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Motility in Scenedesmus quadricauda.

Roman Zielinski

MOTILITY IN SCENEDESMUS QUADRICAUDA

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by

Roman Zielinski

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

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THESIS ACCEPTANCE

Accepted for the faculty of the Graduate College, University of Nebraska, in partial fulfillment of the requirements for the degree Master of Arts, University of Nebraska at Omaha.

Thesis Committee

Name	Department
T. V. Price	
D. Kelliher	Chemistry

Mark E. Brasher
Chairman

Nov. 13/75
Date

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

MOTILITY IN SCENEDESMUS QUADRICAUDA

INTRODUCTION

The study of algal life cycles is important in terms of taxonomic classification. Reproduction is one of the criteria used in placing algae into different divisions, classes, orders, and families. Growth and cell replication which are involved with life cycles are the "sine qua non" of living organisms (Coleman, 1961).

Results of studies concerned with life cycles in the algae were varied as to the question of how algae reproduce. Early botanists such as Linnaeus considered algae to lack a sexual cycle. Other ideas concerning reproduction of algae include: sexual, parthenogenesis, "subtle vapor", hermaphroditism, conjugation, de novo synthesis, metamorphosis, infusoria, and zoospores (Smith, 1951).

That algae could reproduce by both asexual and sexual means became established by mid nineteenth century. However, the exact mechanisms initiating the sexual part of the life cycle of some algae still need to be worked out.

Factors effecting sexual reproduction in the algae include: temperature, intensity of illumination, duration of light, size of the cells, concentration of dissolved

salts, partial drying followed by moisture, and release of sexual substances by the gametes (Smith, 1951). The range of these factors varies for different algae. Coleman (1961) reported that other factors influencing the cycle include: provision of renewed nutrients, dilution of old metabolic products, lowered concentrations of organisms followed by reduction in secretion products, accumulation of CO_2 in the dark, vitamins, trace elements, and hormones.

Hollenberg (1936) studying Halicystis noted a periodic sexual cycle which was not effected by intensity of illumination, duration of light, temperature or O_2 content. Factors effecting sexual reproduction vary in importance for different algae. No theory as yet satisfactorily incorporates all the data available.

Scenedesmus was first described by Meyen in 1829 (DeAlton, 1894). He described the genus as a colony composed of two to sixteen ovoid or fusiform oblong cells joined together in a single row. Smith (1916) was the first to monograph the genus. He described each cell in the coenobium as having a single pyrenoid located toward the outside wall and a single nucleus toward the inside. Each cell has a peripheral cup-shaped chromatophore which contains a few starch grains. Vacuoles occupy a considerable internal space on either side of the pyrenoid. The cell has a single, large,

chloroplast.

Scenedesmus quadricauda was first described by Turpin in 1835 (DeAlton, 1894). Prescott (1962) describes S. quadricauda as a colony consisting of 2-4-8 oblong cylindrical cells usually in a single row. The outer cells have a long curved spine at each pole. The inner cells are without spines. Cells may vary in size between 3-18 microns in diameter and 9-35 microns in length. There are several different varieties of S. quadricauda that differ in spine length and cell dimensions. This organism was isolated by Trainor and given to the Richard Starr Algal Collection at the University of Indiana (Starr, 1964). The most noticeable characteristic of this variety is its large size, which is 20 microns or larger in diameter and 30 microns or larger in length. The inner cells may also bear spines.

Since its first description and monograph the genus Scenedesmus was considered to reproduce solely asexually by non motile autospores. There was no motile phase in the life cycle. Consequently very little work has been done pertaining to motility in Scenedesmus. Starr (1954) found zoospores for Tetraëdron a genus closely related to Scenedesmus and thought to reproduce solely by autospores. No gametes were observed although a motile phase in the life cycle was discovered.

Trainor (1963) found a motile phase for S. obliquus strain 393. He called these motile cells zoospores. The motile cells were described as having two flagella of equal length, a parietal chloroplast, occasionally a stigma, contractile vacuoles, ro rigid cell wall, and no pyrenoids.

It was noted that motile cells were formed under conditions which stimulated sexual cycles in Chlamydomonas (Sager and Granick, 1954). Nitrogen deficiency and light were necessary to stimulate production of motile cells.

Trainor and Burg (1965) observed motile cells for S. obliquus strain WH 50 and S. dimorphus strains CAB-3, 63-2, and IUCC 746. It was found that storage at low temperatures (15 degrees C.) kept cells motile longer and favored their production. Attempts to subculture the motile cells in different media failed. The cells plasmolyzed. The concept of zoospore was considered premature and the term "obligate gametes" was substituted.

Trainor (1965) found zygotes when two different strains of S. obliquus were mixed. This demonstrated a sexual phase in the life cycle and that the motile cells were gametes. However, it was noted that only Scenedesmus species lacking spines were able to produce motile cells. Scenedesmus species having spines did not produce motile cells.

This experiment was an attempt to determine whether motile cells could be produced by a spine-bearing Scenedesmus species. Scenedesmus quadricauda was placed under conditions found to be necessary to stimulate production of motile cells for species lacking spines. The effects of temperature, nitrogen deficiency, and light were studied to determine if these variables can induce motile cell production in this organism.

METHODS AND MATERIALS

Cultures of Scenedesmus quadricauda IUCC 614 were obtained from the algal collection at the University of Indiana.

Axenic cultures were used throughout the experiment. Innoculations were made by the loop method using cultures of vegetative cells in the log phase of growth. Motile cells were transferred by pipette. Media, glassware, and pipettes were autoclaved. Aseptic techniques were used throughout the experiment.

All cultures were placed in a growth chamber. Light intensity was kept constant throughout the experiment at 400 foot candles. Stock cultures were maintained on proteose agar slants at 21 degrees C. under continuous light.

The following modifications of Beijerinck medium were used: Beijerinck medium with all the nutrients present, with glucose supplementation (0.1%), without nitrogen (ammonium nitrate), without nitrogen but with glucose (0.1%). All Beijerinck media and modifications consisted of 10 ml media in 18 x 150 mm pyrex test tubes.

The following temperatures were used: 13 degrees C., 15 degrees C., 18 degrees C., 21 degrees C., and 25 degrees C.

Photoperiods used (L:D) were: 24:0, 16:8, 8:16 and 0:24.

The following salts were used in addition to the salts present in the Beijerinck medium to test the effects of the components of the medium in their ability to stimulate motile cell production: KNO_3, $(NH_4)_3PO_4$, $(NH_4)_2SO_4$, $Mg_3(PO_4)_2$, KCl, and $Fe_2(SO_4)_3$. All salt substituted cultures were kept at a photoperiod 16:8 at 21 degrees C.

Fifteen cultures were subjected to identical treatments for each different combination of temperature, photoperiod and media used as well as for the different combinations of salts and the average effect was noted. All cultures were checked at daily intervals over a period of two weeks for the appearance of motile cells.

A Spencer Bright-Line Hemacytometer was used to determine the number of cells per cubic millimeter. Numbers are based on the average of 100 counts.

The following media were used to subculture motile cells: Beijerinck, 0.1% glucose supplemented Beijerinck, Bold's, Molisch, Chu's #10, Knop's, Nutrient Broth, 1% Yeast Extract, Proteose Agar, and Yeast Dextrose Agar. Stein (1973) and Pringsheim (1967) provide formulae for the different media. Five cultures of each different media were used. All cultures were placed at a photoperiod 16:8 at 21 degrees C. Cultures were checked daily over a

period of 5 days to note if motile cells could survive and grow in a given medium.

Photographs were taken with a Pentax Spotmatic camera mounted on a phase contrast (Wild model 1420) microscope using a blue filter and Ektachrome film ASA 160.

RESULTS

Description of Motile Cells.

Scenedesmus quadricauda, a spine-bearing species, (Fig. 1) is able to produce motile cells (Fig. 2) under certain conditions. Motile cells have two flagella of equal length, a parietal chloroplast, and no rigid cell wall. Contractile vacuoles may or may not be present since they could not be seen well in unstained or stained cells. Pyrenoids and stigmas were not present. Motile cell dimensions were ca. 5 x 6 microns. Description of motile cells is similar to those seen by Trainor (1963) for *S. obliquus*. I_2KI stain was necessary to see organelles. In natural state motile cells are too active for any organelles to be seen clearly.

Effects of Media.

Cultures grown in complete Beijerinck medium did not produce motile cells within the temperature or photoperiods of this study (Tables I-V). Cultures grown in ammonium free Beijerinck medium were able to produce motile cells regardless of glucose supplementation (0.1%) and photoperiod (except at 0:24) at 15 degrees C. (Table VII), 18 degrees C. (Table VIII), and 21 degrees C. (Table IX). This indicates that motile cells are produced in an ammonium deficient substrate at temperatures below

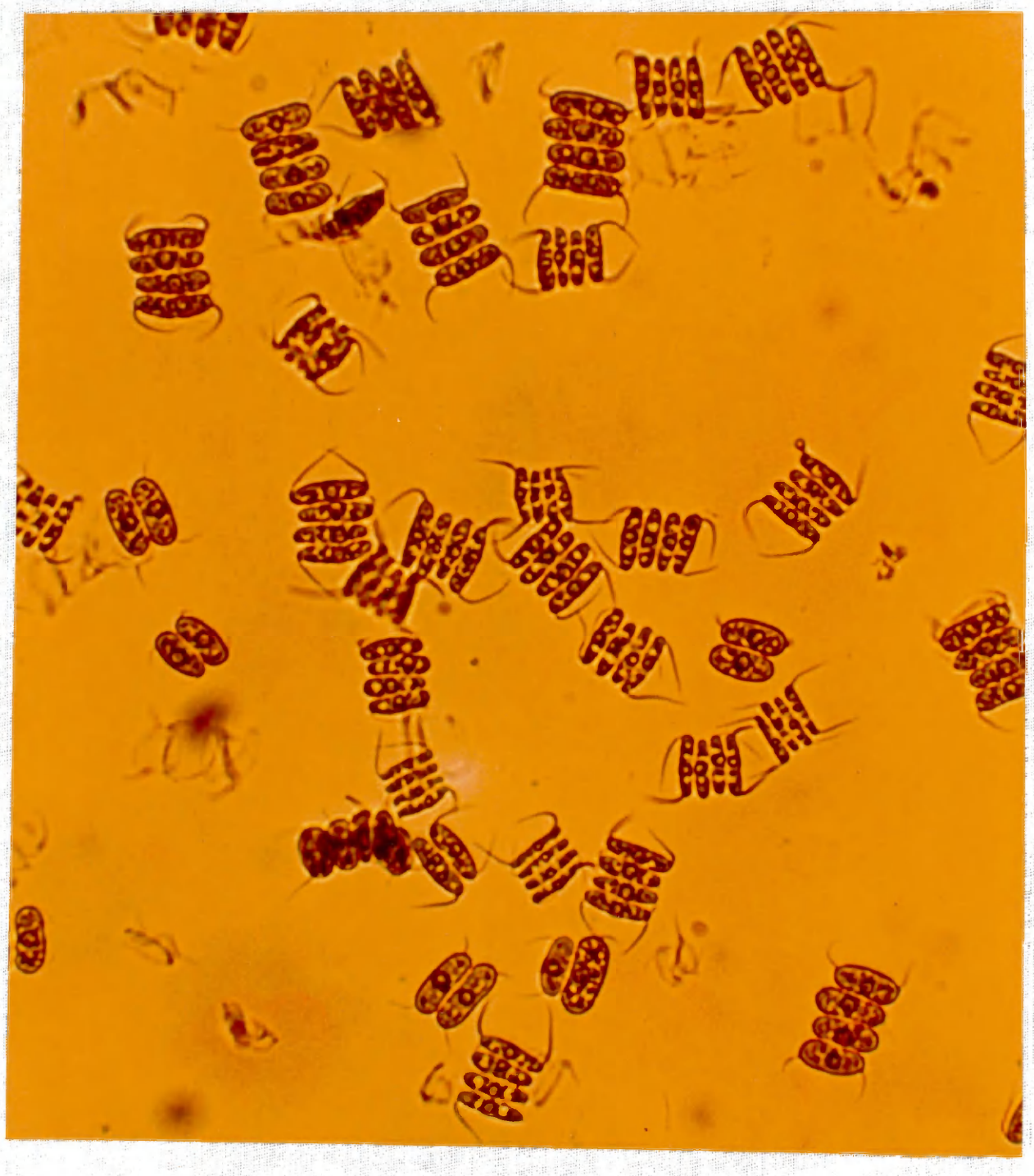

Fig. 1. Scenedesmus quadricauda on Proteose Agar. Cells ca. 800X.

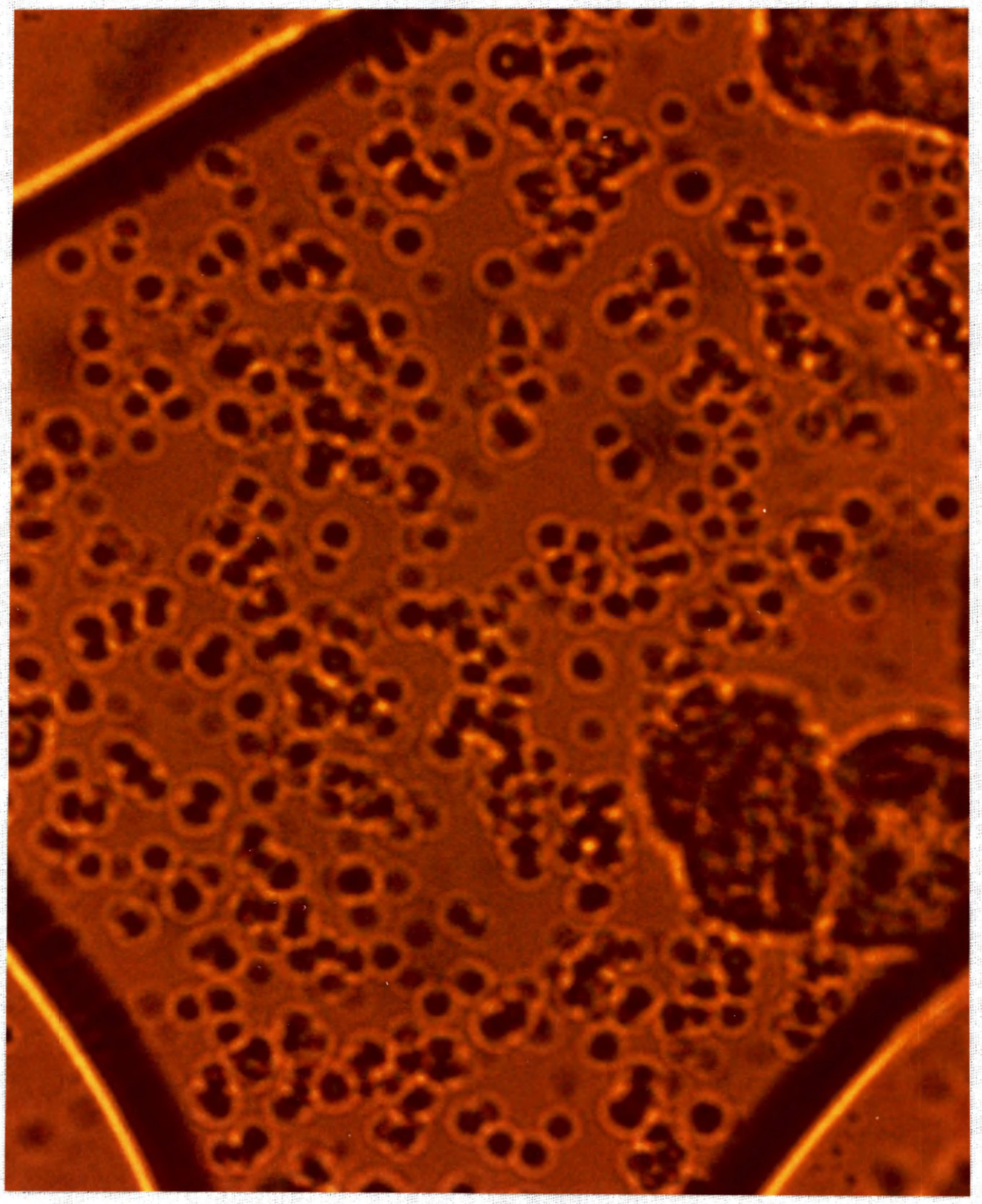

Fig. 2. Motile Cells of Scenedesmus quadricauda.
Cells ca. 1600X unstained.

24° C. and above 14° C.

Effects of Components in Beijerinck Medium.

Components of the Beijerinck medium were eliminated individually as well as different combinations to note the effect on motile cell production (Table XI). Careful substitution of the components revealed NH_4^+ was the determining nutrient component in the medium for motile cell production. Nitrate deficiency did not stimulate motile cell production. This signifies that total nitrogen depletion is not necessary to stimulate production of motile cells and that not all nitrogen sources will stimulate production of motile cells when they become depleted from the medium. A quantitative measurement for different concentrations of NH_4^+ and other components of the medium was not determined.

Effects of Temperature.

Nitrogen deficiency by itself is not solely responsible for motile cell production. Temperature was also a factor, since at temperatures below 14 degrees C. (Table VI) or above 24 degrees C. (Table X) motile cells were not produced even under nitrogen deficient conditions. The time from inoculation when motile cells are first observed decreases with an increase in temperature (Figure 3).

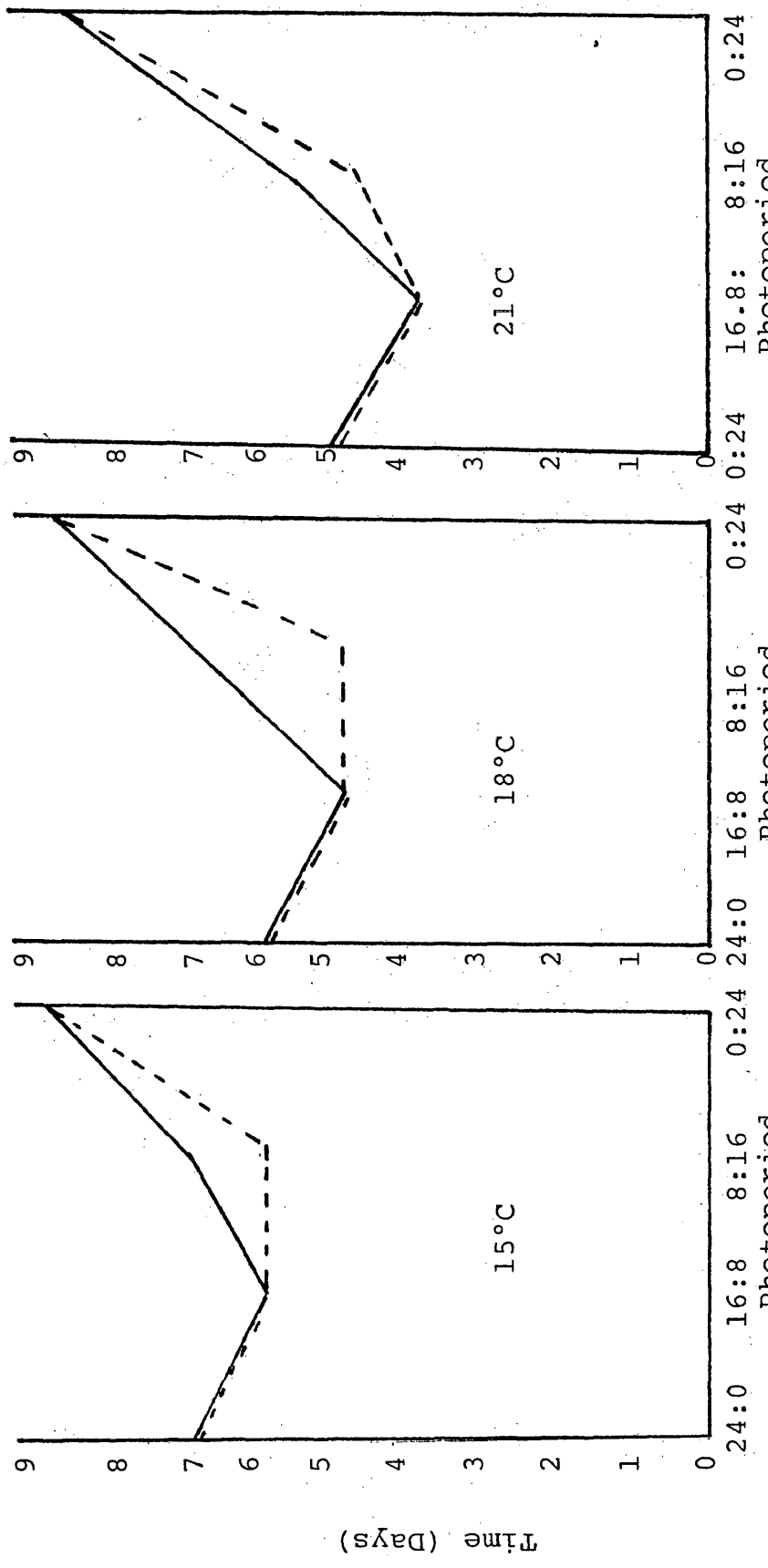

Figure 3. Comparison Photoperiod, Temp., Glucose Supplementation vs. Time from inoculation when motile cells first observed.

Table I. *S. quadricauda* in Beijerinck Medium at 13 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table II. S. quadricauda in Beijerinck Medium at 15 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table III. S. quadricauda in Beijerinck Medium at 18 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table IV. S. quadricauda in Beijerinck Medium at 21 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table V. S. quadricauda in Beijerinck Medium at 25 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table VI. S. quadricauda in Nitrogen Free Beijerinck at 13 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table VII. S. quadricauda in Nitrogen Free Beijerinck at 15 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	7	+	150
	-	7	+	280
16:8	+	6	+	350
	-	6	+	400
8:16	+	6	+	200
	-	7	+	220
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table VIII. S. quadricauda in Nitrogen Free Beijerinck at 18 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	6	+	750
	-	6	+	820
16:8	+	5	+	1000
	-	5	+	1230
8:16	+	6	+	670
	-	7	+	730
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table IX. S. quadricauda in Nitrogen Free Beijerinck at 21 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	5	+	5900
	-	5	+	6200
16:8	+	4	+	9400
	-	4	+	10000
8:16	+	5	+	5100
	-	6	+	5400
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table X. S. quadricauda in Nitrogen Free Beijerinck at 25 degrees C.

Photoperiod (L:D)	GS	Time (Days)	MC	No. MC/cu. mm.
24:0	+	14	-	0.0
	-	14	-	0.0
16:8	+	14	-	0.0
	-	14	-	0.0
8:16	+	14	-	0.0
	-	14	-	0.0
0:24	+	14	-	0.0
	-	14	-	0.0

+ = present

- = absent

GS = Glucose Supplementation

MC = Motile Cells

Table XI. Effects of Components in Beijerinck Medium in Stimulating Motile Cell Production for Scenedesmus quadricauda.

NH_4^+	NO_3^-	K^+	HPO_4^{-2}	Mg^{+2}	SO_4^{-2}	Fe^{+3}	Cl^-	MC	T	No. MC/cu. mm.
-	+	+	+	+	+	+	+	+	4	9500
+	-	+	+	+	+	+	+	-	14	0.0
+	+	-	+	+	+	+	+	-	14	0.0
+	+	+	-	+	+	+	+	-	14	0.0
+	+	+	+	-	+	+	+	-	14	0.0
+	+	+	+	+	-	+	+	-	14	0.0
+	+	+	+	+	+	-	+	-	14	0.0
+	+	+	+	+	+	+	-	-	14	0.0
+	+	-	-	-	-	-	-	-	14	0.0
+	+	+	-	-	-	-	-	-	14	0.0
+	+	+	+	-	-	-	-	-	14	0.0
+	+	+	+	+	-	-	-	-	14	0.0
+	+	+	+	+	+	-	-	-	14	0.0
+	+	-	-	+	+	+	+	-	14	0.0
+	+	+	+	-	-	+	+	-	14	0.0
-	-	+	+	+	+	+	+	+	4	9900

+ = present

- = absent

MC = Motile Cells

T = Time (Days)

No. MC/cu. mm. = Number Motile Cells/Cubic millimeter

Effects of Light.

Whether light is a factor in motile cell development still needs to be determined. Cultures grown in complete darkness and checked at daily intervals over a period of two weeks did not develop motile cells regardless of temperature, glucose supplementation, or nitrogen deficiency. However, vegetative cells did not grow well as evidenced by chlorophyll deficiency and cells exposing xanthophyll and carotenoid pigments. More experimentation is necessary to determine the role of light in motile cell production.

Best results for motile cell production in terms of photoperiod occurred at L:D cycle of 16:8. This signifies that continuous light does not necessarily give best results for motile cell production.

Effects of Glucose Supplementation.

Glucose supplementation did not effect motile cell production except where the photoperiod was 8:16 L:D (Figure 3). This indicates glucose is an indirect factor for motile cell production and becomes more important with a decrease in duration of light except at total darkness where it becomes insignificant again. In S. quadricauda with an increase in darkness glucose supplementation is favored for motile cell production. Quantitative effects of glucose was not determined.

Best Conditions to Induce Motility.

When motile cells were produced there was a difference in the numbers produced. Greatest numbers were produced at a photoperiod 16:8, without glucose supplementation, under ammonium free conditions at 21 degrees C. (Table IX). The least number of motile cells produced occurred under similar conditions as above except the temperature was 15 degrees C. (Table VII).

Cells remained motile longer at 15 degrees C. (one week) than at 21 degrees C. (3 days). Motile cell production was favored at 21 degrees C. as opposed to the other temperatures used (Figure 4).

Growth rates for vegetative cells on proteose agar (Figure 5) and Beijerinck medium (Figure 6) showed that the average life span of a culture was 18 days. The average life span of a nitrogen deficient culture was 9 days (Figure 7). The time from inoculation when motile cells first appeared showed that motile cell production is favored from cultures in the stationary or declining phase of growth.

Effects of Subculturing.

Attempts to subculture motile cells on the following media failed: Beijerinck, 0.1% glucose supplemented Beijerinck, Bold's, Molisch, Chu's #10, Knop's, Nutrient Broth, 1% Yeast Extract, Proteose Agar, Nutrient Agar, and

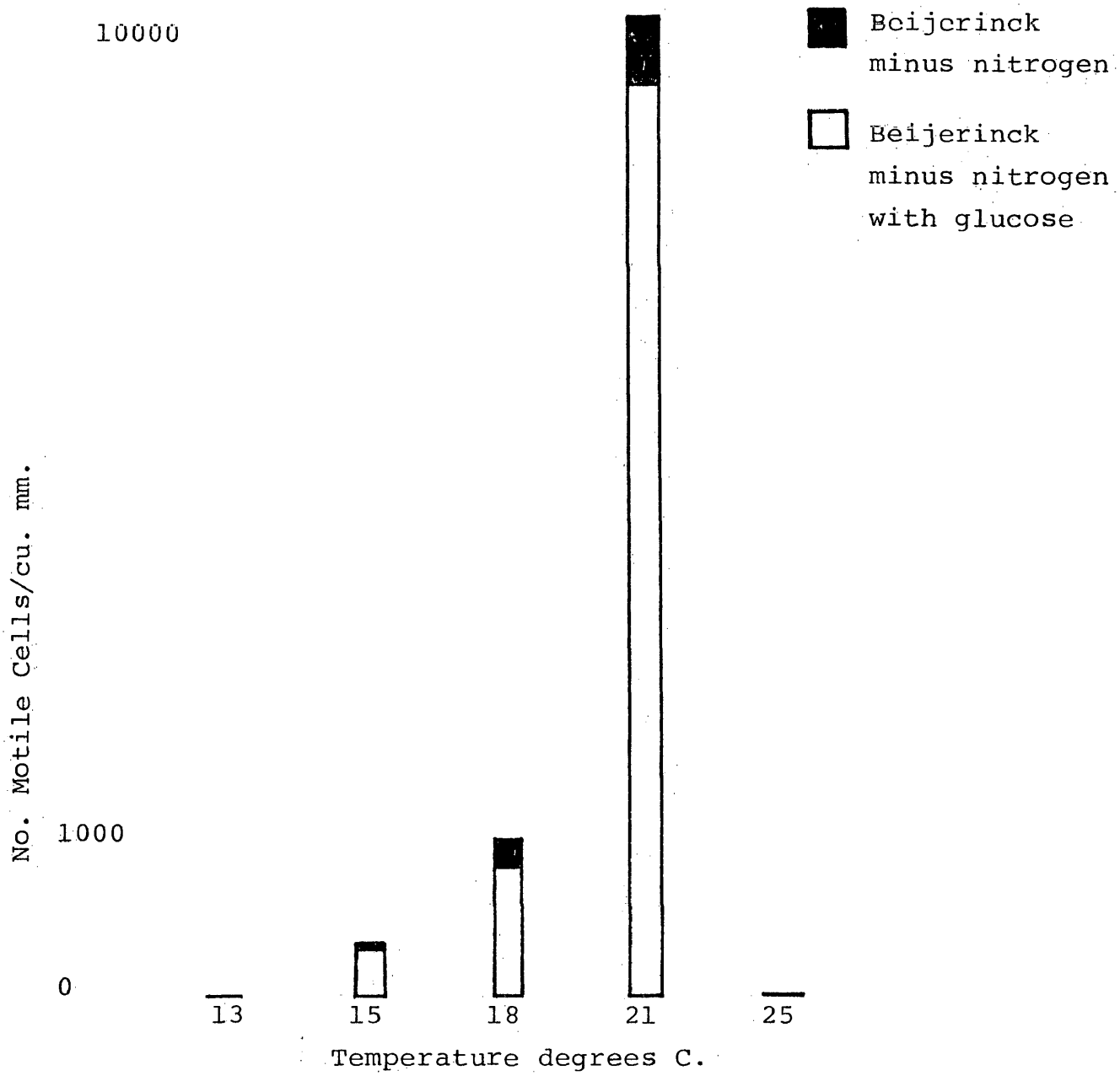

Fig. 4. No. Motile Cells/cu. mm. vs. Temp.

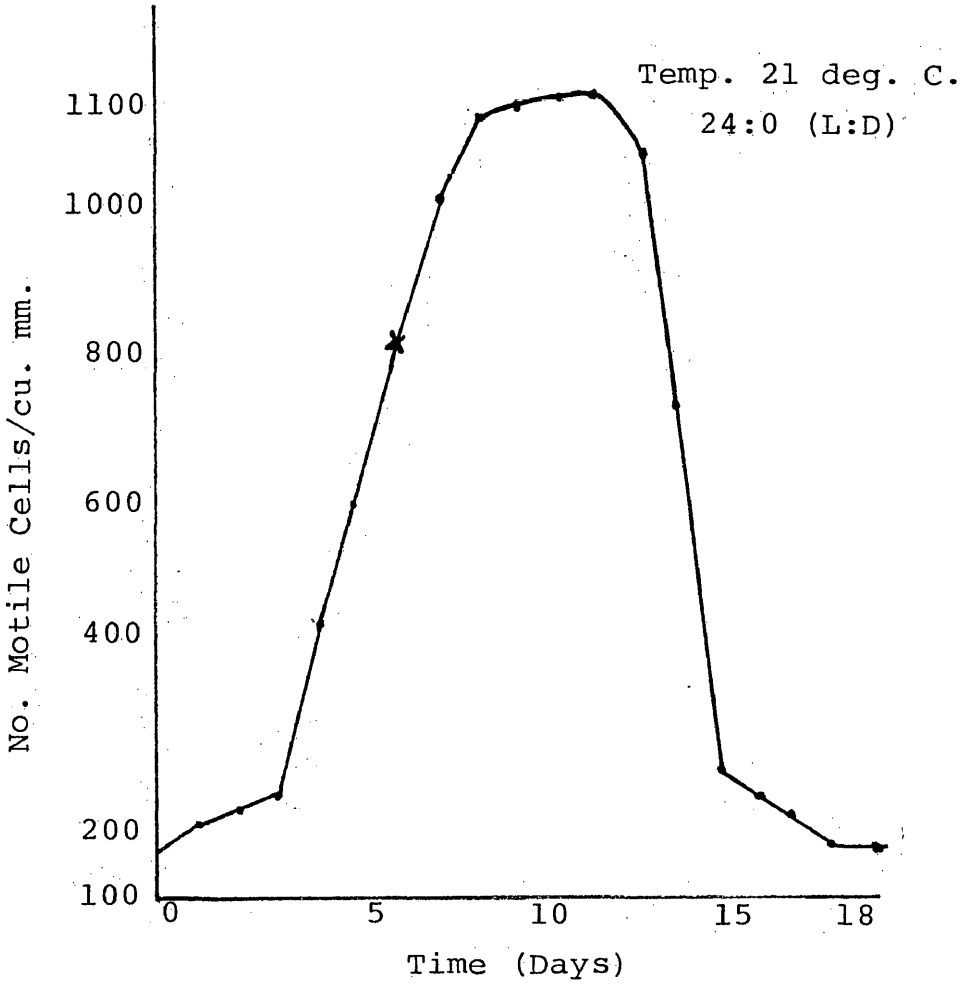

Fig. 5. Growth Rate S. quadricauda on Proteose Agar.

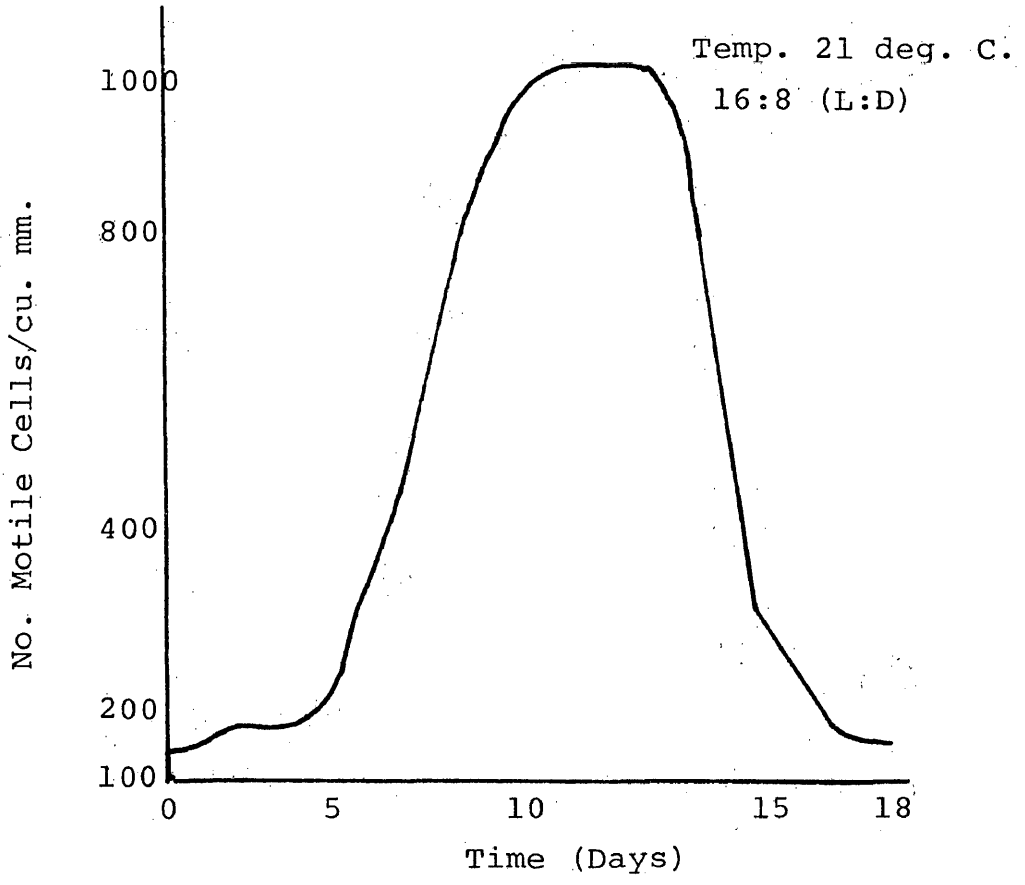

Fig. 6. Growth Rate S. quadricauda in Beijerinck Medium.

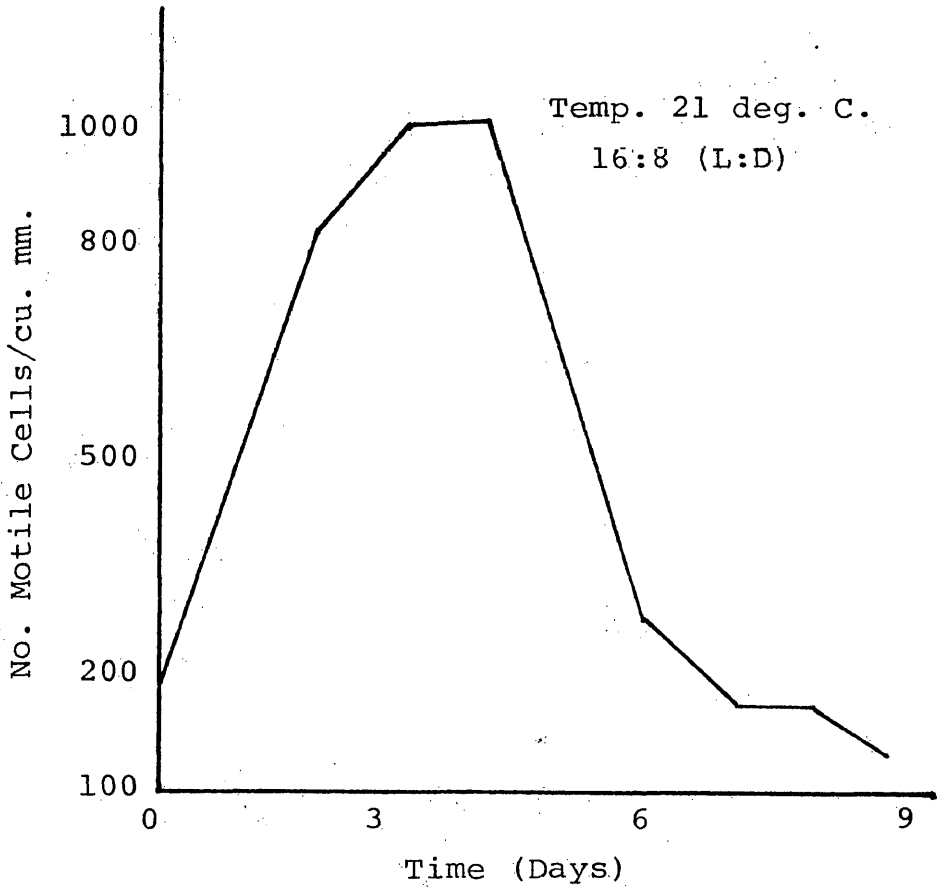

Fig. 7. Growth Rate S. quadricauda in nitrogen free Beijerinck.

Yeast Dextrose Agar. The motile cells plasmolyzed. Motile cells in the nitrogen free Beijerinck medium did not live longer than one week. Further study for pH and osmotic pressure is indicated.

Problems for Further Study.

Based on the information provided by this study it was not possible to determine whether motile cells are zoospores or gametes. No "relative sexuality" was observed (Hartman, 1934). No plasmogamy, karyogamy, zygotes, or attempts to form clumps or pairs were observed.

More work needs to be done in terms of mating competence or crossing motile cells with different strains and species to note whether motile cells behave like zoospores or gametes. Also more work needs to be done on the quantitative aspects of the different factors involved in motile cell production for Scenedesmus as well as other possible factors involved in motile cell production.

DISCUSSION

Since 1829 when the genus Scenedesmus was first described it was noted that the species could be placed into two general morphological categories. Those species that are ovoid and those that are fusiform.

Most fusiform species lack spines while most ovoid species have spines. Trainor and Burg (1965) suggested that the ability to produce motile cells might be one essential difference which may be linked to morphology between Scenedesmus species lacking spines which tend to have a fusiform to lunate shape from spine-bearing Scenedesmus species which have oblong or ovate shaped cells.

This experiment shows that at least one spine-bearing species is capable of producing motile cells. Motile cells of S. quadricauda were similar in appearance to motile cells of non spine-bearing species. This would indicate that the ability to produce motile cells is not an essential difference between spine-bearing and non spine-bearing species.

Fritsch (1935) placed Scenedesmus in the order Chlorococcales family Coelastraceae. This family is characterized by its members having no motile cells. With the finding of motile cells (Trainor, 1963, 1965) the genus

was placed in the family Hydrodictyaceae. This family is characterized by having strictly colonial members capable of forming zoospores and gametes.

Whitford (1969) places the genus Scenedesmus in the family Scenedesmaceae. Unlike members of the Hydrodictyaceae which tend to be multinucleate and produce a chloroplast which is diffuse in mature cells, Scenedesmus is uninucleate with a well defined plastid that is not diffuse. Whitford's Scenedesmaceae are characterized as reproducing solely by autospores. The motile cells discovered in this study indicate that a revision of this family or other taxonomic adjustments may be necessary.

Klebs (Trainor, 1959) noted that semi-starvation was necessary before certain strains of Chlamydomonas would produce gametes. Sager and Granick (1954) found that only ammonium nitrate depletion was necessary for gametogenesis in Chlamydomonas. Compounds such as NH_4Cl, $NaNO_3$, urea, and glutamine can act as sole nitrogen sources but zygote formation will not take place. Trainor (1963, 1965) also found that ammonium nitrate depletion stimulated motile cell production in S. obliquus. This study found that ammonium nitrate deficiency will stimulate motile cell production but only a deficiency of NH_4^+ is actually necessary (Table XI).

Moewuss (1938) noted that only certain wavelengths at the blue end of the spectrum are necessary for motile cells to be produced in Chlamydomonas. Sager and Granick (1953) found the light requirement to be a function of nutrient depletion. Sager and Granick (1954) found that light was necessary to produce cells competent to mate. Light was involved in photosynthesis which promoted anabolic processes in the cell which ties up any remaining free nitrogen.

Lewin (1956) observed that the light for gamete production has an action spectrum similar to that of photosynthesis. Gamete production was inhibited by phenylurethan a photosynthetic inhibitor. In this study it was noted that only nitrogen deficient cultures in the presence of light were able to produce motile cells. Other studies by Hoyt (1927), Spessard (1930), Couch (1932) using different genera found that light was not necessary for the liberation of gametes. More experimentation is necessary to determine the function of light in motile cell production for Scenedesmus quadricauda.

In this study an increase in temperature up to 21 degrees C. resulted in an increase in the number of motile cells produced (Figure 4). Motile cell production was favored at higher temperatures. These results agree with the work of Forester and Wiese (1957) who found that

an increase in temperature up to a certain point results in an increase in production of motile cells in Chlamydomonas.

Trainor and Burg (1965) noted that in Scenedesmus obliquus motile cell production was greatest and favored at lower temperatures (15 degrees C.). The same temperature tolerance range for motile cells exists for Scenedesmus obliquus and Scenedesmus quadricauda. Temperature comparison for motile cell production in S. obliquus and S. quadricauda cannot as yet be explained.

In this study motile cells were produced at the end of the log phase when the growth rate was declining (Fig. 7). If motile cells are gametes, these results would agree with the work of Coleman (1958) who noted that the appearance of mating competence is most often correlated with the end of the log phase of growth. Growth rate declines when mating ability appears at a maximum. Trainor and Burg (1965) noted that with an increase of vegetative growth in S. obliquus there was a decrease in the number of motile cells produced.

The role of temperature and illumination in mating behavior is not yet completely understood in Scenedesmus. Further experimentation is necessary.

Why motile cells for spine-bearing Scenedesmus species were not found in previous studies is not known. Previous studies by Trainor (1963, 1965) were conducted

under continual illumination which the present study shows is not the best lighting conditions to stimulate production of motile cells. Time of experimental observation when motile cells were first observed was within three days of inoculation in the previous studies. Under the best conditions for motile cell production in this study, four days were required before motile cells were first observed. The present study indicates that vegetative cells in the log phase of growth is not the time to observe motile cells. This may not have been the case in previous studies.

Consideration of some of the many variables involved in motile cell production suggests possible reasons why motility was not observed in spine-bearing Scenedesmus species previously.

SUMMARY

Scenedesmus quadricauda, a spine-bearing species, was found to possess the ability to produce motile cells under certain conditions.

Best conditions for stimulating motile cell production were found to be a nitrogen-free Beijerinck medium at a temperature of 21 degrees C., at photoperiod 16:8 (L:D), and from vegetative cells that were not in the logarithmic phase of growth.

Analysis of the Beijerinck medium revealed that only ammonium deficiency was necessary in terms of nutrient depletion to stimulate motile cell production.

Temperature range for motile cell production was 14 degrees C. to 24 degrees C. More experimentation is necessary to determine the role of light in motile cell production. Attempts to subculture motile cells to different media failed. The presence of plasmolyzed cells indicates more experimentation in terms of pH and osmotic pressure is necessary.

Motile cells were similar in appearance to those found in non spine-bearing species. Further experimentation is necessary to determine whether motile cells are zoospores or gametes.

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