THE TUCKER MEMORIAL SYMPOSIUM ON PHYTOPHTHORA

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Introductory Remarks

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Commemorating Dr. C. M. Tucker's 23 years of faithful, unselfish service to the academic life of the University of Missouri, the agriculture of that state, and the science of Plant Pathology, this symposium on the genus *Phytophthora* was convened. It was part of the ceremonies dedicating Tucker Hall, the new home of the Botany Department, of which Dr. Tucker served for 18 years as Chairman.

Development and Reproduction of Phytophthora

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In the 38 years since the publication of Tucker's monographic treatment of the genus *Phytophthora* (77), research efforts at a number of institutions have contributed to understanding the factors involved in development, reproduction, and pathogenesis of species of this genus. *Phytophthora* spp. cause devastation ranging from the familiar late blight of potato to the less well known but equally destructive black pod disease of cacao and numerous serious root rot and canker problems.

We will point out some of the major advances in the study of these pathogens, with emphasis on factors involved in the development and germination of sporangia, chlamydospores, and oospores; the role of these structures in pathogenesis; and studies of saprophytism. Where appropriate, relationships of these various contributions to control of these pathogens will also be developed.

SPORE DEVELOPMENT AND GERMINATION.—*Sporangia.*—Sporangia in the genus *Phytophthora* were defined years ago by Blackwell as organs of vegetative reproduction (10). Their capacity to germinate by two different methods has intrigued research workers since the first description of zoospore formation by DeBary in 1860 (19). Zoospore production provides an effective means of intensifying the disease-producing capacity of these fungi in a remarkably short time.

Waterhouse, in one of her early publications on *Phytophthora* (83), identified the factors affecting production of sporangia (or conidia as they were termed) as moisture, oxygen, light, temp, and nutrition.

The amount of moisture required for production of sporangia varies with the species; with some species, production is higher when mats are merely wetted with water; in others, sporangia are only formed in free water. Leonian (48) commented that the chief limiting factor was access to atmospheric oxygen. Generally, sporangia are more abundant on the surface of a liquid medium, indicating the importance of oxygen, though data are not yet available on effective concentrations of oxygen. Root infection by *P. cinnamomi* increased in well-aerated nutrient solution as compared to solutions with reduced oxygen, indicating the significance of oxygen in formation of sporangia and zoospores, since infection in that system depends upon zoospores (18). Tsao (72) showed that *P. parasitica* formed chlamydospores but no sporangia when the fungus was submerged in deep water, and commented that sporangia were not formed because of reduced aeration.

Light has effects on sporangium production varying from stimulation to inhibition. In general, however, sporangia are produced more abundantly in continuous light and in alternating light and darkness than in complete darkness (50). The contributions from West Virginia are significant here (28, 50, 56). *P. palmivora* responds to light, with 10 times the numbers of sporangia produced in light (fluorescent light, 200 ft-c) as compared to dark, in our tests. Brasier (12) and others have shown similar effects with *P. palmivora.*

Variability in response to light was also indicated by Harnish (28), who showed that at least some light was required for, or increased the formation of, sporangia by *P. cactorum, P. heveae,* and *P. himalyensis,* while total darkness favored sporangia in *P. hibernalis* and *P. syringae.* Blue light is sometimes more stimulatory than light of longer wavelengths (1, 28, 56). Relations between light and nutrients are evident; Aragaki & Hine (1) reported, for example, that light did not affect sporangium production in *P. parasitica* (we consider their isolate to be *P. palmivora*) when green papaya fruit were used as a substrate, while on vegetable juice agar, blue light was stimulatory.

Temperature is one of the limiting factors in the process of formation of sporangia as well as in their germination. Species differ widely in sporangium production in relation to temp as they do in the response

of vegetative growth to temp, which has been assigned some taxonomic significance (77, 78). Often there is a broad optimum, as with *P. cinnamomi* where the optimum temp for sporangium production, mycelial growth, and root rot development coincide. Temperature responses are useful in forecasting outbreaks of late blight and thus increasing the efficacy of fungicidal sprays. *Phytophthora* can also be eliminated from infected seeds (42) or from nursery soils by selective heat treatment.

Lillian Hawker (31) commented that "Nutrition is perhaps the most important single factor in growth and reproduction." The most significant development on production of sporangia in recent years is the discovery by Hendrix (33, 34) that sterols are required for formation of sporangia in *P. lateralis, P. capsici, P. cactorum, P. infestans, P. palmivora, P. parasitica,* and *P. parasitica* var. *nicotianae*. He commented that normal sporangia are not produced by any species in the absence of sterol. Most fungi synthesize sterols, whereas species of Pythiaceae do not. Hendrix (35) also demonstrated a relationship between light and sterol in this process; *P. palmivora* required light and cholesterol for formation of normal, typical sporangia. It did not produce sporangia in the dark without sterol; in the dark with sterol some sporangia were produced, but they resembled *P. parasitica* more than *P. palmivora*. In light without sterol, only a few abortive sporangia formed.

With some species, incubation of mycelial mats in specific salt solutions is needed for sporangium production. Wills (84) demonstrated that *P. parasitica* v. *nicotianae* formed sporangia more abundantly in soil extract, in concentrated tap water, and in a synthetic salt solution which contained carbonates of several cations than in distilled water. Kennedy & Erwin (39) reported that the production of sporangia by *P. megasperma* from alfalfa was enhanced by incubation of the fungus grown in V-8 juice agar in an inorganic nutrient solution.

A few species of *Phytophthora,* notably *P. cinnamomi* and *P. fragariae,* differ from others in requirements for production of sporangia. Hickman noted the occurrence of sporangia of *P. fragariae* on strawberry roots in wet soil (36), and described production of sporangia in nonsterile substrates such as pond water (38). The only consistent method for sporangium production in *P. cinnamomi* until recently required the use of nonsterile soil extract or other nonsterile liquid media. This method was described originally by Mehrlich (54), and we have elaborated on this (85, 89). In 1959 (89), a microbial agent or agents was implicated in the production of sporangia by *P. cinnamomi,* since sterilization of the nonsterile medium by autoclaving or filtration removed the stimulatory factor. In later research in our laboratory and elsewhere, specific soil bacteria, including species of *Chromobacterium* (87) and *Pseudomonas* (16, 52), were shown to be associated with stimulation of sporangium production by *P. cinnamomi.* The precise role of the bacteria in this process is not known.

In recent work in our laboratory, Chen developed the first method for production of sporangia by *P. cinnamomi* in axenic culture. Although Rands, in his original description of the species in 1922 (62), reported formation of sporangia by washing 8- to 10-day-old cultures in distilled water, no one since has been able to obtain similar results. Chen & Zentmyer (17) obtained abundant sporangia in sterile culture when young (16-36 hr), vigorously growing cultures grown in nutrient media (dilute pea or V-8 broth, or a sucrose-asparagine-β-sitosterol synthetic medium) were washed thoroughly in a salt solution. The sporangia were normal in that they released zoospores following a period of chilling, and infection occurred when roots were inoculated with these zoospores.

The relationship between the production of sporangia under these conditions and production in the nonsterile media has not yet been determined. Perhaps instead of supplying some essential substrate to initiate sporangium production, as has been previously postulated, bacteria in the nonsterile media may be removing nutrients, thus producing an effect similar to nutrient depletion by extensive washing. This may be another manifestation of the "Klebs principle" based on Klebs' (40) classic studies on reproduction in *Saprolegnia.*

Germination of sporangia.—Sussman & Halvorson (70) define germination as a process that leads to the first irreversible stage recognizably different from the dormant organism, as judged by physiological and morphological criteria. The bimodal germination capacity of members of this genus provides adaptability to changing environmental conditions. The principal environmental factors involved in this process are moisture, temp, and nutrition, with indirect germination by zoospores only occurring in the presence of free water. In 1911, Melhus (55) first demonstrated that a lowering of temp stimulated zoospore formation; most of his studies involved *Albugo* (*Cystopus*), but he also made observations with *P. infestans*. Fawcett & Klotz (25) in 1934 showed that a reduction in temp stimulated zoospore formation in *Phytophthora*. Subsequent work indicates that a reduction of temp for 15 to 90 min, followed by a return to the original temp, results in the most effective production of zoospores. Without chilling, direct germination commonly occurs. Brief exposures to high temp (40 C) accelerate direct germination in *P. infestans* (71).

Uppal (80) showed that low oxygen concentration stimulated indirect germination of sporangia. Vujičić & Colhoun (81) recently reported that high O_2 concentration inhibited indirect germination of sporangia of *P. erythroseptica,* but stimulated direct germination. Age of sporangia is also related to method of germination, with young sporangia commonly producing zoospores more readily than old sporangia. Nutritional influences have been noted also, with sterols required for normal production of zoospores by sporangia. Several authors (2, 29) report a somewhat anomalous situation in that direct germination is stimulated by plant extracts or plant parts.

Chlamydospores.—The chlamydospore as it occurs in the genus *Phytophthora* is somewhat different from the chlamydospore in the Ascomycetes and Fungi Im-

perfecti. In *Phytophthora* it is terminal or intercalary, usually globose, and not necessarily thick-walled; it germinates in media typically by many germ tubes or in some circumstances by producing a sporangium. Little attention was paid to this spore form in *Phytophthora* until recent years.

In *P. cinnamomi,* the chlamydospore is a common and important spore which germinates primarily by forming many germ tubes. Chlamydospores are formed in abundance in rich nutrient media such as V-8 or potato-dextrose broth. Hendrix (34) showed that *P. cinnamomi* and *P. lateralis* formed chlamydospores on synthetic media in the presence but not in the absence of cholesterol. Little has been published on light effects. In our studies with *P. cinnamomi,* light does not appear to influence the production of chlamydospores; however, numbers of chlamydospores of *P. palmivora* are approximately 4 times greater in light than in continuous darkness.

Tsao (72) reported that formation of chlamydospores by *P. parasitica* was best at relatively low temp (15-18 C), whereas the optimum temp for mycelial growth of this fungus is considerably higher.

Germination of chlamydospores of *P. cinnamomi* in vitro depends on an exogenous source of organic N; many amino acids and exudates from avocado roots stimulate germination (59). Sugars were not effective in stimulating germination in vitro. Tsao & Bricker (74) reported similar results with *P. parasitica* in vitro. In soil, however, sugars, amino acids, and exudates from avocado roots provided the stimulus for germination of chlamydospores of *P. cinnamomi,* indicating that there may be an interaction with the microbial population of the soil (58). Germination is commonly by multiple germ tubes; however, with low nutrient concentration, some sporangia were formed (59).

Chlamydospores are formed by *P. cinnamomi* in host roots and in soil (32, 44, 57). McCain et al. (53), by use of sieves, found these spores in pineapple soils infested with *P. cinnamomi.*

The chlamydospore is a primary unit of survival for *Phytophthora* spp. in soil, and with the heterothallic species may play a more important role than oospores. Chlamydospores have been recognized in a number of species, including *P. arecae, P. boehmeriae, P. cactorum, P. cinnamomi, P. citrophthora, P. colocasiae, P. lateralis, P. nicotianae* var. *nicotianae, P. nicotianae* var. *parasitica* (*P. parasitica*), and *P. palmivora.*

In the control of *Phytophthora*-induced diseases, we must therefore consider not only factors affecting sporangia, zoospores, and oospores, but also chlamydospores.

Oospores.—What physiological requirements are known for production and germination of oospores? Oospores have been produced on a variety of natural media such as pea broth, V-8 juice agar, and oatmeal agar, but prior to 1963 were not produced on synthetic media. Leonian & Lilly's early work (49) on oospore production by *P. cactorum* in an extract of garden peas is significant. It was not until 1963 and 1964 that scientists from three laboratories reported that certain sterols such as cholesterol, stigmasterol, and β-sitosterol were necessary for formation of oospores in *Pythium* and *Phytophthora* (23, 30, 33). The research by Klemmer & Lenney (41) on stimulation of sexual reproduction in *Pythium, Phytophthora,* and *Pythiogeton* by lipids is also of interest.

The role of sterols on oospore production has been reviewed by Lilly (50), and need not be repeated. The actual role of different sterols in the development process is not known, though the effect may be on the cell membrane (3). Schlosser & Gottlieb (67) reported that mycelium of *Pythium* spp., when grown in the absence of sterols, did not contain sterols but was capable of incorporating exogenous sterol. When the mycelium contained sterol, it became sensitive to the polyene antibiotic filipin, and since they detected leakage of phosphates through filipin-treated mycelium, they postulated that sterols had been incorporated in the cell membrane. Elliott (22) recently reported that cholestanol competed with cholesterol for possible sites of action in *P. cactorum,* commenting that both substances promote vegetative growth and oogonium development, but differ in their ability to promote oospore development. Only cholesterol stimulated oospore development.

Oospores eventually germinate, but the percentage that germinate at any one time is usually low and variable. The effect of sterols on germination of oospores is poorly understood. Romero & Erwin (63, 64) showed that addition of β-sitosterol to cleared V-8 juice agar not only increased the number of oospores of *P. infestans* but also the percentage of germination from 20 to 34%. Oospores of homothallic species such as *P. cactorum* (6), *P. erythroseptica* (46), and *P. megasperma* var. *sojae* (24), however, usually germinate more quickly and in greater abundance than those of heterothallic species.

The reason for dormancy of oospores has not yet been discovered, but as early as 1934, Blackwell (8) postulated that dormancy was associated with the nature of the oospore wall, stating that (i) it possesses a thick inner wall composed possibly of hemicellulose and a thinner outer wall composed of "fungus cellulose" which is practically impermeable; (ii) the substances in both layers are in a colloidal state; (iii) germination cannot take place until the spore has matured and the wall is rendered permeable to oxygen and water; (iv) external agents are effective in inducing germination either from small cracks in the wall or bringing about a change in the colloidal state of the wall; and (v) the change in the cell wall in relation to germination comes from the inside.

Sussman (69) used Blackwell's paper (9) on the germination of oospores of *P. cactorum* as an example in explaining different stages in the dormant oospore. He distinguished constitutive dormancy, "a condition wherein development is delayed due to an innate property of the dormant stage such as a barrier to the penetration of nutrients, a metabolic block or the production of a self-inhibitor" from exogenous dormancy, "a condition wherein development is delayed because of unfavorable chemical or physical conditions of the environment". Dormancy in *Phytophthora*

oospores is probably constitutive. However, until more is known about the effect of physical and chemical factors, there is no assurance that exogenous factors are not also important.

In Fig. 1 we show the developmental sequence in the germination process of oospores from a cross between the A^1 and A^2 isolates of *Phytophthora drechsleri.* Germination of oospores from these isolates was first reported by Galindo & Zentmyer (26) in 1967. These isolates were crossed on V-8 juice agar, incubated in dark for 10-15 days, and exposed to light in the laboratory 2 hr/day for 7 days. Oospores were then incubated in distilled water for 2-6 days. The average percentage of germination varied from 20-60% in different experiments.

In Fig. 1-A the oospore has differentiated within the oogonium after fertilization and become shrunken in size or aplerotic. The cytoplasm contains numerous spherical bodies. The thin inner oospore wall is plastic and follows the shape of the spherical bodies in the cytoplasm. In Fig. 1-B, the thickness of the oospore wall has increased. In the center of the cytoplasm, a large spherical body with sharply defined edges has formed. Later (Fig. 1-C) in the fully dormant oospores, the texture of the cytoplasm becomes finer and the central globule becomes larger. At this stage, the spore is dormant. The incubation period may vary from several days to several months at this stage.

Exposure to fluorescent light at this stage is necessary for germination (6, 7, 65). Blackwell also advocated lowering the temp to about 1 C to break dormancy of *P. cactorum* oospores (9). Our work to date with *P. drechsleri* and *P. megasperma* var. *sojae* indicates that lowering the temperature to 1 C does not increase the percentage of germination.

In Fig. 1-D, the oospore is in the active pregermination stage about 2-5 days prior to germination. The central globule has begun to disintegrate. The cytoplasm is no longer homogeneous and has lost the fine texture. The gradual pitting and erosion of the inner oospore wall is a definitive indication of impending germination.

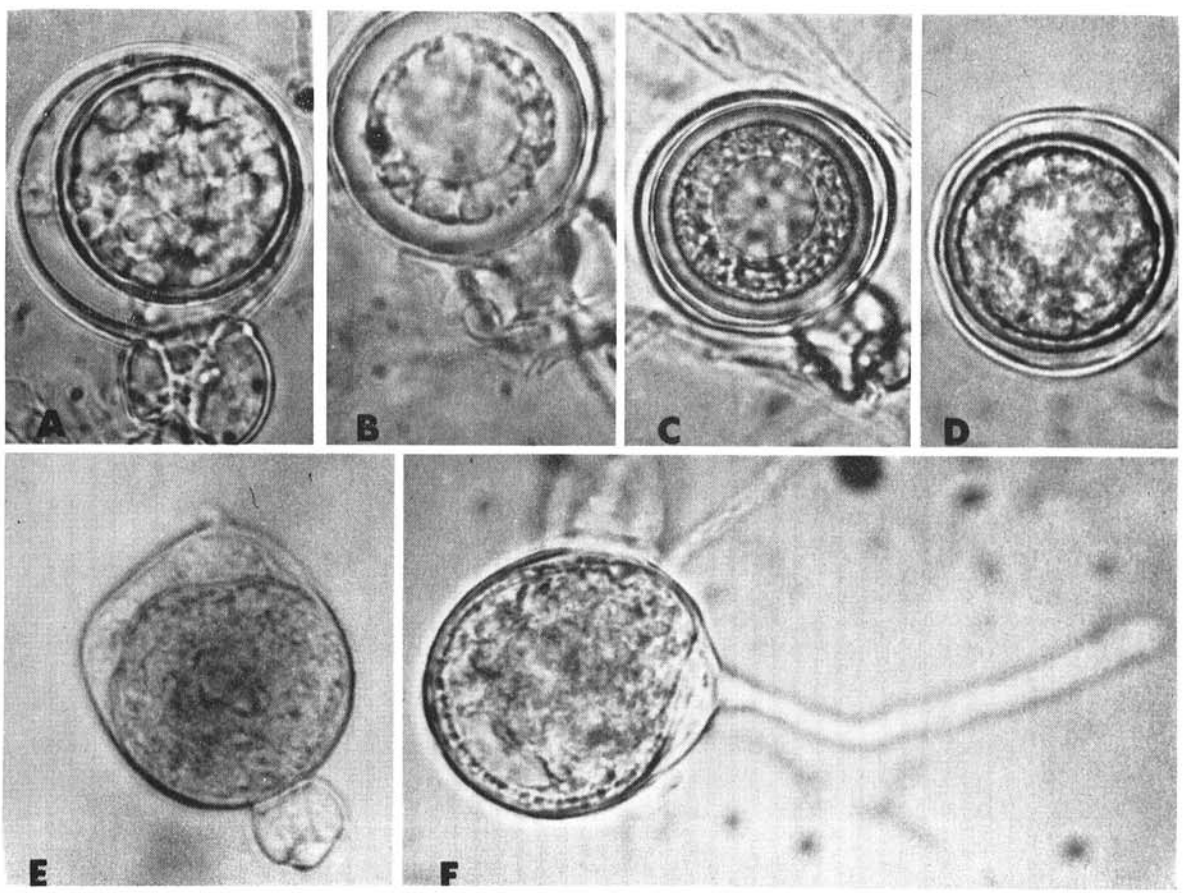

Fig. 1. Photomicrographs of several oospores of *Phytophthora drechsleri,* P208 (A^1) × P209 (A^2), in different stages of development prior to germination. **A)** Postfertilization stage. The oospore has differentiated and become shrunken within the oogonium; the cytoplasm is made up of large spherical bodies; the antheridium is empty. **B)** The oospore wall has become thicker and fully differentiated. A large spherical body has appeared in the center. **C)** Dormant stage. The spherical body persists but the texture of the cytoplasm has become finer. **D)** Pregermination stage. The texture of the cytoplasm has become coarser, suggesting a change in physiology. The inner oospore wall has begun to erode. **E)** Germination. The oospore has swelled to fill the oogonium, and the germ tube has passed from the oospore through the oogonial wall. **F)** The germ tube has extended to several times the diam of the oospore.

The oospore begins to swell, suggesting inbibition of water. When germination has begun (Fig. 1-E), the oospore has nearly filled the oogonial cavity and the germ tube emerges from the oospore and presses against the oogonial wall. In the last photograph of this sequence (Fig. 1-F), the germ tube has emerged and shortly thereafter will either terminate in a sporangium (64) or become a mycelial thallus.

The role of light in the germination process has been studied extensively by the West Virginia group (6, 7, 56, 65). Surprisingly, continuous light in the developmental stage suppressed oospore production (65). After the oospores formed, however, exposure to light from fluorescent lamps (more precisely light in the blue spectrum) was required for germination. Romero & Gallegly (65) reported that oogonium germination of *P. infestans* (27, 63) (now accepted by both authors as probably true oospore germination) was stimulated by light. Romero and Erwin (64) observed that 15% of the oospores of *P. infestans* germinated following exposure to light from fluorescent lamps for 2 hr a day for 7 days, as compared to no germination of oospores matured in the dark.

Brasier (12, 13) recently reported that heterothallic isolates of *P. palmivora* formed oospores preferentially at 20 C, while the optimum temp for growth was 25 C. In detached leaves of *Piper nigrum*, oogonial formation was inhibited by light. He postulated that these two limiting factors may explain why oospores of this fungus are seldom found in nature.

The genetic proneness of oospores from certain crosses to germinate is also an important factor. Forty-eight per cent of the oospores from two different crosses of *P. infestans* 473 (A^1) × 445 (A^2) and 473 (A^1) × 477 (A^2) germinated, compared to only 6% germination from the cross 63B (A^1) × 60A (A^2) (63, 64).

If dormancy of oospores has survival value for the fungus in the absence of a susceptible host, it would seem that dormancy might have been an evolutionary trend explaining why progenies of some crosses germinate well and others do not. Thus, for genetic studies of *Phytophthora* it may be important to catalog isolates that produce the highest number of readily germinable spores. For ecological studies, dormancy must be considered to be an important factor favoring the perpetuation of *Phytophthora* species.

Since genetics is covered by Gallegly in this Symposium, we will dwell only on the pathological implications of the sexual stage. From the crosses of 471 (A^1) (race 1,2,3,4) × 445 (A^2) (race 0) of *P. infestans*, Romero & Erwin (63, 64) reported recovery in the A^1 mating type of the following races: 0; 1; 2; 3; 1,3; 1,4; 3,4; 2,3,4; 1,2,4; and 1,2,3,4, and in the A^2 mating type of 0; 1; and 1,4. These data indicated that recombination had occurred. Thus, the sexual mechanism of transmitting variation could be responsible for the rearrangement of genes for pathogenicity. Cultural variation of progeny was also common in progeny of this cross. Recombination in progeny of the same isolates was confirmed by Laviola (27).

Production of auxotrophic mutants for methionine and tryptophan from the ultraviolet irradiated zoospores of *Phytophthora* was reported by Castro et al. (14). Germination of oospores from crosses of these isolates indicated recombination of factors governing requirement for these amino acids. The fact that auxotrophic mutants were obtained in this heterothallic species makes it doubtful that the zoospores could be diploid.

Despite the proof of cultural and pathogenic variation in progeny of a cross of *P. infestans*, the report of Castro & Zentmyer (15) indicates that germinated oospores may not always be viable. In this study of oospores from crosses of A^1 and A^2 clones of F_1 individuals from Romero's 473 × 445 cross, only four of 620 germinated oospores survived and these were abnormal in production of sporangia. A high percentage of bursting of hyphae was associated with an apparent inherent weakness in the hyphal membrane. Based on this work, it was proposed that the criteria necessary for determining "compatibility" of A^1 and A^2 isolates be extended to include establishment of a colony by germinated oospore progeny.

Interspecific crosses of heterothallic species have resulted in production of oospores. Savage et al. (66) reported that *P. infestans* had the potential to produce oospores when A^1 or A^2 types were crossed with nearly all of the heterothallic species. Barrett (4, 5) was probably the first to report oospores from a cross of *P. drechsleri* and *P. infestans*, and even reported that some of the oospores germinated. Unfortunately, no subsequent report followed his brief abstracts.

We recently reported (88) that A^1 and A^2 cultures of *P. cinnamomi*, *P. parasitica*, *P. palmivora*, and *P. drechsleri* intercrossed and produced oospores. However, germination occurred only in the *P. drechsleri* A^1 and A^2 cross.

These failures to obtain germination of interspecific crosses are negative evidence and cannot be taken as conclusive that interspecific hybridization is not possible. It is an intriguing basic as well as extremely practical possibility that the sexual stage could be the source of recombinants possessing new and different host ranges and perhaps even physiological characteristics such as temp tolerance.

Why continue to study the germination of oospores? Aside from the intellectual challenge to understand the physiological processes governing germination, the study is practical in that the fullest range of variability of which a species is capable can be studied. This is important for the study of variation in pathogenicity and virulence as well as for the study of taxonomy. Snyder & Hansen (68) made a strong plea for a full understanding of the morphologica variability within a species before taxonomic conclusions can be made. Also, for control of diseases caused by *Phytophthora* it is important to understand the significance and the capabilities of oospores in perpetuating the pathogen.

SAPROPHYTISM.—Species of *Phytophthora* have not been generally considered as effective saprophytic competitors in soil; most of the research reported on saprophytic behavior bears this out, with notable exceptions including our studies with *P. cinnamomi* (90). A report in 1941 by Blackwell et al. (11) indicated the

possibility of *Phytophthora* spp. existing as water molds; they reported an isolate obtained from a pond by means of apple fruit to be *Phytophthora megasperma*. To our knowledge, this type of occurrence has not been studied further. Klotz et al. (43) reported occurrence of species of *Phytophthora* in irrigation ditches receiving water from infested groves. This situation is somewhat different from the occurrence in ponds.

DeBruyn's studies (20) have been cited as providing an example of saprophytic existence of several species of *Phytophthora* in soil (*P. syringae, P. erythroseptica,* and *P. infestans*). These studies, however, were all conducted in sterile soil, and the broad extrapolation that these fungi can survive similarly in natural soil is unjustified.

Legge (47) in 1953 demonstrated the occurrence and survival of several species of *Phytophthora* in soil in the form of oospores and sporangia, with viability of oospores up to 1 year and survival of the pathogen in the absence of a host for 9 months. She proposed that survival by means of oospores seemed the most likely means. Hickman (37) reported that *P. fragariae* survived in nonsterile soil for over 1½ years.

Our studies (90) with *P. cinnamomi* showed long persistence (up to 6 years) of the pathogen in the absence of a host when naturally-infested soil was stored in moist condition at 20 C, but survival for only a few weeks when the soil was permitted to dry to 2-3% moisture content. Survival for the 6-year period was at least to some extent in remnants of small roots, probably as chlamydospores. We also found that the fungus had some competitive saprophytic ability, invading wheat straw and dead avocado roots in nonsterile as well as sterile soil, particularly when the water content was maintained at near saturation. In our tests, *P. cinnamomi* grew up to 3 cm into nonsterile soil from a food base.

In contrast to our results with *P. cinnamomi,* several authors have reported poor or no invasion of organic matter and little or no movement of mycelium through nonsterile soil. Kuhlman (44) reported poor invasion of Douglas fir twigs by *P. cinnamomi* in nonsterile soil, and no spread through nonsterile soil. Lacey (45) noted that *P. infestans* mycelium grew only in sterilized soils, and commented that it is highly improbable that this pathogen can survive overwinter as a saprophyte because of the competition from other microorganisms. Vujičić & Park (82) found that *P. erythroseptica* made very limited growth (6 mm) through nonsterile soil from a food base, and that it did not colonize dead plant material in the soil; they reported oospores and sporangia formed in soil. Tsao (73) in his studies of *P. parasitica,* has found that this fungus does not grow in soil because of the general lack of available food sources; he obtained no growth from fiberglass squares containing mycelium when these were placed in natural soil, and found that the mycelium soon formed resistant reproductive structures or lysed. The antibiotic media (3-P, PV, and more recently, P_{10} VP) developed by Eckert & Tsao (21), Tsao & Menyonga (75), and Tsao & Ocana (76) provide a more effective technique than has been available in the past for studying occurrence and survival of species of *Phytophthora* in soil.

Turner (79) found that *P. palmivora* made limited growth from a food base into either sterile or nonsterile soil, but, as in our results with *P. cinnamomi, P. palmivora* competed successfully with other soil flora in colonization of pieces of unripe and mature cacao pods.

Members of this genus can survive for many years, either in decaying infected tissue or in the form of resistant structures in the soil. A number of workers have reported formation of chlamydospores and/or oospores in soil, with the following species: *P. cinnamomi* (44, 53, 58); *P. erythroseptica* (82); *P. parasitica* (72); *P. cactorum* (47); *P. cryptogea* (47); *P. porri* (47); and *P. megasperma* (47). Studies of Ocana & Tsao (60, 61) and Tsao & Ocana (76) on isolation of species of *Phytophthora* from soil indicate survival by means of nonmycelial propagules, as higher dosages of pimaricin (100 ppm) inhibit recovery of these fungi from soil, while lower dosages (10 ppm or less), which permit germination of chlamydospores and other spores, permit isolation of *Phytophthora* from field soil.

The matter of competitive saprophytic ability is less certain. Our studies of *P. cinnamomi* indicate the potential, especially under high soil moisture conditions that favor development of *Phytophthora*. Infected roots can provide a food base from which *Phytophthora* can develop; it would seem that studies which stress the fact that no growth occurs unless a food base is present are somewhat unrealistic. Mycelium can spread from diseased roots to some extent. Saprophytic activity of this type can thus increase the pathogenic capacity of a fungus such as *P. cinnamomi*. Mycelial invasion of nonwounded roots takes place, with invasion of avocado roots occurring in the region of elongation in a similar manner to the attack by zoospores (86). Marx has recently shown that mycelium of this fungus has the capacity for invasion of pine roots (51).

This concludes our presentation of recent and current information on development and reproduction in species of *Phytophthora*. Considerable progress has been made by many research workers in studying this group of fungi, but much remains to be learned.

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