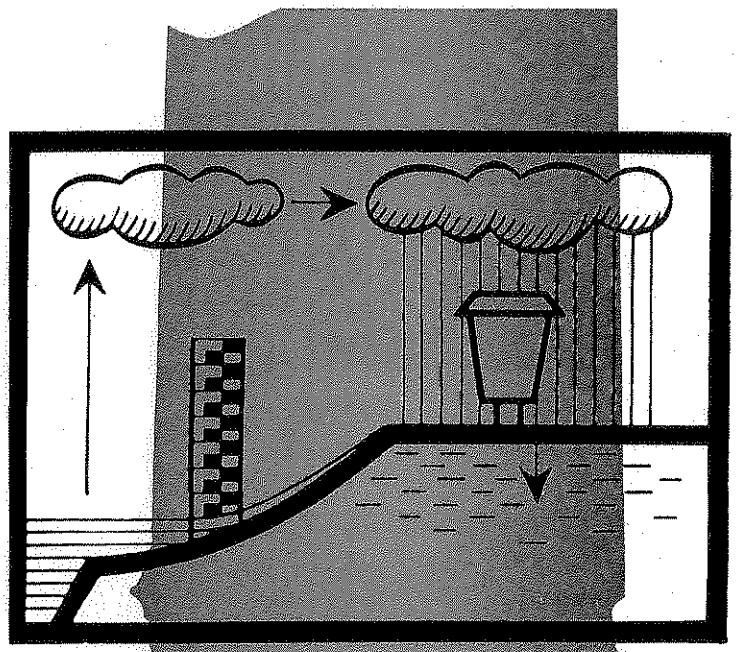
TECHNICAL REPORT 154

INTEGRATION OF MANAGEMENT METHODS WITH THE LIFE CYCLE AND ECOLOGY OF THE FILAMENTOUS ALGA, *PITHOPHORA*



by

Steven W. O'Neal

David F. Spencer

Carole A. Lembi

April 1983

PURDUE UNIVERSITY
WATER RESOURCES RESEARCH CENTER
WEST LAFAYETTE, INDIANA

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Purdue University, West Lafayette, IN 47907

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Project personnel were Steven W. O'Neal (Postdoctoral Associate), David F. Spencer (currently Assistant Professor at I.U.P.U. at Indianapolis), and Carole A. Lembi (Associate Professor, Purdue University).

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ABSTRACT

Pithophora oedogonia is a green, filamentous alga in the order Cladophorales (Chlorophyceae). It is a free-floating organism forming thick, heavy mats of vegetation that can fill in small impoundments, shallow lakes, and coves and channels of larger lakes and reservoirs. It is generally considered to be a serious managment problem because of its apparent resistance to standard algicides. In Indiana the alga overwinters as vegetative filaments and akinetes (nonsexual, thick-walled spores). Akinetes comprise approximately 21% of the overwintering free-floating biomass. Akinetes and overwintering filaments germinate to produce new vegetative filaments in the spring in response to an increase in water temperatures from 10 to 20 C and a replenishment of nutrients (NO_3 and PO_4) from winter and spring rains. Net photosynthesis rates increase from a low of 0.91 mg O_2 g^{-1} dry wt h^{-1} in midwinter to a high of 37.0 mg O_2 g^{-1} dry wt h^{-1} in mid June and July. Biomass accumulation lags behind that of photosynthesis peaking in late September-early October. Akinete formation in late summer-early fall occurs in response to a decline in NO_3 and PO_4. Akinetes are also present on the bottom sediments. Their germination occurs approximately a month after germination of akinetes in the free-floating mats. The lag may be due to the slower warming of the sediments. The spring akinete germination period is an extremely critical one because this is the time when most algicide (e.g., simazine and copper sulfate) treatments are made. Our studies of the akinete germination process show that germination and initial germ tube elongation are dependent on the respiration of internal stored materials and are simazine-insensitive. Simazine, a photosynthetic inhibitor, does effect germ tube elongation after approximately 21 days

when the transition from metabolism of internal reserves to photoautotrophic metabolism occurs. Germinating akinetes (in comparison to akinetes and vegetative filaments) are also relatively insensitive to copper sulfate. The I_{100} value for germinating akinetes is 6.8 uM Cu^{++}; for akinetes, 3.7 uM Cu^{++}, and for vegetative filaments, 0.9 uM Cu^{++}. The inherent tolerance of germinating akinetes to simazine and copper sulfate plus the extended germination period due to sediment akinete germination may be a major reason for the ineffectiveness of spring algicide treatments. Other alternatives such as copper treatments in late winter prior to akinete germination are being explored. Simazine appears to be relatively ineffective for Pithophora control. Although photosynthesis in vegetative filaments is inhibited by simazine, the alga appears to be protected from phytotoxic effects by low light intensities at the lower depths and within the algal mats and by an ability to survive on stored internal materials until simazine residues have dissipated from the water.

INTRODUCTION

Pithophora oedogonia is a green, filamentous alga in the order Cladophorales (Chlorophyceae). It is a free-floating organism forming thick, heavy mats that can completely fill in small impoundments, shallow lakes, and coves and channels of larger lakes and reservoirs. It is generally acknowledged to be a severe management problem in fish-rearing facilities, farm ponds, and shallow lakes throughout the southeastern and midwestern sections of the United States.

One of the major problems in managing excessive growths of *Pithophora* has been the apparent resistance of the organism to treatment with standard algicides. The major objective of this research was to determine whether the application of algicides could be integrated into "susceptible" stages in the life cycle of the organism. Before this could be achieved, however, the life cycle itself had to be elucidated. The research reported here is divided into the following sections: I.) Life cycle and environmental parameters regulating the life cycle, II.) Effects of algicides on specific stages in the life cycle with emphasis on A.) simazine and B.) copper sulfate, and III.) Conclusions regarding possible approaches for the management of *Pithophora*.

I. LIFE CYCLE AND ECOLOGY OF *PITHOPHORA*

Pithophora Wittrock 1877 is placed in the green algal order Cladophorales because it consists of multinucleate cylindrical cells united end to end in branched filaments. Each cell of *Pithophora* is enclosed by a thickened stratified wall containing cellulose and chitin (Pearlmutter and Lembi, 1980) and contains a reticulate chloroplast with numerous pyrenoids.

It differs from its more widely studied relative, Cladophora, by its branching pattern (Pearlmutter and Lembi, 1980) and by the fact that Pithophora does not undergo sexual reproduction. Most species of Pithophora are free-floating in contrast to the major weedy species of Cladophora which develop attached to a substrate.

Pithophora does form thick-walled, asexual spores known as akinetes. Akinetes are present throughout the life cycle of the organism but are more prevalent during the fall, winter, and early spring months (Lembi et al., 1980). The presence of thick-walled structures such as akinetes, like the seeds of terrestrial plants, could presumably protect the organism from the phytotoxic effects of algicides or aquatic herbicides. The natural purpose of the akinete has been variously described as providing the organism with a means of overwintering, surviving desiccation when filamentous mats are stranded above the shoreline (Fritsch, 1907) and surviving conditions of nutrient depletion (Ernst, 1908). Lembi et al. (1980) reported that akinetes are formed in large numbers when filamentous mats are stranded. Although themselves quite susceptible to death by desiccation, akinetes can survive both summer and winter drawdowns as long as they are protected from complete desiccation. This is usually the case because the filamentous mats in which the akinetes are formed are thick enough to retain sufficient moisture even during the driest summer months.

In addition to being found in the free-floating, filamentous mats, akinetes are also found in abundance in the sediments of ponds and lakes where they are deposited in the fall and winter (Lembi et al., 1980). Any attempt at integrating chemicals into the life cycle of the organism would have to take into account the periodicity of akinetes in the sediments.

Materials and Methods

Culture studies. Pithophora oedogonia (Mont.) Wittrock 1877 was isolated into culture by Pearlmutter and Lembi (1980). Stock cultures were maintained in C1(II) medium modified by deletion of B7 and C13 trace element solutions (Gerloff and Fitzgerald, 1976). Standard culture conditions used in growing the stocks were as follows. Illumination was provided by a 61 cm cool white fluorescent tube with an 18:6 L:D cycle and photosynthetically active radiation (PAR) of 15 uEinsteins m^{-2} s^{-1}. Temperature was maintained at 20 $\pm$ 1 C. Changes in nitrogen and phosphorus content of the medium or of light and temperature conditions are indicated in the text.

Laboratory measurements of photosynthetic rates are described in Section IIA.

Field studies. Field studies were conducted in Surrey Lake, a small shallow man-made lake in Bartholomew Co., central Indiana (T9N, R6E). The lake was constructed in 1945 and has supported extensive growths of Pithophora oedogonia since at least the late 1960's. Water flow is from south to north (Fig. 1). The inflowing stream drains a watershed of approximately 1128 ha, 62% of which is agricultural land and pastures; 21% woodland; and 16% residential.

Most of the residential area is within the boundaries of Jewel Village, the homes of which are on septic tank-finger systems that probably contribute significantly to the high nutrient load in Surrey Lake (Lembi et al., 1980). The major soil types in the Jewel Village drainage areas are the Fincastle silt loam and Brookston silty clay loam which are indicative of poorly drained soils such that heavy rains may transport sewage material

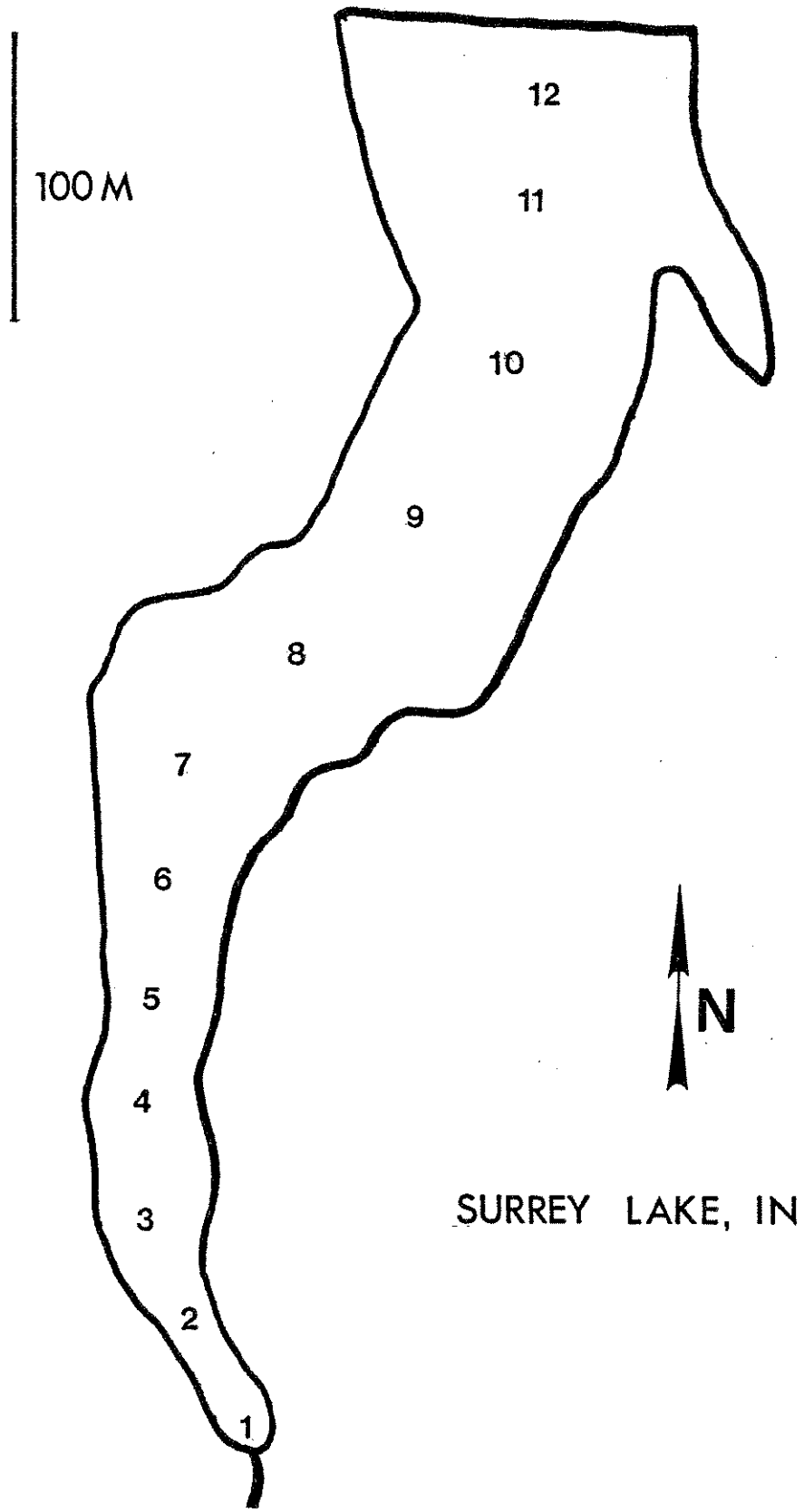

Figure 1. Map of Surrey Lake, Indiana and approximate locations of sampling stations 1-12.

to the lake. Mean water quality values for Surrey Lake are published in Lembi et al. (1980).

For prior studies (described in Lembi et al., 1980; Spencer and Lembi, 1981), sampling stations 1 through 12 were established along the length of Surrey Lake (Fig. 1). Stations 1-7 were located in the shallow or cove end of the lake and averaged 0.42 m in depth. Stations 8-12 were located in the "deep" end of the lake and averaged 2.0 m in depth. There is an outflow through a spillway at the dam or north end of the lake.

Algal biomass samples were routinely collected from the shallow stations using a 30.48 cm diameter aluminum tube. The tube was inserted into the sediment and both floating and submerged algal material was collected. In the deeper part of the lake, floating mats were sampled by hand. Deeper mats were collected with an Ekman dredge. Samples were returned to the laboratory, rinsed to remove sediments and Lemna, placed in aluminum trays and dried for 48 h at 110 C.

Akinete numbers were estimated by taking small clumps of fresh, rinsed alga and blotting them dry. Ten g of this material was added to 100 ml of water and disrupted in a Waring blender at medium speed for 10 min. The resulting liquid consisted of broken Pithophora filaments and intact akinetes. Akinete counts were made on 1 ml aliquots of the solutions dispensed into Sedgewick-Rafter counting chambers (at 40X). The number of akinetes present per gram fresh weight of filaments was then calculated.

In situ measurements of photosynthesis and respiration rates were made on Pithophora mats collected near stations 7 and 11. Small clumps of the alga (30 to 500 mg dry wt) were placed in 500 ml erlenmeyer flasks. Five replicates for photosynthesis and respiration were run at both stations. Respiration was measured in flasks covered with aluminum foil to exclude

light. The flasks were filled with unfiltered lake water collected at the two stations. When necessary the lake water was bubbled with nitrogen to reduce oxygen levels below saturation. The flasks were sealed with rubber stoppers in a manner that avoided trapping air within the flasks.

Initial oxygen concentrations in the water prior to filling the flasks was determined with a YSI Model 53 portable oxygen meter and polarographic electrode Model 5739. Mixing of the water during oxygen determination was accomplished with a small, battery-powered drill with stirring attachment. Rates of photosynthesis and respiration were determined by measuring changes in oxygen concentration in the flasks after incubation. Changes measured in light and dark flasks without Pithophora were used to estimate photosynthesis and respiration by plankton.

The flasks were placed in a wooden rack and returned to the station where the mat was collected. The rack held the flasks just below the water surface. The flasks were incubated for 2 to 5 hours beginning at 1000 to 1300 Eastern Standard Time.

At the end of the incubation period, the algal material was removed from the flasks and kept on ice during transportation to the laboratory. The algal mats were weighed (fresh wt) and dried at 60 C. Rates of photosynthesis and respiration of the Pithophora mats were calculated on a dry weight basis (mg O_2 g^{-1} dry wt h^{-1}).

Various aspects of the lake environment were monitored concurrently with the photosynthesis and respiration study. Water temperatures were monitored at the start and end of the incubations. Light intensity was measured with a KAHLSICO Model 268 WA 310 Submarine Photometer calibrated against a LiCor Model LI-185A Quantum Meter.

Measurements of akinete periodicity in lake sediments were determined on sediment cores collected from station 4. The top layer (approximately 0.5 cm) was removed from the core and placed with water in a 200 ml plastic bottle. In the laboratory, the sediment samples were allowed to settle and the overlying water removed. A weighed amount of sediment (4 to 7 g) was placed in a 250 ml flask and the volume made up to 100 ml with water. The contents were shaken vigorously to mix the sediment and water and 10 ml portions were removed and dispensed into gridded petri dishes. The sediments were allowed to oxidize for 30 minutes so that sediment akinetes could be distinguished from other sediment particles. Counts were taken under a dissecting microscope.

Measurements of Eh and temperature of the sediments and the water were taken concurrently with the sediment sampling. A Sargent-Welch PBL meter with a platinum probe was used for the Eh measurements. Sediment readings were taken at 1 cm intervals to a depth of 5 cm. Temperature measurements were made in the same way using a portable temperature meter and probe.

Results and Discussion

The life cycle of P. oedogonia and regulating environmental parameters are summarized in Fig. 2. Field data on algal biomass (g dry wt m^{-2}), akinete numbers (akinetes X 10^3 g^{-1} fr wt), and net photosynthesis (mg O_2 g^{-1} dry wt h^{-1}) for 1979 through 1981 are presented in Figure 3 and should be referred to as necessary in the following discussion. Water temperature and light intensity measurements at 0.3 m depth for the same years are provided in Figure 4. Nutrient data has been presented elsewhere (Spencer and Lembi, 1981). For ease of discussion, the data will be described according to the seasons of the year with emphasis

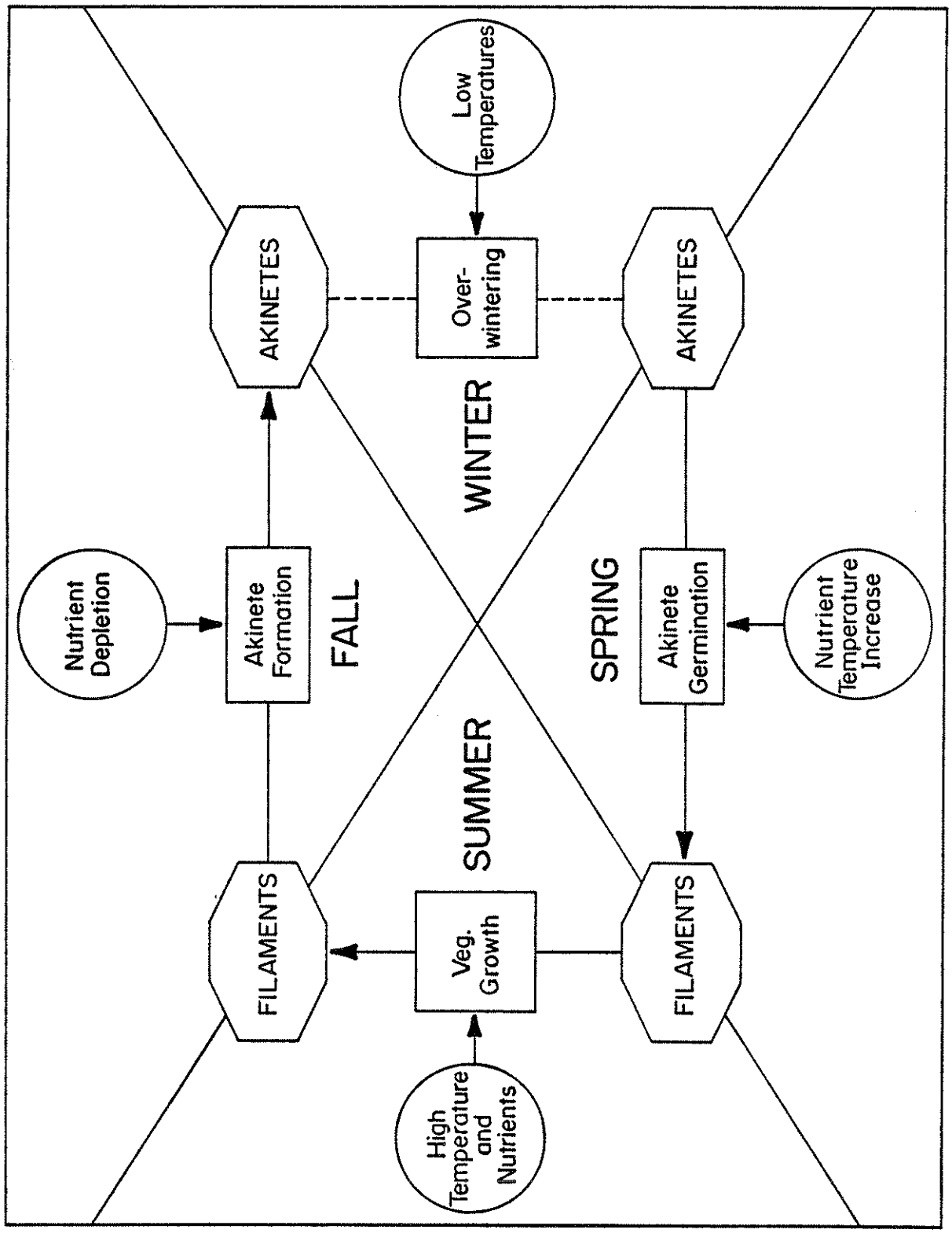

Figure 2. Summary chart of life cycle of Pithophora oedogonia.

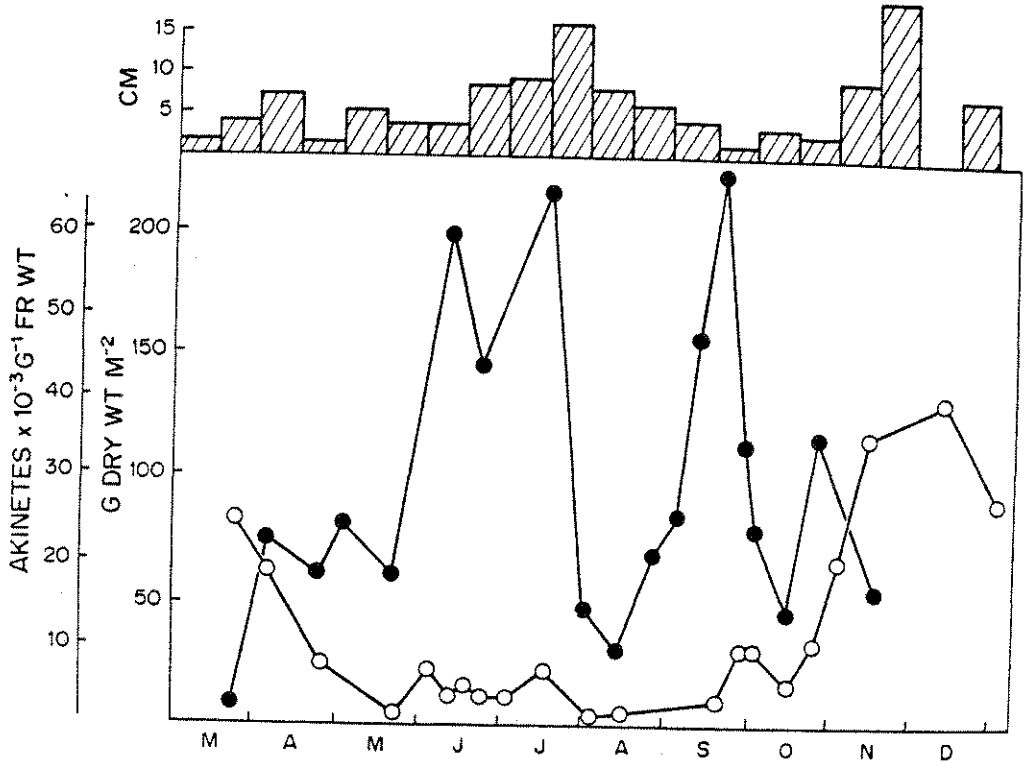

Figure 3A. P. oedogonia biomass (●—●) and akinete numbers (○—○) in Surrey Lake in 1979.

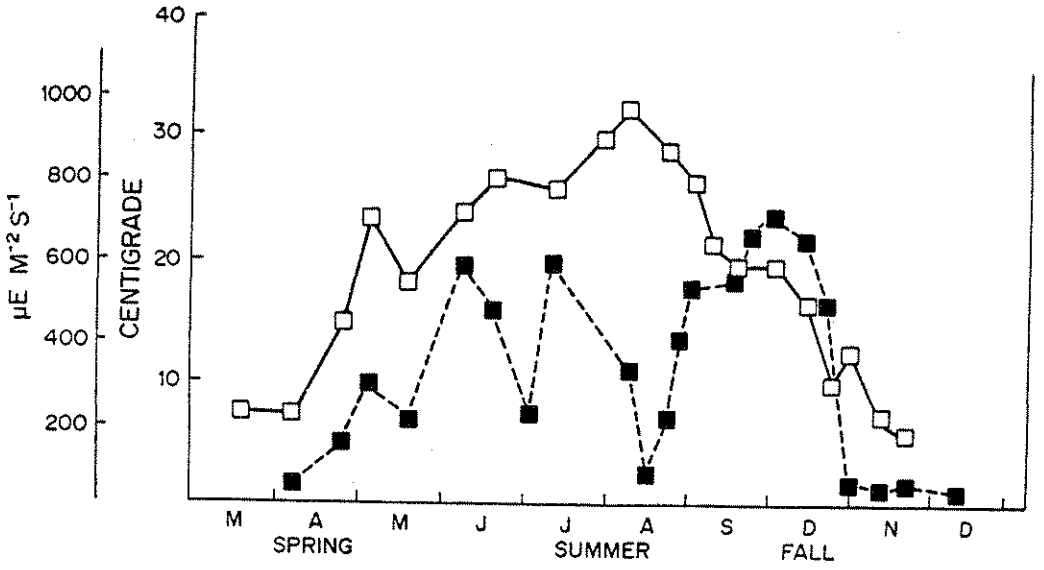

Figure 4A. Water temperature (□—□) and light intensity (■▪▪▪■) measurements at the 0.3 m depth in Surrey Lake in 1979.

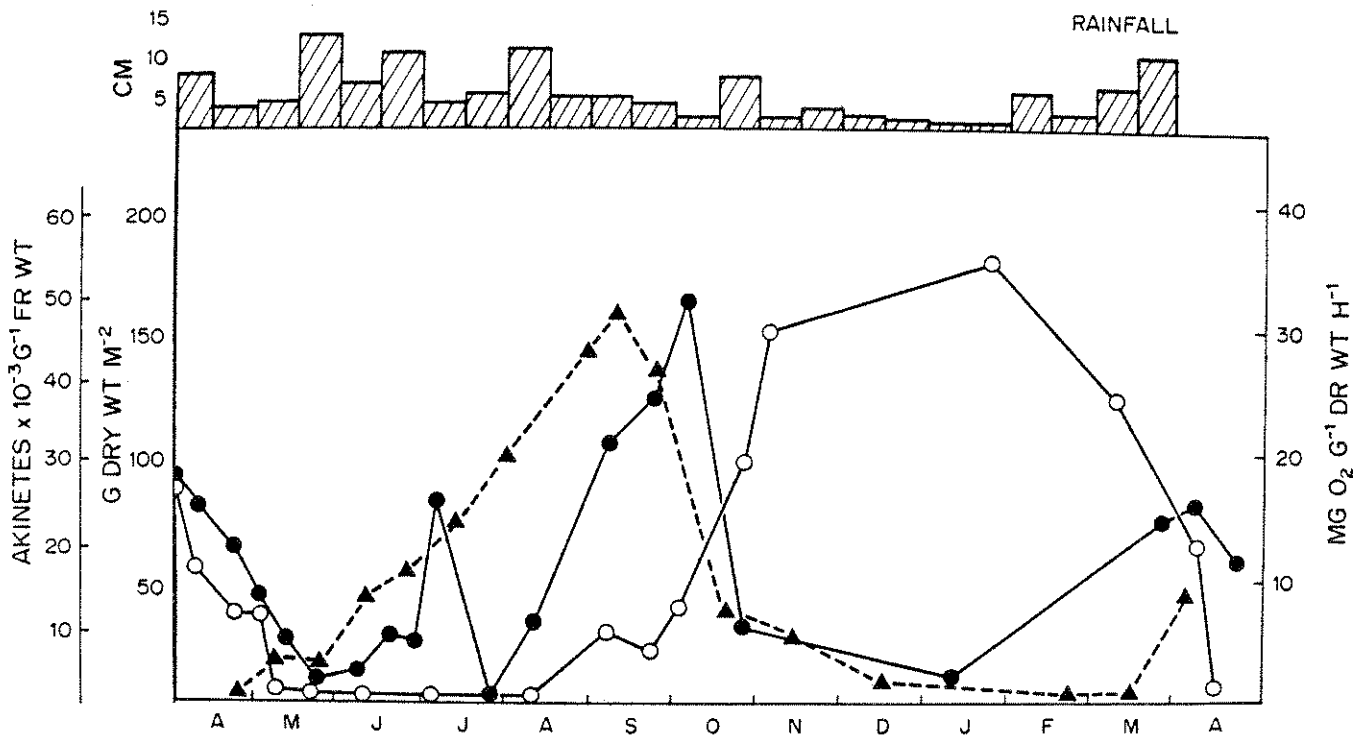

Figure 3B. P. oedogonia biomass (●—●), akinete numbers (○—○) and net photosynthesis rate (▲▪▪▪▲) in Surrey Lake in 1980.

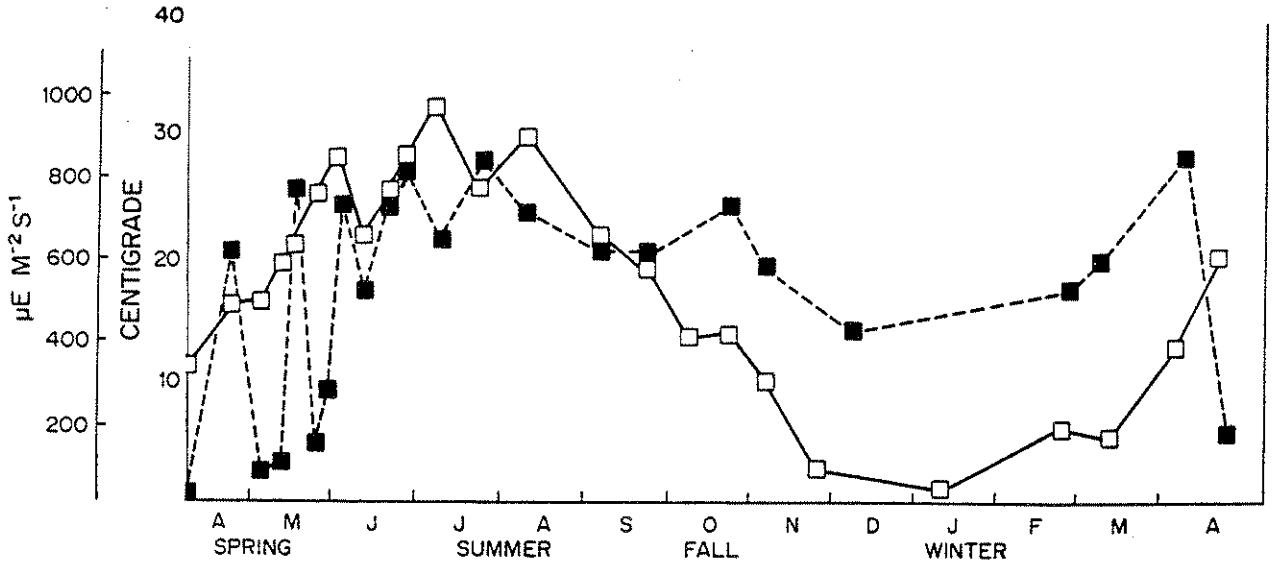

Figure 4B. Water temperature (□—□) and light intensity (■▪▪▪■) measurements at the 0.3 m depth in Surrey Lake in 1980.

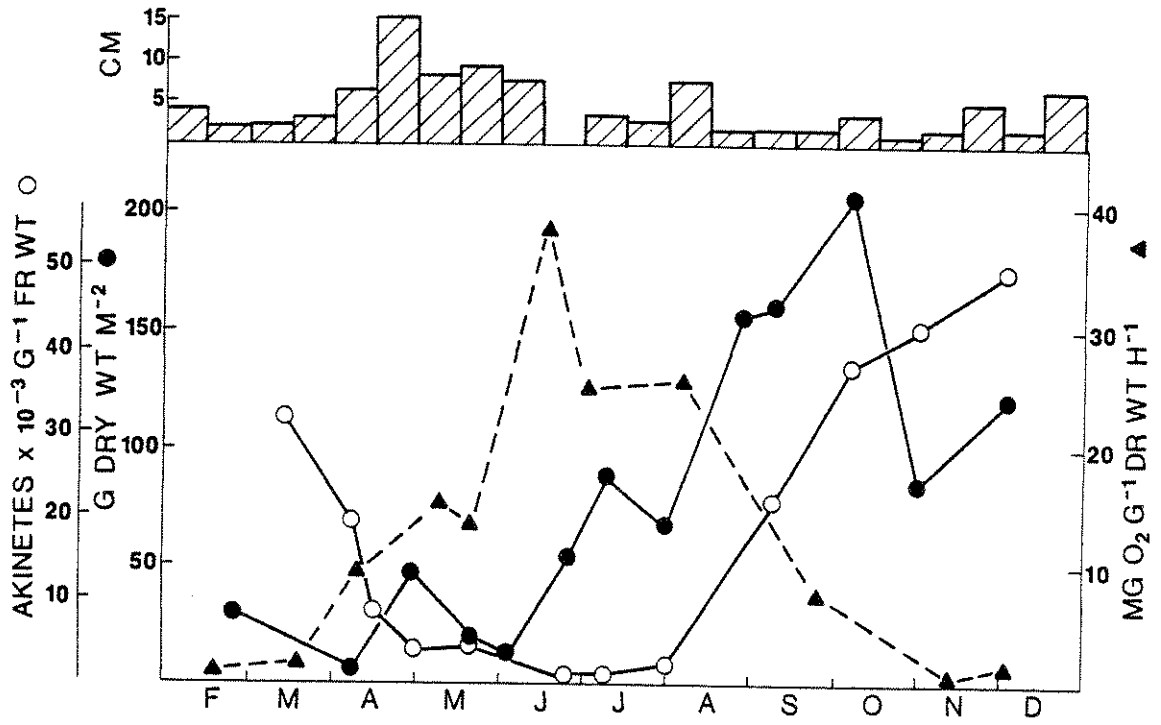

Figure 3C. P. oedogonia biomass (●—●), akinete numbers (○—○), and net photosynthesis rate (▲▪▪▪▲) in Surrey Lake in 1981.

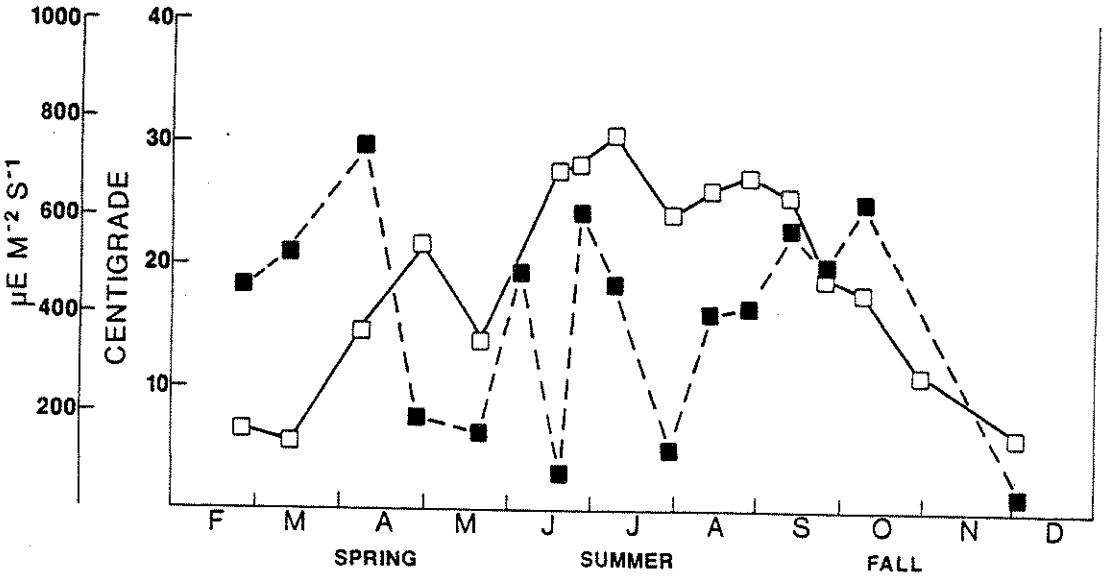

Figure 4C. Water temperature (□—□) and light intensity (■▪▪▪■) measurements at the 0.3 m depth in Surrey Lake in 1981.

placed on the free-floating mats. The last section will be devoted to sediment akinetes and their periodicity.

Winter (November through February). Free-floating mats of P. oedogonia overwinter both as vegetative filaments and akinetes. Akinete numbers in winter range from 30,000 to 50,000 g^{-1} fr wt and compose approximately 21% of the total mat biomass. This is in contrast to midsummer akinete numbers of 200 g^{-1} fr wt which account for only 0.8% of the biomass.

Overwintering akinetes and filaments brought into the laboratory and placed under optimal germination conditions of 20 C and an 18:6 L:D cycle germinate (and sprout in the case of the filaments) indicating that both forms are viable and can serve to reestablish the population in the spring. This observation disputes the theory that akinetes are formed specifically as an overwintering mechanism in temperate climates. Both akinetes (Lembi et al. 1980) and filaments (Table 1) can survive freezing in lake ice and show growth characteristics similar to those of nonfrozen akinetes and filaments collected at the same time.

Table 1. Growth parameters of Pithophora filaments collected in midwinter from Surrey Lake 7 days after transfer to 20C. Values given are $\bar{X} \pm$ SD.

	No. Branches mm^{-1}	Branch Length (mm)	Chlorophyll (% increase)
Nonfrozen filaments	.876 ± .22	9.6 ± 2.7	66
Frozen filaments	.880 ± 4.0	11.1 ± 3.5	54

Although algal biomass is at its lowest point in the winter, values of 10 to 50 g dry wt m^{-2} indicate the presence of a significant amount of algal material. These measurements are probably low in relation to potential biomass since Surrey Lake is susceptible to washout after heavy rain events. The mats maintain a low photosynthetic activity with O_2 evolution rates ranging from 0.91 to 2.33 mg O_2 g^{-1} dry wt h^{-1} as measured at various times in November through March of 1980 and 1981.

Spring (March through June). The three major events occurring in the spring period are 1.) the germination (and sprouting) of akinetes and filaments to produce new filaments (germ tubes) 2.) the rapid elongation of the germ tubes, and 3.) an increase in net photosynthesis rates.

Laboratory studies (Spencer and Lembi, 1981) show that under the water temperature regime, nutrient conditions and photoperiod of Surrey Lake in early spring, the primary factor regulating akinete germination is the increase in water temperature from 10 to 20 C. Analysis of field-collected mats shows a negative correlation ($r=-0.89$) between spring akinete numbers and water temperature. In all three study years, akinete numbers decreased during the period in which water temperatures were increasing from 10 to 20 C (early April to early May, 1979; early April to mid May, 1980; and early April to late April, 1981). Overwintering filaments appear to sprout at approximately the same time as the akinetes.

Following germination and sprouting, germ tubes are observed undergoing rapid elongation to form new filaments. Under laboratory conditions, elongation doubling times of 4 to 6 days for germ tubes have

been monitored (Fig. 14). This is in contrast to doubling times of at least 7 days for mature filaments placed in fresh medium.

Both the germination process and initial germ tube elongation are driven by respiration of internal storage materials rather than by photosynthesis. However, by 14 days after germination, photosynthesis rates increase in relation to respiration and the organism becomes photosynthetically competent (a more detailed description of germination and accompanying metabolic processes is presented in section IIA.). In the field, net photosynthesis rates increase during and/or following the akinete germination period and continue to increase throughout the spring period. Field respiration rates (data not shown) also increase during the germination period and may reflect both the increase in water temperature and the initial utilization of stored internal reserves during akinete germination.

Although physiologically active, the algal biomass in Surrey Lake remains low through the spring period. This is due to washout of the lake from heavy spring rains. The susceptibility of Surrey lake to washout is best illustrated in the bar graph associated with Figure 3A in which a heavy rain event in late July 1979 resulted in a precipitous loss in biomass. In lakes and ponds not subject to such severe washout, a significant increase in Pithophora biomass could presumably occur earlier in the year than it does in Surrey Lake.

Summer (July through September). Photosynthesis rates continue to increase through the late spring and summer. In 1981, photosynthesis rates peaked in mid-June (37 mg O_2 g^{-1} dry wt h^{-1}) but remained at elevated rates into August. Algal biomass peaks later than photosynthesis and its buildup tends to depend on rainfall patterns. Maximum values of

220, 160, and 210 g dry wt m^{-2} were all monitored in late September-early October of 1979, 1980, and 1981, respectively.

The two critical factors regulating growth of the free-floating mats in Surrey Lake are temperature and nutrient concentrations. Photosynthesis rates in Surrey are correlated with temperature (r=0.71) but not with light intensity (r=0.13). Laboratory studies also indicate a linear increase in photosynthesis rates with temperatures as high as 30 C (Fig. 5).

As reported in Lembi et al. (1980) and Spencer and Lembi (1981) laboratory studies of growth rates and nutrient concentrations at 20 C indicate K_s values of 88 uM NO_3 and 3.2 uM PO_4 with an N/P ratio of 27.6. Mean NO_3 and PO_4 concentrations in Surrey Lake from 1978 through 1980 were 88 uM and 7.0 uM, respectively, and appear to be sufficient to support growth in at least some portions of the lake.

Fall (September through November). The fall period is marked by a decline in photosynthetic rates and biomass and by the formation of akinetes. Cessation of growth is probably due to a decrease in water temperature and depleted nutrients although this aspect has not been completely elucidated. Loss in biomass is presumably due to a variety of factors including decomposition and lake washout from fall and winter rains.

The major stimulus for akinete formation appears to be an interaction between ambient PO_4 and NO_3 concentrations (Fig. 6). In laboratory studies, akinete formation occurs under conditions of low phosphate (0 to 0.93 uM) and intermediate nitrate (117 uM) and, to a lesser degree, under conditions of low nitrate (11.7 uM) and high phosphate (9.3 to 93 uM). Exposure of filaments to full strength medium (1171 uM NO_3 and 93 uM PO_4) under temperature and photoperiod conditions similar to those found

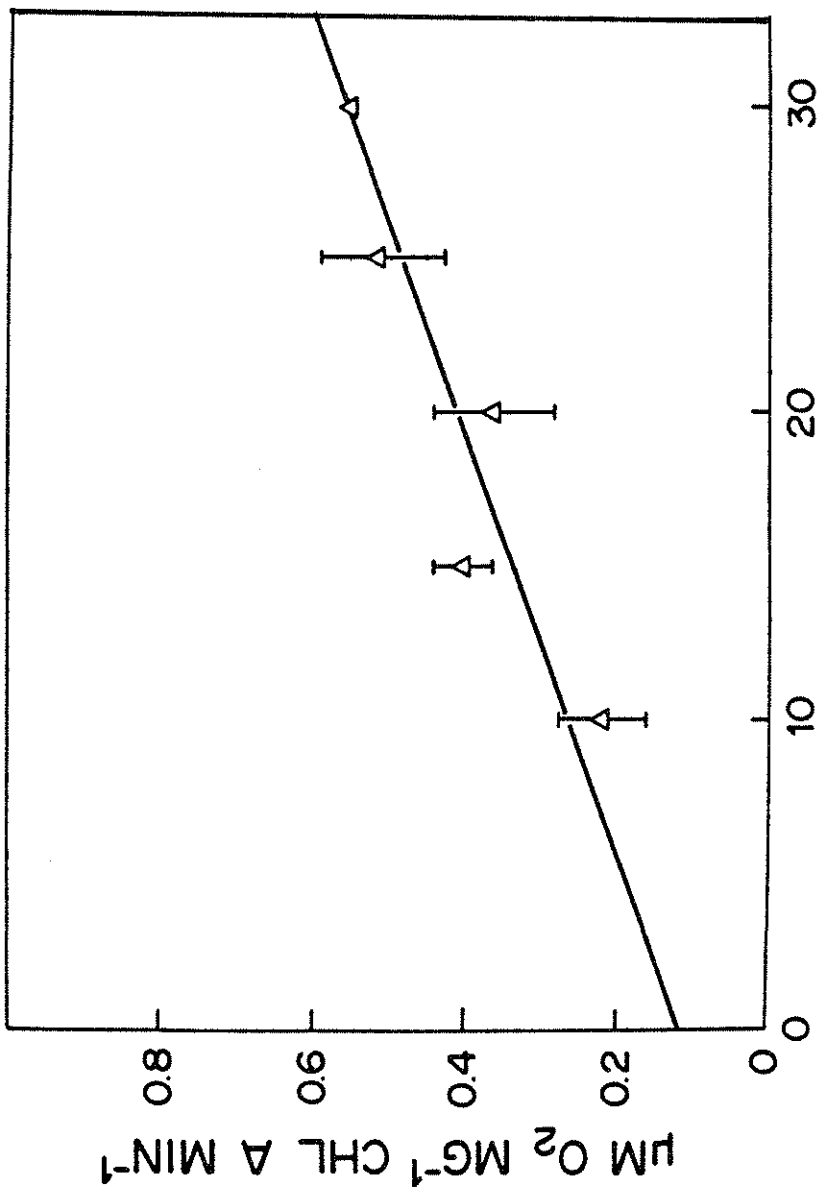

Figure 5. Relationship of P. oedogonia photosynthesis rates and water temperature.

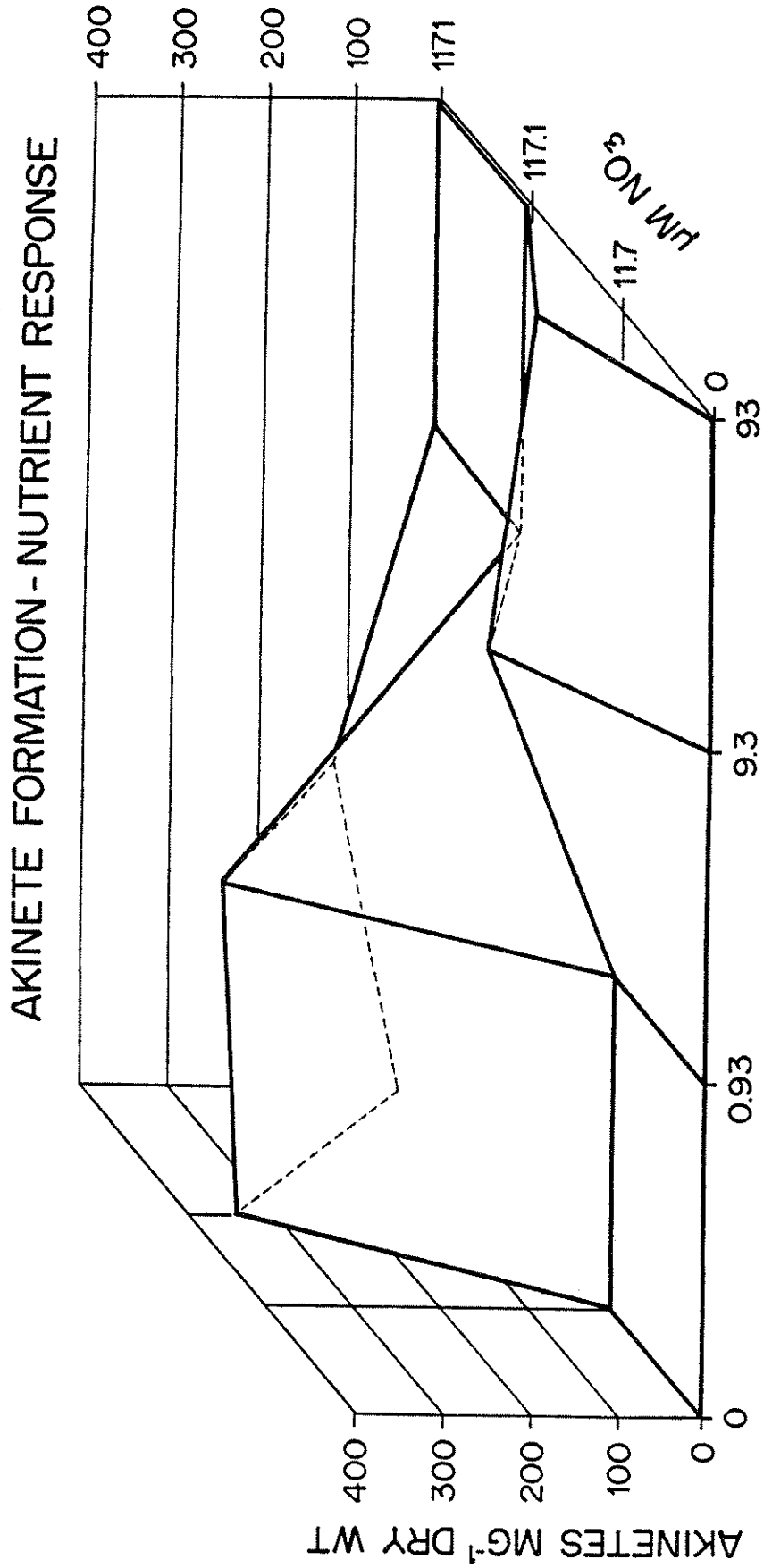

Figure 6. Effect of NO_3 and PO_4 concentrations on akinete formation in P. oedogonia.

in late fall (14 C and 10:14 L:D) does not appear to induce akinete formation. This supports the theory that akinetes form in response to nutrient depletion rather than to the onset of winter.

Germination of the akinetes is prevented during the winter by cold water temperatures. Replenishment of nutrients in the lake from spring rains plus the increase in water temperatures accounts for the germination of the akinetes the following spring.

Sediment akinetes. Overwintering akinetes are present in the sediments at densities as high as 15.2 X 10^6 m^{-2} in the upper cm and are 95 to 100% viable (Lembi et al. 1980). Akinetes in the sediments, like those in the free-floating mats, germinate in the spring. However, germination of sediment akinetes lags behind that of akinetes in free-floating mats by approximately a month (Fig. 7).

There is some evidence that a lag in sediment temperatures may be a cause in the delay in sediment akinete germination. In 1980, akinete germination in the free-floating mats was first observed to occur between March 25 and April 9. Germination was completed by May 28. Water temperatures rose from 9 to 24 C during this period. Sediment akinete germination first occurred between April 22 and May 2; germination was complete by early July. Temperatures in the upper cm of sediment at the time free-floating akinetes were germinating also increased from 9 to 24 C but averaged 16.6 C in comparison to an average of 17.7C for the water.

In 1981, akinete germination in the free floating mats was first observed between April 8 and April 15 with germination completed by April 28. Water temperatures increased from 13.1 to 20 C during this interval. Sediment akinete germination was first observed on April 15 and was not complete until July 3. Temperatures in the upper cm of sediment during the

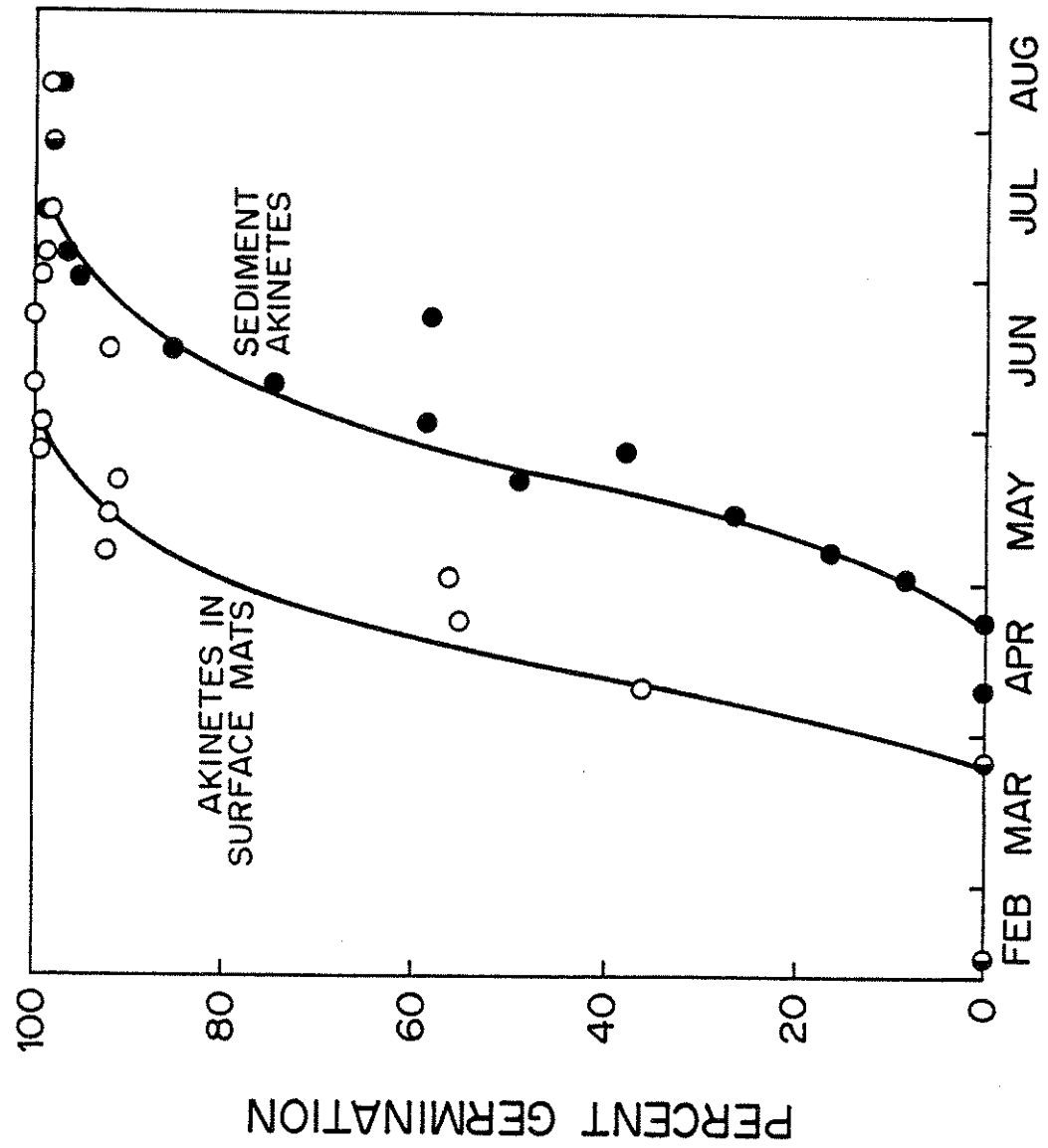

Figure 7. Germination of akinetes collected from free-floating mats and sediment in Surrey Lake in 1980.

period of free-floating akinete germination increased from 11.3 to 17.3 C from April 8 to April 28. Average sediment temperature during this period was 13.6 C in comparison to an average water temperature of 16 C.

Other possible causes for the lag in sediment akinete germination include the presence of chemical inhibitors in the sediments that must be leached out or degraded before germination can proceed, anaerobiosis of the sediments, or limiting light conditions.

Akinetes require oxygen for respiration of stored materials during germination and germ tube elongation since photosynthesis is inoperative during the early stages of germination (see Section IIA). Eh measurements indicated that both the upper cm of sediment and the water directly above it were oxidized during the period in which free-floating akinetes were germinating. This suggests that anaerobiosis was not operating as a deterrent to sediment akinete germination except perhaps in the deeper sediment layers.

There is also ample evidence that light is not a limiting factor. Neal and Herndon (1968) reported akinete germination could occur in darkness. Our laboratory studies indicate that akinetes germinate in blue light at an intensity as low as 0.15 uE m^{-2} sec^{-1}. Our studies also show that akinetes can germinate in the presence of a photosynthesis inhibitor (see Section IIA), another indication of the lack of dependence of akinetes on light in order for germination to proceed.

II. ALGICIDE SUSCEPTIBILITY

Algicides can provide excellent (albeit temporary) control of many nuisance algal species. Two of the most common algicides are simazine (sold as Aquazine®) and copper sulfate. Simazine has been registered for aquatic use since 1975 whereas copper sulfate has been used for algae

control since the early 1900's. Resistance of Pithophora to both compounds has been frequently observed (see below). Although other, more permanent methods of algal control such as nutrient control can and should be practiced, there is no doubt that the use of algicides for the immediate removal of algae will continue to be widespread. When "resistant" algal forms are encountered, the temptation to apply larger, more frequent doses of algicides is great and could result in potentially damaging environmental consequences. The following sections describe experiments intended to explain the basis for algicide resistance in Pithophora and to point the way to more effective and efficient control by integrating these compounds (particularly copper sulfate) into the life cycle of the alga.

II.A. Simazine

Simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) is a photosynthetic inhibitor. It specifically inhibits noncyclic electron transport in photosynthesis and causes chlorosis and necrosis in susceptible plant tissue (Moreland, 1980).

The compound is registered for use against a broad spectrum of aquatic weeds including filamentous algae. However, selectivity among algal groups has been noted. Ratings of field applications suggest that simazine is very effective on genera of blue-green algae (Cyanophyta) but has a lesser effect on genera of green algae (Chlorophyta) (Ellis et al., 1976). Selectivity also appears to exist among the green algae. Ramirez-Torres and O'Flaherty (1976) reported that simazine concentrations of 5 uM inhibited chlorophyll production 42% in the unicellular green alga Chlorella vulgaris but had no effect on the filamentous green alga Stigeoclonium tenue. The same concentration inhibited chlorophyll production in the blue-green alga Oscillatoria lutea by 74%.

Reports from commercial applicators of aquatic herbicides in the midwest (personal communications) indicate a general inconsistency in control of green filamentous algae with simazine and a frequent lack of control of Pithophora. The Aquazine label suggests that higher concentrations of the product may be required for control of Pithophora and Cladophora but inconsistency of control is noted even at these higher dosages.

At least one stage in the life cycle of Pithophora, that of the akinete and/or germinating akinete, could be resistant to simazine. This would be true if, during germination, the organism relies on the utilization of internal food reserves rather than on photosynthesis. As noted in previous sections, little is known of the role of the akinete in the survival of the alga. Even less is known regarding the physiological processes that accompany germination in green algal akinetes. Thus, the first portion of this section will describe the changes in various physiological parameters that occur during akinete germination and the effects of simazine on the germination process. The second portion will deal with the effect of simazine on mature filaments of Pithophora and other green filamentous species.

II. A.1. Physiological Changes during Germination of Pithophora oedogonia Akinetes

Materials and Methods

Stock cultures of Pithophora oedogonia were maintained as described in Section I. Akinetes began to form in 3 to 4 week old cultures and comprised approximately 90% of the biomass after 2 to 4 months. Akinete cultures were stored at 4 C in the dark for at least one week prior to use. Sterile culture procedures were used in handling stock and experimental cultures.

Cultures were not axenic but microscopic examination did not reveal extensive bacterial growth. Filaments were rinsed in sterile medium prior to use in experiments.

Determination of photosynthetic and respiratory rates. Individual clumps of filaments composed of akinetes were transferred to 24 erlenmyer flasks (500 ml) containing 300 ml of fresh Cl(II) medium and allowed to germinate under standard conditions. At 6 intervals (0, 1, 3, 6, 12, 21 days) during germination, 4 flasks were harvested and samples (10 $\pm$ 3.6 ug Chl a n = 24) were taken for rate determinations. After 8 days the remaining cultures were transferred into fresh medium to prevent nutrient limitation. Rates of photosynthesis and respiration were measured as the production and consumption of oxygen (u mol mg^{-1}chl a min^{-1}) with a YSI Model 53 Biological Oxygen Monitor. The oxygen electrode was calibrated with an air saturated sample of Cl(II) medium at the determination temperature of 25 $\pm$ .1 C. Prior to rate determinations a sample of Cl(II) medium was bubbled with N_2 to reduce the oxygen content. Photosynthetic rates of the algal clumps were measured in the reduced oxygen medium at a light intensity of 100 uEinsteins m^{-2} s^{-1} (PAR) provided by a GE infrared flood lamp. Respiration rates were measured in the dark immediately following photosynthetic determinations.

Chlorophyll was extracted by grinding samples in 5 ml of 90% acetone (4 C) and quantified (ug $sample^{-1}$) according to the method of Wetzel and Westlake (1969). In addition, chlorophyll content of the filaments on a unit dry weight basis (ug chl a mg^{-1}dry wt.) was determined by blotting the clumps dry and taking the fresh weight prior to extraction. Replicate portions of the filaments were weighed (fresh wt.) and dried at 60 C. After weighing to determine dry weights, the proportion of dry

matter in the replicate clumps was calculated. The proportion of dry matter was used to convert the fresh weights of the extracted filaments to dry weights.

Determination of storage compounds. Individual clumps of filaments composed of akinetes were transferred to 15 erlenmyer flasks (500 ml) containing 300 ml of fresh C1(II) medium and allowed to germinate under standard conditions. At intervals (0, 3, 6, 12, 21 days) during germination 3 cultures were harvested for analysis. After 8 days the remaining cultures were transferred into fresh medium to avoid nutrient limitation. Harvested akinete material was dried (60 C) and ground individually with a mortar and pestle. Samples of ground material were weighed (approximately 10 mg) and analyzed for starch, lipid and protein. Starch and protein samples were washed repeatedly with warm 80% ethanol to remove chlorophyll and soluble sugars (Jensen, 1962). Starch was extracted from the washed sample with 29% perchloric acid (PCA) at 4 C (Jensen, 1962) and quantified by the phenol-sulfuric acid method (Kochert, 1978a). Protein was extracted with 1N NaOH at 100 C (Kochert, 1978b) and quantified by the coomassie brilliant blue dye binding method (Kochert, 1978c). Lipid was determined by the dichromate reduction method (Kochert, 1978b).

Effects of simazine. Individual akinetes were isolated by disrupting filaments for 60 s in 100 ml of water with a Waring blender. Using a finely drawn out pasteur pipet, 20 akinetes were dispensed into each of 8 petri dishes (100 mm x 20 mm) containing 50 ml of C1(II) medium. Four dishes were treated with simazine (25 uM) with the remaining dishes acting as controls. Both treatment and control dishes contained 0.5% DMSO. No effect of DMSO on the germination of the akinetes was noted. The akinetes were incubated under standard conditions and determinations were made at

intervals during germination for percent germination and germination tube length. A dissecting microscope equipped with an ocular micrometer was used to take the measurements. Fresh medium containing the appropriate treatment compound was supplied after 17 days to avoid nutrient limitation.

Effects of cyanide. The effect of cyanide, a respiratory inhibitor, on the germination of Pithophora akinetes was investigated and compared with simazine using akinetes isolated as described above. Akinetes were uniformly suspended in 75 ml of C1(II) medium by constant stirring. One ml aliquots of akinete suspension were pipetted into each of 12 screw top vials (20 ml) containing 10 ml of either a) C1(II) medium (controls), b) 20 uM KCN in C1(II), c) 20 uM KCN plus 20 uM simazine in C1(II), or d) 20 uM simazine in C1(II). Replicate 1 ml aliquots of akinete suspension were analyzed for chlorophyll to give the initial biomass inoculated. The tubes were capped tightly to prevent loss of CN by volatilization (Yu et al. 1981) and incubated under standard conditions. At 10 day intervals 4 tubes were harvested and the medium in the remaining tubes replaced with fresh medium containing the appropriate treatment compound(s). Changes in total chlorophyll in the tubes was measured after 10, 20 and 30 days. In addition, one of the four tubes from each treatment on days 10 and 20 was examined to determine percent germination and length of germination tubes of randomly selected akinetes.

Statistical analysis. Data sets were analyzed by either the Student's two sample t-test or a one way ANOVA and Student Newman Keuls multiple range test. The level of significance in all analyses was $P = .05$.

Results

When transferred to germinating conditions, Pithophora akinetes underwent a two day lag period during which no visible changes occurred (Fig. 8A). Germination tubes first appeared after three days (Fig. 8B) and most akinetes germinated by day-6. Between day-6 and day-14 germination tubes elongated rapidly (Fig. 8C). Elongation of germination tubes continued and was accompanied by cell and branch formation around day-21 (Fig. 8D).

Initial rates of apparent photosynthesis and respiration in Pithophora akinetes were low (Fig. 9) and not significantly different from each other. At the end of day-1 respiration rates increased by 110% and remained at this elevated level for the next 12 days. After 13 days respiration rates declined to a level on day-21 that was similar to the low initial rate. Photosynthetic rates remained at the low initial level for the first three days. On day-6 photosynthetic rates began to increase and reached the highest measured rate on day-21 that was 290% greater than the initial rate.

The ratio of apparent photosynthesis to respiration (P/R) did not change significantly during the first 13 days of germination remaining around one (Fig. 10). However, the P/R ratio less than one that occurred around day-3 was real, since respiration rates were significantly higher than rates of photosynthesis at this time. Between day-13 and 21, P/R ratios increased rapidly ($P<.05$).

Chlorophyll content in ungerminated akinetes averaged 2.9 ug chl a$\cdot mg^{-1}$ wt (Fig. 11). No change occurred until day-6 ($P>.05$) when chlorophyll content began to increase. A maximum chlorophyll content of 9.9 ug chl a mg^{-1}dry wt was recorded on day-13 ($P<.05$). Water content of

Figure 8A-D. Sequence of visual changes during germination of Pithophora oedogonia akinetes A. Ungerminated akinetes. Scale = 100 um. B. Protrusion of germination tube (arrow) after four days. Scale = 100 um. C. Germination tube during elongation (day-9). Scale = 500 um. D. Formation of primary branch (arrows) after 20 days. Scale = 500 um.

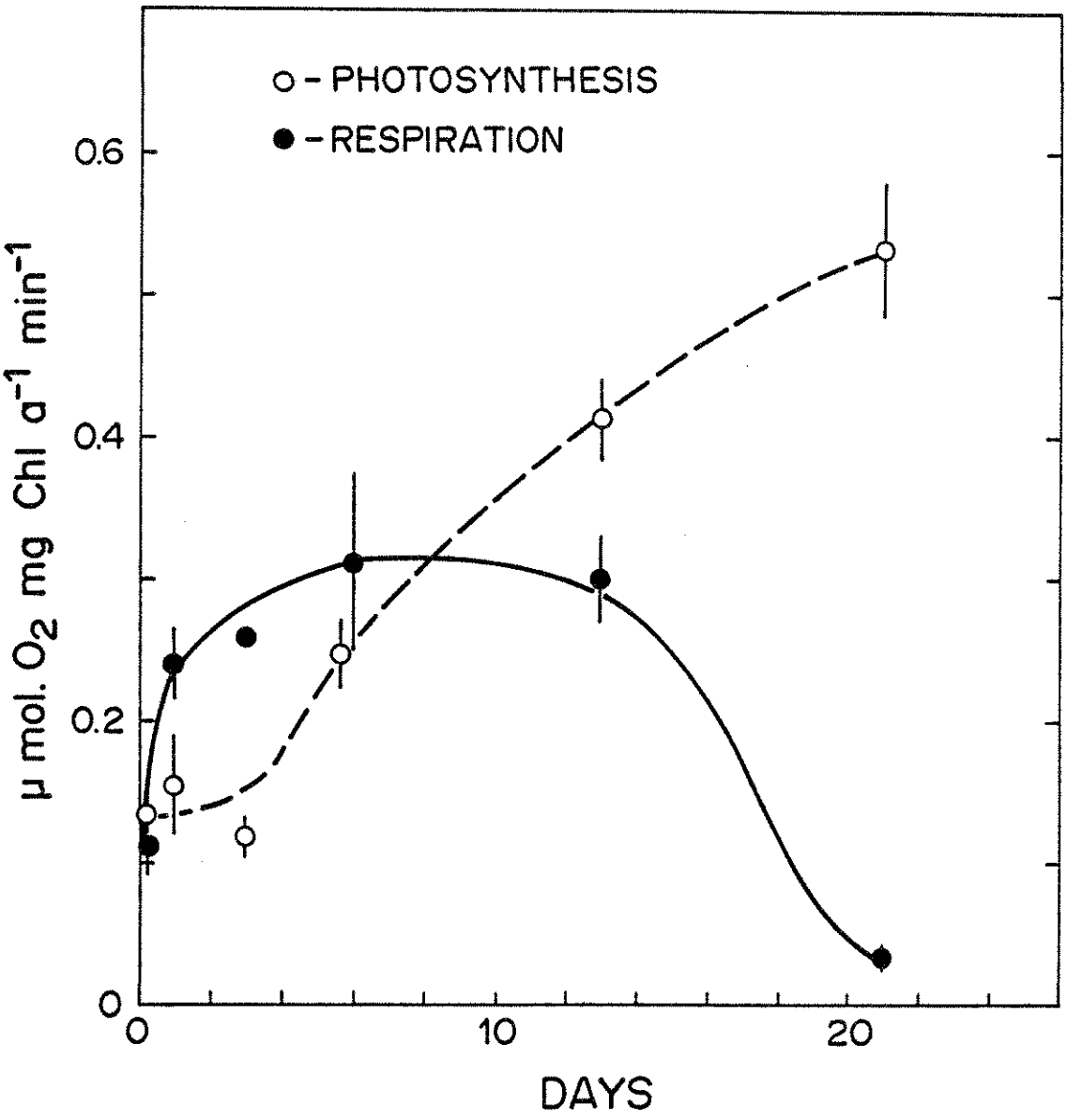

Figure 9. Rates of photosynthesis and respiration during germination of P. oedogonia akinetes. Values are $\bar{X}$ + 1 SE (bars), N = 4.

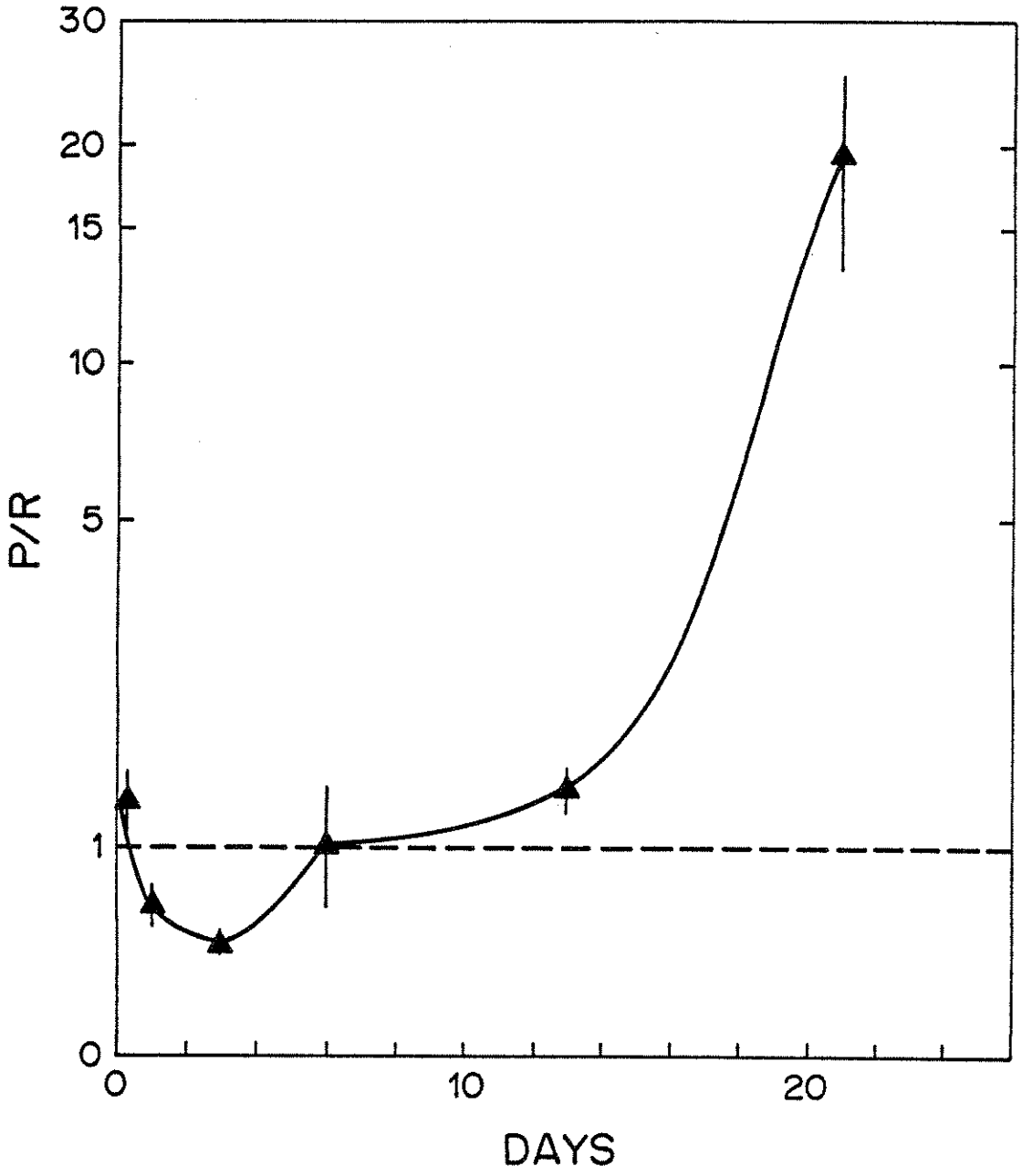

Figure 10. Changes in ratio of photosynthesis to respiration (P/R) during germination of P. oedogonia akinetes. Values are $\bar{X}$ + 1 SE (bars), N = 4.

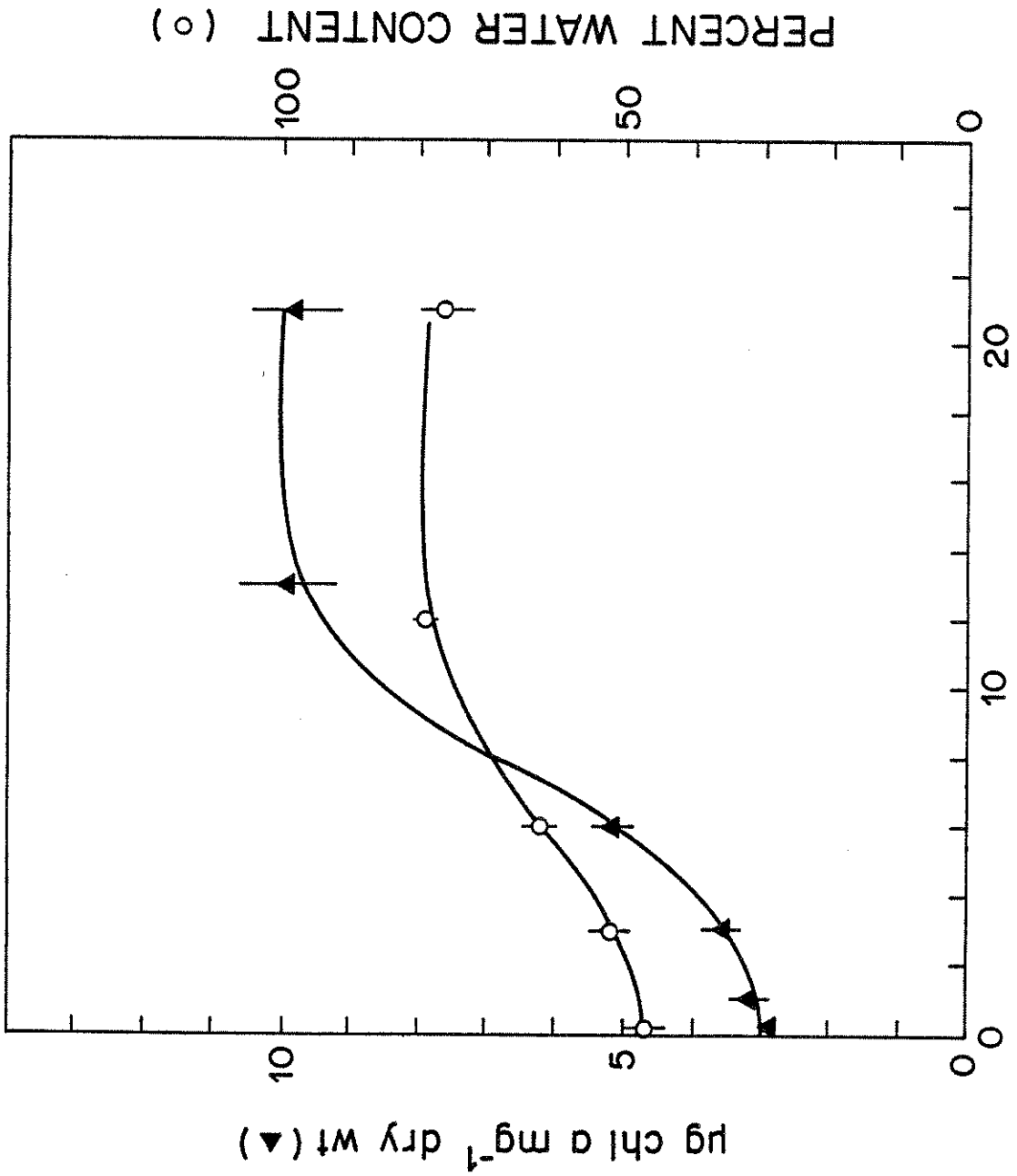

Figure 11. Changes in chlorophyll and water content during germination of P. oedogonia akinetes. Values are $\bar{X}$ + 1 SE (bars); N = 4.

ungerminated akinetes was 47% (Fig. 11). Imbibition began around day-6 and was completed by day-13 when water content reached 79% ($P<.05$).

Initially akinetes contained 330 ug starch mg^{-1}dry wt., 150 ug lipids mg^{-1}dry wt., and 30 ug protein mg^{-1}dry wt. (Fig. 12). The initial levels of starch and lipid were maintained through the first six days of germination ($P>.05$). Starch and lipid content fell 40 and 35%, respectively, between days 6 and 12 ($P<.05$). No further decrease in the content of these constituents was detected after day-12. Protein content remained constant until day-22 when a 27% increase was detected ($P<.05$).

Simazine did not prevent germination of Pithophora akinetes (Fig. 13). Both control and simazine treated akinetes germinated rapidly between days 3 and 6. In fact, simazine stimulated protrusion of germination tubes. All simazine treated akinetes germinated by day-10 but control akinetes did not reach 100% germination until day-14.

A similar stimulatory effect of simazine was observed on the initial elongation of the germination tube (Fig. 13). After 10 days, germination tubes of simazine treated akinetes averaged 900 um longer than the controls ($P<.01$). After day-14, simazine inhibited further elongation of germination tubes relative to controls ($P<.05$). Maximum length reached in the presence of simazine was 5430 ± 1400 um ($\bar{X} \pm SD$, $n = 54$) compared to 7750 ± 2745 um ($\bar{X} \pm SD$, $n = 26$) in controls.

Akinetes germinated in the presence of both KCN (42% on day-20) and KCN plus simazine (56% on day-20); however, elongation of germination tubes was severely inhibited in these treatments (Fig. 14). Mean germination tube lengths of akinetes treated with KCN and KCN plus simazine were 135 um and 154 um, respectively, on day-20, compared with 2246 um for simazine treated and 10778 for control akinetes ($P<.05$). In this experiment, elongation in

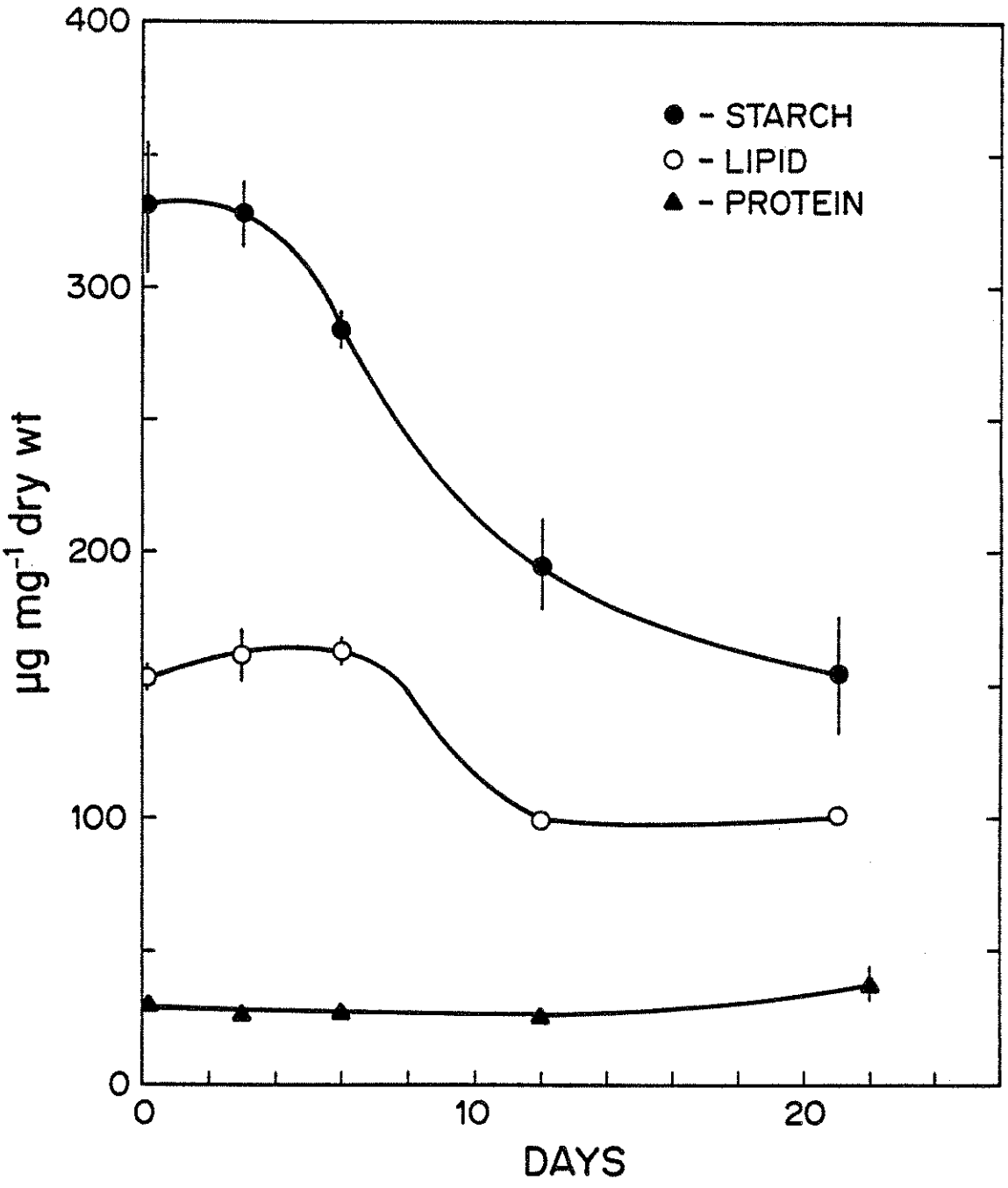

Figure 12. Changes in starch, lipid, and protein content during germination of P. oedogonia akinetes. Values are X ± 1 SE (bars); N = 4.

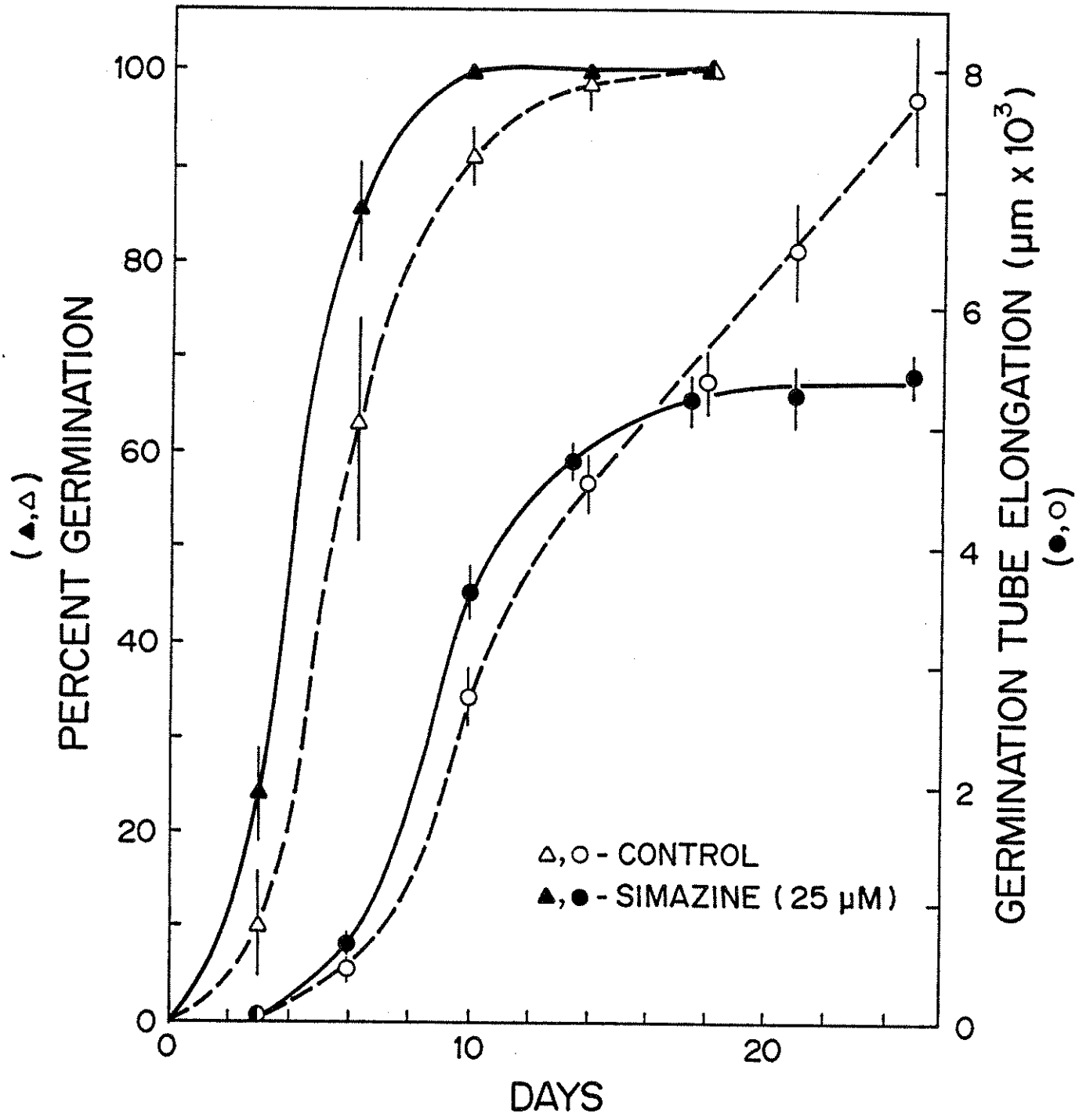

Figure 13. Effect of 25 uM simazine on germination (triangles) and elongation (circles) of germination tubes of P. oedogonia akinetes. (Controls are open symbols, simazine treatments are closed symbols). Values are $\bar{X} \pm 1$ SE (bars).

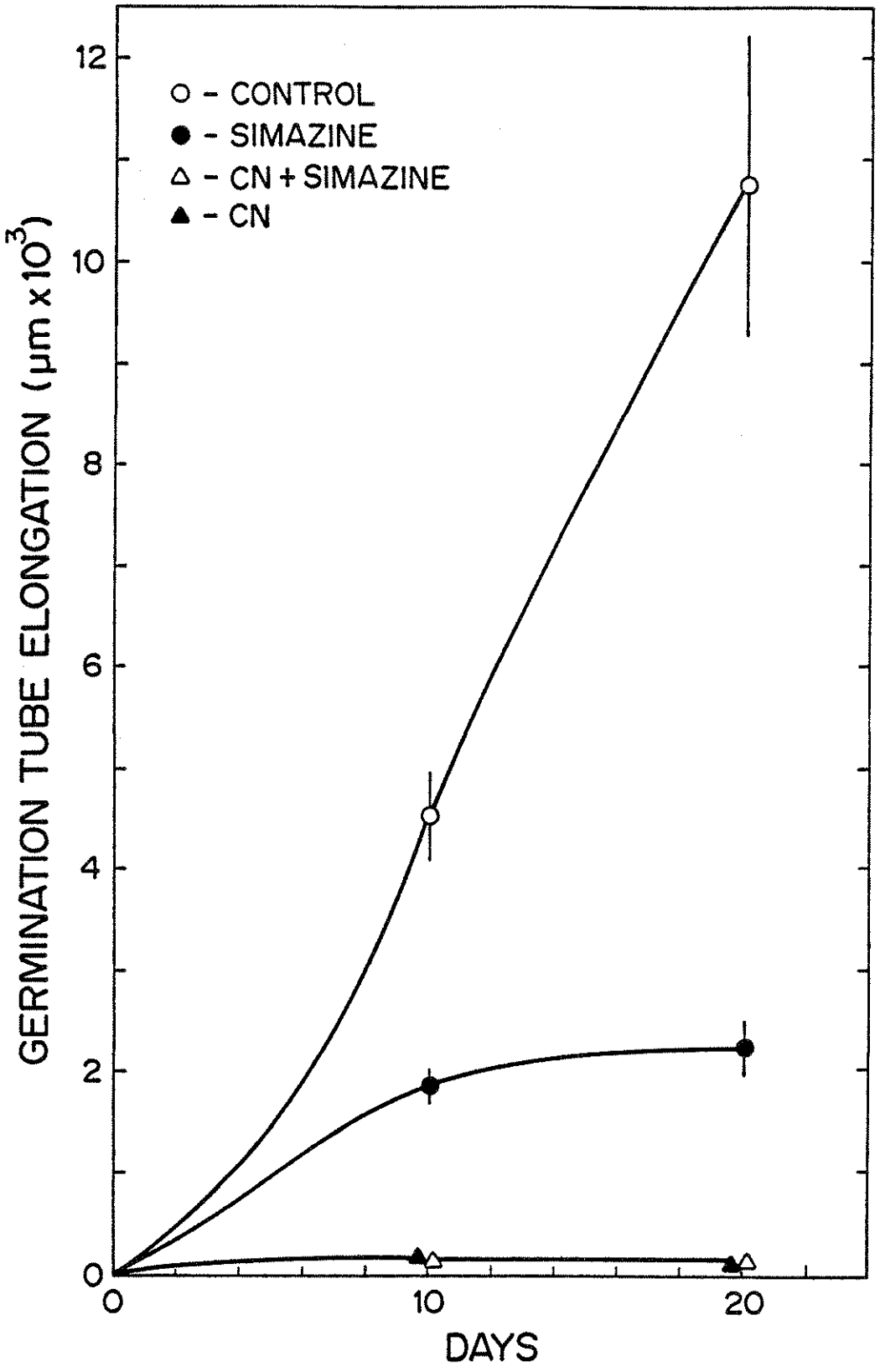

Figure 14. Germination tube elongation in response to cyanide, cyanide + simazine, and simazine treatments. Values are $\bar{X}$ + 1 SE (bars).

the simazine treatment was inhibited after 10 days. Germination tube elongation in the simazine treatment was less than in the previous experiment and was probably attributable to lower respiration rates caused by low oxygen concentrations in the sealed tubes.

Changes in total chlorophyll also indicated that KCN strongly inhibited germination tube elongation (Fig. 15). Chlorophyll in control and simazine treatments increased by similar amounts during the first 10 days ($P<.05$). After 20 days, the chlorophyll increase in simazine treated cultures had slowed relative to controls ($P<.05$) and showed little change after 30 days ($P>.05$). Chlorophyll in KCN and KCN plus simazine treated cultures remained at the initial level for 20 days ($P>.05$). After 30 days these cultures exhibited a significant decline in chlorophyll indicating that death was occurring.

Discussion

The germination process in akinetes of Pithophora oedogonia involves progressive physiological changes that result in a shift from metabolism of internal food reserves to photoautotrophic assimilation. Germination can be divided into four phases based upon observed changes (Fig. 16).

Phase I encompasses approximately the first 24 hours of germination and is characterized by a rapid rise in respiratory rates. This increase is probably analogous to the initial respiratory rise in germinating seeds which is attributed to the activation of mitochondrial enzymes associated with the citric acid cycle and electron transport (Bewley and Black, 1978). Phase II (approximately day-2 to day-6) is marked by protrusion of the germination tube. High respiratory rates, low photosynthetic rates, and P/R ratios less than 1 indicate that metabolism of internal reserves supports germination during this phase. However, lipid and starch content

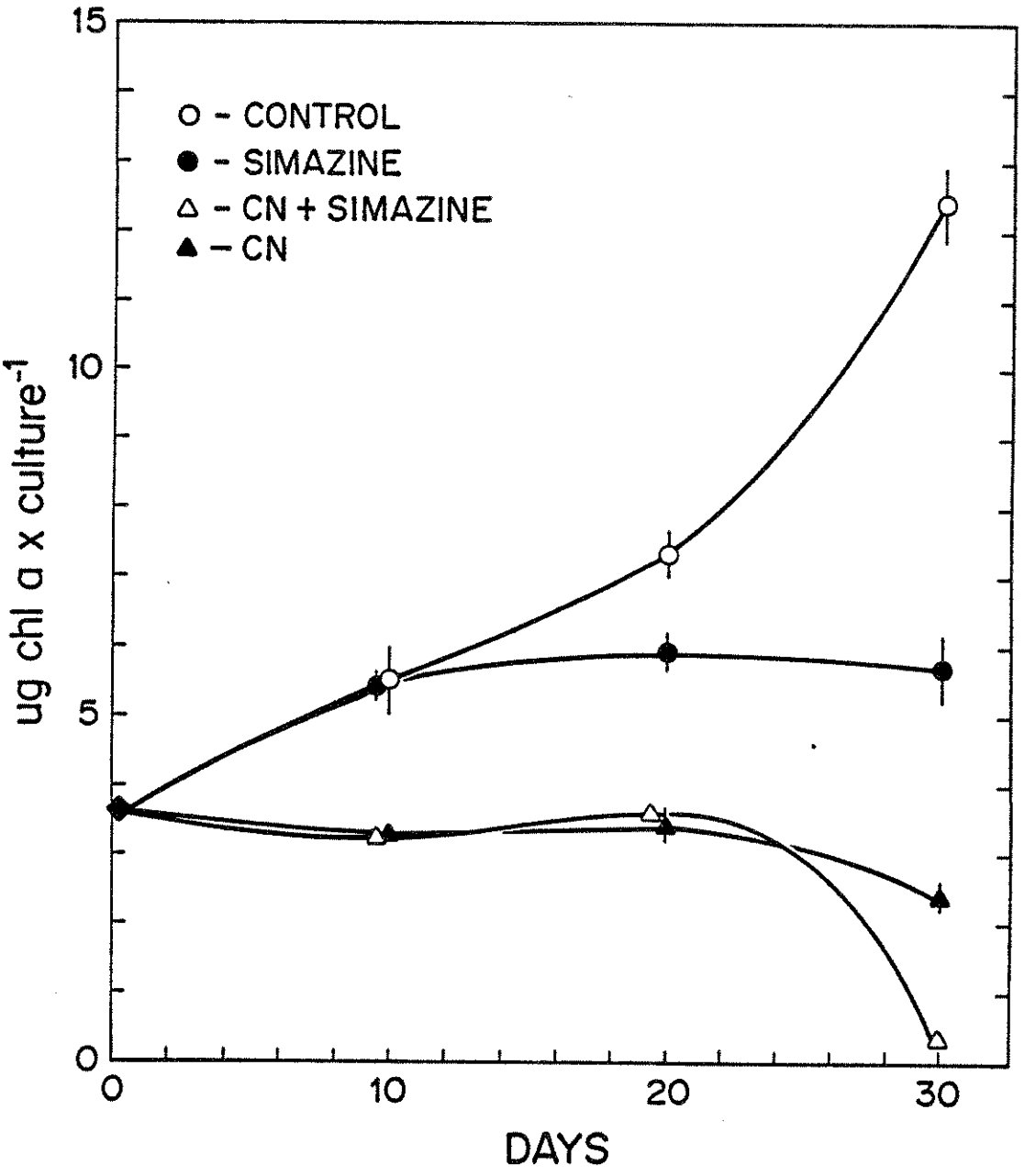

Figure 15. Biomass (chl a) changes in cultures of akinetes in response to cyanide, cyanide + simazine, and simazine treatments. Values are $\bar{X} \pm 1$ SE (bars), N = 4.

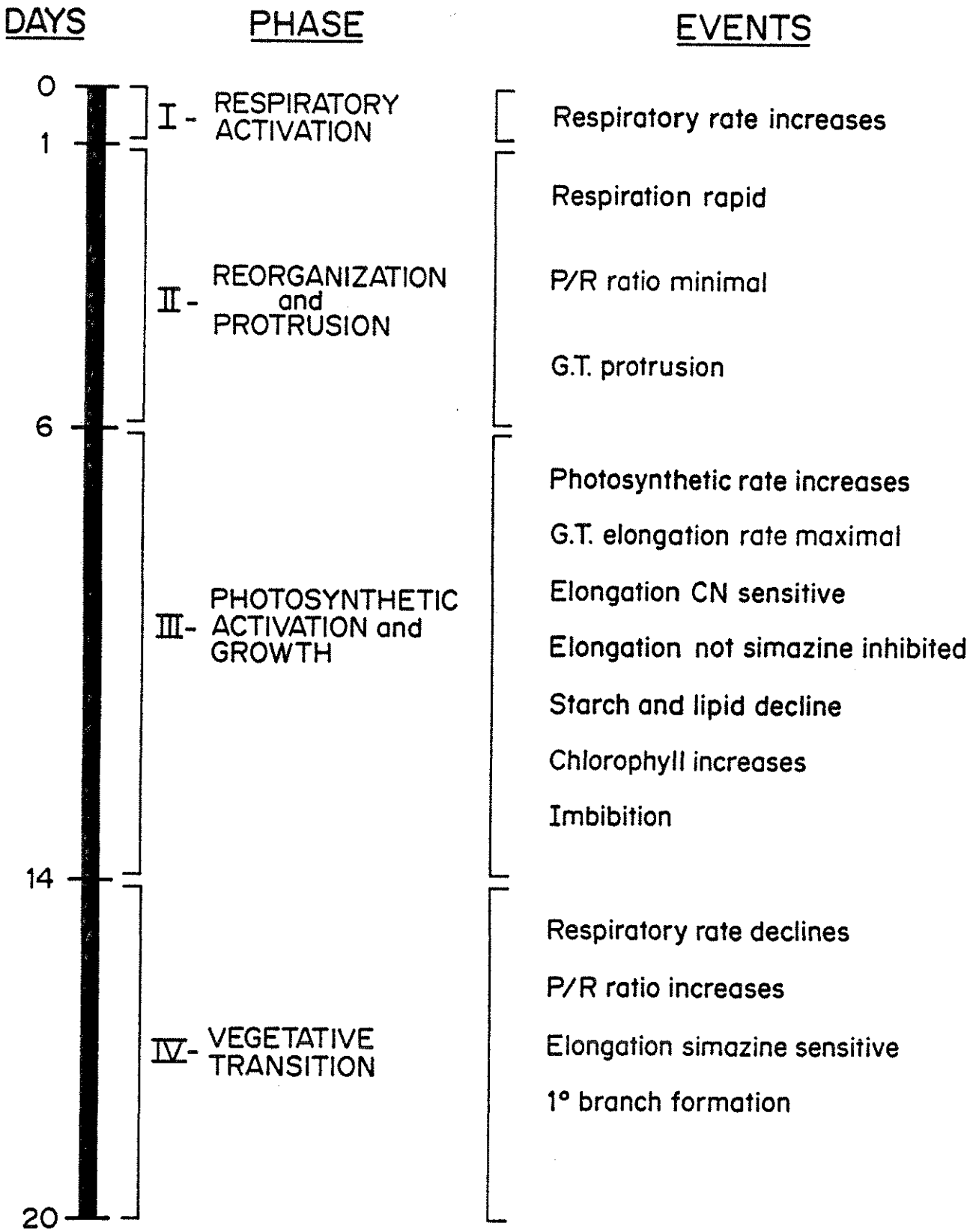

Figure 16. Summary diagram showing the approximate time course of events in the germination of Pithophora akinetes (20 C; 15 u einsteins m^{-2} s^{-1}; 18:6, L:D).

was not significantly reduced during this period suggesting that other reserve compounds, such as soluble sugars, were being respired. Since cyanide did not prevent protrusion of the germination tube, a cyanide-resistant respiratory pathway may be involved during early stages of germination (Hommersand and Thimann, 1965, Yentur and Leopold, 1976). Phase II appears to be a period during which cellular systems are reorganized in the process of forming the germination tube.

Rapid elongation of the germination tube during phase III (approximately day-7 to day-14) was not inhibited by simazine but was sensitive to cyanide. Lipid and starch content declined during this period indicating that utilization of internal reserves supported germination tube elongation. General activation of cellular processes is also indicated by imbibition during this period. Initial activation of photosynthetic pathways as indicated by increasing photosynthetic rates and chlorophyll content also occurred during phase III. Inhibition of photosynthesis by simazine stimulated rather than inhibited elongation of the germination tube during this period (Fig. 14). Blockage of photosystem II by simazine may prevent the further formation of photosynthetic enzymes and chloroplast membranes resulting in diversion of reserve material from the activation of photosynthesis to the elongation process.

Phase IV of the germination process (approximately day-15 to day-20) is marked by the transition from metabolism of internal reserves to photoautotrophic metabolism. The P/R ratio increased sharply during this period due to the decline of respiratory rates and a continual increase in photosynthetic rates. Elongation of the germination tube during phase IV was sensitive to simazine indicating that respiration of internal reserves was no longer capable of supporting further development. Starch and lipid

content stabilized during this period as respiratory demands on reserves were reduced. The formation of primary branches around day-20 probably marks the beginning of the vegetative portion of the life cycle.

The respiratory dependent germination process of Pithophora oedogonia akinetes is advantageous to the alga in several ways. It permits germination to occur under conditions that are suboptimal for photoautotrophic metabolism, e.g., in the sediments and within the free-floating mats. Light is likely to be restricted in both sediment and mat microenvironments. Gordon et al. (1980) reported that light intensity reached the compensation point of photosynthesis within 10 mm of the surface of mats of a marine species of Cladophora. A similar light attentuation with respect to photosynthesis was found in mats of P. oedogonia (Fig. 20). Utilization of starch and lipid reserves permits Pithophora akinetes to produce germination tubes in excess of 5 mm. This should allow a larger portion of the akinete populations within algal mats and the upper hydrosoil to reach areas more favorable for photosynthesis and continued growth. The end result would be a greater biomass contribution by akinetes to the Pithophora standing crop.

The other advantage of respiratory dependent germination is that Pithophora akinetes are not inhibited by simazine treatment during the first 14 days of germination. In fact, our data indicates that germination rates may actually be enhanced by simazine treatment. In the field, akinete germination occurs in the spring as water temperatures increase to 20 C. This is also the period when most applications of aquatic herbicides and algicides are made (most labels recommend treatment at water temperatures bewtween 18 and 21 C). Thus, at least a portion of the overwintering population of Pithophora may be protected from the phytotoxic

effects of simazine when the compound is applied during the germination period.

II.A.2. Effects of Simazine on Mature Filaments of *Pithophora* and Other Filamentous Algae

The purpose of this portion of the study was to investigate the effect of simazine on photosynthesis of mature filaments of *Pithophora oedogonia* and two other species of mat-forming, filamentous chlorophytes, *Cladophora glomerata* and *Spirogyra jurgensii*. A non-filamentous species (*Ankistrodesmus braunii*) was also studied. These algal genera were selected for comparative purposes because Ellis et al. (1976) reported that visual ratings of lake applications of simazine indicated *Spirogyra* was "very sensitive", *Pithophora* and *Cladophora* were "sensitive" and *Ankistrodesmus* was "resistant" to this herbicide. Effects of recommended application rates of simazine on growth was determined for the filamentous species and on effort was made to distinguish between growth inhibitory (algistatic) and phytotoxic (algicidal) effects. The role of the filamentous algal habitat in modifying simazine effects was also studied.

Materials and Methods

Isolation of the strain of *Pithophora oedogonia* used in the present study was described in Section I. Cultures of *Cladophora glomerata* were obtained from J. P. Hoffman, University of Wisconsin. Cultures of *Spirogyra jurgensii* and *Ankistrodesmus braunii* were obtained from the Culture Collection of Algae at the University of Texas at Austin. All stock cultures were maintained as described in Section I. Algal cultures were not axenic but microscopic examination did not reveal extensive bacterial growth. Algal filaments were rinsed in sterile medium prior to use in experiments.

Photosynthesis inhibition by simazine. Small clumps of filamentous algae were isolated from stock cultures and placed individually into the sample chamber of a YSI Model 53 Biological Oxygen Monitor. The sample chamber was filled with 5 ml of C1(II) medium previously bubbled with N_2 to reduce the oxygen content. Ankistrodesmus was tested by pipetting 5 ml of a stock culture into the sample chamber. Simazine dissolved in dimethylsulfoxide (DMSO) was injected into the sample chamber with a microsyringe to provide a range of simazine concentrations (0.5 to 5.0 uM). Concentrations of DMSO did not exceed 0.5 percent of the resulting solution. The effect of 0.5 percent DMSO on algal photosynthesis was tested and was not significant. The oxygen electrode was calibrated with an air-saturated sample of C1(II) medium. Photosynthesis rates (umoles O_2 mg^{-1}chl a min^{-1}) were measured before and after simazine injection at a temperature of 25 $\pm$ 0.1 C and light intensity of 100 uE m^{-2} s^{-1} (PAR). Four replicates were evaluated at each simazine concentration for each of the algal species. Percent inhibition of photosynthesis was plotted against simazine concentration. Data on percent inhibition were transformed ($\arcsin\sqrt{x}$) and fitted by linear regressions forced through the origin. Slopes of the regression lines were compared by analysis of covariance at a significance level of $P = 0.05$. I_{50} concentrations and their associated 95 percent confidence intervals were derived from the regressions.

Growth inhibition and development of phytotoxic symptoms. Clumps of the filamentous algae were inoculated into 250 ml flasks containing 150 ml of modified C1(II) medium. Ankistrodesmus was not evaluated in these experiments. Half of the flasks were treated with simazine dissolved in 0.75 ml of DMSO. The resulting simazine concentration of 5 uM was

approximately the application rate recommended for use in lake treatments. The remaining flasks were treated with 0.75 ml of DMSO and acted as controls. Care was taken to obtain equal size inocula by visually estimating the size of algal clumps placed in ceramic spot plates. Initial biomass inoculated was quantified by chlorophyll extraction of six to eight replicate clumps. Chlorophyll a was measured according to the method of Wetzel and Westlake (20). Simazine treated and control flasks were incubated at 20 ±1 C and three light intensities (15, 100, 400 uE m^{-2}). Four simazine treated and four control flasks were harvested and chlorophyll a content measured at 15 day intervals for 45 days. An additional two flasks subjected to simazine for 45 days were transferred to simazine-free medium to observe possible recovery. Growth experiments for each alga were duplicated at each light intensity. Treated cultures were visually monitored for development of phytotoxic symptoms (chlorosis and necrosis). Changes in chlorophyll a were used as an index of algal growth in response to the herbicide (Ashton et al., 1966; Ramirez-Torres and O'Flaherty, 1976). Chlorophyll a content over time was subjected to analysis of variance and Student-Newman-Kuels multiple range test ($P = 0.05$). Mean chlorophyll-a levels were converted to percent change in biomass according to the formula:

$$\text{Biomass change} = (C_t - C_o)/C_o \times 100$$

where C_t was the chlorophyll a content at the time of sampling and C_o was the initial chlorophyll a content.

Availability of light in algal habitats. Light penetration was measured at Surrey Lake. Light intensities were measured at depth intervals of 0.3 m on June 17 and June 25, 1981 with a KAHLSICO Model 268WA310 Submarine Photometer calibrated against a LiCor Model LI-185A Quantum

Meter. Surface light intensities were similar on both days but lake turbidity differed greatly.

Light penetration into mats of Pithophora oedogonia was measured on algal material collected from Surrey Lake. The method used was similar to that of Gordon et al. (1980). Thin layers (2 to 3 mm) of mat were spread in 9 cm petri dishes. The dishes were stacked to provide increasing thickness of mat. An incandescent reflector flood lamp (75 W) was positioned to give an intensity of 2000 uE m^{-2} s^{-1} (PAR) at the mat surface. Light intensities were measured at five positions beneath the algal mats. Measured intensities were corrected for absorbance by the petri dishes.

Algal response to starvation. The filamentous algal species were subjected to starvation conditions for periods of up to 60 days. Algal clumps were inoculated into flasks wrapped in aluminum foil to exclude all light. Inoculation, culture conditions, biomass measurements and statistical analysis were identical to those described previously in the growth inhibition experiments.

Results and Discussion

Inhibition of photosynthesis by simazine. Photosynthesis rates of all algal species tested were inhibited within 30 s of simazine injection. This suggests that simazine rapidly penetrated the cells to reach active sites within the chloroplasts. The effect of simazine concentration on photosynthesis inhibition is shown in Figure 17. Spirogyra was most susceptible to simazine inhibition, followed by Pithophora, Cladophora and Ankistrodesmus. The order of susceptibility to photosynthesis inhibition matches simazine susceptibilities reported for these species based on visual ratings of lake treatments (Ellis et al., 1976.). I_{50}

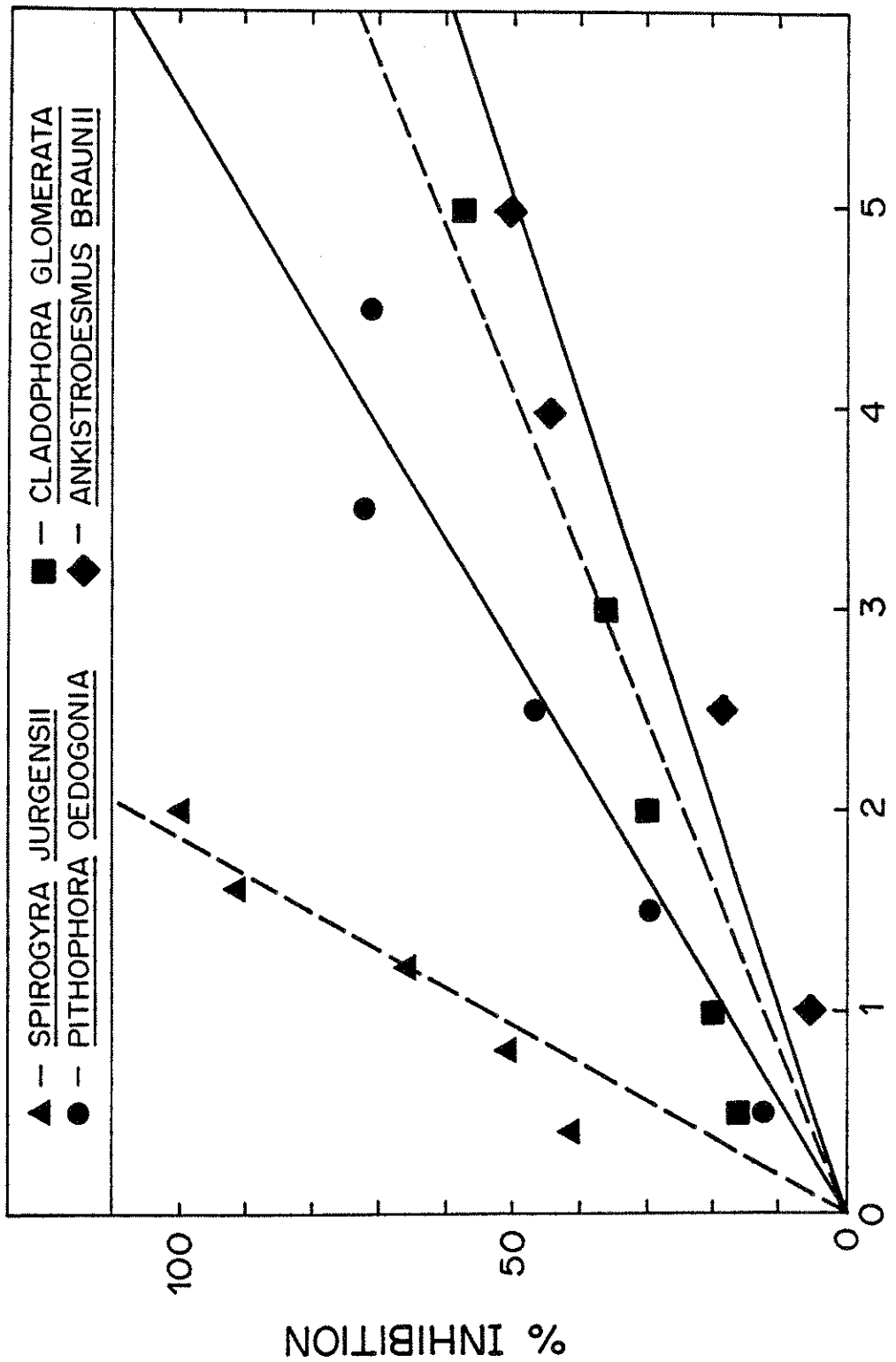

Figure 17. Percent inhibition of algal photosynthesis rates of Spirogyra, Pithophora, Cladophora and Ankistrodesmus at selected simazine concentrations. Symbols indicate the mean of four replictes. Analysis of covariance (0.05) of regression slopes revealed the following inhibition order: Spirogyra > Pithophora > Cladophora = Ankistrodesmus.

concentrations were calculated from the regression lines. The I_{50} value for Spirogyra was 1.1 uM simazine. Values were considerally greater for Pithophora (3.0 uM), Cladophora (3.8 uM) and Ankistrodesmus (4.7 uM). The results show that 3 to 5 times the concentration of simazine is required to inhibit photosynthesis of Pithophora, Cladophora, and Ankistrodesmus compared to Spirogyra. I_{50} concentrations in the present study are similar to published values for Chlorella (1.9 uM) (Zweig et al., 1963)) and Scenedesmus (4.5 uM) (Bishop, 1962).

Inhibition of growth and development of phytotoxic symptoms. Simazine (5 uM) strongly inhibited growth of the algal species relative to controls under all light conditions tested (Figs. 18A, B, C). However, growth response to simazine and development of phytotoxic symptoms varied with light intensity and species. Biomass of simazine treated Spirogyra declined 45 percent ($P<0.05$) after 45 days in cultures incubated at a light intensity of 15 uE m^{-2} s^{-1} (Fig. 18A). No visual phytotoxic symptoms (chlorosis and necrosis) were noted at this low light intensity suggesting that the biomass decline was caused by starvation due to the blockage of photosynthesis rather than from the lipid peroxidation associated with the blockage of photosynthesis (Ashton and Crafts, 1981; Dodge, 1982). Cladophora biomass did not change significantly at 15 uE m^{-2} s^{-1} ($P>0.30$) whereas Pithophora biomass increased 75 percent ($P<0.05$). Biomass of Cladophora and Pithophora probably did not decline because photosynthesis was only partially inhibited at the simazine concentration used in the growth studies (Fig. 17). All algal species exhibited rapid increases in biomass (345 to 616 percent) when transferred to simazine-free media for 15 days ($P<0.05$).

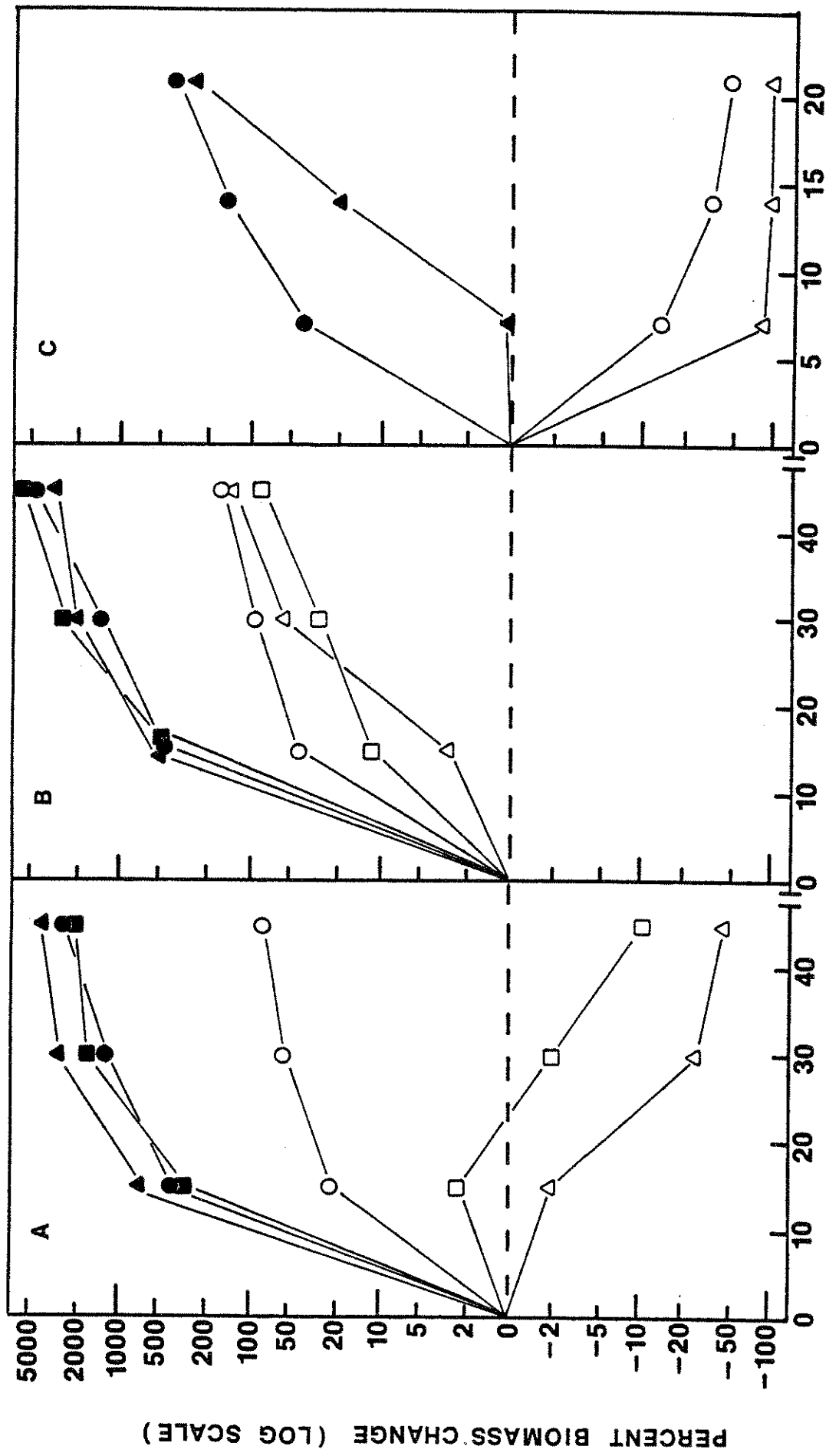

Figure 18. Biomass changes of Spirogyra (△ ▲), Pithophora (○ ●) and Cladophora (□ ■) in control (closed symbols) and 5 uM simazine treated (open symbols) cultures. A. PAR = 15 uE m^{-2} s^{-1}. B. PAR = 100 uE m^{-2} s^{-1}. C. PAR = 400 uE m^{-2} s^{-1}, note that C time scale differs from A and B.

Increasing light intensity to 100 uE m^{-2} s^{-1} resulted in biomass increases ($P<0.05$) for all algal species in the presence of simazine (Fig. 18B). The chlorophyll increase measured for Spirogyra was unexpected since photosynthesis is completely blocked by 5 uM simazine (Fig. 17). The mechanism by which light stimulated the chlorophyll increase is not apparent but may be related to a light induced respiration of energy reserves (Voskresenskaya, 1979). No phytotoxic symptoms were noted on any of the algal species at this light intensity and algal material transferred to simazine-free media showed rapid increases in biomass (435 to 760 percent).

Phytotoxic symptoms developed rapidly in simazine treated cultures of Spirogyra and Pithophora at 400 uE m^{-2} s^{-1}. Development of phytotoxic symptoms was accompanied by a 100 percent reduction in Spirogyra biomass ($P<0.05$) within 14 days of exposure (Fig. 18C). No recovery occurred when transferred to simazine-free media for 15 days. Pithophora was less sensitive to simazine showing a more gradual development of phytotoxic symptoms and decline in biomass. Pithophora biomass was reduced by 50 percent after 21 days of exposure ($P<0.05$) and recovered gradually (35 percent increase over 15 days) when transferred to simazine-free media. Growth of Cladophora was inhibited at the highest light intensity used (400 uE m^{-1} s^{-1}) and could not be tested for simazine toxicity.

The growth studies conducted at various light intensities show relatively high light intensities are required before simazine phytotoxicity occurs in the filamentous algae tested. A toxic interaction between atrazine and similar light intensities was reported for oats (Avena sativa) (Ashton, 1965). A high light intensity requirement for

phytotoxicity could significantly limit the effectiveness of simazine as an algicide for filamentous, mat-forming algae.

Light availability in algal habitats. The typical habitat for filamentous, mat-forming algae are shallow bodies of water and littoral areas of larger lakes. These shallow water habitats are subject to high levels of turbidity that reduce light penetration into the water column. For example, in Surrey Lake algal mats are frequently found on the lake bottom at depths of 0.5 to 1 m. Figure 19 shows light intensity - depth profiles for Surrey Lake on two dates that presented different turbidity conditions. The critical light intensity required to produce phytotoxicity by simazine is between 100 and 400 uE m^{-2} s^{-1} (Fig. 18). Based on this critical light intensity range, algal filaments would not be subjected to simazine injury if located deeper than 46 to 79 cm under low turbidity condition or 11 to 28 cm under high turbidity conditions (Fig. 19). In addition, floating algal mats sink when treated with triazine herbicides (Robson et al., 1976) due to loss of photosynthetic activity and would be removed from high light intensities at the surface.

Light intensities are also greatly reduced within the floating mats formed by filamentous algae such as Pithophora. Figure 20 shows that 90 percent of incident light (PAR) is absorbed within 3 mm of the surface of a mat of Pithophora. Similar reductions in light intensity have been reported in mats of Cladophora (Gordon et al., 1980.). Algal filaments beneath this surface layer of mat would be shielded from light intensities that produce simazine injury.

Algal response to starvation. Although algal filaments may be protected from peroxidation phytotoxicity of simazine (Ashton and Crafts, 1981; Dodge, 1982) by low light intensities, starvation due to blockage of

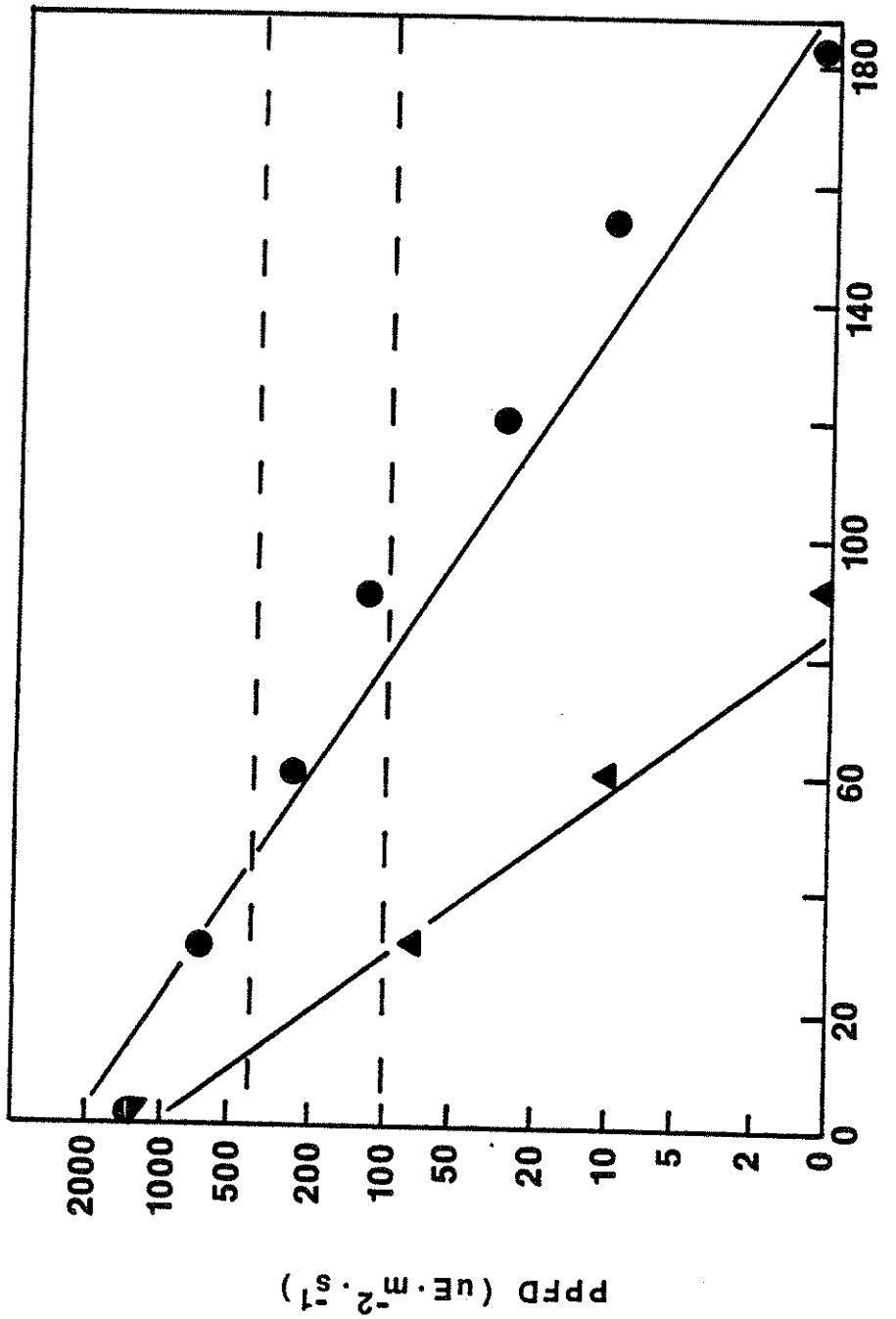

Figure 19. Light intensity - depth profiles in Surrey Lake, Columbus, IN on two dates in 1981 (June 17 -▲, June 25 -●). Dotted lines define the range containing the critical light intensity required to produce simazine toxicity in filamentous algae.

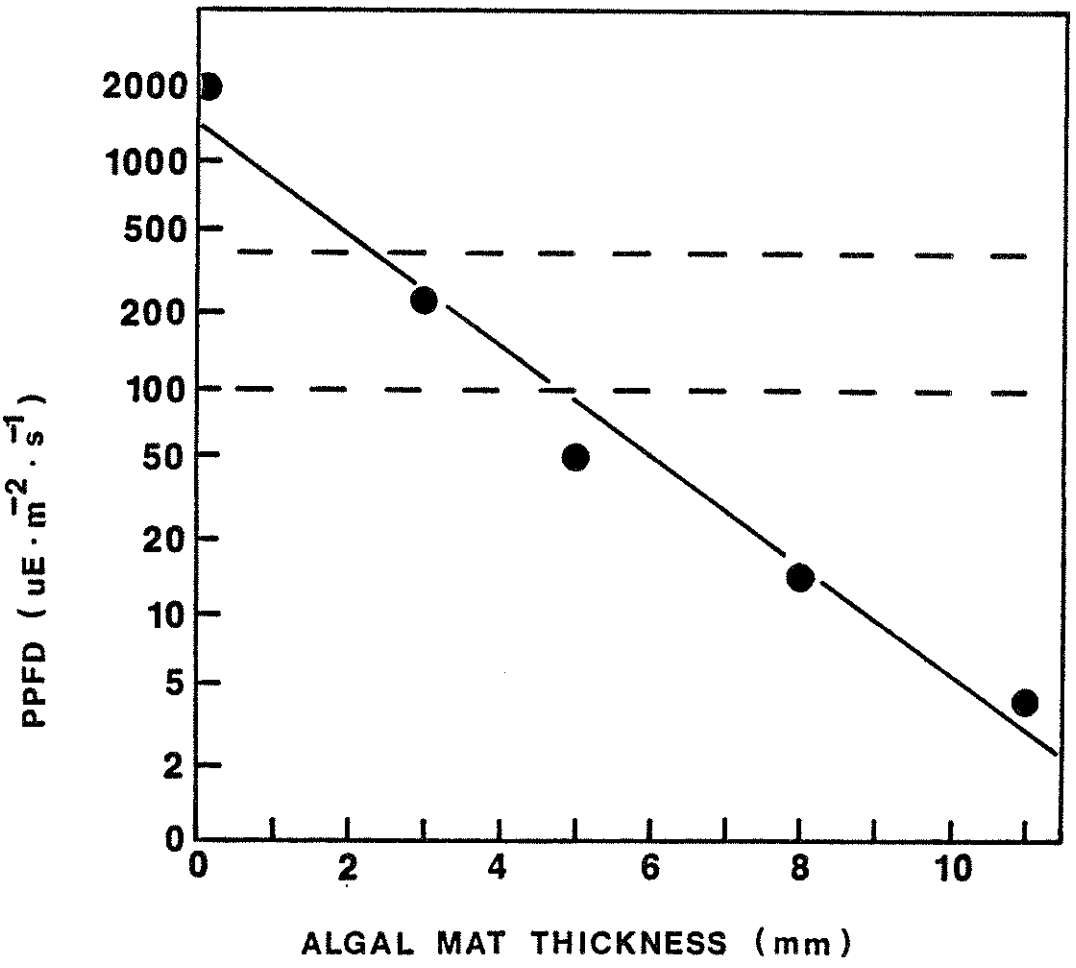

Figure 20. Reduction of incident light (PPFD) within a mat of P. oedogonia. Dotted lines define the range containing the critical light intensity required to produce simazine toxicity in filamentous algae.

photosynthesis is still a possible mode of algicidal activity. When incubated in the dark, Spirogyra biomass declined 76 percent over a 60 day period (Figure 21). In contrast, Pithophora and Cladophora showed a reduction of only 19 percent which was not significant ($P>0.05$). Filamentous algae such as Pithophora and Cladophora appear capable of surviving long periods under non-photosynthetic conditions. Spirogyra is more susceptible to starvation and may be adversely affected by long-term exposure to simazine at low light intensities (Fig. 18A).

Although filamentous green algae, including Pithophora, have been reported to be sensitive to simazine (Ellis et al., 1976), results of our studies demonstrate relatively high light levels (approximately 400 uE m^{-2} s^{-1}) are required to produce algicidal activity by recommended concentrations of simazine in the filamentous algae studied. Thus, the effectiveness of simazine as an algicide against Pithophora and other filamanetous algae in natural situations will depend on turbidity conditions within the lake, the depth distribution of algal vegetation, and the thickness of algal mats. In addition, filamentous algal species appear to vary in their susceptibility to simazine. Pithophora is less affected by simazine at high light intensities than Spirogyra. In addition, Pithophora and Cladophora are less sensitive to photosynthesis inhibition by simazine and are better able to survive long periods of non-photosynthetic conditions than Spirogyra.

II.B. Copper Sulfate

The "resistance" of Pithophora to copper sulfate was noted as early as 1924 (Tiffany, 1924). Eipper (1959) found Pithophora to survive copper sulfate at concentrations 15 to 20 times greater than those for other

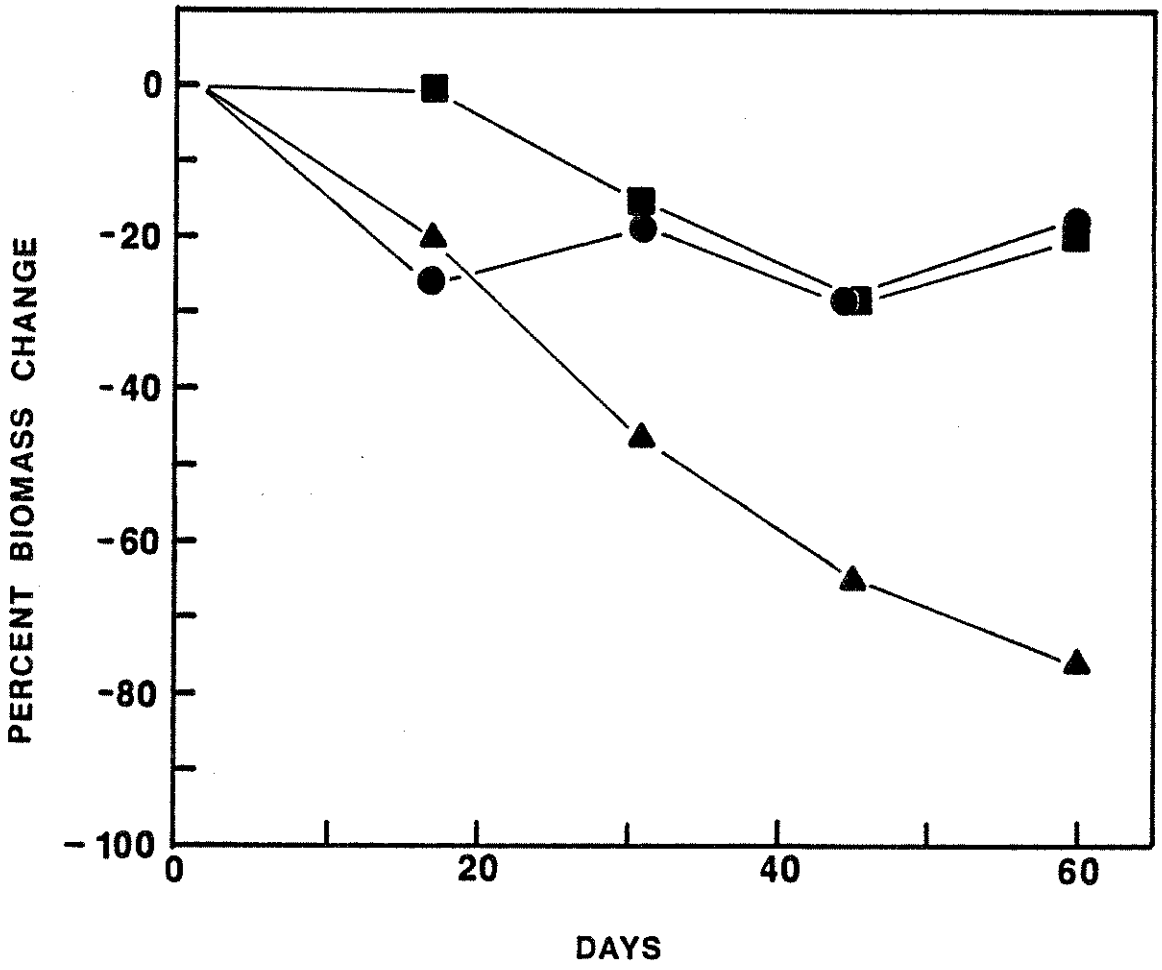

Figure 21. Biomass changes of Spirogyra (▲), Pithophora (●) and Cladophora(■) incubated in the absence of light.

algae. Literature from the Phelps-Dodge Corporation, a major manufacturer of copper sulfate, also notes the difficulty in controlling Pithophora with copper.

In this portion of the study, the susceptibility of akinetes, germinating akinetes, and vegetative filaments of Pithophora oedogonia to copper toxicity was evaluated.

Materials and Methods

Cultures of P. oedogonia were isolated and maintained as described in Section I. Akinetes began to form in three to four week old cultures and comprised approximately 90% of the biomass after 2 to 4 months without transfer to new medium. Individual akinetes were isolated by disrupting filaments for 60 s in 100 ml of water with a Waring blender. The akinetes were washed by suspending the disrupted filaments repeatedly in fresh medium and pouring off the supernatant which contained cellular debris. Akinetes were stored at 4°C in the dark for at least one week prior to use.

Copper sulfate was dissolved in CL(II) medium that lacked organic chelators (EDTA and citrate). Serial dilutions provided Cu^{++} concentration of 0.8, 4 and 8 uM. Known amounts of biomass of the three life stages were innoculated into 15 ml, conical, pyrex centrifuge tubes containing 5 ml of the copper solutions. Controls were run in Cl(II) medium without added copper. Four replicates were run at each Cu^{++} concentration.

Standardization of the initial inocula was accomplished in the following manner for each life stage. Vegetative filament inocula were visually estimated by placing clumps of similar size in ceramic spot plates. Isolated akinetes were suspended in 100 ml of medium by a magnetic

stirbar. One ml of the akinete suspension was transferred to each centrifuge tube and the excess medium removed before addition of the copper solutions. Germlings were obtained by pipetting 1 ml of akinete suspension into centrifuge tubes containing standard CI(II) medium and allowing the akinates to germinate for 7 days prior to treatment with copper solutions. Replicates (6 to 8) of the initial inocula were taken for quantification of biomass. Biomass was measured as chlorophyll a determined by the method of Wetzel and Westlake (1969).

The life stages were exposed to the copper solution for 24 h at 20 + 1 C and 60 uE m^{-2} s^{-1} (PAR). At the end of the exposure period the algal material was washed once with copper-free medium and transferred to disposable petri dishes (100 mm by 20 mm) containing 50 ml of copper-free medium. Treated algal material was incubated for two weeks at 20 $\pm$ 1 C and 18 uE m^{-2} s^{-1} to determine the response to the copper treatment. At the end of the response period, biomass (chlorophyll a) in the petri dishes was determined.

I_{100} values (copper concentration that inhibited growth 100 percent) were estimated from a graph in which mean chlorophyll a content and associated standard deviations were plotted against copper concentration.

Results and Discussion

The life stages of P. oedogonia varied in their response to copper with germinating akinetes appearing to be the most copper-tolerant of the three stages (Fig. 22). Akinetes were somewhat less tolerant while vegetative filaments showed the greatest copper sensitivity. At 8 uM (the highest Cu^{++} concentration tested) the vegetative filaments were almost completely bleached and dead whereas the chlorophyll content of the

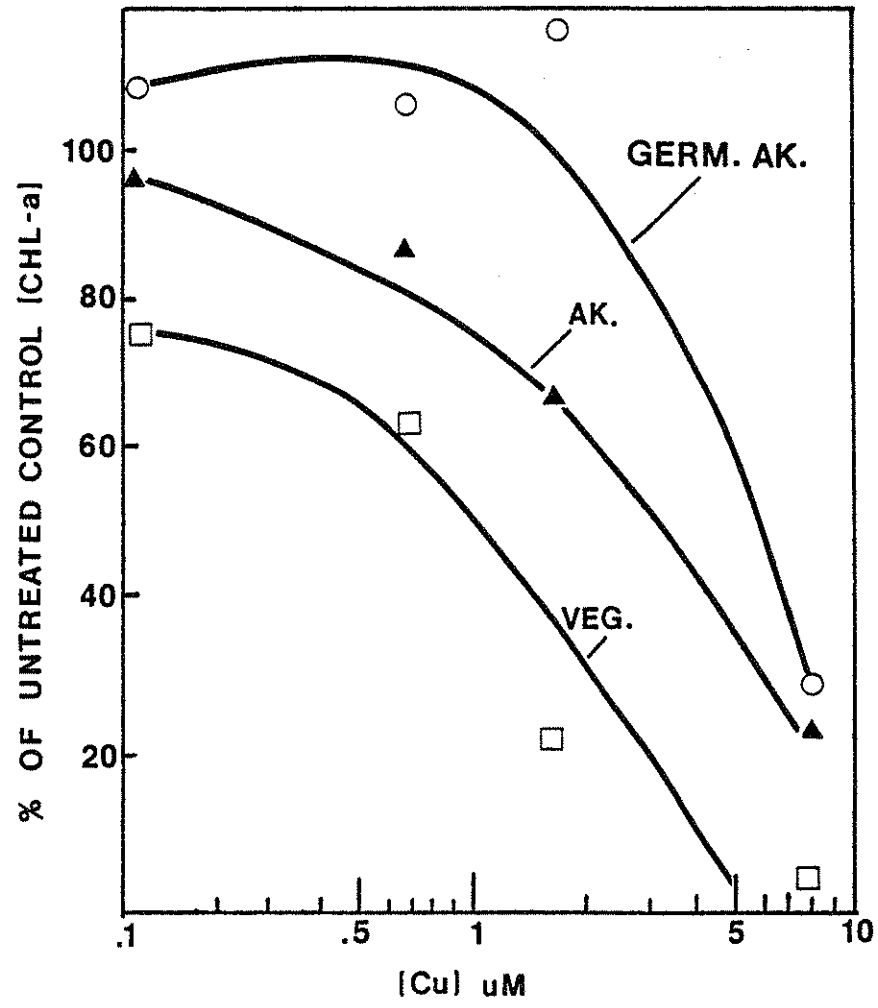

Figure 22. Effect of copper on biomass (chl-a) changes in cultures of akinetes, germinating akinetes, and vegetative filaments.

germinating akinetes was still 30% that of untreated controls. At 1.6 uM, the majority of vegetative filaments were also bleached whereas germinating akinetes remained green and showed even greater growth than the controls. The I_{100} value (copper concentration which resulted in no increase in chlorophyll over initial chlorophyll content) for germinating akinetes was 6.8 uM Cu^{++}, 3.7 uM Cu^{++} for akinetes, and 0.9 uM Cu^{++} for vegetative filaments.

The finding that germinating akinetes were almost twice as resistant to copper as akinetes and more than 7 times as resistant as vegetative filaments was unexpected. In terrestrial plants seedlings are usually the most susceptible of the life stages to herbicidal activity. The reasons for the greater tolerance of germinating akinetes to copper is not readily apparent. Copper has been reported to act by inhibiting photosynthesis (Steeman Nielson, et al. 1969). Although germinating akinetes are not actively photosynthesizing and could theoretically be protected from the effects of copper, the algicide has also been reported to affect respiration as well as other general metabolic processes (Cedeno-Maldonado and Swader, 1974; Mukherji and Das Gupta, 1972).

Another potential mechanism for resistance may relate to the potential of the alga to bind copper to the cell wall. As noted in Lembi et al. (1980), copper is loosely bound to the cell wall of Pithophora and can either be lost to the culture medium or redistributed to new cell wall material during growth (e.g., during akinete germination). Since germ tube elongation occurs at a more rapid rate than elongation of vegetative filaments (Section I) the production of new cell wall binding sites may cause a redistribution of the copper and result in a dilution of the copper concentration in the cell wall. In akinetes, which are essentially

quiescent structures, and slow growing vegetative filaments, copper may saturate cell wall binding sites allowing toxic amounts to penetrate and enter the cytoplasm. However, more study is required before the exact tolerance mechanism of germinating akinetes to copper can be established.

III. MANAGEMENT APPROACHES

We believe that life cycle studies of Pithophora oedogonia can provide insight into the usefulness of various management approaches, particularly those involving algicides. For example, although simazine is recommended for Pithophora control our results suggest that the control obtained is a cosmetic one at best. Since simazine inhibits photosynthesis and oxygen evolution, the mats lose their buoyancy and sink. Low light intensities at the greater depths and within the mats presumably protect the alga from the phytotoxic, membrane-disrupting effects of simazine. In addition, and in contrast to other algae such as Spirogyra, Pithophora can survive at low light intensities for long periods of time (>60 days) drawing on its internal food reserve. Although simazine residues can be found in water for as long as 9 to 12 months after the initial application, Mauck et al. (1976) reported dissipation of initial application concentrations of 5 uM to less than 1 uM in ponds within 30 days. This level should permit the organism to regain its ability to photosynthesize and cause the mats to once again become buoyant. Indeed, this has been the observation of numerous commercial applicators; i.e., initial knockdown of the alga, the presence of algal mats on the bottom sediments, and reappearance of the alga within a month or two of application. The only way in which simazine can be effective in this situation is if the compound is applied continuously and the concentration maintained at levels high enough to keep the alga at the bottom and eventually starve it out. This is extremely

difficult to do since most ponds and lakes are susceptible to periodic washouts and certainly from an environmental standpoint, would not be a desirable practice.

Some applicators believe that simazine treatments of ponds may be selecting for Pithophora; i.e., removing the more susceptible species such as Spirogyra and leaving the Pithophora. If this is indeed the case, it is obvious that a thorough algal survey of a body of water will be required to determine that Pithophora is not present before simazine is applied. The resistance of germinating akinetes to photosynthetic inhibitors such as simazine also suggests that this compound is not a desirable method of control.

Although the reason for germinating akinete tolerance to copper probably differs from that of simazine, the implications are much the same. Copper treatments, like those of simazine, are often made when water temperatures reach 20 C. However, this also appears to be the period in the life cycle when the inherent tolerance of the organism to copper is at its greatest. The lag in sediment akinete germination by at least a month extends this period of copper tolerance into the early summer. Treatments made later in the season to vegetative filaments are probably ineffective because of the tremendous amounts of biomass that can accumulate and thus dilute the effects of the copper. The other alternative, that of applying copper when water temperatures are still cold prior to akinete germination, would coincide with a period of moderate copper tolerance and low biomass. Although treatments in the winter or early spring are generally ineffective on most aquatic plants, there may be promise in this approach for Pithophora control. A preliminary study of akinetes treated with copper at 20 C and at 10 C showed no significant differences in effects between the

two temperatures. Continued research, with emphasis on field trials, is being conducted to determine whether copper applications at the low water temperatures and other environmental conditions of late winter-early spring will provide a viable treatment alternative for Pithophora control.

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