

CHAPTER 16. The Physiology of Growth: Saprotrophic Species

If one can judge reflectively it appears that nutrition of the water molds has been a popular subject of study. Indeed, taken in its totality the literature leaves more than just a vague impression that nutrition has been looked upon as synonymous with physiology. This view is debatable, of course.

The Saprolegniaceae in common with many other fungi are rather efficient machines for the conversion of substrate to substance given the proper conditions. Ample evidence is to be found in the overflow from their metabolism -- the accumulation of such compounds as fats, carbohydrates, and organic acids, and the increases in dry weight and colony size. It happens, however, that measures of the efficiency are done in the laboratory where the water molds hardly can be said to live a precarious existence. Thus, the realities of their physiological processes may be far removed from the observable responses seen in the luxurious surroundings of the plated medium.

In this chapter we consider chemical and physical agents influential in the vegetative growth of the saprophytic water molds. Internal factors such as enzymes and metabolic pathways are for the most part treated in Chapters 22 and 23 (biochemistry), although this separation of subject matter admits to artificiality. Subsequent chapters will deal with the physiological aspects of reproduction. The chemical and physical parameters surrounding hyphal branching are treated elsewhere (Chapters 6, 13).

Actively growing cultures of water molds reach their maximum growth yield rather quickly under favorable conditions then decline (as measured by a loss in mycelial dry weight). Darnaud (1972a) visualized that this pattern of colonial development took place in three phases, namely, active growth, stationary phase, and autolysis. To Barbier (1969), however, growth of *Achlya flagellata* (= *debaryana*) in a liquid nutrient medium involved six periods, if all aspects of its development were taken into account. The latent or lag period of some three days duration was one in which the fungus did not influence the medium. There then followed the onset of growth (3-8 days long), and a subsequent period of maximum growth, extending from eight to thirty days. During this time, reproduction reached its maximum. In the interval between 30 and 90 days, Barbier found, the fungus matured, a transformation accompanied by an appreciable consumption of oxygen. In the period between three and seven months colonies of *A. flagellata* were in a stable phase without displaying additional growth or reproduction. At seven months the period of senescence commenced. For the most part, investigators experimenting with water molds seem not to have carried their physiological observations beyond the onset and peak periods of mycelial growth. Those who first explored the nutrition of water molds concerned themselves chiefly with fungal growth on plant or animal extracts or decoctions (Kauffman, 1908; Chaze, 1924, 1925) or to single substitutions of mineral or metal salts in various media. Schouten (1901) concluded that these fungi could use vegetable as well as animal materials for nutrition, but because animals (or their parts) were put into water to "catch" the organisms, he argued, they obviously "preferred" animal matter

for nutrition. Such reasoning has since been proven fallacious. Moreover, the differences recorded for growth rates (or amounts of mycelium produced) by isolates in various experiments on carbon or nitrogen requirements are highly suspect in many of the early reports. Often the investigators made single-source substitutions in basal media containing peptone or hemoglobin, for example, hence the efficacy of a particular compound could in no way be determined. At the other extreme, there is a report by Talukdar and Baruah (1952) to the effect that *Saprolegnia ferax* would not grow on media containing sucrose, glucose, starch, maltose, lactose, xylose, glycerin, gelatin, or ammonium nitrate. They used each compound alone in the medium, and, moreover, may have autoclaved them beyond recognition.

While there is no doubt that the Saprolegniaceae can utilize a wide variety of substrates for nutrition, they are probably not as versatile metabolically as the bacteria. Miller and Ristanović (1975) sought information on nutritional variability in the Saprolegniaceae by growing representatives on several standard media used in the biochemical characterization of bacteria. Their data show that members of the family cannot be separated and recognized taxonomically by their growth on such media, unlike the common heterotrophic bacteria. They noted, however, that isolates of *Achlya* grew more slowly on the various media than did representatives of *Saprolegnia*, but differences among species were not evident.

THE CHEMICAL ENVIRONMENT -- NUTRITIONAL FACTORS

There is ample evidence that members of the Saprolegniaceae generally range far afield in their ability to use carbon and nitrogen sources, and so far as is known, all are heterotrophic for these two elements. The inability of these fungi to use particular energy sources may reflect some genetic or environmental impairment.

CARBON SOURCES

Table 25 records some of the carbohydrates that have been reported to be used -- or not used in some cases -- by the water molds. This illustrative tally intentionally is not complete for it does not display degrees of response to the compounds. Moreover, when published reports are compared among themselves they are in general very heterogeneous in the amount and detail of information they convey. The same may be said for the data in Table 26 recording some of the instances in which water molds were found to utilize organic nitrogenous compounds for necessary carbon. Some of the published accounts of carbohydrate utilization must be interpreted with caution or even skepticism. This is particularly true where investigators used defined mineral salts media, fortified them with peptone and then used the concoction to test for the efficacy of single sugar substitutions.

Various organic substrates other than those listed in Tables 25 and 26 are used to some degree by particular water molds: gelatin (Gleason, *et al.*, 1970), inulin (Thakur Ji, 1972), ethanol (Reischer, 1951b), glycerol (Whiffen, 1945), and C₁₇-C₂₀ aliphatic alcohol fractions of hydrocarbons (Bilař *et al.*, 1965). In addition, certain organic acids may

supply the necessary carbon for growth as, for instance, acetic, lactic, pyruvic, succinic, and tartaric (Volkonsky, 1933a). Among organic compounds not used by certain species of Saprolegniaceae are dulcitol and tartaric acid (Dayal, 1960), inulin (R. K. Saksena and Bhargava, 1941; contradicts the report by Thakur Ji, 1972, although with another species), galactose (when used as the sole carbon source; Gleason, Rudolph, and Price, 1970), pentoses (Dayal, 1960; Bhargava, 1945c), and alcohols (Bhargava, 1945c). *Achlya bisexualis*, Darnaud (1972a) has found, can utilize as sole carbon sources acetate, fatty acids, and lipids. *Saprolegnia delica* (= *diclina*), on the contrary, is evidently unable to hydrolyze lipids (R. K. Saksena and Bhargava, 1941).

Beraud (1936-37) demonstrated that an unidentified *Saprolegnia* utilizes d-glucose much more readily than the levrorotatory form, and uses glucose "selectively" in a mix of glucose and fructose. Four species, *Achlya aplanes* (see taxonomic account), *Isoachlya* (= *Saprolegnia*) *unispora*, *I.* (= *Saprolegnia*) *toruloides*, and *S. parasitica* (= *diclina*) also use glucose preferentially. Dayal's (1961a) observations affirm that a mixture of glucose, fructose, lactose, galactose, and sucrose does not support any appreciable increase in growth in certain water molds over that enhanced by glucose alone. The carbon requirements of seven species of Saprolegniaceae were recorded by Moreau and Moreau in a lengthy paper published in 1938. Their data are too extensive to summarize conveniently, but suffice it to say that they called special attention to the many minor morphological changes that presumably were associated with the utilization of particular carbohydrates, cations, and anions.

The study by Faro (1972b) on glucans in *Achlya ambisexualis* and *A. heterosexualis* is singled out for special note. The former species could utilize stored glucan (a β -1, 3-glucan, but lacking mannitol) in the absence of an exogenous carbon source. In a medium without a supplier of carbon, but containing the chelating agent dimethylsulfoxide, *A. heterosexualis* continued to grow. Faro concluded that glucose is converted by these *Achlyas* into β -1, 3-glucan that is then sequestered.

NITROGEN SOURCES

Evidently the water molds *in toto* are somewhat more fastidious in their nitrogen requirements than in the carbon sources they utilize. With two reported exceptions, none of the species in the family utilizes nitrate or nitrite nitrogen (Bhargava, 1945b; Dayal, 1966; Darnaud, 1972a; Sorenson, 1964; Volkonsky, 1934). One exception is *Dictyuchus monosporus* which is said to be able to use sodium nitrate as the sole source of nitrogen. The second exception is an isolate of *Saprolegnia delica* that apparently is capable of growing on a medium containing sodium or calcium nitrate, and can use atmospheric nitrogen (Slifkin, 1963); this latter point is debatable. Among other nitrogenous substrates that support growth of some species are ammonium compounds (Slifkin, 1963, 1964; Bhargava, 1945b; Dayal, 1961e; Reischer, 1951b; R. K. Saksena and Bhargava, 1941; Sorenson, 1964; Whiffen, 1945), casein hydrolysate (Griffin *et al.*, 1974), casamino acid (Slifkin, 1963), various organic nitrogen sources (Darnaud, 1972a), urea (Dop, 1905b; Slifkin, 1963), and a number of amino acids (Dayal, 1961c; Thakur Ji, 1969; Volkonsky, 1934).

As is evident from the selected examples in Table 27, various species of the family are noticeably diversified as to the amino nitrogens¹ they can assimilate, and most of the isolates that have been tested use a variety of compounds. *Brevilegnia unisperma* var. *delica* (= *minutandra*) and *Calyptrolegnia achlyoides* are exceptions and quite particular in this regard. The latter can use only a few amino forms of nitrogen, and not even the commonest amino acids are instrumental in providing necessary nitrogen for the former. The sharp contrast (Table 27) in amino acid utilization reported by Sorenson (1964) and Thakur Ji (1969) for *B. unisperma* var. *delica* remains unexplained. Possibly there are physiological differences among strains or individuals of the species, but one would not expect these inconsistencies to be of such a magnitude. Other water molds also vary in their response to amino nitrogen, as the reports by Dayal (1961e) and Fowles (1976) illustrate, but there are not necessarily prominent differences among strains of the same species (Nolan, 1979). Dayal (1961e) discovered that *Achlya aplanes* propagated in the presence of *dl*-serine or glycine grew exceptionally well, but these same compounds were very poor sources of nitrogen for the growth of *Saprolegnia parasitica*. On the other hand, colonies of *Isoachlya* species (= *Saprolegnia*) developed quite well when *l*-cystine was the available nitrogen source, yet this amino acid was not suitable for *A. aplanes*. Species of *Aphanomyces* also react differently to certain amino acids substituted for glutamate in a mineral salts medium. Histidine, leucine, aspartic acid, and cysteine supported good growth of *A. stellatus*, but not of *A. cochlioides*, Fowles (1976) reported. Methionine inhibited *Aphanomyces* sp., but advanced the growth of *A. stellatus*, and was superior in this regard to 18 other sources tested. Two geographical strains (isolates) of *S. australis* utilized the same 17 ninhydrin compounds from casamino acid when propagated on a medium containing glucose (Nolan, 1979).

Some of the literature on the utilization of amino acid mixtures by water molds conveys contradictory information. Dayal (1959) added five amino acids to a basal mineral salts medium (with glucose), and determined uptake chromatographically. Arginine was used completely by *Saprolegnia parasitica* in six days, and by *Isoachlya unispora* and *I. toruloides* in 13 and 14 days respectively (the data supplied in reference to *Achlya aplanes* are contradicted in the published report). In these species, then, these amino acids were used preferentially. Not so in the experimental work reported by Gleason (1973b). The amino acids he supplied to *Saprolegnia* sp. were taken up at different rates, but there was no diauxic effect.

The available data on nitrogen requirements suggest that members of the Saprolegniaceae cluster together in Robbins' (1937) Group III. Fungi in this category (of four) are designated as ammonium organisms because they use ammonium salts or organic nitrogen for their supply of this element.

¹ The optical rotation property of particular amino acids (and other compounds) incorporated into media is not always stated by authors. In instances where the rotation is not designated we assume it is the *l*-form since these are incorporated into proteins.

COMBINED CARBON AND NITROGEN SOURCE RESPONSES

Very little is known about the conglomerate influence of carbon and nitrogen sources on vegetative growth in the water molds. Bhargava (1945b) simply stated that none of the five species he tested would grow in an inorganic salts medium incorporating sodium acetate and glycine. A far more extensive account on this subject was published in 1951(b) by Reischer. She propagated several species -- but singled out *Achlya klebsiana* (= *debaryana*) for emphasis -- in a basal medium (Reischer, 1951a) that combined certain TCA cycle acids with various nitrogen sources. Sodium acetate supported growth of *A. klebsiana* with or without glucose, but did not do so in the species with which Sorenson (1964) experimented. Sodium succinate contributed to enhanced mycelial production by *A. klebsiana* when either ethanol or glucose was used as the substratum, but Faro (1971) reported that sodium glutamate served both the carbon and nitrogen requirements of *A. heterosexualis*. Reischer (1951b) found that none of several acids tested would substitute for the succinate and support *A. klebsiana*: acetic, benzoic, crotonic, gluconic, glutaric, glycolic, itaconic, *p*-hydroxybenzoic, sorbic, or tartaric. All eleven species that she tested grew well in the basal medium with fumarate or malate and ammonium nitrogen, but did not do so if nitrate nitrogen was substituted. Two species were recalcitrant toward glucose: neither *Brevilegnia unisperma* nor *Dictyuchus monosporus* produced generous amounts of mycelium in the presence of glucose, but did so where ethanol was the substrate. Reischer's observations on these two species are contradicted by the reports (Table 25) on glucose utilization provided by Alabi (1967), Thakur Ji (1972), and Whiffen (1945). Differences in other constituents of the basal media used by these investigators may well account for the discrepancy. In any event, for all test species except *B. unisperma* and *D. monosporus* the only suitable carbohydrate source, Reischer found, was glucose (or a glucose-containing compound) coupled with a useable form of nitrogen. Machlis (1953a), in a critique of Reischer's (1951b: table 1) experimental work, pointed out that all the isolates she used grew to some degree in the absence of organic acids if there was a high pH in the culture medium initially.

Two water mold species display special nutritional problems with respect to carbon and nitrogen sources. *Calyptralegnia achlyoides* produced measurable mycelium, Sorenson (1964) found, only if cystine or cysteine were substituted for methionine, or if amino acids (arginine in particular) were used in place of ammonium chloride to provide nitrogen. Glucose, methionine, and NH_4Cl sufficed on single substitution as proper sources for carbon, sulfur, and nitrogen, respectively, but *C. achlyoides* grew only after extended incubation if these compounds were incorporated together in the medium. *Brevilegnia unisperma* simply would not grow (Sorenson, 1964) in any chemically defined medium, or in the presence of EDTA or buffers.

Nolan (1975b, 1976) studied in depth the growth of *Saprolegnia ferax* and *S. megasperma* in a basal medium supplying lactalbumin hydrolysate or casamino acids (with or without glucose), and incorporating ninhydrin-positive compounds. The chief data to emerge from his experiments with *S. megasperma* appear in Table 28. When *S.*

ferax was propagated in the presence of lactalbumin hydrolysate and glucose, all but four of 35 ninhydrin-positive compounds were utilized. Unsuitable growth nutrients for this species were cysteic acid, taurine, glycerophosphoethanolamine, and cystathionine. The latter two could not be used by *S. ferax*, however, if the basal medium contained glucose (Nolan, 1976).

It is chiefly through Sorenson's (1964) work that information on the influence of the carbon:nitrogen ratio on growth of water molds is extant. Except for *Brevilegnia unispurma* and *Calyptralegnia achlyoides* all of the species he used grew best when the C:N ratio was 25:1, and poorest when it was 5:1. Chong (1973) chose precisely this 5:1 ratio for the propagation of *Saprolegnia diclina* to study its growth and reproduction in culture. The carbon fraction is limiting (Sorenson, 1964) at the lower concentrations to the growth of some water molds, and, within limits, as the nitrogen component decreases (in proportion to carbon), the yields of mycelium rise. In *Achlya flagellata*, however, the C:N ratio evidently has no significant influence either on mycelial growth or lipid content (Clausz, 1979) of the hyphae. A somewhat different approach to the part the C:N ratio plays in growth was taken by Ristanović and her associates (1973). They used five species (Seymour, 1970, recognized only two of them as valid), and determined at two-day incubation intervals the values for dry weight biomass, glucose uptake, and nitrogen utilization in a chemically defined liquid medium. Measurements of these three parameters showed that all five species [*Saprolegnia delica*, *S. diclina*, *S. ferax*, *S. kauffmaniana* (excluded name; see systematics section), *S. mixta* (= *ferax*) had a one-day lag phase, followed by a rapid growth increase during the third to fifth day of culture. Two isolates reached maximum growth at seven days, while the remaining three attained this point at nine days. The pH of the medium in all cases became successively more acidic during the period when most of the biomass was accumulating, but it rose toward neutrality or alkalinity as the peak biomass was reached.

SULFUR AND OTHER ELEMENTS

Among the earliest inquiries into sulfur metabolism in water molds were those of Volkonsky (1932a, b; 1933a, b; 1934). While his experiments lacked the elegance that was to come with later technology, his were the first to employ strictly synthetic media. None of the saprolegniaceous fungi he tested used inorganic sulfur (mainly in the form of potassium sulfate), but grew well in the presence of the organic sulfur cysteine. Volkonsky applied the name parathiotrophic to those water molds that would not develop in a medium with sulfate sulfur alone.

With two exceptions, Volkonsky's chief conclusion (1933b, 1934) from his studies has been confirmed. Members of the family cannot use the oxidized form of sulfur, but will utilize readily organic sources such as cysteine, methionine, or thiourea (Darnaud, 1972a; Machlis, 1953a; Reischer, 1951a; R. K. Saksena and Bhargava, 1941; Whiffen, 1945; Sorenson, 1964). Dayal (1961f) contended that *Achlya aplanes* and *Saprolegnia parasitica* grew well in a medium containing sodium thiosulfate. *Brevilegnia gracilis* had long been

troublesome because unlike all other Saprolegniaceae, it was reported (Bhargava, 1945a) to utilize sulfate sulfur. As it happens, this species is a *Pythium* and can be removed from consideration.

The inorganic sodium sulfide evidently provides sulfur for certain water molds. Dayal (1961f) discovered that two species of *Isoachlya* (= *Saprolegnia*) could use this form. Four species of *Achlya*, *Thraustotheca clavata*, *T. primoachlya* (= *Achlya primoachlya*), and *S. diclina* can as well extract their needs for sulfur from sodium sulfide (Sorenson, 1964).

The only available data on the carbon:sulfur ratio and its influence on the growth of water molds are those published by Sorenson (1964). The best growth of the isolates he studied occurred when the ratio was 40:1 or 80:1. The literature is nearly barren on the subject of requirements by water molds of elements other than carbon, nitrogen, and sulfur. Dop's (1905b) contention that two species of Saprolegniaceae would grow in various concentrations of several inorganic salts is meaningless because he utilized peptone broth in propagating the fungi.

Bhargava (1945a) maintained that phosphorus was essential for the growth of water molds, but this has not been explored further. A phosphate concentration of 0.005 M was optimal for the species with which Whiffen (1945) worked. According to Darnaud (1972a) calcium is essential for the growth of *Achlya bisexualis*.

VITAMINS

There is reliable evidence from a number of sources that water molds are autotrophic for vitamins. Indeed, this physiological feature has been cited as one which tends to set these fungi apart from neighboring groups (*see* Chapter 25). It is quite likely that sufficient -- although minute -- amounts of growth-promoting substances are supplied among the impurities of some medium constituents. Bhargava (1946a) failed in attempts to propagate certain water molds when he used purified agar in media for nutritional testing. He assumed that the aqueous pyridine treatment used in the purification of the solidifying agent removed biotin. Thiamin and other vitamins, he found, were not needed exogenously, and, in fact, could impede growth of the water molds he tested.

SALINITY

It has been established beyond reasonable doubt that some members of the Saprolegniaceae do not recognize the boundary created by salinity, and there is good evidence that gemmae may function in this "resistance" to a salinity stress (Padgett, 1980). The enterprising work of Höhnk, whose principal contributions are reviewed in the chapters on ecology, was instrumental in revealing something of the occurrence and distribution of water molds in naturally saline waters. It is almost certain that in such an environment these fungi encounter hindrances of various sorts yet they do seem to exist there. Laboratory work on growth in response to salinity (or percentages of sea water) must be consulted to provide explanations for the ecological distribution of these

freshwater fungi in salt water. While not barren on this subject, the literature is by no means generous (Duniway, 1979).

The very early work (1939) by Höhnk on salinity tolerance by saprolegniaceous fungi demonstrated that hyphae respond morphologically to increased levels of salinity in the growth medium. In a moderate salinity of 13.85 ppt hyphae were dwarfed, thin rather than stout, and tended to develop catenulate, gemma-like cells. Later, Höhnk (1952d) reported that *Saprolegnia ferax* and *Aplanopsis terrestris* grew in seawater at salinities of 25 ppt and 18 ppt respectively, but the hyphae of both species were severely retarded at these high salinities, and of course were sterile (Höhnk, 1953a). Three species -- *Achlya diffusa*, *Dictyuchus monosporus*, and *S. diclina* -- were tested by TeStrake (1958, 1959) for their growth (and reproductive) responses to the combined effect of temperature and salinity *in vitro*. The three reacted in a similar, although not identical manner (TeStrake, 1958), and on the basis of preliminary test results, TeStrake (1959) chose *Dictyuchus monosporus* as the subject for further experimentation. An isolate of this species was grown on cornmeal agar dissolved in filtered seawater of various salinities, and the specimens then were subjected to a series of incubation temperatures. *Dictyuchus monosporus* did not grow on media of 10.7 or 22.5 ppt salinity at temperatures of 10-20 °C or 35 °C; at 25 ° and 30 °C, however, it produced colonies at 22.5 ppt, but not at 29.8 ppt. *Dictyuchus monosporus* also developed (at 15°, 20°, and 25 °C) on plated media with seawater concentrated to 89.4 ppt. Sterilization of the medium likely had reduced the true concentration of salts perhaps by absorption and coagulation or as the result of some heat-induced chemical change. Under laboratory conditions, colonies of *S. diclina* are known to grow in salinities as high as 30.4 ppt, at temperatures above 5 °C, provided the mycelium is established on hempseed before being exposed to a saline environment (Wu, 1979).

To test the hypothesis that the growth of oomycetous fungi could be tempered by salinity, J. L. Harrison and Jones (1974) cultured some thirty water molds on agar and in broth media that provided seawater concentrations of 0-70% (0-24.5 ppt). The cultures were incubated at 10-30 °C, and their growth quantified in terms of colony diameters or dry weights of mycelium. Harrison and Jones recorded the salinities as percent seawater, and referred to "optimum salinities" for growth. By "optimum salinity" is meant that percentage of seawater, incorporated into a growth medium at which best growth of the fungus occurs (E. B. G. Jones, communication, 1978). These investigators contended that salinity tolerance had to be measured in terms of optimal rather than minimal growth since modest or miniscule mycelial development in high salinities did not perforce reflect increased tolerance. A summation of their very extensive data follows.

With the exception of *Isoachlya toruloides*[†], the species were relatively uniform (at all temperatures) in their salinity optima of 0-10% seawater. The optimum for the *Isoachlya* depended upon temperature: at 10 ° and 15 °C, it was 0% seawater, but at 20 ° and 30 °C, the optimum fell at seawater concentrations of 10% and 20%, respectively. The data Harrison and Jones uncovered for the majority of the water molds used in the experimental work indicate that an increasing temperature does not necessarily raise

the watermold's tolerance to a saline environment. Of course, some species were more sensitive to increasing "saltiness" than others: *Achlya klebsiana* and *Dictyuchus monosporus* grew progressively less extensively as the salinity of the medium was raised.

From their data J. L. Harrison and Jones (1974) were able to group the species they used, by genera, into three clusters representing decreased growth tolerance to percent seawater. The pythiaceous fungi displayed the highest tolerances (bearing out earlier observations by Höhnk on the natural distribution of these fungi in relation to salinity). A second cluster included members of *Protoachlya*, *Saprolegnia*, and *Isoachlya*[†]. These were more saline-tolerant than the third group of representatives, those in *Achlya*, *Aphanomyces*, *Dictyuchus*, *Leptolegnia*, and *Thraustotheca*. For example, nearly all species of *Saprolegnia* tested had growth optima in media containing 10–50% seawater. With one exception, all isolates of *Achlya* grew optimally only at 0% seawater. When the various specimens were propagated at 20 °C in liquid culture with successively greater percentages of seawater, a similar grouping of taxa could be established. Species of *Saprolegnia* were the most tolerant of increased amounts of seawater, and those of *Achlya* the least. Harrison and Jones concluded that nutritional level does in fact influence salinity tolerance *in vitro*, thus supporting TeStrake's (1958) earlier contention.

There is an intriguing parallelism between salinity tolerance of the species groups projected by Harrison and Jones, and Höhnk's (1935a, 1968) proposal that sporulation patterns reflect propensities toward an aquatic or a terrestrial existence. The most saline-tolerant species -- in *Saprolegnia* (Harrison and Jones, 1974) -- also are as a group the more typically aquatic (Höhnk, 1935a), and the least tolerant are those with leanings toward a terrestrial existence. Mere coincidence or factual relationship?

GROWTH REGULATORS

Information on the effect of growth regulating substances on the mycelial development of watermolds comes from a few sources based on experimental work on some twenty species. According to Wolf (1937b), *Achlya bisexualis* could grow on potato-dextrose agar containing α -naphthalene acetic acid in a concentration as high as 1:60,000. There was almost no mycelial production by *Saprolegnia ferax* at the same level. In the experiments by Leonian and Lilly (1937) heteroauxin at all concentrations obstructed development of *S. diclina* hyphae, and thus this compound hardly could qualify as a growth-promoting chemical. None of the species which Murdia (1939b) exposed to phenylacetic acid or β -indolyl 3-acetic acid was stimulated toward increased growth, and above particular concentrations, these substances were actually inhibitory. The species tested by Murdia included *A. dubia*, *Protoachlya intermedia*, *Thraustotheca clavata* and two representatives of *Aphanomyces*. Not all watermolds react in like fashion to a given growth regulator, as Greulach and Miller (1956) demonstrated. Mycelium of *S. diclina*, *T. clavata*, *A. hypogyna*, and *Aphanomyces laevis* was inhibited by maleic hydrazide, but that of *Geolegnia inflata* and *Dictyuchus* sp. was not.

The investigation by R. F. Elliott (1967a) on kinetin, 6-benzylamino purine, indole-3-acetic acid, gibberellic acid, and the purines adenine and hypoxanthine is the most complete of the few studies on the effect of growth regulators on water molds. At low concentrations both kinetin and 6-benzylamino purine had no effect on the growth of *Saprolegnia australis*, but as the amounts of these were increased, mycelial development by the fungus was retarded and finally inhibited. Adenine and hypoxanthine did not influence growth (within the concentrations used), and the same was true for IAA and GA. Adenine counteracted the prohibiting effect of kinetin when these two compounds were applied together to the mycelium of *S. australis*.

An observation reported by Bhargava (1946a) may be noted briefly in passing. His experiments demonstrated that *Phytophthora erythroseptica* and *Phycomyces blakesleeanus* grew better on media incorporating staling products from the aqueous culture of water molds than on substrates containing their mycelial extracts.

STEROLS

The sexual regulatory function of steroids in dioecious *Achlyas* is treated in Chapter 21, but these compounds have also been investigated for possible roles in modifying vegetative growth of saprolegniaceous fungi. Hendrix (1965, 1966, 1975) has been a primary contributor to this aspect of water mold nutrition. *Achlya flagellata*, *Isoachlya unispora*, and *S. ferax* are not stimulated substantially by exogenous cholesterol. *Achlya bisexualis* and *A. ambisexualis* evidently are very inefficient in the uptake of cholesterol and sitosterol. Neither of these dioecious species produces (Hendrix, 1975) detectable esters when sitosterol is supplied to the mycelium, but slight quantities are formed from cholesterol. Hendrix suggested that cholesterol but not sitosterol was a normal component of these *Achlyas*.

THE CHEMICAL ENVIRONMENT: TOXINS, ANTIBIOTICS, AND INHIBITORS

Prolonged work with cultures of members of the Saprolegniaceae demonstrates convincingly that these fungi have a metabolism which is easy to modify by exposing them to various toxic substances; resulting changes ordinarily are reflected in alterations in growth rates and hyphal texture. Little is known of the specific ways by which toxic compounds affect the mycelium, and, in fact, no concerted attack on this problem has been launched. It is well to recognize that chemicals thought to be toxic may not necessarily be so. Gleason (1974), for example, found that respiration in a *Saprolegnia* sp. was not sensitive to cyanide, although this response clearly was correlated with age of the fungus.

Studies on the influence of the chemical environment on growth of water molds for the most part have simply explored the tolerances of test species to various compounds. Farr and Paterson (1974) determined the inhibitory effects of mannitol, cyanide, zinc, and two benzene sulfonate surfactants on growth and reproduction of

Achlya caroliniana. Within a concentration range of 2–60 $\mu\text{g mL}^{-1}$ p,p'-DDT has been found (Hodkinson and Dalton, 1973) to enhance growth of three species of Saprolegniaceae, but the magnitude of stimulation is related to the nutritional composition of the medium on which the fungi are grown. It has been hypothesized (Hodkinson and Dalton, 1973) that the growth stimulant effect of DDT perhaps can be traced to its use (by the fungi) as an organic carbon source, or alternatively to its influence on membrane permeability. The effect of various concentrations of malachite green and acriflavine (see also Chapter 29) on growth of water molds was explored by R. L. Martin (1968a). Starkov and Petrushova (1960) have experimented in a like manner with the suppressive influence of natural and synthetic mustard oils on growth.

The earliest efforts specifically directed at toxicity of common compounds ordinarily thought of as supporting growth were those of Moreau and Moreau (1936a-e, 1937a, 1938). They investigated the influence of water, sugars (and glycerin), and various ions on the development of fungi, and while their approach was crude by later standards (and the media not chemically defined) some points emerging from their research are at least of historical interest.

The most general of the studies by the Moreaus was that published in 1936(e) on the effect of mineral waters from various sources on growth (and reproduction) of three species of *Achlya*. They concluded that any source of water which permitted the fungi to develop well represented a "balanced solution". As surely would be expected, increasing concentrations of glycerin and sugars in the culture medium led not only to structural anomalies in the hyphae but also resulted in severe limitations to growth. Indeed, the Moreaus (1936b) looked upon sugars as toxic (in certain concentrations), and even "rated" them as to degree of toxicity -- the C₆ sugars were more damaging to the well-being of the water molds than the C₁₂ ones. Cations and anions, similarly, were growth limiting to various degrees (*A. colorata* was the test organism), Moreau and Moreau (1936c, 1937a, 1938) reported, with the bivalent ones being less so than monovalent ones. Curiously -- or perhaps only coincidentally -- the toxicity of the alkaline metal salts varied inversely with their atomic weight. The Moreaus directed much attention to the interacting influence on growth among inorganic salts incorporated into media. They prepared media containing two-member mixtures of inorganic salts in reverse order of concentration such that the highest proportion of one was paired with the lowest of the other. In these "antagonistic pairings" they propagated their test species. In each instance of paired ionic forms, one particular combination of the concentration of both salts in tandem augmented growth. The succeeding combination, however, moderated growth. The Moreaus believed that the toxicity of one salt counteracted that of another. There are obvious alternative explanations to this sweeping generalization, of course.

Experiments yielding precise data on the effect of toxic chemicals on water mold growth appear in a few papers published subsequent to modern discoveries in biochemistry. Two examples suffice. Timberlake and Griffin (1973) reported that cycloheximide and puromycin are detrimental to the development of *Achlya bisexualis*. The reduced growth associated with exposure of cultures of this fungus to these

inhibitors was accompanied by failure of *l*-proline to be incorporated into acid-insoluble protein. Although *dl-p*-fluorophenylalanine did not inhibit net incorporation of *l*-proline into the mycelium of *A. bisexualis* it did prevent the activity of alkaline phosphatase. The direct effect of this compound on growth, then, was to exclude synthesis of a functional enzyme. The spores of *A. bisexualis* appear to be capable of acquiring a resistance to cycloheximide (Griffin, *et al.*, 1978) if they previously have been exposed to sublethal levels. This resistance (or perhaps it is an adaptation) is lost, however, after a single transfer of spores to a medium lacking the inhibitor.

Four species of the Saprolegniaceae have been tested for their sensitivity to antibiotics. Moreau (1952) simply noted that *Saprolegnia ferax* was sensitive to the metabolic products of *Penicillium brevi-compactum* Dierckx. *Aphanomyces laevis* was not inhibited by pimarinin in concentrations up to 125 ppm, or by streptomycin sulfate at 400 ppm, according to Schäufele and Beiss (1973). This species exhibited decreased growth (over that in controls) in the presence of the sodium salt of penicillin, but actually showed better growth at 100 ppm than at 50 ppm. *Achlya* sp., Hutchinson (1967) reported, had a slight suppressive effect on the growth of gametophytes of *Pteridium aquilinum* (L.) Kühn suspended above actively growing mycelium. Volatile metabolites presumably given off during the growth of the fungus accounted for the inhibition.

Filtrates from cultures of 26 moniliaceous fungi and six dematiaceous ones were tested by Ristanović (1970b) for possible antagonistic effects to five species of Saprolegniaceae. Nearly 47% of the Deuteromyetes used were antagonistic (only one was a dematiaceous species, *Humicola brevis* Gilman) to the growth of the water molds. Only the filtrate from cultures of *Penicillium rugulosum* Thom inhibited all the water mold test species. Ristanović suggested that *Saprolegnia ferax* was the most "resistant" species to antagonist fungus filtrates while *S. diclina* and *Dictyuchus monosporus* were the most "susceptible" ones.

An unusual instance of the effect of chemicals on four species of Saprolegniaceae was uncovered by R. Mitchell and Wirsén (1968). They placed mycelial mats of the water molds in natural seawater with and without *Agarbacterium* sp. (*Flavobacterium* species, according to Weeks, 1974). In every instance, the hyphae were lysed readily in 3–5 days in the presence of the bacterium, and in 5–7 days in raw seawater. The authors suggest that seawater harbored a specific microflora which "attacked" the hyphae of the test species. Moreover, since all the test species were lysed by metabolites or enzymes from the *Agarbacterium* the investigators concluded that the taxa obviously were closely allied in hyphal wall chemistry.

Park's (1963) experiments with some nonsaprolegniaceous fungi are worthy of mention. By physically separating but chemically manipulating "producer" and "receiver" cultures, he demonstrated that production of an inhibitor that modifies growth as well as morphogenesis is common among fungi. Whether or not species of Saprolegniaceae possess such a compound remains to be investigated in depth, but one study suggests that some representatives may be so equipped. Uličná and Nemeč (1973) extracted (with organic solvents) the mycelium of a water mold identified simply

as *Saprolegnia*, and discovered that the resulting material had an anti-helminth action against *Turbatrix aceti*.

THE PHYSICAL ENVIRONMENT

The part played by physical conditions on the growth of water molds has been less rigorously explored than that of factors in the chemical environment. For the most part, investigators describing species or recording their occurrence have been content to note the temperature of incubation of their cultures, and little else. Studies on the physiology of growth of the water molds more often than not are carried out with single preselected temperature and pH regimes (Reischer, 1951a) that are deemed to be most suitable for growth.

TEMPERATURE AND pH

If the scanty available data (Table 29) are any indication, mycelium production by species of the family seems to be favored by somewhat higher temperatures than does their reproductive capacity (Chapters 19, 20). One obvious exception is the isolate of *Pythiopsis cymosa* collected by Nolan and Lewis (1974). Their fungus was found in the colder waters of a Newfoundland stream, and might perhaps be a strain selectively adapted to low temperatures. In our experience, temperatures approaching 35 °C usually support delicate, inferior colonies composed of weakened, flexuous, often irregular hyphae.

There evidently are strain differences within species of water molds with respect to temperature and pH tolerances, but this hardly has been explored extensively. From an analysis of mycelium dry weight determinations Nolan (1979) concluded that a Newfoundland isolate of *Saprolegnia australis* appeared to be more suited than a North Carolina specimen of the same species to an acidic environment. The latter strain, however, was more tolerant of high temperatures of incubation, a characteristic that seemed compatible with its temperature and climate existence.

The growth tolerance ranges and optima with respect to the hydrogen ion concentration of the medium are known but for a few saprotrophic species of Saprolegniaceae (Table 30). As the scanty available data indicate, the water molds survive over a wide range of acidity and alkalinity, but appear to grow optimally in an acid medium. Oogonium production (Chapter 20) likewise seems to be favored by a slightly acid environment. Reischer (1951a) suspected that reports of water molds growing in media of low pH were incorrect because the ingredients of the medium could influence the results of tests. The whole subject of the influence of pH on, for example, the uptake of medium constituents by water mold mycelium is unexplored, and, indeed, the alleged retarding or stimulating effects of pH may well be due to pH-induced chemical changes or chelations in the medium.

The changes brought about by metabolites that the fungi themselves secrete into the growth medium are also far from being analyzed and understood. Some of the

studies of this aspect of watermold growth have produced curious conclusions. Lilienshtern (1924), for example, decided that *Saprolegnia monoica* (= *ferax*) changed its growth medium from acid to base only when the initial pH of that medium was in the range of pH 4-5. In the presence of "fermentable" sugars and some forms of nitrogen, Volkonsky (1933a) reported, *Saprolegnia* sp. did not acidify the medium in which it grew. On the contrary, the growth of the fungus in such instances neutralized the medium.

Only Barbier (1971b) has been critically attentive to the relationship between hyphal growth and pH. He established a series of cultures of *Achlya flagellata* in water that was initially adjusted to pH ranging from 4.5 to 7.9. In four days of incubation, mycelium was well developed in cultures at 4.5 and 5.5, but for those exposed to higher pH levels, the onset of growth was postponed. However, by the end of 21-30 days of incubation, all cultures had grown equally vigorously and extensively in spite of initial delays. Thus, decreasing acidity simply appeared to lengthen the lag period in the growth of the fungus. Barbier (1971b) interpreted these results to mean that in a basic milieu, the fungus first had to synthesize adaptive enzymes before it could begin to grow.

OXYGEN AND OTHER FACTORS

Although by the beginning of the 1900's mycologists had on occasion remarked that species of the Saprolegniaceae were abundant in nonpolluted, relatively clear water, this idea certainly was not universally accepted. Dop (1905a, b), it appears, was the first to explore the growth of watermolds in conditions of "less than usual" available oxygen. It was he, in any case, who reported that in a culture environment to which hydrogen had been added *Saprolegnia thureti* (= *ferax*) would grow provided glucose rather than peptone was the available substrate. He regarded the fungus as facultatively anaerobic, but in retrospect it is clear that his experimental method certainly did not support that conclusion. In an attempt to correlate ecological distribution of watermolds with environmental factors) Suzuki (1961b, i) experimented with growth of several test species in relation to oxygen tension. None of the isolates grew well (if at all) at 0.56 mL L⁻¹ oxygen, but did develop vigorously at an oxygen level of 7.06 mL L⁻¹. The comments by Barbier (1969) on the ecology of the Saprolegniaceae are pertinent. He argued that since the watermolds grow in stagnant water rich in plant debris they must be adapted to reduced oxygen conditions. This conclusion was reinforced, Barbier thought, by the fact that watermolds cannot utilize elemental sulfur.

Darnaud (1972b) experimented with osmotic pressure and its effect on the growth of *Achlya bisexualis*. He grew the fungus in a defined medium but modified the glucose or NaCl concentration. With increasing amounts of either of these constituents -- recalling the toxic effects of sugars as proposed by the Moreaus -- the dry weight of the mycelium decreased. Internal metabolic reactions, Darnaud believed, modified constantly the hypha's endogenous osmotic concentration, and adjustments to these changes were reflected in the magnitude of growth. Darnaud did not consider that

either nutritional deficiency or failure in uptake and transport could cause the growth reduction that occurred concomitant with increasing osmotic concentration in the surrounding medium.

As will be evident in a later chapter the influence of illumination on reproduction in the Saprolegniaceae has been explored to some small degree. Alabi (1967) reported that light had no effect on the growth of *Achlya dubia*, but a later study by Prabhuji (1979) yielded contradictory data. The latter found that growth of *Isoachlya luxurians* (= *Saprolegnia luxurians*) and *Brevilegnia subclavata* was inhibited in the dark or when the mycelium was exposed to ultraviolet for two minutes prior to dark incubation. Unfiltered white light (400 ft. c.), on the other hand, stimulated growth in *B. subclavata*, while blue light had the same effect on *I. luxurians*. Prabhuji uncovered some evidence for photoreactivation in the mycelium of these water molds. Maurizio (1899) much earlier had noticed that reduced light had no influence on the growth of several species of *Achlya* and *Saprolegnia*.