Isolation and Chemical Composition of Oospore-Oogonium Walls of *Phytophthora megasperma* var. *sojae*

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SUMMARY

The entire sexual apparatus (oospore-oogonium-antheridium, or ooa) of *Phytophthora megasperma* var. *sojae* was isolated in large quantities free of mycelium. The cells were fractured in a Teflon tissue homogenizer and the wall fraction was washed with a cytoplasmic dialysate to prevent agglutination and a tendency to adhere to glassware. The wall preparations were a mixture of oospore and oogonium walls (oow) and probably unrecognizable antheridial wall fragments. The walls constituted 47% of the dry weight of the ooa. Insoluble glucans comprised almost 80% of the wall; the main component was non-cellulosic β -glucan(s) linked mainly through the 3-position. Less than 10% of the oow was cellulose. In addition to glucose, there were small amounts of mannose and glucosamine in the wall polymers. Proteins comprised about 12% of the oow; the most abundant amino acids were arginine and glycine. Hydroxyproline, a component of oomycetous hyphal walls, was also present. Readily extracted lipids varied from 2.8 to 5.9%, while bound lipids comprised 5.4 to 5.9% of the wall. Most of the readily extractable lipids of the ooa were found in the walls whereas bound lipids were mainly in the cytoplasmic fraction. Sterols (0.025%) were detected in the readily extracted lipid fraction. Phosphorus amounted to 0.55% of the oow. There was substantial similarity in gross chemical composition between oow and hyphal walls of *Phytophthora* spp.

INTRODUCTION

This work is part of an investigation of the biochemistry of wall differentiation in *Phytophthora*. Previous reports from our laboratory dealt with the structure and biochemistry of walls from hyphae, sporangia and cysts of various *Phytophthora* spp. (Bartnicki-Garcia, 1966; Bartnicki-Garcia & Lippman, 1967; Zevenhuizen & Bartnicki-Garcia, 1969; Tokunaga & Bartnicki-Garcia, 1971*a, b*). The present investigation of the composition of oospore-oogonium walls is an initial effort to define the biochemical basis of oosporogenesis in *Phytophthora*. These studies may also permit an assessment of the suspected roles of these walls in oospore dormancy and germination. Blackwell (1943*b*) attributed oospore dormancy to the special properties of oogonium and oospore walls. This is a commonly held view, though the mechanism of oospore dormancy has yet to be elucidated (Zentmyer & Erwin, 1970).

Phytophthora megasperma var. *sojae*, the causal agent of a root and stem rot of soybean, was chosen because it produced large quantities of oospores in homothallic cultures. Oospores as such could not be isolated. However, the entire sexual apparatus (Fig. 1), oospore-oogonium-antheridium (ooa), was readily prepared free from mycelium (Partidge & Erwin, 1969). The chemical determinations reported below pertain to a mixture of

Fig. 1. Cell walls in the sexual-reproductive apparatus (OOA) of *Phytophthora megasperma* var. *sojae*. og = Oogonium wall; os = oospore wall; a = antheridium wall. To facilitate observation of wall relationships, the specimen was mounted in 1N-KOH and stained lightly with I_2 . The alkali caused the oospore wall to swell and the oogonium wall to spread out smoothly over the oospore wall. Bar = 10 μ m.

oospore and oogonium walls (oow), which we could not separate from each other, and probably unrecognized fragments of antheridial walls.

A preliminary report of this work was presented earlier (Lippman, Erwin & Bartnicki-Garcia, 1971).

METHODS

Microbiological techniques. A single-oospore isolate of *Phytophthora megasperma* var. *sojae* (P405, race 1) was maintained on V-8 juice agar slants. The fungus was grown in 90 mm Petri dishes containing 10 ml of V-8 juice agar at 25 °C in the dark for 7 days followed by exposure for 7 days to light (2150 lux) for 4 h daily. Oospores were formed abundantly under these conditions. The cultures were frozen and stored at -20 °C before harvesting.

Isolation of the sexual apparatus. The entire sexual reproductive apparatus consisting of the oospore, oogonium and antheridium (OOA) was removed in large numbers from associated mycelium (and agar) by repeated cycles of homogenization and centrifugation. All isolation procedures were performed at 4 °C. The contents of 10 frozen culture plates were homogenized in 100 ml water with a Waring blender for 2 min, diluted to 500 ml with water, and centrifuged for 1 min at 1500g. The resulting sediment contained an upper layer of agar and mycelium and a lower layer of OOA. These layers were separated with a Pasteur pipette. The agar-mycelium layer was rehomogenized and sedimented until an OOA pellet was no longer obtained. The combined OOA pellets were homogenized in a Sorvall Omni-mixer (50 ml chamber) at 10000 rev./min for 1 min. The homogenate was diluted with water and centrifuged at 1500 g. Contaminating agar and mycelial fragments sedimented above the OOA

Fig. 2. Purified sample of oospore-oogonium-antheridium structures, OOA, of *Phytophthora megasperma* var. *sojae*. Stained with I_2 . Bar = 40 μ m.

Fig. 3. Purified sample of oospore-oogonium walls, oow, of *Phytophthora megasperma* var. *sojae*. Stained with I_2 . Bar = 40 μ m.

pellet and were removed with a Pasteur pipette. The procedure was repeated until the OOA pellet was free of mycelium and agar (Fig. 2). The samples were then lyophilized.

A second simpler procedure for OOA isolation was developed and the samples thus obtained were used for some of the analytical work (sterol analysis). The contents of four culture plates were homogenized in 100 ml cold water for 4 min in a Sorvall Omni-mixer. Small portions of the homogenate were forcibly washed through a double layer of nylon net (100 mesh) with cold water. After the filtrate was centrifuged for 2 min at 1000g, the supernatant and the fluffy white upper sediment containing mycelium and agar were discarded. The tan-coloured OOA pellets were collected. The homogenization and filtration procedure was repeated until the material retained by the filter no longer contained OOA. Combined OOA pellets were centrifuged and the upper white sediment discarded. The OOA was again homogenized, filtered, and centrifuged. To remove small amounts of agar and mycelium, the OOA pellets were sonicated in a Raytheon Sonic Oscillator (Waltham, Massachusetts, U.S.A.) for 2 min at maximum intensity (OOA resisted sonication) and centrifuged for about 1 min at 1000g; the supernatant and white upper sediment were discarded and the OOA pellet lyophilized.

Preparation of oospore and oogonium walls (OOW). Lyophilized OOA samples were suspended in water and broken in 100 mg batches with a 17 ml capacity Teflon Potter-Elvehjem tissue grinder (Kontes Glass Co., Vineland, New Jersey, U.S.A.) driven by an overhead stirrer for about 10 min. The broken-cell suspension was centrifuged at 75 g. To prevent agglutination, walls were resuspended and washed in a cytoplasmic dialysate instead of water or buffer. (The cloudy supernatant from the first cell breakage was dialysed against 15 vol. distilled

water and the outside dialysate used to resuspend the walls). The pellets were homogenized twice in the tissue grinder and sedimented at 75 g; each sedimentation was followed by several washings with cytoplasmic dialysate. To remove small particles and lipid-like globules, the suspension was subjected to mild sonication for three 5-min intervals (Cole-Parmer Ultrasonic Cleaner, Model 8845-4) each followed by centrifugation at 200 g. After the final sonication, the walls were washed by centrifugation until the supernatant was no longer turbid. The walls were then washed in distilled water to remove traces of the cytoplasmic dialysate (Fig. 3). All breakage and purification procedures were performed at 4 °C. The wall preparations were lyophilized and stored over P_2O_5 .

Chemical analysis. Total hexosan was determined by the anthrone procedure (Dreywood, 1946), with glucose as a standard.

The hexoses were characterized as alditol acetates (Sloneker, 1972) by the following procedure. The walls were soaked at 25 °C in 22 N- H_2SO_4 for 3 h. The acid was then diluted to 0.85 N (1.00 mg arabinose internal standard was added to one of the replicates) and hydrolysis was continued at 97 °C for 4 h. Acid was neutralized by the addition of $BaCO_3$ and the precipitated $BaSO_4$ was removed by filtration. Sugars were reduced with $NaBH_4$ for 1 h. The sample was then acidified by addition of Dowex-50W-X2, hydrogen form (Bio-Rad Laboratories, Richmond, California, U.S.A.). The sample was filtered and evaporated to dryness. Methanol was added and evaporated 3 times to remove all traces of methyl borate. Alditol acetate derivatives were prepared by heating for 20 min at 97 °C with equal volumes of acetic anhydride and dry pyridine. Solvents were removed by addition of toluene and evaporation to dryness. The sugars were dissolved in chloroform and separated by g.l.c. on a glass column (200 × 0.3 cm) packed with 3% (w/w) ECNSS-M on chromosorb (100 mesh) by means of a Perkin-Elmer 990 Chromatograph.

Total nitrogen (Dumas) was assayed by Elek Microanalytic Laboratories, Torrance, California, U.S.A.

Total protein was determined by the ninhydrin-hydrindantin method (Moore & Stein, 1954) on a 6 N-HCl acid hydrolysate as previously described (Bartnicki-Garcia & Nickerson, 1962).

Quantitative amino-acid analyses were done on 6 N-HCl hydrolysates according to Spackman, Stein & Moore (1958) with a Beckman Spinco automatic amino-acid analyser. Separate analyses were performed on the 1 N-KOH-soluble and the KOH-insoluble fractions of defatted (ether-ethanol extracted) oow and the results combined. Hydroxyproline was determined independently in 6 N-HCl hydrolysates as follows. Radioactive [$2-^{14}C$]hydroxyproline (Cal Atomic, mixed isomers; specific activity 18.5 Ci/mol), 50000 d.p.m. was added as a marker to the neutralized hydrolysate. The amino acids were first separated by descending paper chromatography in a phenol-water system [liquified phenol:0.2 M- K_2HPO_4 (10:1, v/v), containing 8-hydroxyquinoline (100 mg/l)]. The isotope was located by using a Vanguard Autoscaner (LaGrange, Illinois, U.S.A.) and rechromatographed on paper with butan-2-ol-formic acid-water (7:1:2, by vol.). The radioactivity was eluted from the paper with water and hydroxyproline assayed chemically by using the method of Stegemann & Stalder (1967).

Ammonia was determined from a 6 N-HCl hydrolysate in Conway microdiffusion units by using the Nessler reaction (Ammonia Color Reagent, Sigma Chemical Co., St Louis, Missouri, U.S.A.). 2-Amino sugar was detected in 6 N-HCl hydrolysates during the amino-acid analysis and also by the Elson-Morgan method (Tracey, 1955).

Free and bound lipids were extracted as previously described and determined gravimetrically (Bartnicki-Garcia & Nickerson, 1962). Sterols in the lipid fractions were assayed by a modification of the Liebermann-Burchard colorimetric assay (Jack, 1964).

Table 1. *Chemical composition of the oospore-oogonium wall of Phytophthora megasperma var. sojae*

Component	Method	Wall dry weight (%)
Hexosan	Anthrone	77.9
Protein	Ninhydrin	11.45
Total lipid	Gravimetric	9.96
Amino sugar	Elson-Morgan	0.43
Phosphorus (P)	Semidine-HCl	0.55

Total phosphorus was determined by the semidine-HCl procedure (Dryer, Tammes & Routh, 1957).

Chemical fractionation. The walls were extracted with 1 N-KOH at 25 °C for 30 min. The alkali-soluble supernatant was dialysed against distilled water and the non-dialysable fraction assayed for carbohydrates and proteins. The alkali-insoluble material was then extracted with Schweizer's reagent (cuprammonium hydroxide) (Jayme & Lang, 1963) for several days under an atmosphere of nitrogen at 1 °C in the dark. The soluble fraction was separated by centrifugation and the solubilized cellulose was reprecipitated by acidification with glacial acetic acid.

Enzymatic hydrolysis. Purified exo- β -1,3-glucanase from a Basidiomycete, QM806, was kindly supplied by D. E. Eveleigh of Rutgers, The State University, New Brunswick, New Jersey, U.S.A. A mixture of endo- β -1,3- and endo- β -1,4-glucanases from Streptomyces, QMB814, was the generous gift of E. T. Reese of U.S. Army Laboratories, Natick, Massachusetts, U.S.A. Both enzymes were used in a ratio of 1 mg enzyme to 10 mg wall sample. Exo- β -1,3-glucanase digestion was carried out in 0.05 M-sodium acetate buffer, pH 4.8, and the endo- β -glucanases in 0.05 M-sodium citrate buffer, pH 5.75. Digestion of the walls was carried out at 50 °C for 42 h. The enzymes were inactivated at 97 °C for 5 min and the soluble supernatants assayed for hexose by the anthrone procedure. Five mg of oow, digested with 1 mg acid phosphatase from potato (Calbiochem, La Jolla, California, U.S.A.) in 1 ml 0.05 M-sodium acetate buffer, pH 4.5, for 10 h at 37 °C, were assayed to determine phosphate solubilized.

X-ray diffraction patterns were obtained in a Norelco 2.5 kW generator with a 114.6 mm Debye-Scherrer powder camera using CuK_{α} radiation.

RESULTS

Our isolate of *Phytophthora megasperma* var. *sojae* produced abundant sexual reproductive structures, OOA, on V-8 agar (Fig. 1). The oogonium diameters ranged from 30 to 40 μm (Fig. 2). The yield of OOA from 100 Petri dishes was about 750 mg dry wt.

Preparations of oow contained oospore and oogonium walls in various stages of fragmentation and probably some unrecognizable pieces of antheridium walls (Fig. 3). There were occasional unbroken OOA and hyphal fragments, but these were thought to be insignificant. Purified wall samples retained the light brown colour of the intact oospore preparations. The final yield of oow was approx. 40% of the dry wt of the OOA.

Carbohydrate. Hexose polymers comprised almost 80% of the dry weight of oow of *Phytophthora megasperma* (Table 1). Three hexoses were identified in oow hydrolysates: glucose (97.4%), mannose (2.4%), and traces of galactose. For comparative purposes similar determinations were made on hydrolysates of whole OOA. A comparison of mannose

Table 2. *Fractionation of polysaccharides of oospore-oogonium walls of *Phytophthora megasperma* var. *sojae**

Fraction	Total carbohydrate (%)	Wall dry wt (%)
KOH-soluble	1.0	0.8
KOH-insoluble		
Schweizer-soluble (cellulose)	8.7	6.6
Schweizer-insoluble (non-cellulosic glucan)	90.3	68.3

Fig. 4. X-ray diffraction patterns of regenerated cellulose from oospore-oogonium walls (top) and from Whatman paper (bottom).

content in the walls (1.65 % dry wt) with that in the whole oospores (1.1 % dry wt) indicated that at least 80 % of the mannose in the oospore must be part of the wall if it is assumed that the walls are 47.3 % of the dry wt of the intact oospores (see below). In contrast, a similar comparison of galactose values indicated that the trace amounts of this sugar found in the wall hydrolysates were most probably of cytoplasmic origin.

An Elson-Morgan determination on a 6 N-HCl hydrolysate revealed only a small amount of hexosamine (0.43 % wall dry wt). A glucosamine peak (0.25 % wall dry wt) was identified during the automatic amino-acid analysis. This glucosamine appeared mainly in the 1 N-KOH-insoluble fraction of the oow. Only a trace of the glucosamine was found in the 1 N-KOH-soluble fraction.

The glucans were characterized by both solubility (Table 2) and enzymic hydrolysis. Only 1 % of the oow polysaccharides was soluble in dilute alkali. Cellulose (extracted with Schweizer's reagent and recovered by reprecipitation upon acidification) comprised only 6.6 % of the oow (anthrone determination). The identity of this material was confirmed by X-ray diffraction (Fig. 4). The main portion of these walls (68 %) was a highly insoluble non-cellulosic glucan.

Microscopic comparisons of oow extracted with Schweizer's reagent and unextracted samples failed to disclose any differences indicative of a preferential location, if any, of cellulose in the oogonium or the oospore wall. However, these examinations allowed us to conclude that the principal component in either the oospore or the oogonium walls was a non-cellulosic β -glucan.

When oow samples were digested with a crude mixture of endo- β -1,4 and endo- β -1,3-

Table 3. Amino acid composition of the oospore-oogonium wall proteins of *Phytophthora megasperma* var. *sojae**

Amino acid	Moles (%)	Wall dry wt (%)
Alanine	10.14	0.49
Arginine	9.95	0.94
Aspartic	9.35	0.67
Glutamic	8.15	0.65
Glycine	20.88	0.85
Histidine	0.48	0.04
Hydroxyproline†	3.66	0.26
Isoleucine	1.41	0.10
Leucine	4.22	0.30
Lysine	3.15	0.25
Methionine	0.74	0.06
Phenylalanine	1.79	0.16
Proline	5.61	0.35
Serine	7.37	0.42
Threonine	7.73	0.50
Tyrosine	2.54	0.25
Valine	2.83	0.18
Total	100.00	6.48

* Values represent the combined total of alkali-soluble and -insoluble protein fractions from defatted samples.

† Determined independently.

glucanases or purified α -D-glucanase, 50% of the wall glucans were dissolved, thus indicating a preponderance of β -1,3- linkages. Microscopic examination of oow stained with alcian blue (E. Lippman and S. Bartnicki-Garcia, unpublished) showed no ostensible change in the oogonium walls but there was substantial digestion of the oospore walls, particularly by the α -D-glucanase.

Protein. Values of total protein as determined by the ninhydrin procedure (Table 1) were in agreement with a total nitrogen value of 2.01%, equivalent to 12.5% protein (dry wt basis). Only about one-fifth of the wall protein was soluble in 1 N-KOH at room temperature.

The amino-acid composition of oow proteins is shown in Table 3. The total amount of amino acids recovered in the automatic analysis was only 6.5% (oow dry wt). This lower yield was probably the result of destruction of amino acids as evidenced by humin formation and a relatively high NH_3 content in the hydrolysate (0.35% wall dry wt). On a molar basis, the most abundant amino acids were glycine, alanine, arginine, and aspartic and glutamic acids. Hydroxyproline, an amino acid characteristic of hyphal walls of oomycetous fungi (see Bartnicki-Garcia, 1968), was present.

Lipids. Lipid values for ooa and oow are shown in Table 4. It follows from these data and the finding that the oow comprises 47% of the total ooa weight, that about 25% of the total lipid content of the ooa is in the walls. Furthermore, the wall contains most of the readily extractable lipids of the oospore whereas the bound lipids are largely found in the cytoplasmic fraction. Sterols were detected colorimetrically in the readily extractable lipids (0.025% dry wt) but not in the bound lipids.

Miscellaneous. Phosphorus represented 0.55% of the wall dry weight. When digested with acid phosphatase, oow released neither phosphate nor carbohydrate into the medium.

Proportion of wall in the oospore-oogonium. The total wall volume in the oospore-

Table 4. *Lipid content of intact oospore-oogonium-antheridium structures (OOA) and oospore-oogonium walls (OOW)**

Lipid	Dry wt (%)	
	OOA	OOW
Readily extracted	2.8 ± 0.8	4.2 ± 1.7
Bound	16.4 ± 5.4	5.7 ± 0.2
Total	19.2 ± 4.9	9.9 ± 1.7

* Values are the means of four determinations ± standard deviation.

oogonium was measured as the difference between total oogonium volume and cytoplasmic volume. To exclude as much as possible the seemingly empty space between oogonium and oospore walls, only those cells with the oogonium wall tightly adpressed against the oospore wall (for at least 50% of the cell circumference) were scored. Accordingly, the total wall volume was $64.74 \pm 5.24\%$.

To estimate the dry-weight percentage of wall in intact OOA, a sample of OOA was thoroughly disrupted with a Potter-Elvehjem tissue grinder in 0.1 N-NaOH. The wall fraction was quantitatively separated by centrifugation and its carbohydrate content measured by anthrone (37.8% of the initial OOA dry wt). Since the unextracted OOW contained 77.9% hexosan (Table 1), the total amount of walls in the OOA was calculated to be 47.3%. This value agrees with the yields of purified OOW (37 and 42% dry wt) obtained from two different OOA batches. The latter values are, of course, minimal estimates attributable to small losses during handling.

DISCUSSION

This appears to be the first report on the chemical composition of isolated oospore-oogonium walls of Oomycetes. There have been several studies on the nature of these walls in the genus *Phytophthora*, but they were mainly cytochemical observations (Mangin, 1895; Dastur, 1913; Blackwell, 1943*b*), the validity of which is open to question (see Aronson, 1965). Electron microscopy of sexual reproductive structures of various Oomycetes (Howard & Moore, 1970; Gay, Greenwood & Heath, 1971; Heath, Gay & Greenwood, 1971; Vujić, 1971) provides little information on the structural organization of these walls.

Ballistic disruption (Bronwill MSK with 0.45 to 0.50 mm ballotini beads), the preferred method for fungal cell breakage in our laboratory, was ineffective for breaking OOA; it resulted in some walls being broken into minute fragments (difficult to recognize microscopically and difficult to separate by centrifugation) while numerous cells remained unbroken. In contrast, a Teflon tissue homogenizer, which was unsuccessful for smaller spheroidal cells such as yeast or sporangiospores of *Mucor rouxii* (unpublished observations), fractured essentially all OOA and yielded wall pieces that could easily be isolated. The method worked well with OOA, probably because of the relatively large size (30 to 40 μ m diam.).

The tendency of wall preparations to agglutinate after a few washings is an annoying problem frequently encountered in cell wall purification (Bartnicki-Garcia, 1966; Bartnicki-Garcia & Reyes, 1968). Agglutination increases sample loss and interferes with the elimination of debris by differential centrifugation. Washing the walls with various solutions did not alleviate this problem. Since agglutination did not occur during the first two washings with distilled water, we suspected that some solute from the fungus cytoplasm was

preventing agglutination. Accordingly, a dilute cytoplasm dialysate (diffusible fraction) was prepared and found to eliminate the agglutination problem during wall washing. The nature of the active agent(s) is not known.

Glucans comprise almost 80% of the oow. The bulk of the wall is composed of a non-cellulosic β -glucan(s) linked mainly through the 3-position. Less than 10% of the oow is cellulose. Preliminary data (P. Bardalaye and S. Bartnicki-Garcia, unpublished) indicate that this glucan is also a highly branched molecule like that found in the mycelial walls (Zevenhuizen & Bartnicki-Garcia, 1969). In addition to glucose, minor amounts of mannose and glucosamine are present. The glucose, mannose and glucosamine values are comparable to those in the mycelial walls of *Phytophthora* and *Pythium* (Bartnicki-Garcia, 1966; Mitchell & Sabar, 1966; Cooper & Aronson, 1967; Novaes-Ledieu, Jiménez-Martínez & Villanueva, 1967; Sietsma, Eveleigh & Haskins, 1969; Tokunaga & Bartnicki-Garcia, 1971*b*). The role of the minor sugars in the walls is not known, but they could be the connecting links of glycoprotein complexes as has been found for glucosamine in the cell wall mannans of *Saccharomyces cerevisiae* (Sentandreu & Northcote, 1968).

It was earlier claimed by Mangin (1895), Dastur (1913) and Blackwell (1943*b*) that cellulose was present in oospore and oogonium walls. Our results confirmed that cellulose is indeed present in the oow, but the precise location of the cellulose in either or both of these walls was not determined. Although our findings pertain to a combined analysis of oogonium and oospore walls, it was possible to conclude that the main constituent of both the oogonium and the oospore wall is a non-cellulosic β -1,3-glucan. This may represent the callose and/or the unknown compound reported earlier in oow (Mangin, 1895; Dastur, 1913; Blackwell, 1943*b*).

The chemical composition of the oow of *Phytophthora megasperma* was qualitatively similar to that of the vegetative or sporangial walls of related species of *Phytophthora* (Crook & Johnston, 1962; Bartnicki-Garcia, 1966; Aronson, Cooper & Fuller, 1967; Bartnicki-Garcia & Lippman, 1967; Novaes-Ledieu, Jiménez-Martínez & Villanueva, 1967; Zevenhuizen & Bartnicki-Garcia, 1969; Michell & Scurfield, 1970; Tokunaga & Bartnicki-Garcia, 1971*b*) and differs only quantitatively. Significant differences in the relative proportions of components do exist, notably increased lipid and protein at the expense of a lower glucan content. The oow proteins of *P. megasperma* also differ appreciably from those of the mycelial walls of *P. cinnamomi*. In mycelial walls, threonine, glutamic acid and serine are the principal amino acids; glycine and arginine, the most abundant amino acids in oow, did not predominate in the vegetative walls. The amino-acid composition of the oow proteins was comparable to that found in most other fungal cell walls (Bartnicki-Garcia, 1973), except for the presence of hydroxyproline (commonly found among oomycetous fungi, see Bartnicki-Garcia, 1968) and the unusually high arginine content.

The phosphorus content of the oow (0.55%) was several times higher than that found in the hyphal walls of *Phytophthora cinnamomi*, *P. parasitica* (Bartnicki-Garcia, 1966) and *P. heveae* (Novaes-Ledieu, Jiménez-Martínez & Villanueva, 1967) and the cyst and sporangial walls of *P. palmivora* (M. C. Wang, unpublished data). Unlike that in cytoplasmic phosphoglucans (Wang & Bartnicki-Garcia, 1973), the phosphorus in the oow was not removed by phosphomonoesterases, and its nature is uncertain.

The oow contained a large amount of lipid in both free and bound forms. Lipids may be bound to the cell wall and play a structural role, e.g. they may contribute to the stiffness of the walls in *Saccharomyces cerevisiae* (Hurst, 1952). However, exhaustive extraction for lipids did not produce alterations in the characteristic morphology of the cells. Another possibility is that they confer hydrophobic properties to the walls which could have

implications in dormancy and germination. These conjectures are worthy of further attention.

The oow constitute about 47% of the dry wt of the intact ooa. This is one of the highest percentages of wall material in fungal cells. Ostensibly, the thicker oospore wall is the major component of the oow.

Unequivocal roles for the oogonium and oospore wall cannot at present be ascertained; however, on germination, the internal portion of the oospore wall is markedly eroded (Blackwell, 1943*a*; Henry & Stelfox, 1968; Satour & Butler, 1968; Zentmyer & Erwin, 1970; Erwin & McCormick, 1971) suggesting that a main role for the oospore wall is that of carbohydrate storage. The oospore wall probably supplies much of the carbon and energy needed for germination while the oogonium wall probably performs protective functions, e.g. resisting lysis by soil micro-organisms (Honour & Tsao, 1973).

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