

CHAPTER 19: The Physiology of Reproduction: Asexual

As the pioneering research cited in the previous chapter demonstrates, the kinds and concentrations of nutrients affect the development of the asexual apparatus of water molds. The beginning efforts to study the relationship between nutrition and morphogenesis, not only of the sporangia but of the sexual apparatus as well, were severely hampered by lack of attention to precise chemical definition of the media in which the species were grown and tested. With time, experimental and observational work on the physiological aspects of asexual reproduction became more refined. The explanations for some morphogenetic events in the life of water molds, however, are still elusive.

THE SPORANGIUM

NUTRITION AND CHEMICAL CONSTITUENTS OF THE MEDIA

Most of the experimental efforts with various media constituents have centered on the influence of the particular compounds on vegetative growth by members of the Saprolegniaceae (Chapter 7). Yang and Schoulties (1972), for example, only incidentally mentioned that isolates growing on a chemically defined medium produced many sporangia and formed correspondingly few oogonia. Working with a synthetic basal medium Whiffen (1945) demonstrated that various concentrations of peptone and glucose affected sporangium production (Table 34). Two conclusions seem warranted from her data. First, peptone could serve as the sole carbon source in supporting asexually reproducing mycelium of most test species. Second, water molds responded in accord with the Klebsian hypothesis that high concentrations of nutrients are unfavorable for asexual reproduction.

A few studies have explored the effect of specific carbohydrates, mineral and metallic salts, and particular ions on asexual reproduction in the water molds. Lounsbury (1930) tested 25 inorganic salts in 0.02 and 0.01 molar concentrations (as M/50 and M/100, respectively) for their effect on asexual development in *Protoachlya paradoxa*. Some compounds were lethal, but of those that were not, chemicals such as sodium nitrate, sodium nitrite, disodium phosphate, magnesium chloride, and magnesium nitrate inhibited sporogenesis. Sporangia developed in the presence of potassium chloride, magnesium sulfate, monosodium phosphate, and potassium nitrate, among other salts. Spore discharge by *P. paradoxa*, however, was prevented by 0.02 M concentrations of all inorganic compounds tested except KH_2PO_4 . A few years earlier, Lenz (1924) had studied some of these same compounds as agents causing plasmolysis in young sporangia of a nonsexual *Saprolegnia*. He reported that differentiation of cellular units could sometimes occur in plasmolyzed sporangia, but he offered no supportive illustrations. Very extensive data on sporulation, chiefly by *Achlya colorata*, were published by Moreau and Moreau in 1938. Certain general features of their data emerge. Mycelium in solutions of glycerin in concentrations of

three or four percent was deficient in sporangia. Polysaccharides were more “toxic” to sporangium development than were monosaccharides, and ions in low concentrations influenced sporangium formation in various ways. For example, as the concentration of particular compounds in the medium was increased, the immediate effect was to repress sporangium production by *A. colorata*. Kotova’s (1971) work on several nitrogen and phosphorus sources for growth and reproduction by *Aphanomyces euteiches* confirmed most of the conclusions reached by the Moreaus.

The role of ions in morphogenesis of *Aphanomyces euteiches* was investigated in a meaningful way by J. E. Mitchell and Yang (1966). They determined that Ca^{2+} was essential for primary spore differentiation, and the highest degree of sporulation occurred in a basal medium with calcium supplemented by K^{+} or Mg^{2+} . Sporangia on mycelium of *A. euteiches* in 0.05 M CaCl_2 produced spores, but these did not discharge. It is important to recognize that, on the contrary, only low concentrations of potassium, calcium, and magnesium in the growth medium (Unestam, 1968b) favored spore formation in *A. astaci*. Thioglycollic acid elicited sporogenetic responses of *A. euteiches* similar to those induced by calcium, and although most metallic ions inhibited sporogenesis in this species, zinc was an exception. Spore development by *A. euteiches* was enhanced in solutions of zinc at 10^{-6} or 10^{-7} M. The study by J. A. Lewis (1973b), however, was contradictory. He found zinc to be toxic in low concentrations, as were copper ($5 \mu\text{g mL}^{-1}$ prevented sporogenesis) and calcium in high concentrations. The effect of washing mycelium of *A. euteiches* to induce spore formation was also explored by J. E. Mitchell and Yang; this aspect is treated in a later section.

Sporulation by *Aphanomyces euteiches* is influenced (Hoch, 1972) by glucose and mannitol uptake, and Schäufole and Beiss (1973) demonstrated that increased spore production by cultures of *A. cochlioides* was concomitant with enhanced levels of nitrogen in the medium. Confirming evidence for the stimulatory effect of nitrogen on spore production appears in the study by Humaydan and Williams (1978) on *A. raphani*. They observed that although 0.01% peptone in the medium did not support sporangium development, a level of 0.5% favored hyphal growth with a maximum of spore development.

Members of the Saprolegniaceae are not usually thought of as inhabitants of saline water (see review by Duniway, 1979), but some have been collected (TeStrake, 1959; Padgett, 1978a) in estuarine waters. For these “freshwater” fungi to maintain themselves in saline waters, they must at some time reproduce successfully. It is J. L. Harrison and Jones (1971, 1975) who have done most to explore the salinity tolerance of water molds *in vitro*. In freshwater and in seawater solution of 10% (*i.e.*, $V_{\text{seawater}}/V_{\text{total}} \times 100$), at 10, 15, and 20 °C, asexual development in *Saprolegnia parasitica* (= *diclina*) was normal. Although sporogenesis took place in mycelium immersed in 20% (7 ppt) seawater at 15 ° and 20 °C, the filaments were abnormal in several ways, and the sporangia on hyphae at 10 °C incubation did not form spores. At all incubation temperatures, in solutions of 30% and 40% seawater, the mycelium of *S. parasitica* produced sporangia, but there was no sporogenesis. Culture water having more than 40% seawater suppressed asexual reproduction.

A much more inclusive set of experiments on the effect of salinity on asexual development by saprolegniaceous fungi was reported by J. L. Harrison and Jones in 1975. They extended their earlier (1971) study to include observations on 17 species of water molds. Sixteen of these generated sporangia (though spore release was not typical in all cases) in 10% seawater. The single exception was *Achlya klebsiana* (= *debaryana*) which reproduced only in freshwater. The same sixteen species [members of *Achlya*, *Isoachlya* (= *Saprolegnia*), *Protoachlya*, *Saprolegnia*, and *Thraustotheca*] produced some nonfunctional sporangia on mycelium grown in 20% seawater, but in the isolates propagated in 40% seawater, only those of *I. intermedia* (= *S. intermedia*) and *S. parasitica* showed any evidence of sporangium delimitation. In none of the species was asexual reproduction normal in solutions equivalent to 7.0% NaCl.

Conflicting results on the effect of salinity on sporulation in some water molds appear in the accounts by Höhnk (1939) and TeStrake (1959). In a *Saprolegnia* isolated from soil, sporulation was normal when the fungus was grown in 0.11 ppt salt water (Höhnk, 1939). In water at a salinity of 7.09 ppt, sporangia were abnormal and did not sporulate, and mycelium held at 13.85 ppt (pH 7.2-8.0) failed to produce any sporangia. TeStrake (1959), however, found that sporulation in three other species of Saprolegniaceae was regulated both by temperature and salinity. The water molds she studied sporulated in water at 22.5 ppt salinity at 30 °C, at 10.7 ppt when the incubation temperature was 35 °C, but formed spores only in water at salinities of 3.6 and 0.3 ppt, at temperatures of 20 ° or 25 °C and 15 °C, respectively. TeStrake proved that the planonts were viable (germination in freshwater) after exposure to salinities as high as 29.8 ppt.

Data from an unpublished thesis by Wu (1979) indicate that at least in her isolate of *Saprolegnia diclina* any measurable salinity in the surrounding culture water created less than optimum conditions for sporangium formation and sporogenesis. The maximum salinity at which functional planonts of this species were produced was 2.2 ppt at 25 °C, and 6 ppt at 30 °C. Sporangia were produced by *S. diclina* in water at salinities of 2.2-30.4 ppt -- at incubation temperatures of 5-20 °C -- but none cleaved spores under these conditions.

Using various osmotica Herr (1971) discovered that there was a lower respiration rate -- measured by oxygen uptake -- by the mycelium of *Aphanomyces euteiches* when sporulation was induced than when the hyphae lacked sporangia. Herr also found experimental data to support the view that the range of water potentials in which sporulation could occur (see also Chapter 7) was related to the nature of the potential. Spore production by *A. euteiches* was more limited when mycelium was exposed to solutes (ψ_{π}) than to the matric water potential (ψ_m) resulting from imbibitional forces of cell colloids and cell wall capillary forces.

By means of a replacement culture technique, Griffin (1966) discovered that the calcium ion was essential for sporogenesis (see Chapter 7 for an account of the morphological events of the process) in a dioecious *Achlya* (a E strain of *A. bisexualis*). The optimum concentration of calcium chloride for sporangium differentiation was 0.1 mM, and salts of magnesium, sodium, or potassium could not be substituted for the

Ca²⁺. In concentrations as low as 0.001 mM, EDTA prevented sporangium induction (Griffin, 1966). Data recorded by Suzuki (1961g) on the effect of minerals (including calcium) on sporulation by two water molds simply show that both species were equally well adapted to media with a high mineral content. Subsequent studies based on calcium-stimulated asexual cell development were to explore more fully the physiological aspects of sporogenesis.

Applying actinomycin D to CaCl₂-induced sporangia of *Achlya* sp. (mating strain of *A. bisexualis*) Griffin and Breuker (1969) determined that DNA-dependent RNA synthesis was necessary if sporangium differentiation was to occur in the hyphal tip. Ribosomal RNA synthesis continued throughout the differentiation process until spore release, but during the same period there was no detectable degradation of protein or nucleic acids. By measuring such factors as total nucleic acid and protein content of the mycelium, rates of amino acid uptake and incorporation, and protein turnover, Timberlake and his associates (1973) demonstrated that *A. bisexualis* actively synthesized protein during sporangium differentiation at a rate greater than that at sporulation. The amino acids required for synthesis came from the degradation and reutilization of preexisting protein. Protease activity rates paralleled those of protein degradation and synthesis, and cycloheximide (capable of blocking protein synthesis) could prevent any of the events in the differentiation process from occurring.

Calcium induction of sporangia also has been used to investigate the activities of acid phosphatase, α -mannosidase, and ribonucleases associated with sporangium differentiation in *Achlya bisexualis*. O'Day and Horgen (1974) disrupted hyphae of this species, obtained microsomal and lysosomal fractions, and then assayed by gel electrophoresis cell extracts for acid phosphatase activity. During spore development by *A. bisexualis* there was a continual rise in the activity of this enzyme. The highest rates of activity were measured at 4-5 hours after calcium induction, a time corresponding to the period of septum formation and spore cleavage. Acid phosphatase, O'Day and Horgen found, also accumulated extracellularly during sporangium development. Both actinomycin D and cycloheximide prevented any increase in acid phosphatase activity. Timberlake *et al.* (1973) had demonstrated that protein synthesis takes place during sporangium differentiation; the data from the study by O'Day and Horgen suggest that acid phosphatase arises during these events.

There is little α -mannosidase activity detectable in the vegetative mycelium of *Achlya bisexualis*, but as sporangium differentiation is initiated (following calcium induction) and the events leading to spore release proceed, the action of this enzyme steadily increases (Horgen and O'Day, 1975). Mannosidase reaches a peak of activity at the time of septum formation, then declines. A chemical that inhibits RNA -- actinomycin D -- effectively blocks α -mannosidase activity at any time during the sequence of events in differentiation up to about 4-5 hours after induction (accumulation of α -mannosidase during differentiation requires RNA synthesis). Cycloheximide also obstructs the increase in activity of this enzyme suggesting (Horgen and O'Day, 1975) that protein synthesis is necessary for α -mannosidase production.

Ribonuclease activity is also evident during differentiation in calcium-induced sporangia of *Achlya bisexualis*. Sutherland *et al.* (1976) examined the effect of Actinomycin D and cycloheximide on RNase activity during the six-hour period that coincided with sequential events (Fig. 12) in sporangium development (measurements of poly-A and pulse-labeled RNA). Prior to the onset of sporangium differentiation in *A. bisexualis*, RNase activity was high, but the level dropped about 44% at the beginning of morphogenesis. The level of this enzyme was lowest at the time of septum formation, then steadily increased during sporogenesis, up to the moments preceding spore release. Both actinomycin D and cycloheximide obstruct changes in activity of RNase, an essential link in the degradation, turnover, maturation, and processing of cellular RNA (Sutherland *et al.*, 1976).

LéJohn and associates (1978) have shown that there is an increase in dinucleoside polyphosphates (the HS compounds reported by LéJohn *et al.*, in 1975) in mycelium of *Achlya* sp. at sporulation. Nucleosides and *l*-glutamine reduce the cellular levels of HS, and sporulation is prevented. The dinucleoside polyphosphates possibly act as sensors of a nitrogen deficiency. Evidently all three HS compounds must increase simultaneously for sporulation to occur in *Achlya* sp. since selectively blocking the individual HS fractions inhibits sporulation. Proof is still lacking (LéJohn *et al.*, 1978), however, that these polyphosphates have a regulatory function, although they may serve as pleiotypic regulators (LéJohn *et al.*, 1979).

The first investigators to record any substantial data on lipid metabolism in water molds in relation to asexual development were S. W. T. Law and Burton (1976a, b). They considered that the asexual life cycle of *Achlya* sp. occupied a time span of 27-30 hours, beginning with spore germination, followed by development of a mycelium, and ending with the release of spores from newly formed sporangia. Their studies took into account changes in the content, turnover, metabolism, and composition of lipids during morphogenesis (the activity of lipids in germinating spores of this *Achlya* is considered in a later section). At the onset of differentiation in the mycelium¹ lipid synthesis increased (incorporation of labeled Na acetate), and during the genesis of sporangia the capacity of the fungus to incorporate the labeled acetate into lipid rose about threefold. Lipid synthesis, then, appears to proceed rapidly during this phase of morphogenesis. In the course of sporulation by the fungus, fatty acid synthetase activity and fatty acid oxidation levels peaked, and concurrently, lipid synthesis was at its maximum.

The main feature of lipid metabolism in *Achlya* sp. during asexual morphogenesis is the change that takes place in the content and amount of that portion of the total lipids representing neutral fractions. The spores have about 62% neutral lipid; the amount falls sharply to 8% during thallus growth, then rises again to 55% at

¹ In their papers Law and Burton refer to "sporulating cells" as opposed to "vegetative cells." Since they used a coenocytic organism, the former designation means that hyphae macerated to make "cell-free" extracts were ones bearing sporangia (S. W. T. Law, communication).

the onset of sporulation. The chief components of the neutral lipid fraction are triglycerides (20%) and free fatty acids (50%). During sporulation by *Achlya* sp. the level of the latter fraction increases (over that in the undifferentiated mycelium), and presumably these compounds are incorporated into glycerides. Fatty acids present in sporulating hyphae, but not in vegetative ones, are hydroxyhexadecanoic and eicosatetraenoic acids (the latter in a very high proportion) and an unidentified long-chain acid fraction (S. W. T. Law and Burton, 1976b).

Studies on the biochemistry of asexual reproduction in some water molds argue that sporangium induction and the events leading to sporulation are accompanied by very specific and subtle but critical chemical changes. These are, in sum, RNA and protein synthesis, increase in acid phosphatase and α -mannosidase (hydrolytic enzyme) activity, and a decrease and rise in the activity of ribonuclease. A prominent but unexplained event in sporogenesis is the dedifferentiation phase (Chapter 7). Possibly the various biochemical activities accompanying asexual development proceed through and include the homogeneous phase, hence none serves to single out this particular morphogenetic change.

NONNUTRIENT FACTORS IN ASEXUAL REPRODUCTION

Although the majority of those investigating saprolegniaceous fungi report the conditions of culture incubation, accounts that deal specifically and precisely with the influence of factors other than those of the substrate are scarce. The existing reports are nearly mute, in fact, in attempting to explain the physiology of sporangium morphogenesis.

Temperature: -- In spite of his very extensive physiological work on members of the family Pieters (1915a) merely reported that high temperatures hinder sporangium formation (in *Achlya racemosa*). Reference to a few selected published reports indicates something of the temperature ranges within which the asexual stage of water molds develops.

In some specimens of *Saprolegnia parasitica* (Lee, 1962) there are no sporangia produced at 0-1 °C, and those developed at 30 °C and above are atypical. Most reports of this species indicate that the optimum temperature for sporangium production is in the range of 20-28 °C (Neish, 1975a, for example), but W. N. Tiffney (1936) reported optimum sporulation by his isolates when they were grown at 15 °C. Hoshina and his associates (1960) found spore production by *S. parasitica* from infected eels to occur in water temperatures of 4.1-17.9 °C. According to Chong (1973), an isolate of *S. diclina* (from fish) produced sporangia optimally in the range of 7-30 °C. A temperature of 20-28 °C seems to be optimum (Llanos and Lockwood, 1960) for sporangium formation by *Aphanomyces euteiches*. In our experience, an incubation temperature between 18° and 22 °C is most suitable for the development of sporangia in the majority of species of Saprolegniaceae, but there are exceptions.

Two investigators, chiefly, have given attention to temperature effects on sporangium morphology. Within the range of 10-25 °C, temperature did not influence sporangium morphology in an unnamed species of *Brevilegnia*, according to Salvin (1942a). At 35 °C, however, the asexual cells were often bent or branched. Mycelium incubated above 25 °C tended to produce sporangia with decreased diameters (in contrast to those on colonies held at lower temperatures). Sporangia of *Thraustotheca clavata* and *Dictyuchus* sp., Salvin found, formed apical discharge papillae at 35 °C; species in these genera do not normally develop such an exit apparatus. Alabi (1967, 1972) studied in some detail the growth and reproductive responses to temperature by *Achlya dubia* and *Brevilegnia bispora* species that release spores in an achlyoid as well as a thraustothecoid (brevilegnoid) fashion. Sporangia were not produced by colonies of either taxon incubated at 4° or 35 °C. Mycelium of both species held at 10-25 °C gave rise to a greater proportion of achlyoid than thraustothecoid sporangia; the reverse was true when the mycelium was grown at 28-32 °C.

pH: -- Most investigators deal rather fleetingly with the influence of acidity or alkalinity on asexual reproduction, seemingly being content to record the pH ranges at which sporangia are formed or are most abundant. As would be expected, extremely acid or alkaline conditions so obstruct vegetative growth that sporangia simply are not formed. A few of the more prominent reports may be cited.

Bhargava (1950) noted that sporangia of *Isoachlya anisospora* var. *indica* (= *Saprolegnia diclina*) were rare in cultures propagated at pH 4.0 and 9.5, and absent from those incubated at pH 3.5 or 10.0. Nonetheless, the asexual cells of the isolate were produced over a very wide range pH 4.5 to 9.0. Somewhat different results with *Saprolegnia parasitica* were reported by Lee (1962). Maximum sporangium production by this species occurred within the pH range of 6.3-7.4, and the upper limit at which these structures appeared on colonies was at pH 8.0-8.3. The most acid conditions permitting sporangium development was pH 4.0-4.1. It may be noted that, in contrast, W. N. Tiffney (1936) had found that pH was not an important factor in sporulation (in a natural habitat) by *S. parasitica*. *Saprolegnia diclina*, isolated from fish by Chong (1973), developed sporangia well in media at pH 5.0 and 6.8, and the incorporation of buffers into the adjusted medium did not inhibit their formation. One of the earliest studies on the influence of pH on a watermold -- *S. monoica* (= *ferax*) -- was that of Lilienshtern (1924). He stated that acid media promoted the genesis of sporangia and in buffered solutions an "antagonism" developed between asexual reproduction and growth (he did not explain this latter comment). In solutions unfavorable to mycelial development, sporangium formation was promoted -- the Klebsian concept expressed as a function of a factor other than nutrition.

Light: -- The literature on the Saprolegniaceae shows that little is known of the influence of illumination on sporangium production by its members. All species that we have cultured produce functional sporangia in diffuse light or in the dark. To be sure, direct light can have deleterious consequences on sporangium induction because

of the increased radiation temperature within the confines of illuminated culture vessels containing media.

According to Llanos and Lockwood (1960), light did not influence spore production by *Aphanomyces euteiches*. Lee (1962) found to the contrary in *Saprolegnia parasitica*. At incubation temperatures lower than optimum for sporulation, exposing mycelium to light increased the number of sporangia produced by this species. Hyphae subjected to alternating light and dark periods produced the most sporangia upon receiving the longest light exposure. *Achlya dubia* responds in a similar fashion, Alabi (1967) has reported. Sporangia of this species "matured" better (sporulated more readily?) when colonies were illuminated continuously than when they were kept in the dark. Up to a point (with respect to the age of the mycelium) the number of sporangia of *A. dubia* increased as the exposure period to light was lengthened.

THE PLANONTS (ZOOSPORES)

It is difficult to judge in many cases reported in the literature whether reference to sporulation, *per se*, by particular water molds is to endogenous sporogenesis, to the spore discharge process itself, or to the activity of the released planonts. In this section we consider the effects of physiological parameters on the pattern of spore release, motility, and germination.

CHEMICAL CONSTITUENTS OF THE MEDIUM

Although it has long been known -- through somewhat parenthetical reports -- that spore discharge in the water molds is influenced by the substratum, Horn (1904) was among the first to explore this aspect of culturing specifically. In weak solutions of inorganic salts some sporangia of the species he used in his study showed clumping of the protoplast, but the mycelium nevertheless sporulated. Irregular cells of various sizes also developed in sporangia in the presence of such compounds, and the typical achlyoid sphere of encysted spores sometimes would not form at discharge. Griffin (1966) reported that the spores from a particular *Achlya* (mating strain of *A. bisexualis*) failed to cluster at the exit orifice when sporangium-bearing mycelium was exposed to 0.001 or 0.01 mM of calcium chloride. At the latter concentrations some spores were sluggishly motile upon release while others simply floated free without aggregating. Nitrogen sources unsuitable for good growth by water molds are known (Fowles, 1976) to favor planont discharge in species of *Aphanomyces*, and the same effect is associated with absence of this element in the growth medium. Liles (1969) propagated a water mold (unnamed except as a member of the "*Thraustotheca* complex") in shake culture to produce spherical mats of mycelium, then put these in water to encourage sporulation. Sporangia of the isolate tended to change with prolonged incubation from achlyoid to dictyucoid discharge pattern. Liles also subjected colonies to various inorganic salts. Calcium was essential for discharge of spores, and neither $MgCl_2$ nor KCl would substitute satisfactorily, although strontium chloride was slightly effective

as a replacement. The achlyoid type of discharge in *Achlya* sp. occurred in colonies incubated in a very restricted range of calcium salt concentration, while dictyucoid sporangia were favored over a much wider range in amounts of this same salt. Maximum discharge potential was achieved in solutions with calcium phosphate, and some form of phosphate was necessary if discharge was to occur at all.

Working with the pea pathogen, *Aphanomyces euteiches*, J. E. Mitchell and Yang (1966) discovered that the sodium ion inhibited motile spore formation (Ca^{2+} was essential for sporogenesis), while the divalent magnesium ion stimulated motility. Colonies incubated in a medium with 0.025 M calcium chloride produced sporangia from which the spores emerged to clump typically at the orifice; these encysted spores, however, germinated only by forming new hyphae.

The principal early physiological work on asexual reproduction by *Aphanomyces astaci* was carried out by Rennerfelt (1936). He noted that blood (from *Astacus astacus*) and horse blood serum in some concentrations promoted sporulation by the crayfish plague fungus. Various chemicals -- copper sulfate, mercuric chloride, anhydrous and hydrated arsenic, among others -- either prevented sporulation at particular concentrations or prohibited spore motility after release of the cells from the sporangium. Such information, however, has not contributed to an understanding either of growth and survival of *A. astaci*, *in vivo*, or of the physiology of sporulation by this watermold.

Seeking factors that might account for the low level of sporulation by *Aphanomyces astaci*, Unestam (1966b, 1969d) experimented widely with medium additives. Of 11 compounds tested, copper ions were the most effective and those of magnesium were least influential in preventing spore motility in this fungus. Calcium functioned as a "protective" factor against the toxicity of EDTA and the ions of sodium, potassium, and lithium. The addition (singly) of various anions or cations to the medium in which *A. astaci* was growing induced the planonts to encyst, and respiratory inhibitors and metabolic products reduced the level of motility by the cells. Spore discharge by *A. astaci* was impeded by some mineral salts incorporated into the growth medium, yet motility was enhanced by calcium and magnesium. Potassium ions (KCl), on the other hand, apparently had some restraining effect on *A. astaci* because motility was strong, Unestam found, only in mineral salts media lacking this element.

It has been demonstrated repeatedly that the addition of toxic compounds to growth media inhibits various aspects of sporogenesis and sporulation by members of the family. One such study, on the effects of dinitroaniline herbicides on *Aphanomyces euteiches*, was reported by Grau (1975). Both trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine) and dinitramine (N^3 , N^3 -diethyl-2,4-dinitro-6-trifluoro-methyl-*m*-phenylene diamine) inhibited motile spore production by sporangia of this species (and, as well, motility, germination, and germ tube length). Liles (1969) explored rather thoroughly the effects of three antibiotics, actinomycin D, puromycin, and cycloheximide, on the pattern of spore discharge displayed by a nonsexual watermold having both achlyoid and dictyucoid release. The actinomycin interfered with achlyoid discharge very quickly, unless the mycelium was exposed to the chemical after

sporangia had formed; in terms of mRNA transcription, this event took place in the period up to four hours after the colony had been transferred to water from the growth medium. The mRNA transcription for dictyocoid discharge was in the range of 3 (or 4) to seven hours after transfer of the mycelium, and Liles' data show that the mRNA associated with this type of discharge occurs after transcription for the achlyoid mechanism that had been effected. Puromycin (very rapidly) and cycloheximide (less rapidly) interfered with protein synthesis by Liles' watermold. The proteins necessary for this isolate to form sporangia with achlyoid discharge were 70% complete in six hours, although transcription was fulfilled in 0-4 hours. Those proteins needed for the mycelium to produce dictyocoid sporangia were assembled in five hours, but the mRNA transcription at this time was only 50% effective. Thus, the enzymes involved in assuring the function of the two types of spore discharge are different, and are not formed in the sporangium at the same time. The effect of cycloheximide was reversible (Liles, 1969).

It is well known that volatile and nonvolatile fungicides, sulfur-containing compounds, and the vapors from cruciferous soil amendments affect the growth and infectivity of *Aphanomyces euteiches* (Lewis and Papavizas, 1971b; Papavizas and Davey, 1963a; Papavizas and Lewis, 1970, 1971). Vapors from decomposing cabbage leaves reduce sporulation by *A. euteiches*, and those of carbon disulfide, methanethiol, and dimethyl sulfide suppress or inhibit spore formation, motility, and even germination (Lewis and Papavizas, 1971a).

GERMINATION

The fate of spores after their release from the sporangia has been little studied save, perhaps, by plant pathologists exploring such circumstances as infection routes and disease severity. Some reports -- Horn (1904) as an example -- dealt with rare occurrences or patterns of spore germination behavior; these can be ignored since none subsequently has been confirmed. The experiments on two species of watermolds by Shcherbak' (1910), though manifestly unrefined, at least yielded some pertinent observations on what was then called diplanetism. *Achlya gracilipes* (= *polyandra* Hildebrand) and *Saprolegnia thureti* (= *ferax*) were propagated in various concentrations of compounds such as mono- and dibasic potassium phosphate, sodium chloride, glycerin, alcohol, and meat extract. As might be expected, increasing concentrations of meat extract (0.25 to 5%) hindered motility, and, in some cases, favored direct germination. The spores of these watermolds exposed to solutions of some mineral salts in certain concentrations -- KNO_3 at 1.0% and KCl at 0.5%, for example -- never developed into the second motile stage. Shcherbak' concluded that diplanetism could be artificially controlled by manipulating the substratum.

Little has been done to determine the direct effects of metabolites on mobility in the watermolds. Incidental to a study on *Phytophthora infestans* Harris and Dennis (1977) subjected motile spores of *Saprolegnia diclina* and *S. parasitica* to some proprietary surfactants and the metabolites rishitin, phytuberin, solavetivone, and anhydro- β -

rotunol from infected potato tubers. These compounds caused spores of the test water molds to lose motility rapidly and to encyst. The spores were not lysed, and the loss of movement was not necessarily accompanied by a loss in viability. The investigators suggested that the motility-retarding effect of the various metabolites was on the function of flagella.

Inhibitors of protein and nucleic acid biosynthesis have a decided effect on spore germination in *Achlya bisexualis*. It has been noted by H. MacLeod and Horgen (1979) that there was no germ tube emergence from spores of this fungus exposed to actinomycin D, α -amanitin, cycloheximide, or puromycin. The latter two compounds blocked protein incorporation, and actinomycin D prevented the incorporation of uridine into RNA. Studies on thymidine uptake by *A. bisexualis* indicate (MacLeod and Horgen, 1979) that DNA synthesis does not occur in spores during the early stages in germination, yet germlings can sporulate (4-6 spores produced) when they are as young as four hours (developmental competence); see also study by Willoughby (1977). Germlings of *A. bisexualis* stained with mithramycin did not show a binucleate condition until they were more than four hours into the germination process.

The physiology of spore germination has been explored in some pathogenic species of *Aphanomyces*. Although the oxidation state of organic and inorganic sulfur in the medium tempers mycelium production by *A. euteiches* (Chapter 17), it does not influence spore germination, according to Papavizas and Davey (1963a). The authors also assayed 60 nonsulfur compounds (amino acids, fatty acids, coenzymes, purines, pyrimidines, and structurally related chemicals) for possible contributions to spore behavior in this same species (Papavizas and Davey, 1963b). Certain amino acids enhanced germination: *dl*-serine, *dl*- α -methylserine, *dl*-serine methylester·HCl, *dl*-*o*-methylserine, *dl*- β -methylaspartic acid, and *dl*-norvaline. On the other hand, *dl*-norleucine only moderated the level of germination. It also has been shown by J. E. Mitchell and Yang (1966) that the potassium ion stimulates germ tube growth in this same species. Some concentrations of calcium and copper are toxic to the spores of *A. euteiches*, and prevent their germination (J. A. Lewis, 1973b).

Aphanomyces astaci has been experimented with extensively to uncover conditions affecting its spore germinability, and something is known also of the enzyme systems associated with germination. Söderhäll and his associates (1978) identified protease in germinated and ungerminated spores of *A. astaci*, but chitinase was not detectable until the encysted planonts had produced a branched germ hypha. Unestam (1969d) analyzed various aspects of reproductive physiology comparatively among a variety of representatives of *Aphanomyces*: *A. laevis*, *A. stellatus*, *A. cochlioides*, *A. cladogamus*, *A. astaci*, and *A. aculeatus* (Fowles, 1967, applied this name, in thesis only, to a fungus isolated from dolphin; later published as *Aphanomyces* sp.). About 90% of the spores of *A. laevis*, *A. cochlioides*, and *A. cladogamus* germinated well in peptone-glucose medium. Yields of germinable planonts were moderated when specimens of these three taxa were exposed to blood serum from two species of crayfish: germination of 56-68% for *A. laevis*, 56-85% for *A. stellatus*, and only 1-4% in the case of *A. cochlioides*. The proportion of germinated spores in strains of *A. astaci* was much lower (6-15%) in

peptone-glucose medium than was measured for the other species tested. However, the serum from *Astacus astacus* and *Pacifastacus leniusculus* (a species resistant but not immune to *A. astaci*) failed to suppress spore germination by the *Aphanomyces*, and the serum from the latter crayfish even supported slightly higher spore germination than did the peptone-glucose medium (Unestam, 1969d: table 2). The various data on sporulation by *A. astaci* show that the frequency of spore germination is low but substrate-independent; this seems contradictory to the rapid spread and virulence of the fungus in *Astacus astacus* in nature (see Chapter 30).

Particular chemical compounds incorporated into growth media are known to influence the degree of spore germination in *Aphanomyces astaci*. Svensson and Unestam (1975) reported that mannitol and the chlorides of calcium, potassium, rubidium, and sodium stimulated germination, as did NaCl in combination with mannitol and mannose. Lithium and cesium chlorides were toxic to the fungus, and in solutions of these salts planonts lost motility but remained reniform.

In their experiments with planont germination in *Aphanomyces astaci* Svensson and Unestam (1975) uncovered a curious side effect: the physical handling of spores had a marked influence on germinability and motility. If spore suspensions were gently stirred (ten rotations), motility was reduced by 90% and germination by 50% when compared with nonagitated suspensions. Centrifugation at 1000 G for five minutes left no motile spores, and only half of the number in the suspension fluid germinated. It has also been shown (Unestam and Svensson, 1971) that the inability of some cultures of *A. astaci* to sporulate is a mutational defect, and therefore is irreversible. The loss of spore motility, on the other hand, can be a reversible event.

Aspects of the biochemistry of lipid synthesis and degradation during spore germination are known in an unidentified *Achlya* studied by S. W. T. Law and Burton (1976a, b). They analyzed fatty acid oxidation, lipid turnover, and incorporation of labeled Na acetate during the 30-hour period of development of the fungus from spore germination to sporangium formation and spore release. The lipid fraction of ungerminated spores was 10% of the dry weight of these cells, but this amount decreased rapidly at germination. Both the lipid and nonlipid content of the spores of *Achlya* sp. was degraded during this process. Most of the lipid in them was in the form of glycerides, compounds that presumably are used during germination. The spores of *Achlya* sp. had little ability to incorporate lipids, and fatty acid synthetase activity was relatively low during germination even though the activity of this enzyme increased during sporulation. The composition of the total lipid in *Achlya* sp. during its asexual reproduction also was analyzed by S. W. T. Law and Burton (1976b). They extracted free fatty acids from neutral lipid fractions, and by photorelectrometry determined quantitatively the compounds of these fractions. The total lipid content of the spores consisted of 62% neutral lipid, 13% phospholipid, and 25% glycolipid.

Thin layer chromatography produced evidence that the chief components of the neutral lipid fraction in this fungus were triglycerides (no mono- or diglycerides), free fatty acids, and cholesterol. In the ungerminated spores there was a higher concentration of free fatty acids than of glycerides, and after germination the levels of

both declined. More than one-third of the total fatty acids in the spores were hexadecanoic and octadecanoic acids.

EFFECT OF TEMPERATURE AND LIGHT

A reading of the record makes it clear that attention has been given largely to the influence of temperature on genesis of sporangia by water molds, and on endogenous spore cleavage, with little emphasis accorded to its possible effects on spore emission. Cotner (1930b) concluded that the optimum temperature for planont liberation in *Isoachlya paradoxa* (= *Protoachlya paradoxa*) was 23-25 °C, with the maximum at which discharge could occur being between 32 ° and 34 °C; the optimum for release of *Achlya conspicua* (= *debaryana*) spores was 25-27 °C, and in *Aphanomyces euteiches* it was 25-29 °C, with no emergence occurring at 31.5-33 °C. In 1925, F. R. Jones and Drechsler reported that although spores were produced by *A. euteiches* at 33-35 °C, they were not motile within this temperature range. However, the released spores were motile in water temperatures of 8-22 °C, but at the lower extremity of this range their production was delayed. The maximum temperature permitting primary spores of *A. euteiches* to move actively was >33 °C, but for motility in secondary planonts to occur the upper limit was 28 °C according to R. T. Sherwood (1958). Approximately the same number of spores was produced by sporangia of this species propagated in the range of 6° to 32 °C.

Spore production and motility by other pathogenic species of *Aphanomyces* also are influenced by temperature. Winner's (1966b) experiments with *A. cochlioides* demonstrated that this species would not sporulate at 4° and 8 °C, but the maximum spore production occurred at 16° and 20 °C. Herr's (1971) findings agree with those of Winner, that is, 16 °C is the best incubation temperature for *A. cochlioides* to insure maximum numbers of motile cells and greatest longevity of them as well. The data from W. E. McKeen's (1949) study indicated that maximum spore formation in this species occurred at a point some 6 °C lower than the temperature optimum for growth, that is, at about 24-26 °C. Spore production by *A. raphani* appears to be optimal at 20-25 °C (Kendrick, 1927). The sporangia of *A. daphniae* are reported (Prowse, 1954a) to release spores only at temperatures below 18 °C.

Noticing that the planonts of *Aphanomyces euteiches*, *A. raphani*, and *A. cochlioides* tended to aggregate when placed in water, Yokosawa and Kuninaga (1978) explored the effect of temperature on this phenomenon. By means of a glass-enclosed circulating water system placed within an aqueous suspension of motile spores these investigators found that the cells aggregated toward the circulating water when it was colder than that of the surrounding suspension. Conversely, clumping of planonts took place in a region away from the circulating system when the water in that system was warmer than the spore suspension fluid. Yokosawa and Kuninaga interpreted these results to mean that the spores aggregated in response to thermal convection and were not themselves thermotactic.

Four species of water molds isolated from diseased rice (*Oryza sativa* L.) seeds and seedlings were propagated at various temperatures by S. Ito and Nagai (1931) and

their sporulation responses observed. Sporangia were produced by *Achlya americana* at incubation temperatures of 12-28 °C, spore emergence occurred at 18-19 °C, and germination commenced at 22-28 °C. At 18-30 °C, sporangia were produced by *A. flagellata*, but within this same range of temperatures the spores only germinated *in situ*. *Achlya flagellata* var. *yezoensis* (= *debaryana*) produced sporangia -- and these formed emergent spores -- at 18-32 °C. Sporangia of *Dictyuchus sterile* (excluded name) were produced by colonies incubated at 23-28 °C, and these cells also sporulated normally within this range.

It is common practice to record water temperatures at times of collection, but only Suzuki (1960f, 1963; there is discord among some information in these two papers although one is but an English version of the other) assembled data on temperatures favoring spore release by saprolegniaceous fungi in natural bodies of water. He reported (1963) the existence of optimum field temperatures (°C) for sporulation: *Saprolegnia monoica*, 6-17°; *Achlya flagellata*, 9-20°; *A. racemosa*, 3-11°, and *Aphanomyces laevis*, 15-17°. Certainly for some of these species, the temperature optima for spore discharge in laboratory culture fall within an elevated range. It is unfortunately not evident how Suzuki derived these data.

The rather crude experiments reported by Strasburger (1878) demonstrated that light had no influence in determining or directing spore motility in a *Saprolegnia*. The role of light as an ecological factor in the distribution and occurrence of water molds is discussed in Chapter 3.

EFFECT OF pH

As would be expected, pH of the culture medium influences the behavior of water mold spores, in particular their movement and release. Three accounts deal chiefly with the influence of hydrogen ion concentration on the type of sporangium with respect to its pattern of spore release.

Achlya treleaseana (= *androgyna*) is a common inhabitant of acid waters such as is found in *Sphagnum* bogs. The species rarely produces sporangia either in gross or axenic culture, but it has been alleged by some authors to have achlyoid discharge while others have pointed to the aplanoid nature of its sporangia. In 1956(b), T. W. Johnson reported certain experiments with this species in which he found that in unbuffered solutions of sulfuric acid and sodium hydroxide, at an initial pH range of 3.7-4.1 and 8.1-9.3, sporangia with the achlyoid discharge pattern were formed. In cultures of this species grown in water with an initial pH range of 3.0-3.4 only aplanoid sporangia developed. An unidentified water mold (possibly an *Achlya*) exhibiting two types of sporangial discharge is also influenced by pH, according to a 1969 study by Liles. He discovered that achlyoid spore release occurred in colonies exposed to water having a pH in the range of 4.9-8.0, but the number of such cases was significantly less when the pH was below 5.0 and above 7.8. The colonies producing dictyucoid sporangia reacted to pH in essentially the same fashion. These data from Liles' study are somewhat at variance with T. W. Johnson's (1956b) results for *A. treleaseana*.

Much is known about *Achlya flagellata* and its response to pH largely because of very intensive studies conducted by Barbier (1969). Mycelium of this species grown in media in the pH range of 4.5-7.9 produced varied quantities of sporangia, and the spore release pattern from these was modified to a considerable degree. Sporangia with achlyoid discharge developed with increasing frequency as the pH of the medium was raised incrementally between 4.5 and 6.7. Colonies incubated at pH 6.7-7.9 yielded only aplanoid sporangia (*A. treleaseana*, by contrast, produced such sporangia at pH of 3.0 - 3.4). In *A. flagellata* the dictyocoid discharge pattern predominated at the lower range of pH that Barbier tested, that is, about 4.5. Spores released in an achlyoid fashion from sporangia germinated *in situ* (while still clustered at the apical orifice) in media with a pH between 5.5 and 7.5.

Pathogenic species of *Aphanomyces* also are sensitive to pH levels in culture. Spores of *Aphanomyces cochlioides* respond to somewhat narrow ranges of pH. According to Schneider (1963), planonts of this species are produced in abundance in tap water at pH 6.0-7.5, with the optimum for maximum yield being in the range of 6.4-6.5. Herr (1971) demonstrated additionally that a substantial range in magnitude of spore production by *A. cochlioides* could be attained by varying the pH of the medium. He counted a minimum of 16×10^3 spores from mycelium produced in a medium at pH 6, and a maximum of 71×10^3 spores when the hyphae sporulated in a medium at pH 8. Fewer spores were produced by *A. cochlioides* in demineralized water than in that from other sources. With demineralized water of a pH range of 5.8-7.5 the best sporulation response was elicited. Planonts produced by colonies of *A. raphani* occurred in distilled water in the pH range of 4.0-5.3 (Humaydan and Williams, 1978), at a temperature of 20 °C, following transfer of the mycelium from a radish-peptone broth. Maximum spore production by *A. astaci* occurred within a pH range of 6.5-7.5 (Unestam, 1966b), and Svensson and Unestam (1975) reported that the optimal pH for spore germination in this species is 7.0-8.0. Rennerfelt (1936) had concluded from his experimental work that the asexual cells of *A. astaci* were motile only at pH 7.2. In an *Aphanomyces* isolated from dolphin (Fowles, 1976), spore discharge is at least partially retarded by culture media having a pH of 4-5.

WATER QUANTITY AND QUALITY: OXYGEN TENSION

A small segment of literature on the Saprolegniaceae calls attention to the influence of such agents as source of culture water, colony age, and staling products on sporulation. One factor common to the propagation of these fungi in water in the laboratory is doubtless the oxygen level, and some culture fluids -- staling water, for example -- have an amount of this element subminimal for proper development. Either by implication, or convenience, oxygen tension has been singled out to explain some reproductive abnormalities expressed by isolates. It is a well known fact that washing mycelial mats (removed from nutrient media) in water or mineral salts solutions is likely to induce sporulation. But it should be recognized that stimulating spores by this means cannot be attributed solely to a high level of oxygen tension in fresh water. For

example, J. E. Mitchell and Yang (1966) reported that untreated lake water consistently induced spore formation in sporangia of *Aphanomyces euteiches*. The factor in that water that was stimulating sporulation was not destroyed either by autoclaving the water or extracting it with ether or chloroform. They found that lake water lost its effectiveness in favoring sporulation only if it was diluted 100 or more fold with distilled water.

The available evidence seems to suggest that oxygen tension may well augment sporulation (Llanos and Lockwood, 1960), at least in young, vigorous cultures. Mycelium of *Aphanomyces astaci* (Unestam (1969d) subjected to aeration either may be stimulated or retarded in spore release depending upon the medium in which the fungus is propagated and tested. One cannot escape the fact, however, that in foul cultures, as, for instance, in cases of heavy bacterial or protozoan contamination where the oxygen level is surely at a low point, there is a tendency for sporangia to retain rather than discharge their spores (Coker, 1923).

Maurizio (1899) was one of the first to comment on the role of oxygen in sporulation by water molds. He reported that depriving these fungi of oxygen neither killed them nor hindered entirely spore motility and germination. The chief effect of the absence of oxygen, Maurizio believed, was to prevent the spores from, as he said, "infecting" new substrates. In any case, he demonstrated that the number of sporangia of a water mold could be reduced by increasing the amount of water in which the organism was grown. Schneider (1963) put the factor of water volume and its possible influence on sporulation in a context of experimentation. He varied the ratio of mycelium mat of *Aphanomyces cochlioides* to the amount of culture water. The maximum production of spores obtained when the ratio of mat to water was 1:3 or 1:4. If mycelium (at age seven days) was removed from the propagation medium and submerged in an amount of tap water (with 120 mg L⁻¹ NaCl) equal to three times the amount of broth used to produce those mats, maximum sporulation resulted. Perhaps, Schneider suggested, the large volume of water simply diluted residual nutrients around the mycelium, and thus favored sporulation.

An isolate of *Brevilegnia* having considerable reproductive variability was studied by Salvin (1942a). This fungus revealed a degree of morphological variability that precluded its identification. Simply by modifying the culture conditions, Salvin could induce in this single-spore isolate characteristics of eight different taxa. The sporangia of the *Brevilegnia* (which he designated "C-2") were particularly variable, notably in relation to the amount of water in which colonies were propagated. With temperature and oxygen held constant (Salvin, 1942a), the more water used in the culture vessels the thinner were the sporangia. Sporangium diameter, in turn, was related to the pattern of spore release: those sporangia with a narrow diameter discharged in a dictyocoid fashion, while those with a wide diameter were typically thraustothecoid. In a later study, T. W. Johnson (1950) repeated with *B. longicaulis* much of Salvin's experimental work, but did not find such variability. Sporangia of Johnson's *Brevilegnia* species were shorter on colonies grown in small amounts of water than in large amounts (for example, 4000 mL), but the magnitude of variation over a

range of both sterile and staling water volumes was very much less than Salvin reported for his *Brevilegnia* C-2.

In one of the most extensive studies of its kind, Salvin (1942b) demonstrated that such conditions as oxygen tension, pH, and staling water factors influence sporangial morphology in species other than those in *Brevilegnia*. He sought to determine which factors might dictate the appearance of achlyoid and thraustothecoid (brevilegnoid) sporangia on the same mycelium of *Thraustotheca primoachlya* (= *Achlya primoachlya*) and *Dictyuchus achlyoides* (= *Achlya achlyoides*). Staling water was most influential in modifying the sporangium discharge pattern. The mechanism of dehiscence in *T. primoachlya*, in glass-distilled water (renewed so that staling products did not accumulate) changed with incubation time. During the first three days of colony growth, all sporangia were achlyoid, then some discharged in a thraustothecoid manner, and by five days all new sporangia produced dehisced as in *Thraustotheca* and *Brevilegnia*. On colonies in water in which another isolate of *T. primoachlya* had previously grown, all sporangia of this species were thraustothecoid. Water in which cultures of a rapidly-growing species of watermold had been propagated inhibited sporulation in *T. primoachlya*. Water from cultures in which an isolate with an intermediate growth rate had been incubated stimulated the development of achlyoid sporangia and reduced the frequency of thraustothecoid ones, whereas liquid from cultures that had supported a slow-growing species (such as *Aphanomyces laevis*) had no inhibitory effect. Colonies of *Dictyuchus achlyoides*, Salvin (1942b) found, responded in essentially the same fashion.

Some diffusing principle in the staling water, Salvin (1942b) reasoned, was influencing sporangium dehiscence in these species of Saprolegniaceae. Moreover, the diffusion time for the active principle would be a partial function of the amount of water in the culture vessels. His experiments confirmed these suppositions. Although ammonia seemed to accumulate in culture water, tests showed that this compound was not the active principle. Concentrated staling water was effective in modifying spore release patterns even when diluted 1:250 and was stable after being autoclaved for 45 minutes. The staling concentrate from *Thraustotheca primoachlya* had three major fractions, an alcohol-soluble one inhibiting achlyoid sporangia, an ether-soluble one having no effect on dehiscence pattern (though it did retard vegetative growth), and an ether-insoluble fraction that prevented formation of sporangia with achlyoid discharge, and additionally induced abnormally extensive and diffuse vegetative growth (Salvin, 1942b). The ether-insoluble fraction also obstructed achlyoid discharge in *Achlya dubia*, *A. flagellata*, and *Dictyuchus achlyoides*.

Factors other than staling water likewise determined the morphology of sporangial dehiscence. For example, Salvin (1942b) recognized that sporangia of *Thraustotheca clavata* and *Dictyuchus* sp. in colonies grown in 50 mL of water at 35 °C developed apical discharge papillae, but otherwise released their spores as was characteristic of members of these genera. Weston (1918) had earlier detected a similar production of exit papillae on sporangia of an isolate of *Thraustotheca*.

In confirmation of Salvin's report, Liles (1969) demonstrated that a shift of discharge pattern occurred in an unnamed water mold, but his study adds little to any explanation of how such alterations occur. In commenting on Salvin's conclusions and his own results, Liles suggested that the causal factor was not necessarily staling products. Since the change in the type of sporangium discharge in the fungi Salvin studied appeared over a time span of several days, it might be supposed that the gradual accumulation of staling products was responsible. Liles, however, offered an alternate explanation: perhaps the staling products acted as chelators to deprive the medium of something necessary for a particular discharge process to be initiated and completed. It seems quite evident, if Liles' assumption has merit, that the influence of staling water is far more complicated than a mere matter of reduced oxygen level or accumulation of some diffusing substance in the metabolites that make up staling products.

GEMMAE

Gemmae may be produced by young, presumably vigorous hyphae of Saprolegniaceae, or in old mycelia surrounded by contamination or metabolic wastes. Precisely what factors (other than genetic) are involved in the appearance and development of these structures are yet to be uncovered.

Kauffman (1908) found gemmae in some of the water molds he investigated to be produced in response to the medium constituency -- hemoglobin and various inorganic salts -- a not altogether surprising discovery. Certainly if Chaze's (1925) data are reliable, especially rich natural-product media (peptone, wort, or fish extract, for example) do not support colonies that produce gemmae. However, it appears that Kanouse (1932: 441) equated gemma formation with bountiful nutrient. She regarded them (in *Saprolegnia parasitica*) as "... a special type of reaction... to transform the almost unlimited food supply into protoplasmic reserves." Salvin (1942a) never observed gemmae on colonies of his *Brevilegnia* C-2 grown in "pure" water, but they were abundant on mycelium propagated in staling water. That such water, presumably harboring a supply of metabolites, does not invariably support gemma-producing hyphae has been demonstrated experimentally (T. W. Johnson, 1950a).

Gemma development is of course influenced by factors other than nutrients (or perhaps toxic or chelating compounds in staling water). Acidity (pH 4.6-6.0) was said to favor gemma production in *Achlya lobata* (A. W. Ziegler and Gilpin, 1954), and a moderately high incubation temperature (21 °C) encouraged abundant gemmae in an isolate of *Saprolegnia* (probably *S. ferax*) studied by Neish (1975a). Calcium in a concentration of approximately 1 mM of CaCl₂ is essential for the differentiation of gemmae by mycelium of the *Achlya* (mating strain of *A. bisexualis*) propagated by Griffin (1966). Chloride salts of magnesium, sodium, or potassium did not support gemma induction in the absence of CaCl₂ (Griffin found that these ions could not replace calcium in sporangium induction).

Some unpublished data on salinity tolerance by gemmae of *Saprolegnia parasitica* were mentioned by H. T. Fuller in a statement accompanying the account published by J. L. Harrison and Jones (1974). Fuller found that gemmae of this species did not germinate when hyphae bearing them were immersed in water at 100% salinity (approximately 35 ppt), but did remain viable at this level for 20 days at 10 °C. In water up to 30% of normal salinity gemmae sporulated. These vegetative hyphal segments germinated by the production of hyphae when exposed to water above 30% but more than 75% of normal salinity. Padgett (1980) has demonstrated by the use of a tidal cycle simulator that some water molds can colonize a suitable substrate in an environment of salinity fluctuation that would restrict sporogenesis or sporulation. In these instances, gemmae function (within limits) as salinity-resistant propagules.