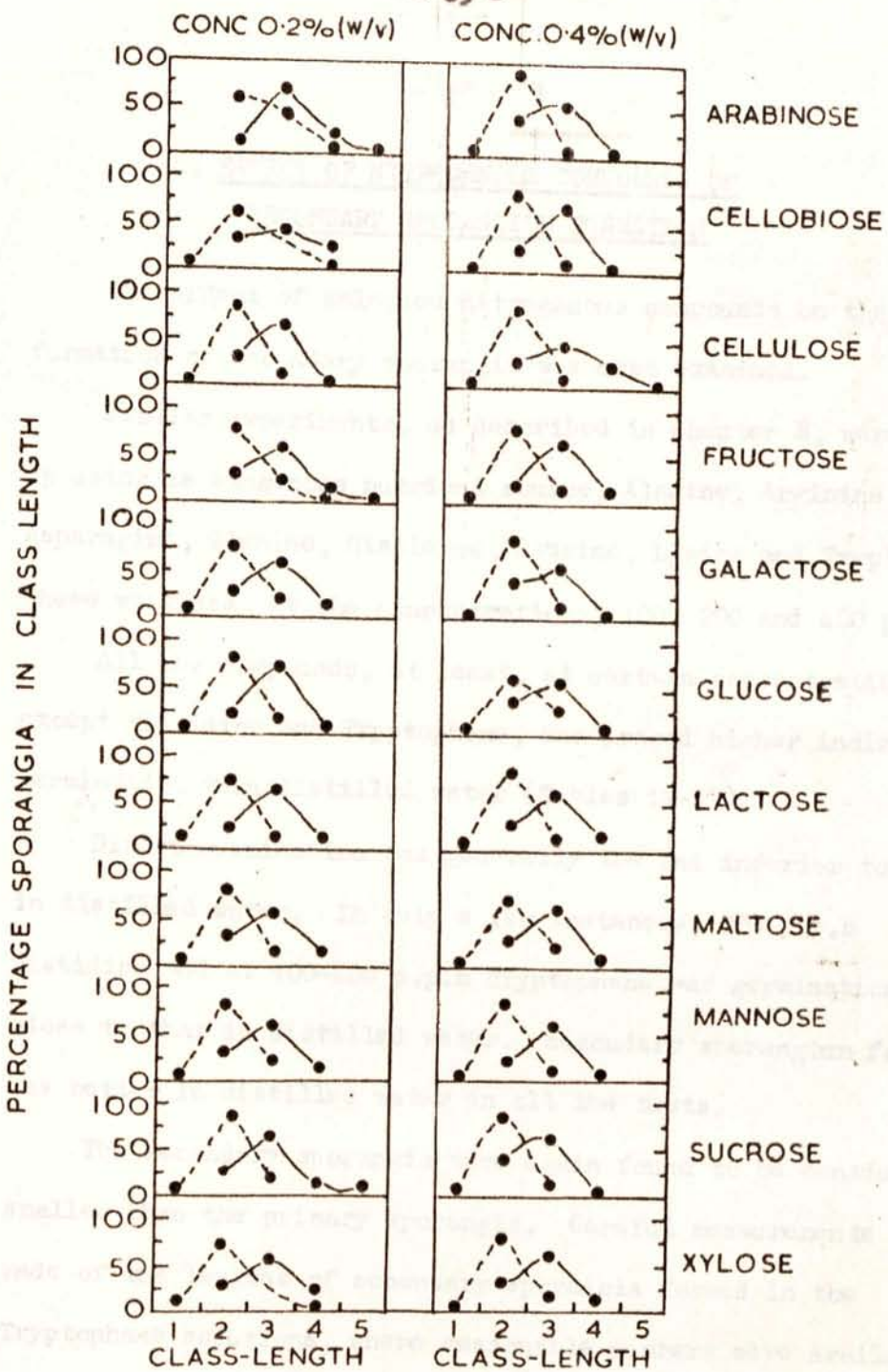

FIG. 6 CLASS-LENGTH OF PRIMARY SPORANGIA OF P. PALMIVORA AND THEIR ASSOCIATE SECONDARY SPORANGIA PRODUCED IN SOLUTIONS OF VARIOUS CARBOHYDRATES AT 30°C. (PRIMARY SPORANGIA, ——; SECONDARY SPORANGIA, - - - - - -)

1: 3·6 – 17·9 μm 2: 18·0 – 32·2 μm 3: 32·3 – 46·5 μm
4: 46·6 – 60·8 μm 5: 60·9 – 75·1 μm

G. EFFECT OF NITROGENOUS COMPOUNDS ON SECONDARY SPORANGIUM FORMATION

The effect of selected nitrogenous compounds on the formation of secondary sporangia was next examined.

Similar experiments, as described in Chapter E, were set up using as exogenous nutrient source, Alanine, Arginine, Asparagine, Glycine, Histidine, Leucine, Lysine and Tryptophane. These were used at the concentrations, 100, 200 and 400 p.p.m.

All the compounds, at least, at certain concentrations, except Histidine and Tryptophane, encouraged higher indirect germination than distilled water (Tables 14-17).

Direct germination was generally low and inferior to that in distilled water. In only a few instances, 200 p.p.m Histidine and at 100-400 p.p.m Tryptophane was germination rate close to that in distilled water. Secondary sporangium formation was better in distilled water in all the tests.

The secondary sporangia were again found to be considerably smaller than the primary sporangia. Careful measurements were made of the lengths of secondary sporangia formed in the Tryptophane solutions, where reasonable numbers were available, to establish the extent of reduction in size.

The largest mean length of 31.0 µm recorded at 200 p.p.m Tryptophane was very close to the mean length of 29.4 µm of secondary sporangia in distilled water, and markedly smaller than the primary sporangia of a mean length of 42.6 µm. (See Table 18)

TABLE 14

Influence of Alanine and Arginine on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Amino acid and Concentration (ppm)	pH	Total No. of Sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single Secondary sporangium	more than one secondary sporangium	no secondary sporangium
ALANINE						
0.0	7.6	2129	36.3	2.1	0.0	2.8
100.0	4.9	2096	59.0	0.0	0.0	1.2
200.0	5.2	2097	41.9	0.5	0.0	2.2
400.0	4.9	2072	34.6	0.2	0.0	2.5
ARGININE						
0.0	7.6	2077	32.3	2.6	0.0	1.5
100.0	5.5	2037	44.8	0.4	0.0	1.3
200.0	5.6	2048	50.1	0.0	0.0	1.0
400.0	5.7	2120	29.8	0.3	0.0	1.7

TABLE 15

Influence of Asparagine and Glycine on Germination of sporangia of *P. palmivora* incubated at 30°C for 24 hours

Amino Acid and Concentration (ppm)	pH	Total No. of Sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
ASPARAGINE						
0.0	7.6	2198	37.2	3.6	0.0	5.5
100.0	5.2	2096	42.8	0.1	0.0	2.2
200.0	5.3	2037	35.1	0.6	0.0	1.3
400.0	5.4	2136	36.5	0.0	0.0	3.9
GLYCINE						
0.0	7.6	2043	87.5	2.1	0.0	3.0
100.0	5.1	2074	97.3	0.0	0.0	0.0
200.0	5.3	2028	99.2	0.0	0.0	0.0
400.0	5.3	2185	96.3	0.0	0.0	1.6

TABLE 16

Influence of Histidine and Leucine on Germination of sporangia of P. palmivora incubated at 30°C for 24 hours

Amino Acid and Concentration (ppm)	pH	Total No. of Sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single secondary sporangium	more than one secondary sporangium	no secondary sporangium
HISTIDINE						
0.0	7.6	2005	14.0	4.1	0.1	1.0
100.0	4.3	2016	14.3	0.0	0.0	0.9
200.0	4.0	2086	11.4	0.0	0.0	4.6
400.0	3.9	2095	1.2	0.0	0.0	2.1
LEUCINE						
0.0	7.6	2050	87.9	2.7	0.0	1.2
100.0	5.3	2003	95.8	0.1	0.0	0.7
200.0	5.2	2042	96.9	0.0	0.0	0.7
400.0	5.2	2008	95.0	0.0	0.0	0.8

TABLE 17

Influence of Lysine and Tryptophane on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Amino Acid and Concentration (ppm)	pH	Total No. of Sporangia observed	% Indirect Germination	DIRECT GERMINATION % Sporangia with		
				Single Secondary sporangium	more than one Secondary sporangium	no secondary sporangium
LYSINE						
0.0	7.6	2264	53.3	4.2	0.0	0.5
100.0	5.1	2076	60.9	0.3	0.0	0.7
200.0	5.2	2138	75.3	0.0	0.0	1.7
400.0	5.6	2055	54.8	0.0	0.0	0.8
TRYPTOPHANE						
0.0	7.6	2084	67.7	2.8	0.0	1.2
100.0	4.8	2415	51.5	2.0	0.0	0.8
200.0	5.2	2033	50.7	2.7	0.0	0.7
400.0	5.3	2407	36.1	0.9	0.0	1.0

TABLE 18

Size of Secondary Sporangia formed in Tryptophane solution by P. palmivora sporangia germinating at 30°C for 24 hours

(Each value is a mean of 50 measurements)

Concentration of Tryptophane (ppm)	Mean length of Secondary sporangia (µm)
Distilled water	29.4 ± 0.8
100	28.9 ± 0.7
200	31.0 ± 1.0
400	26.7 ± 0.5

Mean length of Primary Sporangium : 42.6 ± 0.7 µm

H. EFFECT OF COMPLEX NITROGENOUS COMPOUNDS ON SECONDARY SPORANGIUM FORMATION

The percentage of sporangia germinating by means of germ tubes in most of the carbohydrate solutions (see Tables 5 to 13) and in the amino acid (see Tables 14 to 17) solutions was not high. Secondary sporangium formation was consequently not substantial. It was considered possible that in media that would support a high rate of direct germination, the proportion of sporangium that would form secondary sporangia would increase. The sporangia were, therefore, germinated again in nutrient media using, this time very complex nitrogenous compounds, Peptone and Yeast-extract. Both compounds were found by Clerk (1972) to induce extensive direct germination in sporangia of P. palmivora.

Sporangia from infected cocoa pods were incubated in 1.0 per cent Peptone and Yeast-extract solutions at 30°C and examined for secondary sporangium formation after 24 hours.

The results in Table 19 confirmed that both media were very suitable for direct germination. Peptone supported 57.0 per cent direct germination and Yeast-extract, 67.6 per cent. Very few of the germinating sporangia, however, formed secondary sporangia. In each case, less than 0.5 per cent of the germinated sporangia formed secondary sporangia.

TABLE 19

Influence of Complex Nitrogen Compounds on Germination of Sporangia of P. palmivora incubated at 30°C for 24 hours

Nigrogenous compound (1.0% w/v)	pH	Total No. of Sporangia observed	No. of Sporangia		
			producing zoospores	producing Germ tubes	bearing secon-dary sporan-gia
Peptone	6.6	2512	0	1427	5
Yeast-Extract	7.9	2336	0	1604	6

I. INDUCTION OF SECONDARY SPORANGIA IN GERMINATING SPORANGIA BY SUDDEN WITHDRAWAL OF EXOGENOUS NUTRIENTS

The diversity of spore type in fungi is so great that it is not surprising that the environmental factors leading to their production differ considerably for different species and for the different types of spore produced by the same fungus. Nevertheless, certain fairly general statements may be made as to the factors usually favouring sporulation.

One of the most important factors influencing sporulation of fungi is the concentration of food substances. The concentration most favourable for vegetative growth is almost always too high for maximum spore production. This knowledge has formed the basis for the general practice of mycologists to grow fungi on dilute media in order to induce them to sporulate. This has been particularly successful with aquatic or semi-aquatic fungi. The fungus is grown on a rich medium and then transferred to distilled water, plain agar or a very dilute medium. The classic work of Klebs (1898, 1899, 1900) with the water mould Saprolegnia showed that the

sudden withdrawal of food stimulates the formation of zoosporangia by the fungus. These results and those of similar experiments by other investigators, for example, Kauffman (1908) and Pieters (1915), have led to the development of the common laboratory technique, whereby the water moulds are grown on solid substrates rich in protein, such as ants eggs, dead flies or hempseed, and, when vegetative hyphae have developed, the production of sporangia is induced by transfer to water.

This treatment has also been applied to non-aquatic fungi. For example, withdrawal of food from growing hyphae of some members of the Sphaeropsidales induced sporulation (Leonian, 1924).

So far this treatment has been applied to mycelium only. An experiment was set up to examine whether germ tubes of P. palmivora growing in a nutrient medium would behave in a similar way.

The first investigation was carried out with sporangia which had been germinated in Yeast-extract and Peptone solutions since these media were found to be very suitable for direct germination (see Table 19). Sporangial suspensions prepared with 1.0 per cent Peptone and Yeast-extract solutions were incubated at 30°C for 4 hours, a period sufficient for

direct germination of all germinable sporangia (see Plates 5 and 6). It was noteworthy that the sporangia, generally, produced many germ tubes in the Peptone and Yeast-extract solutions, in contrast to the few germ tubes of sporangia germinating in distilled water.

At the end of the incubation period, described as 'First Treatment' in the tables of results, the sporangial suspensions were poured into sterile centrifuge tubes and centrifuged at a speed of 4,500 revolutions per minute for 5 minutes. The supernatant fluid was discarded and the sediment of germinated sporangia resuspended in sterile distilled water. The suspension was again centrifuged at the same speed for five minutes and the supernatant fluid discarded. This operation was repeated thrice to ensure that all traces of the nutrients were washed from the sporangia.

The washed sporangia were finally re-suspended in sterile distilled water and incubated at 30°C for 24 hours - the 'Second Treatment'.

The percentage of sporangia bearing secondary sporangia was estimated after each incubation, that is, the first and second treatments. The results are presented in Table 20. Controls consisted of sporangia incubated throughout the period of 28 hours in the nutrient solutions.

The results show that after 4 hours' growth only a very small percentage of the sporangia, less than one in a thousand in the Peptone solution and about two in a thousand in the Yeast-extract solution, formed secondary sporangia. These values remained approximately the same in the control suspensions after 28 hours' incubation.

There was, on the other hand, a dramatic production of secondary sporangia by more than 95 per cent of the germinated sporangia which were transferred from either 1.0 per cent Peptone or Yeast-extract solution to distilled water (see Table 20 and Plate 7).

TABLE 20

Subsequent development of Germ tubes of P. palmivora sporangia transferred from Peptone and Yeast-extract Media to Distilled water and incubated at 30°C for 24 hours

FIRST TREATMENT

Incubation in Nutrient Medium for 4 hours at 30°C

Incubation Medium (1.0% w/v)	Total No. of Sporangia observed	No. of Sporangia producing Germ tubes	No. of sporangia bearing Secondary sporangia
Peptone	2121	1641	1
Yeast-extract	2290	1710	4

SECOND TREATMENT

Development after transfer to Distilled water

Sporangia from	% of a total of 800 sporangia with Germ tubes bearing secondary sporangia
Peptone	98.1
Yeast-extract	95.2

Plate 5. Photomicrograph of sporangia of P. palmivora germinating in 1.0 per cent Peptone solution after 4 hours incubation at 30°C showing stage of development at time of transfer to distilled water. x 50.

Plate 6. Photomicrograph of sporangia of P. palmivora germinating in 1.0 per cent Yeast-extract solution after 4 hours incubation at 30°C showing stage of development at time of transfer to distilled water. x 50.

Plate 7. Unstained germinating sporangia of P. palmivora producing secondary sporangia after transfer from 1.0 per cent Yeast-extract solution immediately on germinating, to distilled water at 30°C for 24 hours. x 50.

J. FURTHER EXPERIMENTS ON INDUCTION OF SECONDARY SPORANGIA IN GERMINATING SPORANGIA BY SUDDEN WITHDRAWAL OF THE EXOGENOUS NUTRIENTS, PEPTONE AND YEAST - EXTRACT

The previous experiment was repeated to find out whether the behaviour of the germ tubes produced in Peptone and Yeast-extract solutions would be affected if they were transferred to water and then placed at temperatures other than 30°C. Temperature, a very important environmental factor, fluctuates in the field and the germinating sporangia would be subjected to different temperatures.

The method described in chapter I was closely followed. Sporangia of P. palmivora which had germinated in 1.0 per cent Peptone, and Yeast-extract solutions for 4 hours for the 'First Treatment' were washed thoroughly in sterile distilled water and incubated in sterile distilled water at 20°, 25° and 30°C for 24 hours. Observations made after the two treatments are recorded in Table 21.

Again secondary sporangium formation was poor in the nutrient media. Withdrawal of the nutrients encouraged very high rate of secondary sporangium formation at all three temperatures. The percentage of sporangia initially germinated in both media which formed secondary sporangia in distilled water ranged from 84.1 to 91.4 per cent . There was evidence that 20°C

was slightly less suitable than 25°C and 30°C. Secondary sporangium formation at that temperature was nonetheless very high.

TABLE 21

Subsequent development of Germ tubes of P. palmivora sporangia transferred from solutions of Peptone and Yeast extract to Distilled water and incubated at varying Temperatures for 24 hours

FIRST TREATMENT

Incubation in Peptone and Yeast-extract solutions for 4 hours at 30°C.

Incubation Medium and Concentration (% w/v)	Total No. of sporangia observed	No. of sporangia producing Germ tubes	No. of sporangia bearing secondary sporangia
Peptone : 1.0	2247	1108	1
Yeast-extract 1.0	2202	1588	6

SECOND TREATMENT

Development after transfer to Distilled water

Sporangia from	% of a total of 300 directly germinating sporangia bearing secondary sporangia at		
	20°C	25°C	30°C
Peptone	87.6	91.2	91.4
Yeast-extract	84.1	86.6	85.2

K. INDUCTION OF SECONDARY SPORANGIA IN P. PALMIVORA SPORANGIA GERMINATED IN CARBOHYDRATE MEDIA

The tests with Peptone and Yeast-extract were followed by an experiment in which the sporangia were germinated at 30°C for 4 hours, for the 'First Treatment' in solutions of selected carbohydrates, Fructose, Galactose, Glucose and Mannose and transferred, after thorough rinsing in the usual manner, to distilled water and incubated at 30°C for 24 hours to induce secondary sporangium formation.

Plates 8a and b show germinated sporangia after incubation for 4 hours in 0.4 and 0.8 per cent Fructose solutions, respectively, whilst Plates 9a and b are photomicrographs of secondary sporangia produced in distilled water by sporangia initially germinated in Galactose media.

This experiment was performed to find out whether the nature of the nutrient medium for the 'First Treatment' could influence the extent of secondary sporangium formation. It was possible that either the vigour of the germ tubes or the nature of metabolites synthesised could be related to the type of nutrient. Table 22 contains the results obtained during this investigation.

Very few secondary sporangia were formed in the sugar solutions during the 'First Treatment'. The major observations after subsequent incubation in distilled water for 24 hours were :

(a) In common with observations in Chapter I and J, the germ tubes on transfer from the nutrient media to distilled water grew to some extent before forming the secondary sporangia (see Plates 10a and b).

(b) Germ tubes formed in carbohydrate media did not form secondary sporangia to the same extent as those formed in Peptone and Yeast-extract media.

(c) The highest percentage of sporangia bearing secondary sporangia was 36.7 per cent; observed in 0.8 per cent Galactose medium.

(d) The extent of secondary sporangium formation varied with the carbohydrate. It was poor in the Mannose media with only 8.1 and 8.7 per cent of the germinated sporangia bearing secondary sporangia.

(e) With the exception of Galactose, the two concentrations of the carbohydrate used, 0.4 and 0.8 per cent, had the same effect. The higher concentration of Galactose was superior to the lower concentration.

TABLE 22

Subsequent development of Germ tubes of P. palmivora sporangia transferred from Carbohydrate Media to Distilled water and incubated at 30°C for 24 hours

FIRST TREATMENT

Incubation in Nutrient Medium for 4 hours at 30°C

Incubation Medium and Concentration (% w/v)	Total No. of Sporangia observed	No. of Sporangia producing Germ tubes	No. of Sporangia bearing secondary sporangia
Fructose			
0.4	2085	278	3
0.8	2033	306	5
Galactose			
0.4	2116	90	4
0.8	2090	361	1
Glucose			
0.4	2127	239	4
0.8	2169	232	3
Mannose			
0.4	2084	57	2
0.8	2060	174	2

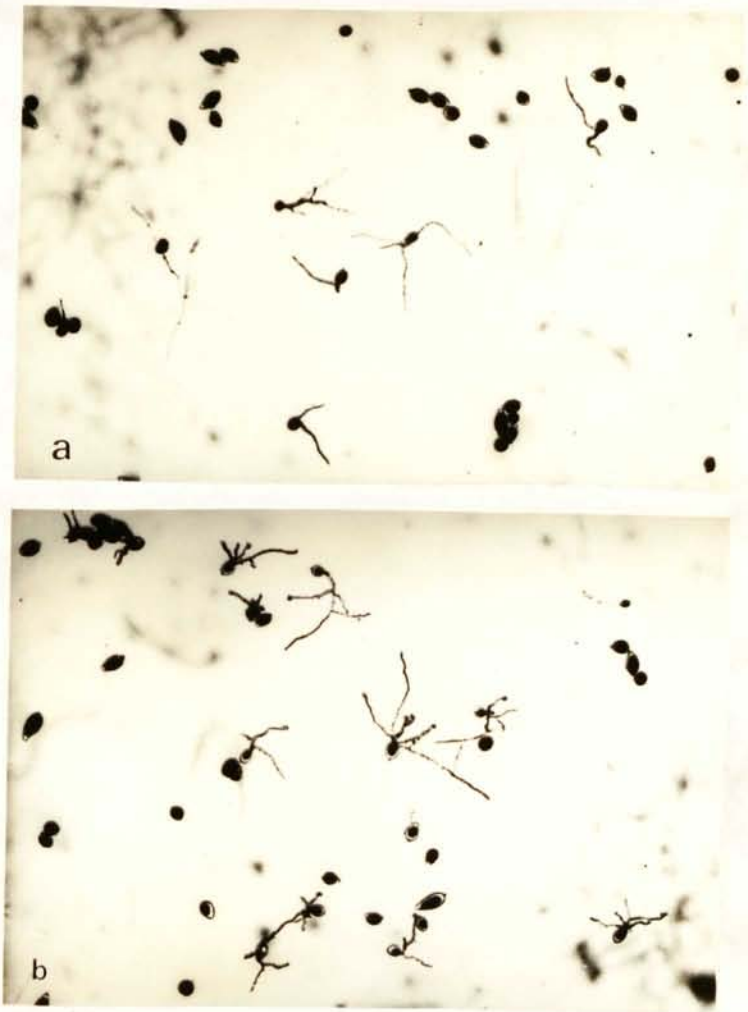

Plate 8. Photomicrographs of sporangia of P. palmivora germinating in 0.4 per cent Fructose (above) and 0.8 per cent Fructose (below) solutions after 4 hours incubation at 30°C showing stage of development at time of transfer to distilled water. x 50

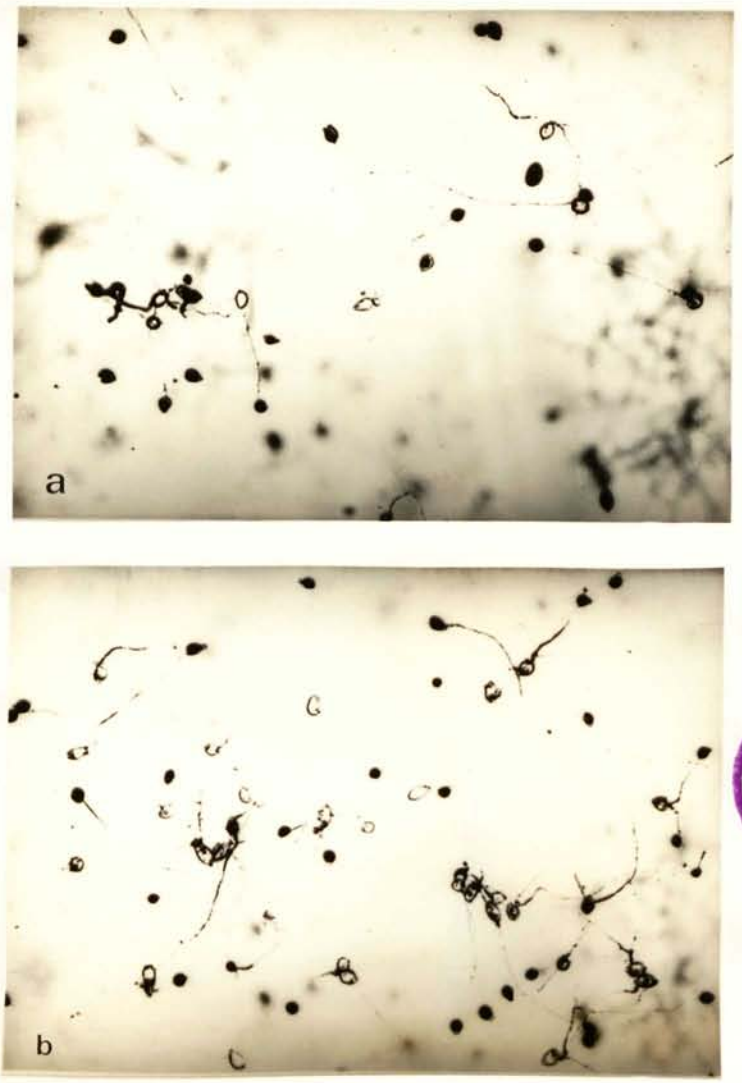

Plate 9. Unstained germinating sporangia of P. palmivora producing secondary sporangia after transfer from 0.4 per cent Galactose (above) and 0.8 per cent Galactose (below) solutions immediately on germinating to distilled water at 30°C for 24 hours.

Plate 10. Photomicrographs of sporangia of P. palmivora germinating after 4 hours incubation in 0.4 per cent Glucose solution (above) and producing secondary sporangia after transfer to distilled water and incubation for 24 hours at 30°C (below).

x 50.

L. INDUCTION OF SECONDARY SPORANGIA IN GERMINATED P. PALMIVORA SPORANGIA TRANSFERRED TO DILUTE MEDIA

The pertinent literature reveals that transfer to water in experiments on induction of reproduction in fungi is often less successful than transfer to a dilute medium (Cochrane, 1958; Hawker, 1950). Because of the well known variation in fungal activity, evidence for this should be sought in every fungus species being investigated. This is, particularly, important in the present investigation in which germ tubes are being used instead of mycelium of other experiments. Secondly, if P. palmivora also behaves in a similar manner, the level of dilution of the nutrient medium most suitable for encouraging induced sporulation must be defined. The results of this experiment would suggest the role the host which contains low concentrations of nutrients (Bimpong, 1968) would play in secondary sporangium formation in the field.

Sporangia of P. palmivora germinated in the following media, Fructose, Galactose, Glucose, Peptone and Yeast-extract at the concentrations indicated in Table 23 for 4 hours at 30°C were incubated, thereafter, at the same temperature in diluted solutions of the same substances and in sterile distilled water. After 24 hours, the percentage of sporangia which have produced secondary sporangia at the various dilutions

was assessed. The results are presented in Table 23.

The results showed that the media for the 'First Treatment' in which sporangia were retained for a total of 28 hours as controls, were unsuitable for secondary sporangium formation, confirming the earlier observations recorded in Tables 20 to 22.

The data in Table 23 show clearly that the concentration for stimulation of secondary sporangium formation should be low. A concentration of 0.2 per cent Fructose, Galactose and Glucose was not very suitable. Galactose supported the highest sporulation of 11.1 per cent. At the same concentration, Peptone and Yeast-extract induced secondary sporangium formation in 29.2 and 22.8 per cent of the germinated sporangia. Higher concentrations would, therefore, be needed to suppress sporulation to the level observed in the original medium of a concentration of 1.0 per cent.

Very low concentrations of some media for the 'Second Treatment' could indeed encourage the formation of more secondary sporangia than distilled water. Thus, the percentage of sporangia with secondary sporangia in distilled water (93.8 per cent) was exceeded by that in 0.01 and 0.05 per cent Yeast-extract solutions, 99.5 and 98.9 per cent respectively. Percentages recorded at these concentrations in the other media were close to those in distilled water.

TABLE 23

Induction of Secondary sporangium in germ tubes of P. palmivora sporangia at 30°C in dilute germination medium after an initial incubation for 4 hours at 30°C in the original medium

Compound and Initial Concentration % w/v	Concentration of Diluted Medium	pH	Observations 24 hours after transfer		
			Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia bearing secondary sporangia
FRUCTOSE (0.4)	0.00	7.6	2018	262	208
	0.01	4.6	2022	206	164
	0.05	5.3	2017	199	151
	0.10	5.6	2043	70	45
	0.20	5.6	2068	112	9
	Original Medium	5.5	2129	175	5
GALACTOSE (0.4)	0.00	7.6	2027	230	146
	0.01	4.5	2036	80	35
	0.05	4.7	2070	63	17
	0.10	4.2	2087	62	14
	0.20	4.2	2272	135	15
	Original Medium	5.5	2032	58	6
GLUCOSE (0.4)	0.00	7.6	2065	222	207
	0.01	4.3	2041	100	68
	0.05	4.3	2071	82	54
	0.10	5.7	2054	58	34
	0.20	5.7	2086	137	14
	Original Medium	4.8	2054	69	2

TABLE 23 (cont'd)

Induction of Secondary sporangium in germ tubes of P. palmivora sporangia at 30°C in dilute germination medium after an initial incubation for 4 hours at 30°C in the original medium

Compound and Initial Concentration % : w/v	Concentration of Diluted Medium	pH	Observations 24 hours after transfer		
			Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia bearing secondary sporangia
PEPTONE (1.0)	0.00	7.6	2090	1919	1901
	0.01	4.7	2095	1821	1811
	0.05	4.7	2012	1848	1831
	0.10	6.4	2071	1397	460
	0.20	4.8	2067	1260	368
	Original Medium	6.6	2096	931	1
YEAST EXTRACT (1.0)	0.00	7.6	2063	1959	1837
	0.01	4.8	2066	1715	1706
	0.05	6.0	2087	1300	1286
	0.10	7.4	2059	962	377
	0.20	6.3	2074	1296	295
	Original Medium	7.9	2080	1085	2

M. EFFECT OF pH OF DILUTE NUTRIENT MEDIUM ON FORMATION OF SECONDARY SPORANGIA

The pH of the medium exerts a definite effect upon the rate and amount of growth and sporulation as well as other processes of fungi. Although pH has definite effects on reproduction, no unitary hypothesis or generalisation is possible; pH effects are exerted in a variety of ways. A most common influence appears to be the generally less favourable acid pH than a neutral or mildly alkaline reaction, but exceptions even to this rule have been reported (Cochrane, 1958).

It is expected that the response of germinated sporangia to dilute nutrients could be overriden by other factors not yet examined during this investigation, such as pH and other organic compounds. It is desirable to know the influence of some of these major factors in the field on sporangia of P. palmivora. In the previous experiment, pH of distilled water was different from that of all media used. The contribution of pH to the response of the germinated sporangia was not known.

Sporangia of P. palmivora germinated for 4 hours at 30°C in 1.0 per cent Peptone and Yeast-extract solutions, respectively, were transferred after rinsing in sterile distilled water to 0.1 per cent solutions of the same compounds adjusted to the different pH's shown in Fig. 7 and incubated at 30°C for 24 hours. The media were adjusted with dilute HCl and NaOH as

necessary. The pH of the sterile media was measured in replicas of exactly the same volume and receiving the same volume of acid or alkali. This avoided introduction of the electrode into the solution that would be used as suspending medium.

The results in Fig. 7 show that P. palmivora was capable of producing large quantities of secondary sporangia over a wide pH range, from pH 5.0 to 9.0. Secondary sporangium formation was very poor in both media at pH 3.0 and this pH was, indeed, the limit at the acidic side, in the Yeast-extract medium. It was not possible to determine the alkaline limit for secondary sporangium formation.

The patterns of response to pH in the two media were identical.

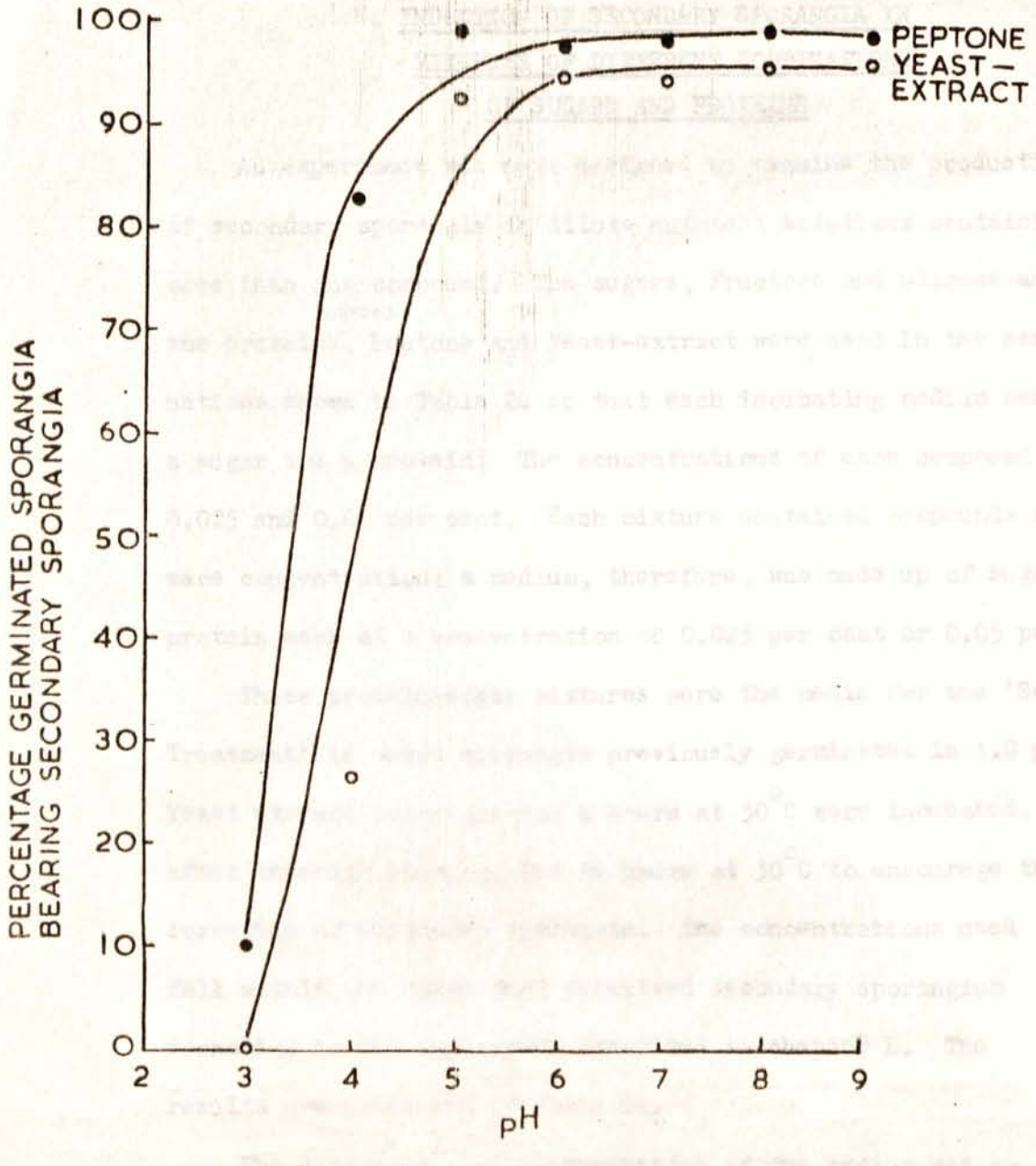

FIG. 7 INFLUENCE OF pH ON THE DEVELOPMENT OF SECONDARY SPORANGIA IN GERMINATED SPORANGIA OF P. PALMIVORA INCUBATED AT 30°C. FOR 24 HOURS IN DILUTE (0·1 PER CENT) PEPTONE AND YEAST-EXTRACT SOLUTIONS

N. INDUCTION OF SECONDARY SPORANGIA IN MIXTURES OF DIFFERENT COMBINATIONS OF SUGARS AND PROTEINS

An experiment was next designed to examine the production of secondary sporangia in dilute nutrient solutions containing more than one compound. The sugars, Fructose and Glucose and the protein sources, Peptone and Yeast-extract were used in the combinations shown in Table 24 so that each incubating medium contained a sugar and a protein. The concentrations of each compound were 0.025 and 0.05 per cent. Each mixture contained compounds at the same concentration; a medium, therefore, was made up of sugar and protein each at a concentration of 0.025 per cent or 0.05 per cent.

These protein-sugar mixtures were the media for the 'Second Treatment' in which sporangia previously germinated in 1.0 per cent Yeast extract solutions for 4 hours at 30°C were incubated, after thorough rinsing, for 24 hours at 30°C to encourage the formation of secondary sporangia. The concentrations used fell within the range that permitted secondary sporangium formation in the experiment described in chapter L. The results are presented in Table 24.

The data show that concentration of the medium was an important factor irrespective of the type of interacting sugar and protein. The percentage of germinated sporangia bearing secondary sporangia in media with constituent compounds of a concentration of 0.05 per cent ranged from 0.8 to 8.1 per cent whilst for media with the lower concentration, the

range was 12.9 to 23.8 per cent.

An examination of the extent of secondary sporangia formation in the presence of the different sugars and proteins, at the lower concentration, stressed an ealier observation that the sugars and proteins at low concentrations exerted almost the same effect. In the Fructose - Yeast-extract, Glucose - Yeast-extract, Glucose - Peptone and Fructose - Peptone media, 19.6, 17.7, 12.9 and 23.8 per cent respectively, of the germinated sporangia formed secondary sporangia.

T A B L E 24

Induction of Secondary Sporangia in germ tubes of P. palmivora sporangia at 30°C in Carbohydrate-protein mixtures.
(Germ tubes developed initially by germinating sporangia in 1.0 per cent Yeast-extract solution at 30°C for 4 hours).

Carbohydrate - Protein Mixture	Concentration (% : w/v)	pH	Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia bearing secondary sporangia
Fructose + Peptone	0.05	4.5*	2003	673	522
	0.10	4.6+	2027	1714	152
Fructose + Yeast-extract	0.05	5.5	2004	1066	260
	0.10	5.4	2001	1747	106
Glucose + Peptone	0.05	5.1	2023	805	120
	0.10	4.8	2036	1886	15
Glucose + Yeast-extract	0.05	5.7	2052	925	206
	0.10	5.8	2058	1621	45
Control : 1.0% Yeast-Extract			2056	1801	8

* Containing 0.025% Sugar and 0.025% Protein.

+ Containing 0.05 % Sugar and 0.05% Protein.

0. INDUCTION OF SECONDARY SPORANGIA IN GERMINATED P. PALMIVORA SPORANGIA TRANSFERRED TO MINERAL SALT SOLUTIONS

The sporangia of P. palmivora would certainly be subjected to another important factor - mineral salts - in the field, and their effects could be important in epidemiology of diseases caused by the fungus.

Numerous observations attest to the influence of mineral nutrition on sporulation. The earlier studies have been reviewed in detail by Foster (1939). As expected, either deficiencies or excesses of particular elements reduce or inhibit sporulation. Fungi have relatively large requirements for phosphorus, potassium, sulphur and magnesium and much smaller but definite requirements for at least five micro-nutrients, copper, iron, manganese, molybdenum and zinc. By a careful and deliberate choice, Potassium dihydrogen Phosphate (KH_2PO_4) and Magnesium sulphate ($MgSO_4.7H_2O$) were selected so that the effect of the macro-elements on secondary sporangium formation could be assessed.

Phosphorus and sulphur occur in known essential compounds, and their major role appears to be constitutive. The other essential mineral elements are today more or less conventionally assigned a catalytic function as activators

or active centres of enzymes. Reasons exist for this general belief : the function of molybdenum in Nitrate reductase and zinc in ethanol dehydrogenase, the occurrence of copper and iron in purified enzymes and the known activating effect of magnesium, manganese and calcium on isolated enzymes.

The two salts, KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ were tested at only one concentration, 5mM. Yeast-extract solution at 1.0 per cent concentration formed the medium for germination of the sporangia. Germination was over the same period of 4 hours at 30°C for the 'First Treatment'. The germinated sporangia were thoroughly rinsed and then suspended in the salt solutions and incubated at 30°C for 24 hours. The results obtained are presented in Table 25.

The mineral salts tested, KH_2PO_4 and $MgSO_4 \cdot 7H_2O$, were both very favourable media at the concentration used, for the development of secondary sporangia. Nearly all the germinated sporangia formed secondary sporangia.

Sporangia lodged in humus would come in contact with far higher level of mineral salts than the concentration tested in this experiment. Another experiment which used higher concentrations of the salts, 10, 20, 40 and 80 mM was set up as a sequel to provide further information on the influence of the salts.

The highest concentration of 80mM was found to be unsuitable for secondary sporangium formation in the Yeast-extract media, and only 16.0 and 25.1 per cent of the germinated sporangia produced secondary sporangia in KH_2PO_4 and $MgSO_4.7H_2O$ media, respectively. Concentrations of 5 to 40mM in the Yeast-extract medium had very good effect - they supported sporulation in all the germinated sporangia. For each concentration level, not less than 2,000 germinated sporangia were observed. In the Peptone solutions, every germinated sporangium formed secondary sporangia at all concentrations (5 to 80mM) of KH_2PO_4 and $MgSO_4.7H_2O$ tested.

TABLE 25

Induction of Secondary Sporangia in germ tubes of P. palmivora sporangia at 30°C in Mineral Salt Solutions

(Germ tubes developed initially by germinating sporangia in 1.0 per cent Yeast-extract solution at 30°C for 4 hours)

Mineral Salt at 5 mM	pH	Observations 24 hours after transfer		
		Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia bearing secondary sporangia
$K H_2 PO_4$	5.2	2001	1716	1704
$Mg SO_4 . 7H_2O$	5.7	2004	1841	1826
Control : 1.0% Yeast Extract		2050	1937	8

P. EFFECT OF KH_2PO_4 AND $MgSO_4.7H_2O$ ON THE FORMATION OF SECONDARY SPORANGIA IN DILUTE PROTEIN AND SUGAR SOLUTIONS

There are innumerable factors in the natural environment which operate together to influence the activities of fungal spores. Only a few of these could be tested in this study. In chapter M, the influence of proteins and sugars acting together was examined and Fig. 7 contained results of experiments to find the effect of pH in two different nutrient media. Information has been provided in this chapter on experiments conducted to investigate the effect of selected sugars and proteins in the presence of the highly stimulatory KH_2PO_4 and $MgSO_4.7H_2O$.

Organic compounds and mineral salts are two very common groups of substances in the field. The profound stimulation of secondary sporangium formation in germ tubes of germinated sporangia of P. palmivora by low levels of KH_2PO_4 and $MgSO_4.7H_2O$ was one of the most outstanding observations of this work. It was considered necessary to find out whether these salts would improve the rate of secondary sporangium formation in organic nutrient media of low concentrations. It was not advisable to extend the test to higher concentrations of the nutrients which have been found to be severely inhibitory.

Freshly harvested sporangia from cocca pods were incubated in 1.0 per cent Yeast-extract solution at 30°C for 4 hours to produce germ tubes. The germinated sporangia, after rinsing, were next incubated in the solutions shown in Tables 26 and 27. Two sugars, Fructose and Glucose and two proteins Peptones and Yeast-extract were used; the sugars at a concentration of 0.05 per cent and the protein, 0.1 per cent. There were three treatments for either a sugar or a protein : one batch of sporangia was incubated in an unamended medium, the second batch in a medium containing 5mM KH_2PO_4 and the third contained 5mM $MgSO_4.7H_2O$. The extent of secondary sporangium formation was estimated after 24 hours' incubation at 30°C. The results of experiments using the sugars are presented in Table 26 and those of the proteins in Table 27.

The effects of the two salts were highly uniform. In brief, interacting low concentrations of any of the organic compounds and 5mM of either KH_2PO_4 or $MgSO_4.7H_2O$ encouraged 100 per cent or near 100 per cent of the germinated sporangia to produce secondary sporangia.

TABLE 26

Induction of Secondary Sporangia in germ tubes of P. palmivora sporangia at 30°C in solutions of Fructose and Glucose amended with KH_2PO_4 and $MgSO_4 \cdot 7H_2O$.

(Germ tubes developed initially by germinating sporangia in 1.0 per cent Yeast-extract solution at 30°C for 4 hours)

Medium	pH	Observations 24 hours after transfer		
		Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia bearing secondary sporangia
0.05% Fructose				
a) Unamended	5.3	2298	1430	1001
b) Amended with 5mM KH_2PO_4	4.8	2006	1799	1796
c) Amended with 5mM $MgSO_4 \cdot 7H_2O$	5.7	2003	1650	1624
0.05% Glucose				
a) Unamended	4.3	2177	1595	959
b) Amended with 5mM KH_2PO_4	4.8	2002	1696	1691
c) Amended with 5mM $MgSO_4 \cdot 7H_2O$	6.1	2021	1774	1771

TABLE 27

Induction of Secondary Sporangium in germ tubes of P. palmivora sporangia at 30°C in solution of Peptone and Yeast-extract amended with KH_2PO_4 and $MgSO_4 \cdot 7H_2O$.

(Germ tubes developed initially by germinating sporangia in 1.0 per cent Yeast-extract solution at 30°C for 4 hours)

Medium	pH	Observations 24 hours after transfer		
		Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia bearing secondary sporangia
0.1% Peptone				
a) Unamended	6.6	2071	1397	460
b) Amended with 5mM KH_2PO_4	4.9	2009	1953	1953
c) Amended with 5mM $MgSO_4 \cdot 7H_2O$	6.5	2014	1899	1899
0.1% Yeast-extract				
a) Unamended	7.9	2059	962	377
b) Amended with 5mM KH_2PO_4	5.5	2001	1924	1924
c) Amended with 5mM $MgSO_4 \cdot 7H_2O$	5.4	2003	1935	1935

Q. INDUCTION OF SECONDARY SPORANGIA IN GERMINATED P. PALMIVORA SPORANGIA TRANSFERRED TO SOLUTIONS OF VITAMINS

The experiments on induction of secondary sporangia so far described have examined the effect of selected examples of major factors in the environment of the sporangia. The organic compounds normally derive from either decomposing organs or from living host plants. In the infection court the effect of exudates of the host is more important of the two.

Carbohydrates and amino acids are the two major groups of components of exudates of the host plant. Although few quantitative estimates of the sugars in plant exudates have been made, fructose and glucose are generally the most abundant (Baker and Sayder, (1965). Amino acids form the most studied group of compounds in exudates. To date more than 20 have been reported in exudates of roots alone. It is likely from the results obtained in the preceding experiments that the concentrations of sugars and amino acids in the exudates would support secondary sporangium formation in already germinated sporangia.

Vitamins form the next major group of compounds in root exudates. Ten vitamins, p- Amino benzoic acid, Biotin, Choline, Inositol, 'M' factor, n- Methyl nicotinic acid, Niacin,

Pantothenate, Pyridoxine and Thiamine, have been identified in exudates from a wide variety of plants (Bhuvaneswari and Sulochana, 1955; Melin and Rana Das, 1954; Meshkov, 1959; Rovira and Harris, 1961; Sulochana, 1962; West, 1939). It was judged desirable to include some of these vitamins in this investigation as test media for the induction of secondary sporangia.

Sporangia of P. palmivora germinated for 4 hours at 30°C in 1.0 per cent Yeast-extract solution were transferred, after thorough rinsing in sterile distilled water, to aqueous solutions of various vitamins and incubated at 30°C for 24 hours. Each vitamin was tested at two concentrations, 200 and 400 pp.m. The results obtained are presented in Table 28.

The vitamins, by their effects, could be separated into two groups. Aneurine hydrochloride (Vitamin B_1), D-Biotin Crystalline, Nicotinamide, (+) - Pantothenic - acid calcium salt, Pyridoxine hydrochloride (Vitamin B_4) and Riboflavin were highly stimulatory. All sporangia with germ tubes produced secondary sporangia.

Cyanocobalamin (Vitamin B_{12}), although suitable for induction of secondary sporangia, was found to be slightly inferior to the others. The percentage of sporangia with secondary sporangia was, however, considerably high at both 200 and 400 p.p.m, (96.9 and 93.5 per cent, respectively). A repeat experiment gave again almost the same values.

TABLE 28

Induction of Secondary sporangium in germ tubes of P. palmivora sporangia incubated at 30°C for 24 hours in solutions of various Growth factors.

(Germ tubes developed initially by germinating sporangia in 1.0 per cent Yeast-extract solution at 30°C for 4 hours).

Growth-factor	Concentration (µg/l)	pH	Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia with secondary sporangia
Aneurine hydrochloride (Vitamin B_1	200	5.4	2053	1857	1857
	400	3.6	2041	1541	1541
D - Biotin crystalline	200	5.4	2034	1722	1712
	400	5.4	2061	1459	1459
Cyanocobalamin Vitamin B_{12}	200	5.5	2050	1628	1578
	400	5.5	2148	1985	1857
Nicotinamide	200	4.5	2036	1751	1751
	400	4.5	2066	1893	1893

TABLE 28 (cont'd)

Induction of Secondary sporangium in germ tubes of P. palmivora sporangia incubated at 30°C for 24 hours in solutions of various Growth factors.

(Germ tubes developed initially by germinating sporangia in 1.0 per cent Yeast-extract solution at 30°C for 4 hours).

Growth-factor	Concentration (µg/l)	pH	Total No. of sporangia observed	No. of sporangia with Germ tubes	No. of sporangia with secondary sporangia
(+)-Pantothenic acid calcium salt	200	4.5	2054	1818	1918
	400	3.9	2097	1897	1897
Pyridoxine hydrochloride (Vitamin B_4)	200	4.5	2058	1748	1748
	400	4.5	2066	1640	1640
Riboflavin	200	4.5	2029	1601	1601
	400	4.5	2074	1837	1837
Distilled water :		7.9	2047	1743	1544

R. CHARACTERISTICS OF THE SECONDARY SPORANGIA

The various pertinent reports in the literature indicate that secondary spores are produced in situations that do not permit harvesting readily. Deliberate studies on the physiology of these spores have, therefore, not been attempted and accounts of the behaviour of secondary spores of fungi are rare. The few observations that have been made on some species suggest that the secondary spores may either possess the same characteristics as the primary spore or may be considerably different.

The primary and secondary sporangia of P. palmivora have the same shape but are conspicuously different in size (see Plates 2 to 4); the secondary sporangia are markedly smaller in size. If reduction in size is accompanied by diminished vigour, the potential of the secondary sporangium as a disease inoculum would be minimised.

Differences between the fungal primary and secondary spores are manifest in many ways. For example, the secondary conidium of Conidiobolus heterosporus differs both in shape and in the manner of discharge (Drechsler, 1953). During asexual reproduction, the conidiophores of C. heterosporus that are extended from hyphal segments seem to be invariably of the stout type familiar in all members of the genus. They widen rather markedly towards the tip on which the single globose conidium is produced. When migration of protoplasm from the

hyphal segment and conidiophore has been completed, the conidium is delimited by a convexly protruding basal septum. Soon afterwards it springs off forcibly, following circumscissile rupture of the peripheral membrane, splitting of the basal partition into two layers, and abrupt eversion of the distal layer.

Instead of germinating vegetatively, the globose conidia, as in numerous related fungi, often develop repetitiously by putting forth a stout conidiophore on which is borne a single secondary globose conidium that springs off forcibly like its parent.

In cultures several days old the conidia of C. heterosporus give rise, on the other hand, to secondary conidia of a different nature, with a distinctive elongated ellipsoidal shape. The ellipsoidal secondary conidium does not spring off forcibly, but become detached on slight disturbance. After falling on substratum already accupied by mycelium of the fungus, they often extend upward a stout conidiophore on which a globose conidium is formed that springs off in the manner usual for spores of that type.

Differences in the structure and behaviour of the primary and secondary sporangia of Basidiobolus haptosporus Drechsler sp.nov. are more dramatic. The primary conidia are colourless, globose or broadly egg-shaped, commonly 25um in

diameter and usually provided with a blunt protuberance. When fully developed, it is forcibly discharged together with the inflated distal portion of the conidiophore. On germination it often produces a secondary conidium at the tip of an erect conidiophore. The secondary conidia are elongated, ellipsoidal, straight or slightly curved, often 50-70µm long and 14.5 - 17.5 µm in width. At the distal end is an apical tubular, beak, 6 - 8 µm long and 2 - 2.5 µm wide with rounded apex surrounded by a glcbose mass of yellow adhesive material 8 - 10 µm in diameter. The elongated secondary conidium often becomes divided by cross-walls into five cells or sporangiophores, each showing a large nucleus near its centre (Drechsler, 1947).

i. Indirect Germination in Secondary Sporangia

The formation and discharge of the zoospores in secondary sporangia of P. palmivora was found to be a reliable character that could be used to compare the functioning of the primary and secondary sporangia.

Sporangia from cocoa pods were incubated in sterile distilled water in Petri dishes at 30°C for 24 hours. Some of the sporangia germinated directly and formed secondary sporangia. The Petri dishes were then transferred, at the end of the incubation period to a temperature of 22°C. Samples of the Petri

dishes were withdrawn at 10 minutes intervals, two drops of formalin added to each suspension to arrest development and examined under the microscope.

Primary sporangia from cocoa pods were incubated along with the secondary sporangia and samples withdrawn at the same intervals to determine percentage indirect germination. The number of sporangia out of at least a thousand which had formed zoospores was recorded. The values obtained were used to plot the graph in Fig. 8.

Zoospore formation commenced early in both types of sporangia. Thus, 10.0 and 8.0 per cent of the primary and secondary sporangia, respectively, produced zoospores within 10 minutes, and almost a quarter of the germinable sporangia formed zoospores within 20 minutes of incubation.

Zoospore formation, thereafter increased rapidly in the primary sporangia and the percentage indirect germination after 30 minutes was 74 per cent. That level of germination was achieved by the secondary sporangia in 80 minutes. This experiment showed that more primary sporangia germinated indirectly than the secondary sporangia and the rate of germination was also faster.

ii. RNA AND RESERVE FOOD SUBSTANCES IN THE SECONDARY SPORANGIA

The slower rate of germination of the secondary sporangia could be attributed to various causes. Among the most important are the level of reserve food substances, nature of enzymes and permeability of the sporangial wall. It was deemed reasonable to begin with an investigation of the amount of the reserve food substances, glycogen, lipids and proteins in the sporangia and the quantity of RNA which is associated with protein synthesis.

Sporangia on albumen - coated slides were used for testing for glycogen, proteins and RNA. The sporangia stained for lipids were put on dry slides.

Drops of sporangial suspension of primary sporangia from cocoa pods were placed on albumen-coated slides and air-dried. They were then treated with appropriate stains indicated under "Materials and General Methods".

The secondary sporangia were produced in large quantities by the method which has been perfected during this investigation. Primary sporangia which had been previously germinated in 1.0 per cent Yeast-extract solution at 30°C for 4 hours were thoroughly rinsed and re-suspended in sterile distilled water Secondary sporangia were readily produced after 24 hours' incubation of the aqueous suspension at 30°C. The suspension

was then centrifuged at a speed of 4,500 revolutions per minute. The germinated sporangia with secondary sporangia formed a scum on the surface of the supernatant fluid. The scum was transferred with an inoculating loop onto either albumen - coated slides or dry slides and appropriately stained after drying in air.

The observations showed that

(a) RNA, , lipids and proteins were present in ~~heavy~~ high concentrations in both types of sporangia.

(b) the reserve food substances and RNA were evenly distributed in the protoplast of both primary and secondary sporangia (see Plates 11 to 14).

(c) the primary and secondary sporangia were stained to the same intensity and apparently contained the same level of endogenous lipids and proteins, and R.N.A. Plates 11a and b are photomicrographs of primary and secondary sporangia stained for RNA, showing the same degree of coloration.

(d) no glycogen was detected in either sporangial type confirming the report by Zevenhuizen and Bartnicki-Garcia (1970) that the cytoplasm of Comycetes contains no glycogen but employs as storage polysaccharides, B-1,3-glucans similar to algal laminarian (Phaeophyta and Chrysophyta) for which the designation mycolaminaran has been proposed.

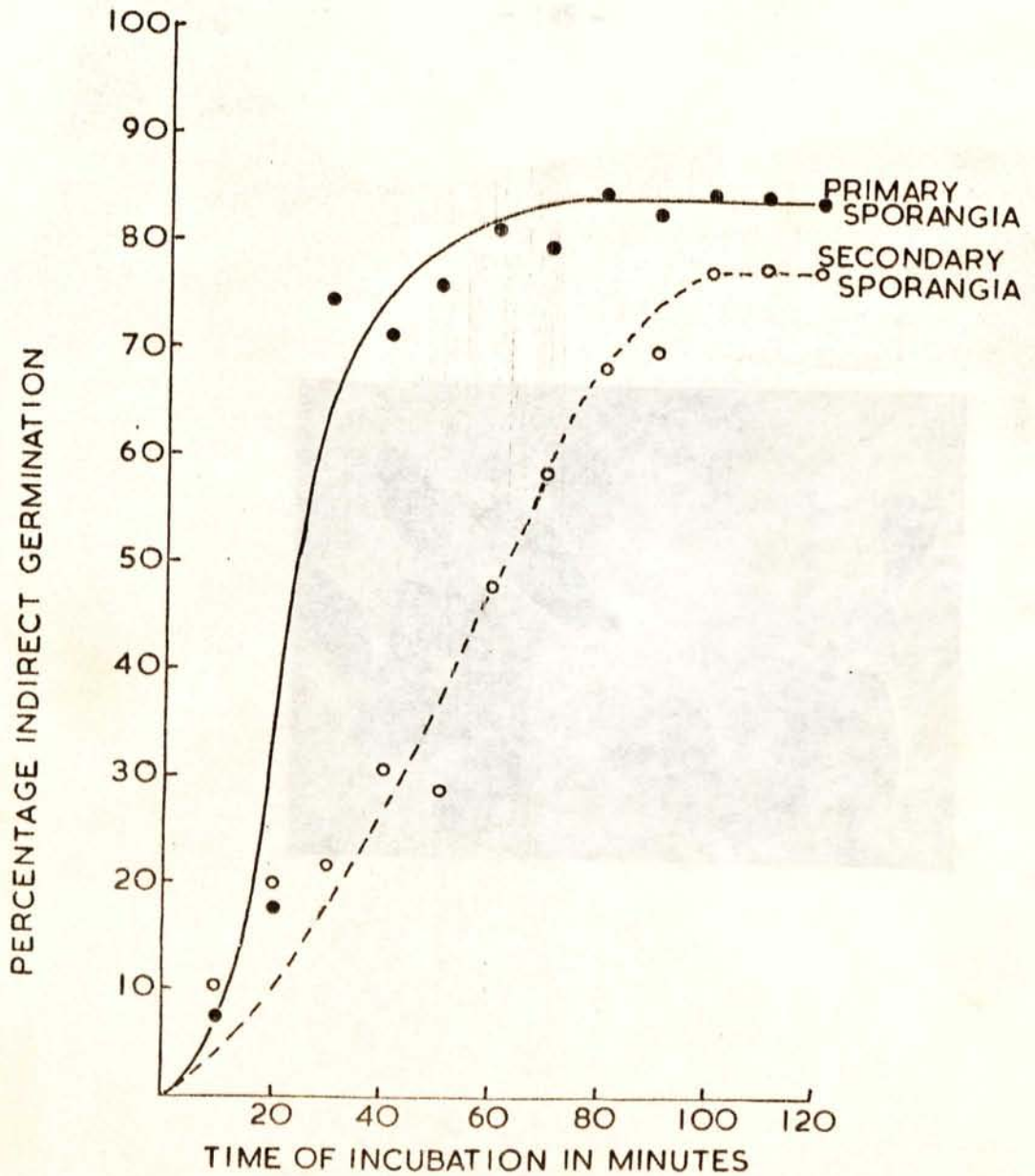

FIG. 8 COMPARATIVE INDIRECT GERMINATION IN PRIMARY AND SECONDARY SPORANGIA OF P. PALMIVORA INCUBATED IN DISTILLED WATER AT 20°C.

Plate 11. Photomicrograph of Primary Sporangia of P. palmivora treated with Korson's Stain to show distribution of R.N.A. x 500.

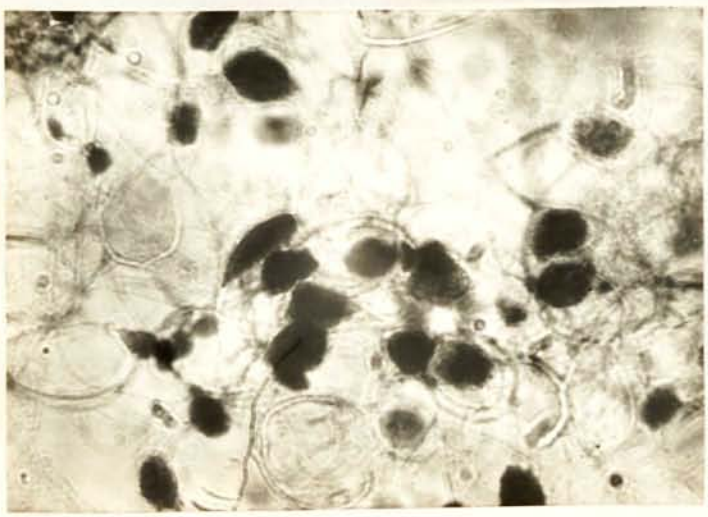

Plate 12. Photomicrograph of Secondary Sporangia of P. palmivora treated with Korsen's Stain to show distribution of R.N.A. x 500.
Note identical concentration of RNA in sporangia of Plates 11 and 12.

Plate 13. Photomicrograph of primary sporangia of P. palmivora treated with Safranin and Fast Green to show distribution of Protein. Note even distribution of protein in sporangium. x 500.

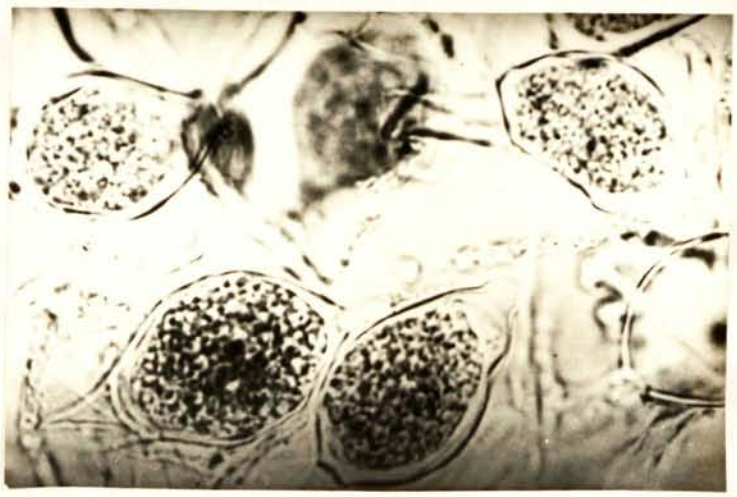

Plate 14. Photomicrograph of secondary sporangia of P. palmivora treated with Sudan III to show presence of Lipids.
Note presence of lipids in the form of globules. x 800.

V. GENERAL DISCUSSION

The economic importance of Phytophthora palmivora lies in its ability to destroy, annually large quantities of both immature and mature cocoa pods, one of the most important cash crops of many tropical countries. In attempts to control effectively this dangerous parasite, various aspects of its biology have been studied. A thorough knowledge of the biology of the fungus is naturally invaluable in the design of any successful control measure, especially if the attack is to be directed at the most vulnerable phase of the pathogen. The importance of the sporangium as a means of dispersal and as infection unit is well known. Without any doubt, whatsoever, the sporangia of P. palmivora are of infinite importance in the epidemiology of black pod disease of cocoa.

The sporangium of P. palmivora plays its role as disease initiator extremely well.

(a) It germinates by forming zoospores that eventually produce hyphae that penetrate the host tissue.

(b) It germinates by forming zoospores which may in turn produce secondary zoospores.

(c) It germinates by producing hyphae that penetrate the host tissue, and

(d) It germinates by producing germ tubes that bear secondary sporangia.

The last habit has great import. If secondary sporangia are developed under conditions unsuitable for hyphal growth, the secondary sporangium becomes a useful survival body. On the other hand, if a sporangium produces more than one secondary sporangium, a very valuable mechanism of rapid turn-over of infection units is available to this pathogen. The object of the present study was to determine more precisely the factors which promote secondary sporangium formation in P. palmivora and to investigate the characteristics of the secondary sporangium as a contribution to the understanding of the value of the secondary sporangium to the fungus and of the biology of the fungus in general.

The development of secondary sporangium has been studied in a number of Phytophthora species. In the collection of descriptions of species of this genus, Waterhouse (1956) gave an account of secondary sporangium formation by some of them. Phytophthora cinnamomi Rands, isolated from a canker in Cinnamomum burmanni Bl., for example, could be considered to possess typical features. The primary sporangia varied in shape from ovoid to ellipsoid or elongate. They were hyaline, thin walled and with broad, flat, inconspicuous papilla. They were averagely 58.0 µm in length and 34.0 µm in width. Germinating sporangia sometimes formed secondary sporangia at the apices of short germ tubes. Occasionally a succession of secondary sporangia, maximum three, was produced in

a series. Such series showed a distinct decline in size of the sporangia in order of production. The first secondary sporangia thus measured averagely 50.0 µm long and 26.0 µm at the greatest width. The second secondary sporangia were averagely 38.0 µm in length, 21.0 µm wide and the mean dimensions of third secondary sporangia were 30.0 µm by 20.0 µm. The secondary sporangia were subtended by germ tubes of varying lengths, ranging from 11.0 to 30.0 µm.

Some of the most unusual sporangia formed by species of the genus Phytophthora were found in Phytophthora primulae Tomlinson, a stem and root parasite of Primula polyantha Mill. The sporangia exhibited great variation in size and shape. Some were single structures, ovoid, bluntly ellipsoid, limoniform or obpyriform, averaging 77.0 by 34.0 µm in size. Other sporangia were compound - compound sporangia - elongate structures consisting of two to eleven spherical or more irregularly shaped sporangial segments, each delimited at one or both ends by septa or hyaline plugs. Any segment could germinate by a germ tube that grew into a hypha or developed a secondary sporangium soon after emergence. The compound sporangia had lengths ranging from 30.0 to 80.0 µm. Germ tubes bearing secondary sporangia were generally short, 10.0 - 80.0 µm long.

During the present investigation, P. palmivora was found to produce secondary sporangia in various fashions. When P. palmivora sporangia were incubated in distilled water secondary sporangia were

produced within 24 hours by some of the sporangia. The primary sporangium produced one to three germ tubes very close to the papilla (see Plates 1 and 2), each bearing a secondary sporangium (see Text Fig. 1, Figs 1 and 2). Germ tubes bearing the secondary sporangia were of variable length. They could be as short as 32.0 um or as long as 240.0 µm (see Plates 3a, b, c). The secondary sporangium in common with those of P. cinnamomi, never attained the size of the mature primary sporangium that produced it. It was always smaller (see Plates 1 to 4). Where a series of two or more sporangia in a chain was formed, each succeeding sporangium was smaller than the preceding one (see Plate 4). Primary sporangium with a mean length of 42.4 µm was markedly larger than the two secondary sporangia, 27.8 and 26.2 µm long, respectively in the chain (see Table 4). The difference between the size of the primary and secondary sporangia was statistically significant. A germ tube of P. palmivora commonly formed only one secondary sporangium and only very few secondary sporangia, 2.6 per cent, germinated during 48 hours incubation and produced another secondary sporangium (see Table 3).

The entire sporangial content of the sporangium of a Phytophthora is not used during germ tube formation and a considerable proportion remains in the sporangium. The drawings Text - Fig 1, Figs 1, 2, 3; Plates 1 and 2 illustrate this in P. palmivora. Ultrastructural studies of directly germinating sporangia of P. palmivora (Clerk 1974) showed more distinctly that the sporangium was never empty when

germ tubes emerged and of the numerous nuclei, more than 20, in the sporangium only two or three migrated into the developing germ tube. Hemmes and Hohl (1970) demonstrated exactly the same phenomenon in their ultrastructural studies on the directly germinating sporangia of Phytophthora parasitica. The smaller size of a secondary sporangium might be the result of developing a sporangium from only part of the contents of the previous one.

Evidence from other studies, however, seem to indicate that the size of secondary spores might probably be just a perculiarity of the secondary spores, and therefore, a natural phenomenon rather than a feature imposed by environmental and internal conditions. This supposition is supported by the inability of extraneous nutrients to influence the size of the sporangia of P. palmivora. The mean length of primary sporangium of 38.1 µm was far superior to the mean length of the secondary sporangia, 27.5 to 32.7 µm, produced by sporangia germinating in solutions of various carbohydrates (see Table 13). Secondary sporangia of closely similar dimensions, 26.7 to 31.0 µm were also produced by primary sporangia incubated in Tryptophane solution (see Table 18).

In certain instances, the secondary spore is even, definitely, morphologically different not only in size, but in shape and form. Secondary conidia of Basidiobolus haptosporus produced by the spheroidal primary conidia were strobiliform or ellipsoidal prolonged at the distal end into a narrow beak which terminate in a relatively large spherical mass of yellow material. The production of secondary

conidium was commonly repeated a second and a third time, each repetition entailing some reduction in size of the spore (Drechsler, 1947). The beak was adhesive and its function was to attach the spore securely to a passing animal to effect probably dispersal, parasitic or even predaceous relationship. Some of the beaked conidia instead of giving rise to another spore externally became divided internally by transverse cross walls. The five resulting cells, though not accurately equal in size showed no great difference in volume. Each of the segments contained near the centre of its vacuolate protoplast a spherical nucleus, which showed a clean hyaline outer layer surrounding a noticeably darker sub-spherical nucleolus. The primary and secondary conidia of Basidiobolus haptosporus are clearly two very different morphological bodies.

Conidiobolus heterosporus has spherical conidia which often germinated on discharge to give rise to secondary globose conidia on long secondary conidiophore. Under certain conditions, however, the primary conidia formed secondary conidia of elongate - ellipsoid shape. This kind of secondary conidia appeared to be more frequent in conidia deposited on glass.

Clerk (1963) described secondary conidia formed by Beauvaria bassiana incubated in distilled water on glass slides and on dry slides placed at 100% R.H. The secondary conidia were oblong -

cylindric, a condition De Bary (1869) described as 'Cylinder conidia'. The primary conidia were, on the other hand, a mixture of globose and oval spores.

Whether the secondary spore would assume the role of a survival body under conditions where the hyphae are vulnerable depends on the characteristics of the spore. If it is a spore with a thick protective wall it could survive for a long time. Such role would be played by the secondary spores of Cercospora arachidicola Hori and Cercospora bougainvilleae Muntandla.

Oso (1972) reported that any cell of the primary conidium of Cercospora arachidicola on glass slide in saturated atmosphere could form a secondary conidiophore, which sometimes, as well as producing a secondary conidium, also gave rise to a vegetative hypha. A primary conidium did not produce more than one secondary conidiophore, which, however, could bear up to two or three conidia. A secondary conidium was lighter in colour than a primary one and there was some variation in its mode of attachment to the conidiophore. No tertiary conidium was observed.

Sober and Martinez (1966) have reported the only other instance of secondary conidium formation in the Cercosporas. In Cercospora bougainvilleae more than one cell could produce secondary conidia. In both instances the secondary conidia possessed spore wall of

similar thickness to that of the primary conidium.

There is evidence of long survival of conidia of the Cercosporas. Teyegaga and Clerk (1972), for example, showed that conidia of Cercospora canescens Ellis and Martin survived under the best storage conditions, 20°C and zero per cent R.H. for 112 days.

Where the fungus species possesses fragile spores as found in the Phytophthoras, production of secondary spores would be a means of multiplying infection units, especially, if more than one secondary spore is formed by the primary spore rather than serving as a body of survival.

An extreme case is provided by Conidiobolus chlamydosporus. Drechsler (1955) described the production of averagely 14 secondary microconidia by a germinating primary conidium of C. chlamydosporus. The colourless globose primary conidia, mostly 15 - 45 µm in diameter produced 2 to 20 colourless, globose or elongate ellipsoidal conidia measuring 8 to 11 µm in diameter. Conidiobolus polytocus conidia has exactly the same features as Conidiobolus chlamydosporus. A germinating primary conidium produced as many as 12 microconidia (Drechsler, 1955b). The microconidia of both species often germinated by producing a single sterigma at the tip of which a microspore was formed. It is probable that the microspores serve mainly in disseminating the fungus through the innumerable interstices

in deposits of decaying plant materials, which the more forcibly propelled primary conidia serve better to spread the fungus on the roomy surfaces.

A sporangium of P. palmivora often produced more than one secondary sporangium. The secondary sporangia cannot be judged to be survival unit because of the general sensitivity of sporangia of the phytophthoras to desiccation.

Warren and Colhoun (1975), in an experiment on Phytophthora infestans (Mont.) de Bary investigating the ability of the sporangia to withstand desiccation, discovered that those exposed to atmospheres of 90% R.H. were quickly killed. Only 3.0 per cent of the sporangia were still viable after 2.5 minutes of exposure and almost all the sporangia lost viability after five minutes. Sporangia exposed for more than 10 minutes became indented and on re-wetting and staining with cottonblue, the cytoplasm did not refill the sporangium. Under dry conditions the sporangia would serve very little or no purpose at all as survival bodies.

The sporangia may, however, survive under conditions other than those related to lack of moisture. Lack of nutrients and, therefore, absence of substrate for the hyphae, could induce secondary spore formation. During this investigation, the sporangia of P. palmivora were germinated in nutrient-rich media, using originally 1.0 per cent Yeast-extract and Peptone solutions. After the sporangia, incubated

at 30°C, had produced very short germ tubes, after 4 hours incubation, they were transferred after washing off any traces of the nutrients into sterile distilled water. The germ tubes, interestingly produced profuse secondary sporangia whilst less than 0.3 per cent out of the germinated sporangia retained in the original nutrient media formed secondary sporangia. As much as 98.1 and 95.2 per cent of the sporangia, initially germinated in Peptone and Yeast-extract solutions, respectively, formed secondary sporangia in distilled water when they were deprived of the nutrients (see Table 20).

When the protein compounds were replaced by carbohydrates, transfer of the germinated sporangia from the nutrient solution to distilled water did not produce development of the secondary sporangia to the same extent. Only 24.1 per cent of germinated sporangia transferred from fructose solutions to distilled water formed secondary sporangia. In similar tests, sporangia germinated in Galactose, Glucose and Mannose solutions for four hours and transferred to distilled water showed reduced rate of secondary sporangium formation of 30.0, 24.0 and 8.0 per cent, respectively. (see Table 22).

These observations are in agreement with reports on other fungi which suggested that lack or reduction in level of nutrients could be one of the factors responsible for secondary spore development.

The conidia of Cercospora arachidicola studied by Oso (1972) formed the secondary conidia in saturated air at 20° - 30°C. Primary conidia on nutrient agar or on fresh groundnut leaves produced only vegetative hyphae and no secondary conidia. Oso concluded that since Cercospora arachidicola produces these secondary conidia on a slide and not on nutrient agar, it could be inferred that the phenomenon of secondary conidia production is a means of ensuring the further propagation of the species in an environment when nutrients for mycelial growth are lacking.

Callaghan (1969) also reported that germinating conidia of Basidiobolus ranarum growing on malt agar medium, invariably developed a vegetative mycelium. In contrast, spores on the surface of the parent colony and on water agar nearly always germinated directly to give a conidiophore bearing conidium. In this instance, nutrients (malt extract) suppressed secondary conidium development on one hand whilst on the other, secondary sporangia were formed in the absence of any nutrients. The track for translocation of nutrients between the mature conidium of Basidiobolus ranarum and the subtending hypha would be blocked by the basal septum of the conidium and the conidium germinating on the parent hypha could be regarded as germinating in humid air in the absence of external supply of nutrients.

Pure water is hardly available in uncontaminated form in nature. In the absence of pure water, however, secondary sporangia could still be formed in large quantities by P. palmivora on evidence obtained in subsequent experiments. It has been shown that when very low concentrations of nutrients were used as growth media for sporangia which had been initially germinated in media of higher nutrient concentration, very high percentage of secondary sporangium formation was often possible. The growth media were low concentrations of proteins, Peptone and Yeast-extract; low concentrations of the carbohydrates, Fructose, Galactose and Glucose and those of the mineral salts, KH_2PO_4 and $MgSO_4 \cdot 7H_2O$.

When sporangia were germinated in 1.0 per cent Peptone solution and then transferred into a diluted medium, 0.05 per cent, of the Peptone solution, as much as 99.1 per cent of the germinated sporangia produced secondary sporangia. Increase in concentration of the medium led to a reduction in the number of germinated sporangia able to form secondary sporangia. Thus only 24.2 per cent of the germinated sporangia formed secondary sporangia in 0.2 per cent peptone solution. Similarly, 99.4 per cent of the sporangia produced secondary sporangia in 0.01 per cent Yeast-extract solution compared to 22.7 per cent in 0.20 per cent Yeast-extract medium after the sporangia had been germinated in 1.0 per cent yeast-extract. (see Table 23).

The situation was not different when low concentrations of carbohydrates were used. In 0.01 per cent Fructose solution, 79.6 per cent of the germinated sporangia produced secondary sporangia compared to 8.0 per cent in stronger medium of 0.2 per cent fructose. In both, 0.4 per cent Fructose solution served as the initial germination medium for production of the germ tubes. Again 47.5 per cent of the germinated sporangia formed secondary sporangia in 0.01 per cent Galactose solution while 11.1 per cent did so in 0.2 per cent galactose solution. After the sporangia had been germinated in 0.4 per cent glucose solution, 68.0 per cent out of the total formed secondary sporangia on transfer to 0.01 per cent glucose solution and 10.2 per cent did so in 0.2 per cent glucose solution (see Table 23).

When carbohydrates were mixed with the proteins at different concentrations a similar picture, as already discussed, was portrayed. Approximately 31 per cent of germinated sporangia incubated in a mixture of Fructose and Peptone, each at 0.05 per cent concentration, produced secondary sporangia compared to 8.8 per cent when concentrations of 0.1 per cent were used. A mixture of Fructose and Yeast-extract each at 0.05 per cent concentration induced secondary sporangium formation in 24.3 per cent of the germinated sporangia in contrast to 6.1 per cent in mixtures

containing 0.1 per cent of each compound. In the glucose - peptone mixture 14.9 per cent germinated sporangia formed secondary sporangia in solutions containing 0.05 per cent concentration of each substance whilst only 0.8 per cent sporangia formed secondary sporangia when the higher concentration of 0.1 per cent was used. The mixture involving glucose and yeast-extract gave results of exactly the same trend (see Table 24).

Seemingly, although reduction of nutrients available to already germinated sporangia or total lack of them would be the primary factor to trigger secondary sporangium formation, the type of compound in very dilute media would possibly play a role. It would be observed that the same concentrations of yeast-extract and peptone (0.01 per cent supported significantly higher rates of secondary sporangium formation, 22.7 and 29.2 per cent, respectively (see Table 23), than fructose, galactose and glucose, 8.0 - 11.1 per cent (see Table 23). Similarly mixtures of the different compounds but at same concentrations induced different levels of secondary sporangium formation (see Table 24). Further support is found from the subsequent experiments that employed mineral salt - sugar mixtures.

Mineral salts, KH_2PO_4 and $MgSO_4.7H_2O$ at 5mM concentration supported rather high secondary sporangium formation of 99.3 and 99.2 per cent, respectively, in already germinated sporangia (see Table 25).

When these mineral salts were used separately to amend 0.05 per cent Fructose and Glucose solutions almost 100 per cent of the sporangia formed secondary sporangia in each case. When the mineral salts were again mixed with 0.1 per cent Peptone and Yeast-extract solutions there was total response of 100 per cent secondary sporangium formation.

In soil, carbohydrates, mineral salts and proteins are available from various sources and principally from decomposing organic materials. Proteins and carbohydrates are also available in pod exudate or pod extract. Bimpong (1969) identified by chromatography four amino acids at the following concentrations in the extract of cocoa pod husk.

Asparagine	:	2000 p.p.m.
Glutamic acid	:	3000 p.p.m
Glycine	:	400 - 500 p.p.m
Tyrosine	:	200 - 300 p.p.m

Neither amino acids nor sugars were detected chromatographically in the exudates at the concentrations used. There is no doubt that such substances exist in the exudate but occurred at too low concentrations to be easily detected by chromatography.

Asomaning (1977) also identified a number of amino acids from the root exudate of 20 day old cocoa seedlings using Amino acid

Analyser, Model JLC - 6 AH. The following amino acids were identified and their quantities estimated :

Alanine	:	0.005 µmol/ml
Asparagine	:	Trace
Glycine	:	0.004
Histidine	:	0.00e
Isoleucine	:	Trace
Leucine	:	Trace
Serine	:	0.012
Threonine	:	0.002
Tyrosine	:	0.015
Valine	:	Trace

There are, therefore, several mineral salts, amino acids and carbohydrates in the environment of the P. palmivora sporangia that will come into contact with them and influence them. The sporangia are formed freely on the infected pod on the cocoa tree and sporangia have been shown to occur in soil as well, produced by hyphae either living saprophytically or parasitically on host roots (Newhall, Diaz and Salazar, 1966; Okaisabor, 1971; Turner, 1961; Wharton, 1955).

It is important that a dilution of the nutrients at a critical stage of germination of the sporangium must occur in nature if the fungus would ever form secondary sporangia at any substantial level because the concentration of some of the substances are likely to be

high, especially, in soil. Sporangia germinating in solutions of amino acids and carbohydrates of even moderately low concentrations and retained in these solutions during growth of the germ tubes hardly formed secondary sporangia in many instances. It could be envisaged that a shower of rain would be the only agent to cause dilution of the compounds; an event which should coincide with the correct stage of germination of the sporangium to be effective.

The results in Tables 5 to 17 showed that in the absence of any dilution of nutrients in the field the chances of substantial secondary sporangium formation are low. Three distinct types of response were discernible. Secondary sporangium formation in solutions of cellobiose, cellulose, glucose, mannose, sucrose and xylose, all the amino acids tested and mixtures of various combinations of fructose, glucose and sucrose, was inferior to that in distilled water. The magnitude of suppression of secondary sporangium formation would be appreciated if it is noted that averagely only 3.9 per cent of the sporangia could form secondary sporangia in distilled water. Arabinose constitutes a group of median response which supported, averagely, a level of secondary sporangium formation, 3.5 per cent close to distilled water. The rest formed a third group that was just slightly superior to distilled water. The respective average percentages of sporangia forming secondary sporangia in these

fructose, galactose, lactose and maltose were 4.7, 4.7, 5.5 and 4.6.

The various amino acids and carbohydrates were used over concentration ranges of 100 to 400 p.p.m. and 0.1 to 0.8 per cent respectively. There were some variations in the behaviour of the sporangia at different concentrations of each compound. Except 0.1 per cent Arabinose (8.3 per cent secondary sporangia formation), 0.2 per cent galactose (15.4 per cent) and 0.2 per cent Lactose (14.4 per cent) the highest values recorded were extremely low.

Some nutrient solutions exhibited no definite observable pattern or were uniformly the same. These included glucose, sucrose and mannose. In each case all the concentrations gave nearly similar values.

The ability of lower concentrations of nutrient solutions to promote secondary sporangium formation in already germinated sporangia may be of benefit. It would enable the fungus to produce secondary sporangia under conditions where the hyphae would not grow adequately. But the phenomenon would only be valuable if phases of low nutrients could alternate with those of high nutrients as good germination needs reasonably nutrient-rich media. A disadvantage will ensue if the secondary sporangia after production fail to germinate and produce yet another crop of secondary sporangia. Dormant spores in soil are favoured substrate of soil

bacteria and lysis of the dormant sporangia, a propagule with very little protection, would readily occur.

It is not only in dilute nutrient solutions that heavy secondary sporangia formation would be found in previously germinated sporangia. Growth factors which are very likely to occur in the infection court of the host plant would most likely also stimulate secondary sporangium formation. A number of growth factors were used at two concentrations, 200 and 400 µg/l as media into which already germinated sporangia of P. palmivora were transferred when the germ tubes were approximately twice the length of the sporangia. All the germinated sporangia placed in Aneurine hydrochloride (Vitamin B1) Nicotinamide, Pantothenic acid calcium salt, Pyridoxine hydrochloride (Vitamin B_4) and Riboflavin formed secondary sporangia within 24 hours at both concentrations. D-Biotin crystalline at 400 ug/l supported secondary sporangium formation in 100 per cent of the germinated sporangia. D - Biotin at 200 µg/l and Cyanocobalamin (Vitamin B_{12}) at 200 and 400 µg/l, however, gave slightly lower, but very high indeed, percentage secondary sporangium formation of 99.4, 96.9 and 93.5 per cent, respectively (see Table 28). Growth substances are likely to occur mostly in the infection court where the sporangium could easily initiate infection. They, therefore, on the whole would play a greater role in epidemiology than nutrients which occur at locations other than the infection court.

It is worthy of note that other substances in growth media which have not been examined in this investigation have been suggested as factors that stimulated secondary sporangium formation. Drechsler (1955a) reported that spores of Conidiobolus polytocus which formed secondary conidia on the culture medium were those that dropped at areas where there were no parent hyphae. This shows that the parent mycelium could produce substances that prevented the formation of secondary conidia or these areas contained less concentration of metabolites exuding from the hyphae. This investigation has shown that in P. palmivora an inhibition by the parent mycelium might not exist, because sporangia still attached to the parent mycelium germinated, even though at low rate of 0.3 - 0.9 per cent, by means of germ tubes and produced secondary sporangia (Table 1). If the parent mycelium produced any such inhibiting substances, it either could not traverse the plug at the base of the sporangium or it was non-volatile.

The pH of the nutrient media and distilled water in the various experiments described did not seem to have influenced the response of the sporangia to the various nutrients and growth factors, and the low rate of secondary sporangium formation recorded in many was a true reflection of the treatment effect on the sporangia. When germinated sporangia of P. palmivora were transferred to yeast-extract solution of very low concentration adjusted to various pH's large quantities

of sporangia produced secondary sporangia over a wide pH range, from pH 5.0 to pH 9.0 (Fig.7). Secondary sporangium formation was only poor at pH 3. The pH's of almost all the media of the tests shown in the tables of results fell within pH 5.0 and 9.0. Even where the pH fell outside this range treatment effect superseded the effect of pH. Solutions of Aneurine hydrochloride (Vitamin B_1) at a concentration of 200 and 400 µg/l had pH's 5.4 and 3.6 respectively and yet 100 per cent of the germinated sporangia formed sporangia in both solutions. The two, Pantothenic acid calcium salt media of pH's 4.5 and 3.9 also gave 100 per cent secondary sporangium formation. All the sporangia in solutions of Riboflavin also produced secondary sporangia even though the pH of the two media was pH 4.5.

Other environmental factors have induced secondary sporangium formation in some fungi. Light encouraged the formation of secondary conidia in Conidiobolus rhyosporus (Dring 1958). Zuchkerman (1957) was able to induce secondary conidium production from the conidia of Endoconidiophora fagacearum (Bretz) by means of X-ray irradiation. There is sufficient evidence to show that P. palmivora sporangia will form secondary sporangia to the same extent in both light and dark. Sporangia which had been germinated in 1.0 per cent yeast-extract and peptone solutions for four hours and transferred to distilled water and incubated at 20°, 25° and 30°C respectively, behaved closely similarly. Out of 800 observed germinated sporangia, in each

treatment, transferred from the peptone solution and incubated at each of the temperatures, 87.6, 91.2 and 91.4 per cent formed secondary sporangia. The corresponding values for yeast-extract were 84.1, 86.6 and 85.2 per cent, respectively. The temperature 20°C was provided by lighted air-conditioned room and 25° and 30°C by electric incubators with no interior lighting.

Further pertinent studies on P. palmivora should examine the effect of osmotic pressure and aeration. Page and Humber (1973) reported that conidia of Conidiobolus coronatus formed secondary conidia when germinated under mineral oil or on a medium, with a high osmotic pressure. It is expected that carbohydrates and mineral salts, especially, will act both through nutrient and osmotic effects.

The importance of the secondary spore, whether it is acting as a survival unit or aiding in multiplying infection units lies in its ability to function in similar manner as the primary spore. There has been no attempt to investigate the physiology of the secondary spore discovered in the numerous fungal species. Observations here, therefore, provide a very significant information on this aspect of the biology of fungi.

Zoospore formation was possible in both primary and secondary sporangia. From the investigation carried out the release of zoospores started early in both types of sporangia. About 10.0 and

8.0 per cent, respectively, of the primary and secondary sporangia released zoospores within 10 minutes. Zoospore formation, however, increased rapidly in the primary sporangia and the percentage indirect germination, after 30 minutes, rose to 70.0 per cent. The same value was, however, achieved by the secondary sporangia in 80 minutes (see Fig. 8).

This investigation was not extended to a comparative study of direct germination of the sporangia due to difficulties of harvesting the secondary sporangia. This is a necessary study in any future pertinent investigations.

Despite the difference in rate of indirect germination, both types of sporangia of P. palmivora seemed to contain the same quantities of R.N.A., lipids and proteins. What will be equally important would be information on the enzyme systems of the two types of sporangia. It is possible that although the same quantities of food reserves may be available to the sporangia, differences in the enzyme components of the two types of sporangia would result in differences in the use of the nutrient reserves and consequently differences in germination rates. There are very well known histochemical tests that would permit assessment of the presence of various enzymes in the sporangia in the same manner as employed in the tests for organalles and reserve food substances. For example, the Wachstein and Meisel technique can be

used to investigate Adenosine triphosphate (Hall, 1969), Gamori method for B - glycerophosphatase (Pearse, 1968) and Cytochrome oxidase (Turian and Bianchi, 1971). The method of Seligman and Rutenberg (1951) has been used to study Succinic oxido-reductase and the Nitro Blue Tetrazolium technique described by Pearse (1968) for Alcohol oxido - reductase.

The sporangium of P. palmivora is too fragile to have any survival value. The importance of the secondary sporangia lies in their acting as a means of producing abundant infection bodies quickly. In the epidemiology of blackpod disease of cocoa, secondary sporangia of P. palmivora might not create any serious problems. The majority of the sporangia produce only one secondary sporangium giving a condition of zero growth population. Furthermore, conditions needed to produce abundant secondary sporangia are too refined to commonly occur in the field. Control measures need not be specially adapted to combat the secondary sporangia. Improvement of known methods of attacking the sporangia would suffice to attack both the primary and the few secondary sporangia and with which they apparently share almost similar physiological characteristics.

VI. SUMMARY

1. Sporangia of P. palmivora attached to the mycelium on cocoa pod could germinate by means of germ tubes in humid air and form secondary sporangia.

2. Secondary sporangium formation in situ at 25°C was rare, involving less than 1.0 per cent of the total number of sporangia observed.

3. Higher temperatures of 30° and 35°C encouraged direct germination of the sporangia.

4. The higher temperature, 35°C, was less suitable for sporangial germination. Only 1.6 to 3.8 per cent of the sporangia produced zoospores at 35°C in contrast to 10.7 to 16.2 per cent at 30°C.

5. More sporangia, 15.5 per cent, germinated by means of germ tubes at 30°C than at 35°C which supported only 7.6 per cent direct germination.

6. A greater percentage of directly germinated sporangia also formed secondary sporangia at 30°C (13.5 per cent) than at 35°C (1.7 per cent)

7. Sporangia germinating in distilled water at 30°C produced one to three germ tubes. The majority of sporangia had only one germ tube

8. Germ tubes bearing secondary sporangia were of variable length; they could be as short as 30.0 um or as long as 242.0 µm

9. The secondary sporangium was always significantly smaller on maturation than the primary sporangium that produced it.

10. Where there was a series of two or more secondary sporangia in a chain each succeeding secondary sporangium was smaller than the preceding one.

11. Secondary sporangium formation in carbohydrate media was limited due to low direct germination of the sporangia in these media at 30°C. The highest percentage direct germination was recorded at 0.2 per cent Galactose; 242 sporangia out of a total 2188. Seventy three per cent of this number formed secondary sporangia.

12. Carbohydrates which supported greater secondary sporangium formation at certain concentrations than distilled water were: Arabinose (0.1 per cent), Cellobiose (0.1 - 0.8 per cent), Galactose (0.1 - 0.2 per cent), Lactose (0.1 - 0.2 per cent), Maltose (0.2 - 0.4 per cent).

13. At the best Cellulose concentration over the range, 0.1 - 0.8 per cent, extent of secondary sporangium formation was close to that in distilled water.

14. Secondary sporangium formation was better in distilled water than in fructose, glucose, mannose, sucrose and xylose media of concentrations of 0.1 to 0.8 per cent.

15. Secondary sporangium formation was also poorer in fructose-glucose, fructose-sucrose, glucose-sucrose and fructose-glucose - sucrose media than in distilled water, although direct germination was higher, 7.8, 8.1 and 14.3 per cent, respectively, in the fructose-glucose, fructose-sucrose and glucose-sucrose media than in distilled water (5.3 per cent)

16. Formation of secondary sporangium on more than one germ tube was not common in the carbohydrate solutions, and only a few, 0.2 per cent Galactose (2.2 per cent), 0.2 per cent Lactose (0.7 per cent) and 0.4 and 0.8 per cent Maltose (1.1 and 0.7 per cent, respectively) provided values greater than those obtained in distilled water.

17. The carbohydrate media were better media for growth of the germ tubes than distilled water.

18. The average length of germ tubes bearing secondary sporangia was 180.0 µm. The longest germ tubes bearing secondary sporangia were found in solutions of 0.1 per cent Galactose (500.0 µm) 0.8 per cent Maltose (425.0 µm)

19. The germ tubes were also longer in comparison to those in distilled water in the fructose-glucose, fructose-sucrose, glucose-sucrose and fructose-glucose-sucrose media. The recorded mean germ tube lengths were 398.0µm Fructose-Glucose, 406.0 and 310.0 µm in Glucose-sucrose and Fructose-Glucose-

sucrose respectively.

20. The sizes of secondary sporangia in the carbohydrate media at concentrations of 0.2 and 0.4 per cent, were similar to those formed in distilled water which were markedly smaller than the primary sporangia.

21. At concentration of 0.2 per cent, the length of secondary sporangia ranged from 26.9 µm, (cellulose) to 32.3 µm Arabinose). The secondary sporangium formed in distilled water was 29.9 µm. The range at 0.4 per cent concentration was 27.5 µm (Galactose) to 32.7 µm (Mannose). The mean lengths of the primary sporangia, 38.1 and 39.3 µm, measured for the two sets far exceeded these values.

22. Indirect germination of the sporangia was greater at 30°C at 0.1 per cent Cellobiose, Lactose and Sucrose and at 0.1 - 0.2 per cent Cellulose and Mannose but lower at the other concentrations within the range 0.1 - 0.8 per cent of these carbohydrates and at all concentrations (0.1 - 0.8 per cent) of Arabinose, Fructose, Galactose, Glucose, Maltose and xylose than in distilled water.

23. Distilled water also supported higher percentage indirect germination than the Fructose-Glucose, Fructose-Sucrose, Glucose-Sucrose and Fructose-Glucose-Sucrose mixtures.

24. Solutions of Alanine, Arginine, Asparagine, Glycine, Histidine, Leucine, Lysine and Tryptophane at 100-400 ppm

were poor media for both direct sporangium germination and secondary sporangium formation. Distilled water was superior as germinating medium to all these amino acids.

25. Zoospore formation was better in solutions of 100 ppm. Arginine, 100-200 ppm. Alanine and Arginine and 100-400 ppm Glycine, Leucine and Lysine.

26. Secondary sporangia formed in 100-400 ppm Tryptophane solutions were 26.7 - 31.0 µm long compared with the length of 29.4 µm of secondary sporangia formed in distilled water and the length of 42.6 µm of the primary sporangium

27. Peptone and Yeast-extract supported 57.0 and 67.6 per cent direct germination, respectively, at 30°C. Approximately, only one out of 300 directly germinated sporangia formed secondary sporangia in each case.

28. A novel method devised to induce secondary sporangium formation was the withdrawal of the nutrient supply to very young germ tubes (2-3 hours old) by transferring them to distilled water or the reduction of the concentration of the nutrient of the germinating medium or the transfer of the germinated sporangia to dilute solutions of completely different compounds for 24 hours.

29. This method allowed the formation of secondary sporangia to approximately the same extent at the temperatures, 20° and 25°C

as well as 30°C.

30. High germination rate for the induction experiments was, specifically, obtained by germinating the sporangia at 30°C in 1.0 per cent Peptone and Yeast-extract solutions for 4 hours.

31. The following media supported good secondary sporangium formation when sporangia germinated for 4 hours in richer medium were incubated in them :

(a) Distilled water : 63.5 - 99.5 per cent

(b) Fructose

0.01 per cent	79.6 per cent
0.05 per cent	75.9 per cent
0.01 per cent	64.2 per cent

(c) Glucose

0.01 per cent	68.0 per cent
0.05 per cent	67.5 per cent

(d) Peptone

0.01 per cent	99.5 per cent
0.05 per cent	99.1 per cent

(e) Yeast-extract

0.01 per cent	99.5 per cent
0.05 per cent	98.9 per cent

(f) KH_2PO_4

5mM	100 per cent

(g) $MgSO_4 \cdot 7H_2O$

5mM	100 per cent

(h) Fructose, 0.05 per :

+ 5mM KH_2PO_4 : 94.2 per cent

+ 5mM $MgSO_4.7H_2O$: 98.7 per cent

(i) Glucose, 0.05 per cent :

+ 5mM KH_2PO_4 : 99.7 per cent

+ 5mM $MgSO_4.7H_2O$: 99.8 per cent

(j) Peptone, 0.1 per cent;

+ 5mM KH_2PO_4 : 100 per cent

+ 5mM $MgSO_4.7H_2O$: 100 per pect

(k) Yeast-extract, 0.1 per cent;

+ 5mM KH_2PO_4 : 100 per cent

+ 5mM $MgSO_4.7H_2O$: 100 per cent

(l) D-Biotin crystalline,

200µg/l : 96.9 per cent

400µg/l : 93.5 per cent

(m) Cyanocobalamin (Vitamin B_{12});

200µg/l : 100 per cent

400µg/l : 100 per cent

(n) Aneurine hydrochloride (Vitamin B_1);

200µg/l : 100 per cent

400µg/l : 100 per cent

(o) Nicotinamide

200µg/l : 100 per cent

400µg/l : 100 per cent

(p) (+) - Pantothenic acid calcium salt;

200µg/l	:	100 per cent
400µg/l	:	100 per cent

(q) Pyridoxine hydrochloride (Vitamin B4);

200 µg/l	:	100 per cent
400 µg/l	:	100 per cent

(r) Riboflavin ;

200 µg/l	:	100 per cent
400 µg/l	:	100 per cent

32. Using the induction method, P. palmivera sporangia previously germinated in 1.0 per cent Yeast-extract solution formed abundant secondary sporangia (by 92.6 to 100 per cent of germinated sporangia) over a wide pH range of pH 5.0 to 9.0 in 0.1 per cent Peptone and Yeast-extract solutions adjusted to different pH levels.

33. There was greater germination rate and higher percentage indirect germination among primary sporangia incubated in distilled water at 20°C than among secondary sporangia.

34. N.N.A., Lipids and Proteins were present in heavy concentrations in both primary and secondary sporangia.

35. It was concluded that conditions required for the production of secondary sporangia in large numbers were too precise to be

common feature of the natural environment. Incidence of secondary sporangium formation would be low in the field. Secondary sporangia of P. palmivora would not therefore play an overly important role in the epidemiology of blackpod disease of cocoa.

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