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Title IDENTIFICATION, CULTURE, AND PHYSIOLOGICAL ECOLOGY OF CRYOPHILIC ALGAE

Abstract approved Redacted for privacy
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The purpose of this investigation was several-fold: 1) identification of species of snow algae from the Cascade Mountains of Oregon and Washington, 2) isolation and culture of snow algae in the laboratory, 3) ecological observations and measurements of physical parameters in the field, and 4) the effect of environmental parameters on growth and photosynthesis of a cryophilic alga in the laboratory.

Snow samples from several alpine areas in Oregon, Washington, and Montana have been examined and found to contain snow algae, often in such abundance as to color the snow surface. Twenty-three species of algae, including three new species (Chroococcopsis nivalis n. sp., Ourococcus cascadensis n. sp., and Scotiella gigantea n. sp.), are described and six species have been grown in unialgal cultures at 5°C.

The physiology of cultured Chromulina chionophila Stein was investigated. The uptake of $NaH^{14}CO_3$ was measured at various light

intensities and temperatures. In this alga, the maximum rate of photosynthesis (at light saturation) occurred at 10°C. At temperatures of 15 to 20°C cells of C. chionophila disintegrate. The effect of metabolic inhibitors on this process of cell disruption indicates the possible presence of an energy requiring active transport of ions in this alga.

The relationship of cryophilic algae to possible Martian life-forms is discussed.

IDENTIFICATION, CULTURE, AND PHYSIOLOGICAL ECOLOGY OF CRYOPHILIC ALGAE

by

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IDENTIFICATION, CULTURE, AND PHYSIOLOGICAL ECOLOGY OF CRYOPHILIC ALGAE

INTRODUCTION

The snow-fields, glaciers, high mountains, and polar regions of the world contain a unique community of snow and ice algae. Many species of algae grow in areas of the Arctic or Antarctic where the temperature range is 20° to -50° C and where the annual precipitation does not exceed that of desert regions. In these areas algae often occur in such abundance as to color the snow surface green, yellow, or red. These cryophilic algae constitute an interesting subject for study because of their obvious adaptation to life under extremely harsh environmental conditions. Furthermore, with the acknowledged possibility of encountering life on Mars, the study of cold-resistant organisms has taken on new significance.

Many investigators have concluded that the dark areas of Mars are composed of some type of vegetation. Alternate theories to explain the dark areas have been proposed (17, 34, 47). However, as Sagan has stated, "The observational evidence suggests the existence in the Martian dark areas of some organisms which darken and change color when the availability of water vapor increases; of some organisms which change their size distribution as the availability of water vapor increases; and of hydrocarbon and aldehyde bonds" (41). Martian organisms, if indeed they exist, probably include simple

autotrophs such as algae.

Mars as a Habitat for Vegetation

One would expect that the most critical environmental parameters affecting Martian autotrophs would be: composition and density of the atmosphere, intensity and quality of solar radiation, temperature, substrate composition, and availability of water.

Atmosphere

Kuiper's observations suggest that the fraction of carbon dioxide by volume on Mars is 2.2 percent, i.e., 14 times more CO_2 on Mars than on the Earth (29). Most investigators regard the remainder of the Martian atmosphere to be composed of nitrogen and a small amount of argon (44).

Temperature

Radiometric measurements by Coblentz and others (4) indicate a range of $+30^{\circ}$ to -101° C at the surface of Mars. The summer temperature reaches its maximum of 25° to 30° C shortly after noon in bright areas. Dark regions are about 8° warmer. The usual night temperature probably reaches -70° C.

Solar Radiation

Solar radiation falling on the surface of Mars amounts to 0.87 gram calories cm^{-2} min^{-1} $\pm$ 20 percent, depending upon the orbital position (54). Carbon dioxide in the atmosphere removes radiation with wavelengths less than 180 mμ and ice haze probably scatters much of the ultra-violet radiation between 180 mμ and 450 mμ.

Substrate

It is generally agreed that the light colored areas on Mars consist of arid desert covered with volcanic dust. Polarization studies of these regions indicate that the soil may resemble the terrestrial common mineral limonite ($Fe_2O_3 \cdot nH_2O$). Kuiper came to the conclusion that Martian soil is composed largely of felsitic rhyolite (a mineral mixture of quartz, silicon dioxide, and alkaline silicates, e.g. $KAlSi_3O_8$). These latter minerals do not differ greatly from volcanic ash from the Oregon Cascade Mountains (38).

Availability of Water

Most investigators agree that the polar caps of Mars are composed of frozen water from a few centimeters to a few feet in thickness. An interpretation of the difference between theoretical and observed surface temperatures due to the carbon dioxide-water vapor

greenhouse effect gives values for atmospheric water vapor of between $1x10^{-3}$ and $2x10^{-2}$ gm cm^{-2} (40). In comparison, atmospheric water vapor over the Arizona desert amounts to about $1x10^{-1}$ gm cm^{-2}. Evidence has been reported which indicates the presence of snow in the Martian atmosphere (28).

Thus, the Martian surface environment possesses an abundant supply of carbon dioxide, light energy is only slightly less than that on Earth, water is available, although in small quantity, and the temperature range does not greatly exceed that found on the Earth. Also, from the present state of knowledge, the range of environmental parameters on Mars does not exclude the growth of many terrestrial organisms. For example, cryophilic or extremely eurythermal terrestrial autotrophs, capable of withstanding considerable dessication, might survive and grow in the Martian environment. Such organisms exist in extremely harsh environments on the Earth. In particular, many species of snow algae grow abundantly in the high mountain and polar regions of the world. In these regions, algae are subjected to daily freezing, thawing, and dessication. Nevertheless, these cryophilic algae often grow in abundance, and in the spring and summer they frequently form visible algal blooms which cover the surface of the snow. Areas of frozen water vapor on Mars, such as around the rims of craters, could provide a habitat for cryophilic autotrophs similar to terrestrial snow algae.

Terrestrial types of snow algae could explain the observational evidence on the dark areas of Mars. Salisbury gives an excellent list of criteria for evaluating known life forms as potential inhabitants of Mars (42). After considering several forms of terrestrial vegetation, he concludes that none of the lower terrestrial vegetation can fulfill these requirements. He therefore proposes that the Martian vegetation consists of highly modified (both biochemically, and morphologically) broad-leafed plants unlike any known terrestrial forms. This suggestion would seem to bring forth more questions about evolution and adaptation than it answers.

However, before eliminating terrestrial vegetation as possibilities for Martian life forms, the cryophilic algae should be examined in the light of the following four criteria given by Salisbury:

1) The suspected organisms must be visible or must form visible colonies which cover the ground rather extensively.

   The cryophyllic or "snow algae" are usually found in large visible colonies covering the surface of the snow or ice. In some areas of Antarctica the growth of Nostoc commune is so luxuriant that areas of algal peat up to six inches in depth are encountered (15).

2) The suspected organisms must account for the color (light-reflecting properties in general) and the observed color changes. The color changes should take place in response to increases in

temperature and atmospheric moisture.

Kuiper's observations at McDonald Observatory indicate that the infrared reflection spectrum of the Martian dark areas is incompatible with that of the higher green plants, but, agrees well with the spectrum of the lower forms such as lichens, or algae (29). However, terrestrial lichens show virtually no seasonal color change, and their growth rate is much too slow to account for the rapid spring and summer increase in the size of the Martian dark areas.

The blue-green algae are the predominant forms found in Antarctica and many areas of harsh environmental conditions. Their color agrees well with the blue-green color of the Martian dark areas. In the spring, as the temperature and moisture (from melting snow) increases, snow algae bloom into a visible covering on the surface of the ice or snow or in pools of cold water.

3) The suspected organisms must account for the observed changes in size and shape of the Martian areas -- that is, they should be able to re-emerge from a covering of yellow dust.

An algal bloom requires only the presence of the correct environmental conditions in order to spread rapidly over the snow or ice.

4) The suspected organisms must exhibit these responses within the Martian environment.

The cryophilic algae thrive in areas of extreme temperatures and little available water. For example, a blue-green algae (Schizothrix sp.) isolated from the Antarctic, showed only a slight decrease in apparent viability after 20 refreezings covering a period of 316 days (15). Consideration of the total amount of water vapor on Mars leads to the conclusion that the height of the Martian vegetation could amount to a layer hardly more than 0.2 mm thick (29). This idea is certainly compatible with a covering of some type of cryophilic algae. Finally, it has been hypothesized that the Martian vegetation is struggling for survival near the end of its planetary life (56). In view of the often held theory that the blue-green algae were the first, and probably will be the last, existing forms of autotrophic life on the Earth (35), the case for their probable occurrence on Mars is again strengthened.

One approach to the question of probable Martian life forms is the study of the growth and viability of terrestrial forms of life under simulated Martian conditions. Very little work has been done along this line beyond the experiments of Kooistra (27) and Fulton (10). These experiments demonstrated that some bacteria will survive and

reproduce under Martian conditions. However, practically no work has been done on the viability of photosynthetic organisms in a Martian environment, and no such experiment has yet been attempted with the terrestrial autotrophs which are best adapted to a Martian-type environment, i. e. the cryophilic snow and ice algae.

Previous investigations of the snow algae inhabiting the southern hemisphere have been made in the volcanic mountains of Ecuador (30) and in Antarctica (9, 58). In the northern hemisphere, studies have been made in Europe, Greenland, and Alaska (19, 20, 21, 22, 23, 24, 25). Snow algae have been reported in British Columbia (49) and in Japan (18). In the western United States, investigations of the cryovegetation have been conducted in Wyoming, the Rocky Mountain region and Olympic National Park (11, 22, 26, 55).

These studies have been of a purely descriptive and taxonomic nature. The purpose of the present study is to learn more about the physiological ecology and growth of cryophilic algae in the field and in the laboratory.

METHODS AND MATERIALS

Collection

Samples of surface snow down to a depth of about 5 cm were collected from several alpine and glacial areas in the northwestern United States (Description of Habitats, p. 49 and Plate 1). Samples were collected in 40 ml polyethylene vials and packed in snow for transportation to the laboratory. In the laboratory, part of each sample was used for inoculation into cultures and part for preservation in formalin:acetic acid:alcohol solution. Identifications of species were made on fresh material when possible, otherwise preserved or frozen samples were used.

Physical Measurements

Estimates of the density of the snow in several samples were obtained by determining the ratio of the volume of fresh snow to the volume of melt water. The pH of the snow melt water was determined in the laboratory using either a Beckman pH meter or "p Hydroin" paper.

Cultures

Algae were isolated, either by plating on agar or by micropipetting, to obtain unialgal cultures. Cultures of algae were grown in a

cold room at 5.° C and illuminated continuously with 40 watt GE "deluxe warm white" fluorescent tubes at an intensity of 0.35 milliwatts cm^{-2} sec^{-1}. Cultures were maintained in liquid culture medium of the following composition:

Major Elements	Mg/liter
$NaNO_3$	250.0
$CaCl_2$	25.0
$MgSO_4 \cdot 7H_2O$	75.0
K_2HPO_4	75.0
KH_2PO_4	175.0
$FeCl_3$ (4 drops of 1.0% solution/l)	

Minor Elements	Mg/liter
$CuSO_4 \cdot 5H_2O$	0.196
$ZnSO_4 \cdot 7H_2O$	0.044
$CoCl_2 \cdot 6H_2O$	0.022
$MnCl_2 \cdot 4H_2O$	0.360
$Na_2MoO_4 \cdot 2H_2O$	0.030

Fifty milliliters of the above solution were dispensed into 125 ml Erlenmeyer flasks and autoclaved for 15 minutes. After cooling, vitamins were added in the following concentrations:

Vitamins	Mg/liter
Thiamin · HCl	0.2
Biotin	0.1
B_{12}	0.1
Folic acid	0.1
PABA	10.0
Niacin	0.1
Inositol	1.0
Ca pantothenate	0.2
pyridoxine	0.1

The final pH of the media was 6.0. Cultures were transferred to fresh media every 20 days.

Photomicrography

Photomicrographs were made under 1000 X magnification. India ink drawings were made directly on the photographic prints and the photographs were bleached out in Lugol's iodine solution to produce black and white drawings (Plates 3 and 4).

Physiological Studies on Chromulina chionophila Stein and Brooke

Experiments on the effects of light intensity and temperature on photosynthesis in an obligate cryophilic alga (C. chionophila Stein)

were carried out in the following manner:

Two to four liter unialgal cultures of C. chionophila were grown for about ten days (early log-phase of growth) under the culture conditions given above. Cell counts were made by first preserving samples in chromic-acetic acid fixer and then counting in a "Fuchs-Rosenthal" chamber. Fifty milliliter aliquots of culture were inoculated into duplicate light and dark bottles and placed in a temperature and light controlled growth-chamber (Sherer-Gillett model GEL 34-7).

Cultures remained in the dark for 90 minutes temperature equilibration. They were then inoculated with 1 ml of $NaH^{14}CO_3$ solution containing 1 microcurie of activity and the lights were turned on. Neutral density filters consisting of various layers of wire screen were used to attenuate light, and rates of photosynthesis were measured simultaneously at different light intensities.

After an incubation period of two hours, the cultures were killed with chromic-acetic acid fixer to stop further uptake of ^{14}C and to prevent cell disruption prior to filtration. The cultures were then vacuum filtered onto membrane filters (0.45 micron pore size) and glued on to aluminum planchets. Radioactivity was determined as relative counts/minute, using an automatic sample counting chamber (Nuclear-Chicago D-47). A total of at least 5000 counts were collected for each sample, and duplicate samples generally agreed to within $\pm$ 5 percent. Net counts/minute/cell (light bottle-dark bottle-

background) were plotted at various light intensities and temperatures. Light intensities within the growth chamber were measured with a radiometer (Kettering-Yellow Springs model 65). Measurements are estimated by the manufacturer to be accurate to $\pm$ 5 percent.

The process of temperature-induced cell disruption which occurs in C. chionophila Stein at 15 to 20° C was investigated using metabolic inhibitors. Cultures of about 30 days age were maintained and observed at 5°C. Inhibitors were added to give concentrations of potassium cyanide (10^{-3} molar), iodoacetic acid (10^{-4} molar or 10^{-2} molar), and sodium-monoflouroacetic acid (10^{-2} molar and 10^{-1} molar). Aliquots were taken at various time intervals and observed under the microscope for cell disruption or loss of motility.

The effects of freeze-thawing on C. chionophila were investigated. Cultures were frozen at -5° C and thawed at +5° C. Cell counts and ^{14}C uptake were then determined as stated above.

RESULTS AND DISCUSSION

The results of this study have been divided into four parts: 1) taxonomy and identification of species of cryophilic algae occurring in the high mountains of the northwestern United States, 2) ecological observations and measurements of physical parameters in the field, 3) isolation and culture of cryophilic algae, and 4) the effect of environmental parameters on growth and photosynthesis of a cryophilic alga (Chromulina chionophila Stein and Brooke) in the laboratory.

SYSTEMATIC LIST OF SPECIES

The systematic list of species given below, for the most part, follows the system of Smith (46). The distribution of the species listed is given in Plate 2.

CYANOPHYTA

I. CHROOCOCCALES

Pilgeria brasiliensis Schmidle (45).
Plate 3, Figs. 1, 2, 3.

The cells are densely packed into hollow ellipsoidal to rounded

colonies of up to 45μ across. They are mutually compressed into 4 to 7 sided cells, which are from 3 to 5μ across. The cell content appears homogenous and is blue-green to grey in color.

This alga was abundant in the sample collected from Mt. St. Helens, Washington. Schmidle found this organism in a brook in Brazil.

This is a rare genus. It is probably closely related to Holopedium or to Coelosphaerium sp. Geitler (14) believes that this is a highly questionable form, which perhaps, is not even a blue-green algae. However, our species appears identical in every respect to the description given by Schmidle. It should be noted that Coelosphaerium Kuetzingianum Nageli has been reported to occur in a small alpine lake at an elevation of 9, 900 feet in the Sierra Nevada Mountains of California (55).

Aphanothece saxicola Nägeli (36).

Plate 3, Fig. 4.
Illustration: Nägeli (36), p. 60, Fig. 2.

The cells lie in all directions within a loose transparent colonial matrix. The colony is of indefinite shape containing up to hundreds of cells. The cells are cylindrical and are 1μ wide by 2. 5 to 4. 5μ long and blue-grey in color.

This alga is, in general, very widely distributed. It has been reported to occur in Antarctica (9), in alpine pools in British

Columbia (50), as well as in hot springs in Wyoming (7).

Gloeocapsa alpina (Nägeli) emend. Brand (2).

Plate 3, Fig. 5.
Illustration: Geitler (14), p. 206, Fig. 98, p. 297, Fig. 99.

The cells are contained in a transparent gelatinous matrix, forming a colony without an evident external sheath, and containing from 9 to 35 cells. The cells are spherical to elongate, 3 to 10μ across, and grey-violet in color.

This alga occurred only rarely in two samples from the Three Sisters Area of Oregon. A few of these colonies have been reported to occur in the snow of Greenland and Europe (19, 25).

Microcystis parasitica Kützz var. glacialis (Fritsch)

Plate 3, Fig. 6.
Illustration: Geitler (14), p. 142, Fig. 63C.

Up to hundreds of cells are contained in a gelatinous colony. The colonies are spherical to ellipsoidal, but are more often irregular, with a gold-brown to transparent colonial matrix. The cells are small, blue-green, 0.7μ across.

This alga occurred in varying abundance in 75 percent of our samples. It has been reported as common in collections from Antarctica (5).

Synechococcus aeruginosus Nägeli (36).

Plate 3, Fig. 7.
Illustration: Smith, G. M. (46), p. 558, fig. 460.

The cells are solitary, ellipsoidal to cylindrical with bluntly rounded ends and are 6 to 9μ wide and 12 to 18μ long. An inconspicuous gelatinous sheath about 1μ thick surrounds the cell.

This alga was found in abundance in one of our samples from South Sister Mountain, Oregon. It is very widely distributed and forms another example of Cyanophyta species that have been reported to occur both in hot springs (7) and in Antarctica (9). This organism has also been found at an elevation of 11, 500 feet in Colorado (16).

II. CHAMAESIPHONALES

Chroococcopsis nivalis n. sp.

Plate 4, Figs. 1, 2, 3, 4.

The cells are seldom solitary, and are mostly contained in spherical to somewhat irregular ellipsoidal masses of up to hundreds of cells within a sporangium. The sporangia are 50 to 100μ across. The cells or endospores are spherical to irregular in shape and are from 3 to 20μ across and dull blue-grey in color. The sporangial wall is transparent to yellow-orange in color and from 3 to 10μ thick. From observation of preserved specimens it appears that, upon

reaching the larger dimensions, the sporangial wall splits open releasing the endospores which then form a more or less pseudoparenchymatous mass.

This alga was first found in a snow sample from South Sister Mountain, Oregon, and was later found to occur in varying abundance in 32 percent of our samples.

This is a rare genus. No tendency toward a filamentous organization is evident, and it would, therefore, appear that the erection of this genus by Geitler (13) is valid. To the author's knowledge, there has been but one other species of Chroococcopsis described. C. nivalis is similar to, and has some characteristics of, both C. gigantea Geitler and Pleurocapsa fuliginosa Hauck. Because of the difference in size and number of endospores, cell size, and habitat, it is described here as a new species.

Chroococcopsis nivalis n. sp.

Cellulae raro singulae, saepius in massis sphericis aut paululum irregulariter ellipsoideis, ex usque ad centurias cellularum intra sporangium compositis. Sporangia 50-100u lat., membranam perlucidam ad flavo-luteam, crassam (3-10u crass.) habentia. Cellulae aut endosporae sphericae inaequalisque, 30-20u lat., caeruleo-cineraea. Nonnulla sporangia fissa, cellulas ad stratum incrustrans formandum liberantia, observata sunt.

Haec species similis C. giganteae Geitler et Pleurocapsae

fuliguinosae Hauck videtur, auctores, autem, ob magnitudinem endosporarum numerumque differentem, atque ob magnitudinem cellularum atque habitatum, eam ut speciem novam constituunt.

Planta in stratis superioribus nivis liquescentis, pH 5, elevatione 2, 400 m. in declivitate versus meridiem aspiciente montis South Sister, Oregon, dicti, reperta; m. Mai., d. 30 1965 lecta.

Myxosarcina amethystina Copeland (7).

Plate 3, Fig. 8.
Illustration: Smith, G. M. (46), p. 569, Fig. 479.

The cells are closely packed into ovoid or subspherical to irregular colonies. Each colony contains from 16 to 30 cells. The cells are irregularly spheroidal to polygonal, and are from 4.5 to 6μ across. The entire colony may be from 14 to 25μ across by 28 to 40μ long. The cells are blue-green to bluish violet. A single large granule may be observed in the center of most cells. Reproduction is by the formation of endospores.

Only two colonies were observed and the identification is not certain. However, this alga appears as another example of the ability of the Cyanophyta to withstand both hot and cold extremes of temperature. This alga was originally found existing in hot springs in Wyoming at a temperature between 37^{o} and 46^{o} C (7).

III. OSCILLATORIALES

Romeria elegans var. nivicola Kol (22).

Plate 3, Fig. 9.
Illustration: Kol (22), p. 186, Fig. 39.

Cells solitary or arranged in short filaments. The cells are long and cylindrical, straight or curved and are 1 to 2μ wide and 4 to 14μ long. The cell contents appear homogenous and are blue-green in color.

This alga was found in samples from Mt. St. Helens and Mt. Rainier, Washington, and has been reported as fairly abundant in the green show of Yellowstone National Park (22). It has also been reported as abundant in one sample from Glacier National Park, Montana (11).

CHLOROPHYTA

I. VOLVOCALES

Chlamydomonas nivalis (Bauer) Wille (58).

Plate 3, Fig. 10.
Illustration: Kol, E. (23), Plate 6, Figs. 42-50.

The cells are solitary or sometimes found in groups and are spherical. Only akinetes (sporelike resting stages) and zygotes were observed. The akinetes are 10 to 25μ in diameter with a transparent mucilaginous envelope varying from 1 to 10μ in thickness. The

zygotes are from 20 to 35μ in diameter and possess a thick transparent cell wall composed of dome-shaped prominences with hexagonal bases. In both the akinetes and the zygotes (zygospores) the cell contents are completely masked by deep red pigment.

This alga was the most abundant species occurring in 75 percent of the samples examined. This organism is, perhaps, the most widely distributed snow alga. Because of its red color, it gives rise to "red snow" on glaciers and snow fields the world over.

Chlamydomonas sanguinea Lagerheim (30).

Plate 4, Fig. 10.
Illustration: Theinemann (51) Pl. 28, Fig. 141.

The cells are solitary and are ellipsoidal to spherical. They are 31 to 45μ in diameter and 44 to 64μ long. The cell wall is transparent and very thick (up to 5μ). The cell contents are completely masked by red pigment. The red color of these cells appears to be slightly more orange-red than the deeper red color of C. nivalis. No motile stages were observed.

This alga was first reported by Lagerheim (30) who found it in the snow of a high volcanic peak in Ecuador. It has since been found in red snow from Europe, Alaska, Antarctica, and in the western United States from Mt. Rainier, Washington, Glacier National Park, Montana, and Colorado (26).

Chlamydomonas yellowstonensis Kol (22).

Plate 3, Fig. s 11, 12, 13.
Illustration: Kol, E. (22) p. 186, Figs. 1-29, p. 189, Fig. s 3-6, 8.

The cells are solitary and are motile or non-motile. The motile cells are ellipsoidal or egg-shaped and are 6 to 10μ wide and 10 to 14μ long and biflagellate. The flagella are about the length of the cell. The cell wall appears thin and there is a circular red eye-spot near the anterior part of the cell. As the cells grow older, they assume a more spherical shape (10 to 34μ in diameter) and lose their flagella. The cell contents are green. Reproduction begins by longitudinal division and the daughter cells are liberated by a gelatinization of the parent cell wall. Isogamous sexual reproduction by fusion of the biflagellate cells to form quadraflagellate zygotes has been reported to occur (6).

To the authors' knowledge this alga has been reported as the causative agent of green snow only twice since its original description. It has been reported as occurring in Colorado (16) and also in the Pacific Northwest (11).

It was found to be very abundant as the causative agent of green snow in one sample (#25) from Mt. Rainier, Washington.

Sphaerellopsis rubra Stein and Brooke (49)

Plate 4, Figs. 8, 9, 10.
Illustration: Stein and Brooke (49), p. 1184, and Pl. I.

The cells are solitary, more or less ellipsoidal and are 25 to 50μ long and 20 to 40μ wide. The cell wall may be very thick and is transparent. The cell contents are completely masked by the presence of red pigment. Vegetative reproduction is by division of the protoplast within the wall to form four to eight spores (49). Two flagella extend from colorless papilla at the apical end of the cell and are about one-half the length of the cell. The original description gives the length of the flagella as equal to, or slightly longer than, the length of the cell. The shorter flagella noted here may be due to the fact that fresh specimens were not observed by us.

This alga was found to be abundant in a snow sample collected on May 23, 1965, from Mt. St. Helens, Washington. We also found it to be abundant in a snow sample collected by S. Roffler from Mt. Ruapehu, New Zealand, on November 13, 1965. In both cases it was the causative agent of red snow. To the authors knowledge, these are the only two records for this species other than the original description from Mt. Seymour, British Columbia. The finding of this species in New Zealand indicates that this alga is probably much more widely distributed than the present literature indicates.

II. TETRASPORALES

Coccomyxa dispar Schmidle (45)

Plate 3, Fig. 19.
Illustration: Smith (46), p. 133, Fig. 70.

The cells are single, or in culture, they may be in colonies of indefinite form, with the cells distributed irregularly within a colonial matrix. They are 2 to 7μ wide and 4 to 11μ long, and are surrounded by a gelatinous sheath. The chloroplast is a longitudinal plate partially encircling the cell. Vegetative cell division is in a plane diagonal to the long axis.

This alga was not found in field collections unless cultured. Holm-Hansen (15) also found that this species was not evident in preserved samples from Antarctica, but appeared in cultures started from the same material.

Ourococcus cascadensis n. sp.

Plate 4, Figs. 20, 21, 22.

The cells are solitary. They may be fusiform and straight, sigmoid, lunate or irregularly bent. Both poles of the cell may be acutely pointed, or one pole may be pointed and the other broadly rounded. They are from 2 to 4μ wide at the center and 10 to 22μ long. A cell contains a single pale green parietal chloroplast, with one or several pyrenoids. Multiplication is by transverse division.

No formation of autospores was observed.

This species was abundant in a sample from Mt. St. Helens, Washington, and was found to occur rarely in three other snow samples from Oregon and Washington (Plate 2).

This species appears very similar to O. bicaudatus which has been recorded from Massachusetts, Kansas and at an elevation of 14,000 feet on Longs Peak, Colorado (16). The present species differs from O. bicaudatus by its smaller diameter (being on the average only half the diameter of O. bicaudatus) and by its growth in snow.

Ourococcus cascadensis n. sp.

Cellulae singulae, fusiformes, rectae ad arcuatas. Aut uterque cellulae polus acutissimus aut unus polus acutus, alter late rotundatus. Pyrenoidea unum ad aliquot. Multiplicatio ut videtur per d divisionem transversam. Cellulae 1-22 μ long., in partem mediam 2-4 μ lat.

Cellulae m. Mai d. 23, 1965, in stratis superioribus nivis liquescentis, elevatione 1,667 m. in monte St. Helens, Washington dicto, c. 33m. versus meridiem et orientem a loco Longview Ski Hut dicto abundabant.

III. ULOTRICHALES

Raphidonema nivale Chodat (6)

Plate 3, Fig. 25.

The cells are solitary or arranged in short unbranched filaments of up to 8 cells. They are cylindrical and one or both ends of the filament tapers to a point. Each cell is 2.5 to 3μ wide and 10 to 20μ long. They are green in color and possess a parietal chloroplast. Small granular bodies (possibly pyrenoids or oil droplets) were observed in several specimens. Reproduction is by constriction and fragmentation.

This alga was found to occur rarely in snow from Mt. St. Helens, Washington, and South Sister Mt., Oregon. It is an ubiqitous cryophilic algae and probably occurs world-wide, although usually not in great numbers.

Stichococcus bacillaris Nägeli (36)

Plate 3, fig. 26.
Illustration: Smith (46), p. 145, Fig. 79.

The cells are solitary or in short filaments. They are cylindrical with rounded poles and are 2.5 to 3μ in diameter and 4 to 8μ long.

This alga was not observed in field collections but appeared in several cultures that were innoculated with snow samples. This is

a common alga which occurs in both snow and in other habitats around the world. It has been reported to occur in snow in Greenland (25) and Alaska (23).

IV. CHLOROCOCCALES

Chodatella brevispina Fritsch (9)

Plate 3, Fig. 23.
Illustration: Fritsch (9), Pl. 19, Figs. 25, 26.

The cells are solitary and ellipsoidal with bluntly rounded ends. They are 12 to 22μ in diameter and 18 to 33μ long. The thick transparent cell wall is uniformly covered with many, short, stout spines protruding up to 2μ. In young cells the spines may be absent or only beginning to develop. Older cells may shed their spines and assume a more spherical shape. The cells are usually completely filled with orange-red pigment.

Fritsch (9) states that he frequently found individuals lying together in groups of four or more, possibly indicating the formation from a common mother-individual; although, he did not observe any stages in the process. We also observed one group of four cells lying together within a transparent sheath. Garric (11) also observed the formation of 4 to 8 aplanospores.

This alga occurred in varying abundance in 28 percent of our samples and in four instances was the causative agent of orange-

red snow. It is a common cryophilic species occurring on snow-fields around the world.

Scotiella cryophila Chodat (5)

Plate 3, Fig. 14.
Illustration: Kol (25), Pl. 2, Fig. s 7-9.

The cells are solitary, spindle-shaped, and are 9 to 12μ wide and 20 to 35μ long. The thick transparent cell wall contains several ribs which run longitudinally from pole to pole. The cell contents are pale green.

Garric (11) found cells of this species with red pigmentation.

This alga appears to be rather widely distributed. It has been found in Greenland (25), and British Columbia (49). It was found, although not abundantly, in 32 percent of the samples in this study.

Scotiella gigantea n. sp.

Plate 4, Figs. 5, 6.

The cells are solitary and spindle-shaped to egg-shaped. They are large and vary from 40 to 46μ wide and 77 to 89μ long. The cell wall is thick and transparent, and contains about 12 ribs which run longitudinally from pole to pole. One end of the cell is more rounded than the other. The cell contents are completely masked by pigment, which may vary from green-brown to dark red in color. One abnormally large cell was observed to be dividing and reproduction

appears to be by division of the cell contents into four daughter cells within the parent cell.

This organism appears similar to Oocystis sp. However; because of the thick transparent cell wall with longitudinal ribs, it is described here under the genus Scotiella.

This alga was found to occur rarely in one snow sample from South Sister Mountain and in another from Mt. Jefferson, Oregon. The most outstanding characteristic is its large size as compared to other species of Scotiella. In discussing the variability found among individuals of S. nivalis Garric (11) noted the presence of one individual measuring 32 to 68μ. It is possible that the individual he observed was actually S. gigantea.

Scotiella gigantea n. sp.

Cellulae singulae, fusiformes, membranam crassam perlucidam habentes. Chromatophorus maxime brunneus, protoplastum omnino implens. Cellulae 40-46μ lat., 77-89μ long.

Haec species in areis parvis residualibus nivis liquescentis in elevatione minima, cum aliis algis nivalibus raro reperta. In declivitate meridionale montis Jefferson, Oregon dicti, admodum infra terminum inferiorem silvestrem, lecta.

Scotiella nivalis (Chodat) Fritsch (9)

Plate 3, Figs. 15, 16, 17, 18.
Illustration: Garric (11)

The cells are solitary and ellipsoidal. They are 14 to 20μ in diameter and 23 to 40μ long. The cell wall is thick and transparent and contains from 6 to 10 longitudinal ribs which may or may not spiral from pole to pole. The cell contents may contain a large orange-red fat body at each pole or the pigment may be distributed throughout the protoplasm.

The individuals of this species may be quite variable as Garric (1965) has noted. The species may actually be composed of several varieties which will not be distinguished here.

This alga was one of the most abundant species present and occurred in 75 percent of our samples. It is widely distributed and occurs on snow-fields and glaciers the world over.

Trebouxia cladoniae (Chod.) G. M. Smith

Illustration: Smith (46), p. 225, Fig. 137.

The cells are spherical to ovoid. They are solitary or within the thalli of lichens. They are 7 to 23μ in diameter with a thin cell wall. The chloroplast of most cells is axial and light green in color. Other cells are colorless and appear to be possibly epiphytic on pollen grains. A few cells contained bright red pigment. Several

cells of 20 to 23μ in diameter were divided into 8 autospores.

This alga was found to be abundant on the surface of snow in one location from South Sister Mountain, Oregon. It has not previously been reported as occurring on snow. Since the area where the collection was made was in a dense forest of fir trees, it is probable that this is not a true cryophilic species. It is likely that lichen fragments were carried by the wind onto the snow surface from the surrounding trees.

CHRYSOPHYTA

I. CHRYSOMONADALES

Chromulina chionophila Stein (48)

Plate 3, Figs. 27, 28, 29.
Illustration: Stein (48), p. 1368.

The cells are solitary or in large non-motile masses of up to several hundred cells. The cells are ovoid; flattened in cross section and are from 5 to 12μ long and 5 to 6μ wide by 2 to 3μ in diameter. There is a smooth membrane surrounding the cell and a single apical flagellum which is about equal to the length of the cell. Older cells in culture lose their flagellum, become spherical, and form gelatinous masses. The cell contains 1 (-4) smooth, 2-4 lobed yellow-brown chloroplast which lacks pyrenoids.

Cells from culture had to be examined immediately, since the warming of the slide caused the cells to disintegrate within a few minutes. It was found that if cells were first preserved in FAA solution, however, disintegration did not take place.

To the authors knowledge, this is the first report of this species since its original description. When snow samples from several locations in Oregon and Washington were innoculated into culture this organism appeared and grew abundantly at 5° C.

EUMYCOPHYTA

Chionaster nivalis (Bohlin) Wille (58)

Plate 3, Fig. 30.
Illustration: Kol (20), Pl. VII.

The cells of this fungus are branched into 3 or 4 arms which radiate from a central axis. The arms are 2 to 4μ thick, with bluntly rounded ends. The entire cell may be from 26 to 37μ from pole to pole. The protoplast is often contracted into the axial region.

This fungus was found to be abundant in only one of our samples, although it did appear occasionally in several other samples. It is widely distributed on the snow-fields of the world.

CULTURES

Algae obtained in unialgal cultures include Chlamydomonas yellowstonensis Kol, C. nivalis (Bauer) Wille, Coccomyxa dispar Schmidle, Chromulina chionophila Stein, Raphidonema sp., and Stichococcus bacillaris Nägeli.

In preliminary trials growth of cultures could not be obtained. It was found subsequently that addition of vitamins was required for growth. Several species appeared to grow for short times and then either died out or were overgrown by other species before they could be isolated. For example, Raphidonema nivale Chodat grew abundantly in several cultures for up to three weeks after the cultures were inoculated with snow. But, they were soon overgrown by the faster growing Chlamydomonas yellowstonensis Kol.

There are undoubtedly many reasons why more species did not grow successfully in culture. The chemical composition of the snow from which the samples were isolated is not known. But, it is probable that the concentrations of nutrient salts in the culture media are much higher than those in snow. Also, compounds such as dissolved organic matter may be present in snow and absent in cultures.

The singularly uniform culture conditions, no doubt, tended by natural selection to eliminate the growth of many species. It is probable that if different light intensities were used during isolation more species would have grown.

ECOLOGY

Distribution of Species

The occurrence of species in the samples has been tabulated (Plate 2). Quantitative sampling has not been attempted and the symbols in Pate 2 represent the estimated relative occurrence of species within each sample. Four of the species listed here (Chroococopsis nivalis n. sp., Ourococcus cascadensis n. sp., Scotiella gigantea n. sp., and Pilgeria brasiliensis Schmidle) have not previously been reported to occur in snow. Two species (Microcystis parasitica Kütz and Synechoccus aeruginosus Nägeli) have been reported to occur in snow elsewhere, but not in the United States. Three species (Coccomyxa dispar Schmidle, Chromulina chionophila Stein and Stichococcus bacillaris Nageli) were not evident in snow samples, but appeared in culture.

Green snow, found in shaded areas of low illumination, was characterized by an abundance of Chlamydomonas yellowstonensis Kol. Red snow was characterized by either Chlamydomonas nivalis (Bauer) Wille or Sphaerellopsis rubra Stein, and orange-red snow by either Chodatella brevispina Fritsch or Scotiella nivalis (Chodat) Fritsch. Heavier concentrations of red snow were frequently found 3 or 4 cm below the snow surface, rather than on the snow surface itself.

One of the more surprising results of this investigation was the discovery in the snow of species of blue-green algae that have previously been found in hot springs. Copeland (7) lists 18 species or varieties of Synechococcus from hot springs, including S. aeruginosus, which we found in abundance in the snow of South Sister Mountain. S. aeruginosus has also been reported from the snow of Antarctica (9) and alpine regions of Colorado (16).

Aphanothece saxicola has been reported from thermal water of 36° C and pH 8.3 (7), and has also been reported from alpine pools (50), and from Antarctica (9). Also, it is interesting that Copeland (7) found Dactylococcopsis antarctica near a geyser in Yellowstone at a temperature of 26° to 34° C. This organism had previously been reported only from the snow of Antarctica (9). As Copeland states, "The presence of this species in Yellowstone is surprising."

Considering the extremely wide range of temperature at which these species occur, one might ask whether these apparently phenotypically identical species, which occur both in hot springs and in snow, are biochemically identical, eurythermal species, or biochemically different organisms (physiological species) with different temperature optima.

A single species may show different temperature optima depending on the temperature at which the alga is grown. For example, Lowenstein (33) showed that Mastigocladus laminosus, originally

found in a hot springs, lost its ability to withstand temperatures in excess of 40° C after being maintained for 5 months at 5 to 8° C. A similar indication of temperature adaptation was noted in Oscillatoria geminata (2).

Water Content and Chemical Composition of Snow

Snow algae generally appear on the surface of the snow in the spring. They may either be present on the surface of permanent snow, until buried by new snow in the fall, or, in areas of seasonal snow, they may completely "disappear" with the snow in the late summer. This annual reappearance of snow algae has not been explained. However, the density and water content of the snow probably constitutes an important seasonal factor in the actual growth of snow algae. During the winter, snow is essentially a desert. Water, locked in the frozen state, is not available for metabolism. Also, any cells that might reach the surface of the snow, are continually buried by new snow falls. However, as spring progresses, the water content and density of the snow increases rapidly due to melting (Plate 5).

Another result of the increasing snow density in the spring is the concentration of nutrient compounds in the liquid interstices of the snow. Rainwater is a mixed electrolyte containing as its major constituents the Na^{+}, K^{+}, Mg^{+2}, Ca^{+2}, Cl^{-}, HCO_3^{-}, and SO_4^{-2} ions,

and the nitrogenous compounds NH_3, NO_2^-, NO_3^-, and N_2. Other constituents include I, Br^-, B, Fe, Al, Si, Cu^{+2}, Zn^{+2}, organic compounds and dissolved gases (O_2, CO_2, and N_2).

The chemical constituents that have been measured are generally in lower concentrations in fresh snow than in rainwater. However, compaction, melting, and ablation during the spring would result in increasing concentrations of these nutrients. Sample number 11, collected from Mt. St. Helens, Washington, contained the largest number of species and was collected subsequent to a light rainfall.

Thus, cessation of new snow, and the increasing availability of water and nutrients (and increasing light intensity) may be important factors in the seasonal growth of snow algae.

pH

The pH of the snow samples ranged between 4.8 and 6.0, but it was not possible to separate species as to their pH tolerances. This tends to confirm the findings of Garric (11); i.e., that the theory of silicotrophic - calcitrophic classification (that green snow flora are found in areas of limestone rock and red snow flora in areas of siliceous rock) does not appear valid.

Light

The intensity of solar radiation above the snow cover may be

exceptionally great due to reflection, especially when a cloud cover is present. Ångström (1) calculated that insolation with an original value of 1 increased to: 1.21 with a snow cover and a clear sky, and to 2.10 with a snow cover and a cloudy sky. Measurement of the incident noon light intensity on a clear day (May 30, 1965) at South Sister Mountain, Oregon, gave a value of 5,000 footcandles.

The intensity and quality of incident radiation passing through the snow depends upon the incident intensity, the albedo, and the water content of the snow. Measurements on the albedo of snow surfaces have been made by previous workers. The albedo for new snow ranges between 75 and 88 percent (43), but for old snow may decline to 43 percent (12). Geiger (12) has summarized the available measurements on the transmittance of radiation through snow. From his values it is evident that about 10 percent of the radiation that penetrates the surface reaches a depth of 30 cm.

With respect to our measurement, assuming an albedo of 50 percent, this means that 2,500 footcandles penetrate the snow surface. Of this amount, 10 percent or 250 footcandles, would reach a depth of 30 cm. Cultures of snow algae have been grown at 100 footcandles. Thus, it appears that enough light penetrates, at least to a depth of 30 cm., for growth of cryophilic algae to take place.

PHYSIOLOGICAL STUDIES ON *CHROMULINA CHIONOPHILA* STEIN

Cell Disruption

C. Chionophila Stein is normally grown in culture at 5°C. When placed at temperatures of 15 to 20°C, cells lose their motility and assume a spherical shape. Within one minute, the cells and then the chloroplasts burst open, spilling their contents into the surrounding media. At 5°C, the same effect was observed within 15 to 30 minutes after the addition of potassium cyanide (10^{-3} molar) in either the light or the dark.

These results might be explained by assuming that *C. chionophila* possesses some type of metabolic pump or form of active transport of ions out of the cell. Active transport in many cases has been shown to require a supply of energy in the form of adenosine triphosphate. It is known that potassium cyanide inhibits electron transport through the cytochrome chain, stopping the production of high energy phosphate, and thus, destroying the active transport of ions. Ions accumulating within the cell would lead to increasing osmotic pressure. Water entering the cell would soon build up enough turgor pressure to disrupt the cell. Inactivation of enzymes controlling active transport at 15 to 20°C, would have the same result.

Iodoacetic acid (10^{-2} molar or 10^{-4} molar) and sodium-monoflouroacetic acid (10^{-1} molar or 10^{-2} molar) did not produce cell disruption either in the light or in the dark. Cells remained intact

and motile at 5°C up to 17 hours after the addition of these compounds. These compounds are inhibitors of glycolysis and unlike potassium cyanide do not inhibit electron transport. Iodoacetic acid inhibits the action of 3-phosphoglyceraldehyde dehydrogenase, whose activity depends on the integrity of the -SH groups of the enzyme protein. Sodium-mono-fluoroacetic acid inhibits enolase, which catalyzes the conversion of 2-phosphoglycerate to enol-2-phosphoglycerate.

Thus, inhibitors of glycolysis do not produce cell disruption at 5°C. It is possible that these inhibitors of glycolysis do not enter the cell. But, either inhibitors of electron transport (at 5°C) of temperatures of 15 to 20°C do produce cell disruption, possibly by inactivating the active transport of ions. It appears that C. chionophila requires electron transport through the cytochrome chain and production of adenosine triphosphate in order to prevent cell disruption.

Freeze-Thawing

When cultures were frozen (-5°C) and then thawed (+5°C) their ability to incorporate ^{14}C was completely destroyed and no net photosynthesis could be measured. Cell counts indicated that every cell in a dense culture was destroyed by freezing.

Thus, it is difficult to understand how these organisms, in nature, can survice and grow on snow, where they are subjected to nocturnal freezing. Some species of Chromulina are known to form statospores (9). It is possible that such spores are more resistant

to freezing than the vegetative cells.

Growth

Several cultures inoculated with snow and maintained at 5° C and a light intensity of 0.35 milliwatts cm^{-2} sec^{-1} produced an abundant growth of C. chionophila Stein. Growth was relatively slow and required about one month to complete a logarithmic curve (Plate 6). Cultures of this organism inoculated at 20° C failed to grow. Cultures grown at 5° C and 2.0 milliwatts cm^{-2} sec^{-1} grew very slowly, and cultures maintained at 0 or 5° C and 5.0 milliwatts cm^{-2} sec^{-1} failed to grow. By replicate counting, cell counts are estimated to be accurate to $\pm$ 10 to 20 percent.

Photosynthesis

C. chionophila Stein obtained maximum photosynthesis at low temperatures and low light intensities (Plates 7 and 8). The photosynthetic maximum (with regard to different light intensities) increased with temperature up to 10° C. At higher temperatures, photosynthesis decreased. Photosynthesis at 0° C was almost 50 percent of the value at 10° C, whereas photosynthesis at 20° C, was only 16 percent of the 10° C maximum.

Thus, it appears that this organism can grow and photosynthesize only at low light intensities and relatively cold temperatures. The

10°C optimum is considerably lower than that found in most algae, which generally occurs between 20 and 40°C, although it has been shown that some organisms may display multiple temperature optima (37).

The rates of photosynthesis per cell were based on cell counts taken at the beginning of each experiment. Cell counts taken at the end of experiments showed that at 15 and 20°C cell numbers were reduced by cell disruption during the experiment. The decrease in photosynthesis at 15 and 20°C was identical with the decrease in cell numbers. That is, at 20°C photosynthesis was only 16 percent of the 10°C value, because only 16 percent of the cells remained viable and intact at that temperature. Thus, temperature appears to act directly by decreasing the number of cells. And, the rate of photosynthesis per cell may not be directly affected.

The very low amount of photosynthesis shown to occur in C. chionophila, even at the moderately warm temperature of 20°C, has important implications for the design of Martian life-probes. Martian life-detection landing instruments such as "Gulliver" (32) are designed to sample the surface of Mars and to measure metabolic activity in a reaction chamber. It is generally expected that at higher temperatures, enzymatic reactions take place at higher and more easily detected rates, and such instruments are provided with a heating coil for the reaction chamber. However, evidence is presented here, that indicates that metabolic activity in a cryophilic organism actually decreases with increasing temperature. Thus, it

is evident that in any Martian life-detection probe designed to measure metabolic activity, the reaction temperature should not greatly exceed freezing.

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APPENDICES

APPENDIX I

LOCATION OF COLLECTIONS

(See Plates 1 and 2)

Three Sisters Wilderness Area, Oregon

#2 - July 4, 1964. South Sister Mountain, red snow in a low area, pH 5, elevation 6, 200 ft.

#6 - March 14, 1965. South Sister Mountain, west side of Fall Creek, in low area, elevation 6, 200 ft.

#9 - March 14, 1965. Ditch next to Sparks Lake, elevation 5, 400 ft.

#17 - May 30, 1965. South Sister Mountain, pH 5, density 50%, elevation 7, 200 ft.

#19 - May 30, 1965. South Sister Mountain, red snow, pH 5, density 63%, elevation 6, 200 ft.

#29 - August 8, 1965. South Sister Mountain, next to Mt. Broken Top Trail, middle of small patch of snow among Douglas Fir trees in shaded area, pH 5, elevation 6, 800 ft.

#30 - August 8, 1965. South Sister Mountain, rapidly melting area on edge of above snow patch.

#37 - August 8, 1965. South Sister Mountain, red snow on snow field below the Lewis Glacier, pH 5, density 65%, elevation 7, 600 ft.

#42 - August 28, 1965. Middle Sister Mountain, red snow at terminal end of Collier Glacier, pH 5, elevation 5, 000 ft.

#43 - August 28, 1965. Middle Sister Mountain, red snow at terminal end of Renfrew Glacier, pH 5, elevation 7, 800 ft.

#44 - September 8, 1965. Middle Sister Mountain, small dirty residual snow patch above Sunshine Shelter, pH 5. 0, density 41%, elevation 6, 900 ft.

#45 - September 8, 1965. Middle Sister Mountain, red snow, permanent snow patch on northeast side of mountain, pH 5. 4, density 59%, elevation 7, 300 ft.

#46 - September 8, 1965. Middle Sister Mountain, red snow, terminal end of Renfrew Glacier, pH 5. 1, density 50%, elevation 7, 800 ft.

#48 - September 8, 1965. Middle Sister Mountain, south side of Renfrew Glacier, surface of dirt covered ice between rocks, pH 5. 6, elevation 8, 700 ft.

#49 - September 8, 1965. Middle Sister Mountain, orange snow, north side of Renfrew Glacier, pH 5. 3, density 50%, elevation 9, 000 ft.

#50 - September 8, 1965. Middle Sister Mountain, orange snow, Middle of Hayden Glacier, pH 5. 3, density 50%, elevation 8, 700 ft.

#51 - September 9, 1965. South Sister Mountain, red snow, terminal end of Carver Glacier, pH 5. 2, density 44%, elevation 7, 300 ft.

#53 - September 9, 1965. South Sister Mountain, dirty snow at south side of Prouty Glacier, pH 5. 3, density 51%, elevation 8, 900 ft.

#56 - September 9, 1965. South Sister Mountain, faint-red snow at lower end of permanent snow field between Prouty and Lewis Glacier, pH 5. 4, density 70%, elevation 8, 900 ft.

#57 - September 9, 1965. South Sister Mountain, lowest residual snow patch in area, northeast corner of lava flow near Green Lake, pH 5. 5, density 51%, elevation 6, 600 ft.

Mt. Jefferson, Oregon

#22 - June 5, 1965. Red snow, south side of the mountain, just below timberline.

Mt. St. Helens, Washington

#11 - May 23, 1965. Red snow, about 100 feet southeast of Lake-view Ski Hut at timberline, elevation 5, 000 ft.

Mt. Rainier, Washington

#24 - July 25, 1965. Red snow, Inter Glacier, very wet rapidly melting, pH 6, elevation 7, 500 ft.

#25 - June 18, 1965. Green snow, near Yakima Park, pH 5, elevation 5, 900 ft.

#27 - June 18, 1965. Red snow, next to beginning of White River Trail at campground, pH 5, elevation 6, 400 ft.

#28 - June 18, 1965. Red snow, near White River Trail, pH 5, elevation 4, 500 ft.

Glacier National Park, Montana

#39 - August 24, 1965. About one mile north of Logan Pass, next to Going to the Sun Highway, small dirty residual snow field, pH 4. 8, density 50%, elevation 6, 00 ft.

#40 - August 24, 1965. Reddish-orange snow, permanent snow field on east side of Going to the Sun Mountain above Roaring Creek, pH 4. 8, density 50%, elevation 8, 500 ft.

APPENDIX II

Plate 1.

Location of collections in the northwestern United States.

Plate 2.

Distribution of species. Symbols represent the relative abundance of each species within each sample.

Plate 3.

Fig. 1, 2, 3. Pilgeria Brasiliensis. 1, mature colony. 2, young colony. 3, solitary cell.

Fig. 4. Aphanothece saxicola, colony of cells.

Fig. 5. Gloeocapsa alpina, colony of cells.

Fig. 6. Microcystis parasitica var. glacialis, colony of cells.

Fig. 7. Synechococcus aeruginosus, solitary cell.

Fig. 8. Myxosarcina amethystina, colony of cells.

Fig. 9. Romeria elegans, filament of four cells.

Fig. 10. Chlamydomonas nivalis, zygote.

Fig. 11, 12, 13. Chlamydomonas yellowstonensis 11, motile cell. 12, vegetative cell. 13, vegetative cell division with 6 daughter cells.

Fig. 14. Scotiella cryophila.

Fig. 15, 16, 17, 18. Scotiella nivalis. 15, spiral trend of cell wall ribs. 16, end view. 17, 18, side view.

Fig. 19. Coccomyxa dispar, cell division.

Fig. 20, 21, 22. Ourococcus cascadensis n. sp.

Fig. 23, 24. Chodatella brevispina, 23, vegetative cell. 24, cell division with four daughter cells?

Fig. 25. Raphidonema nivale, filament of two cells.

Fig. 26. Stichococcus bacillaris, filament of three cells.

Fig. 27, 28, 29. Chromulina chionophila.

Fig. 30. Chionaster nivalis.

Plate 4.

Fig. 1, 2, 3, 4. Chroococcopsis nivalis n. sp. 1, sporangium containing endospores. 2, 3, rupture of sporangia. 4, release of cells to form a pseudoparenchymatous mass.

Fig. 5, 6. Scotiella gigantea n. sp. 5, side view. 6, end view.

Fig. 7. Chlamydomonas sanguinea.

Fig. 8, 9, 10. Sphaerellopsis rubra. 8, vegetative cell. 9, young hypnospore with spines. 10, vegetative cell.

Plate 5.

Average density of snow in alpine and sub-alpine areas of the Pacific Northwest from February to June. Density % represents the volume of snow melt water obtained from a snow core divided by the volume of the core. The data have been averaged over several years from information compiled by the U. S. Department of Agriculture (53).

Plate 6.

Growth curve of Chromulina chionophila at a temperature of 5° C and a light intensity of 0. 35 milliwatts cm^{-2} sec^{-1}.

Plate 7.

Net photosynthesis in Chromulina chionophila at various light intensities.

Plate 8.

Percent maximum photosynthesis (at optimum light intensity) at various temperatures in Chromulina chionophila.

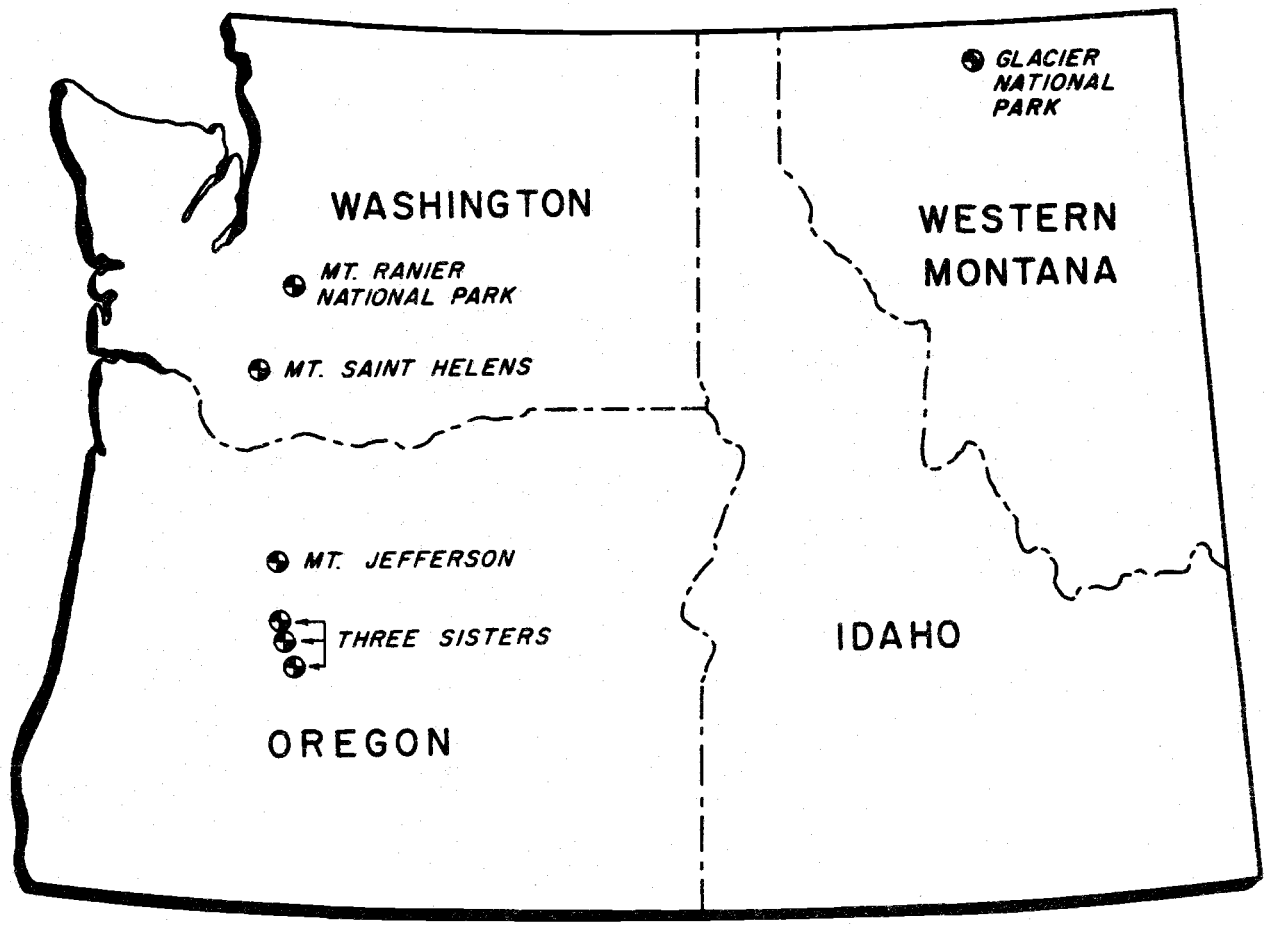

GLACIER
NATIONAL
PARK
WASHINGTON
WESTERN
MONTANA
MT. RANIER
NATIONAL PARK
MT. SAINT HELENS
MT. JEFFERSON
THREE SISTERS
IDAHO
OREGON

Plate 1.

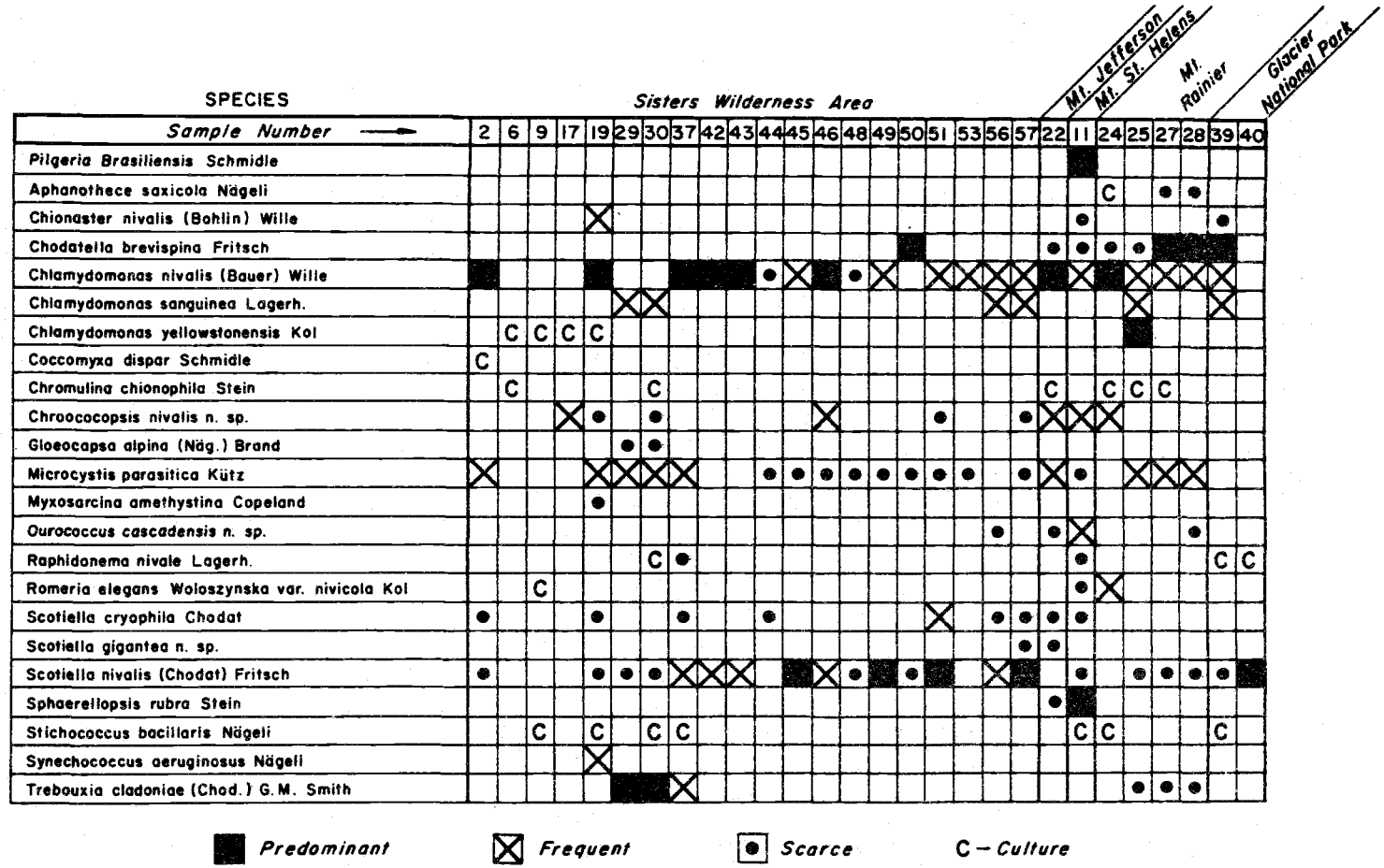
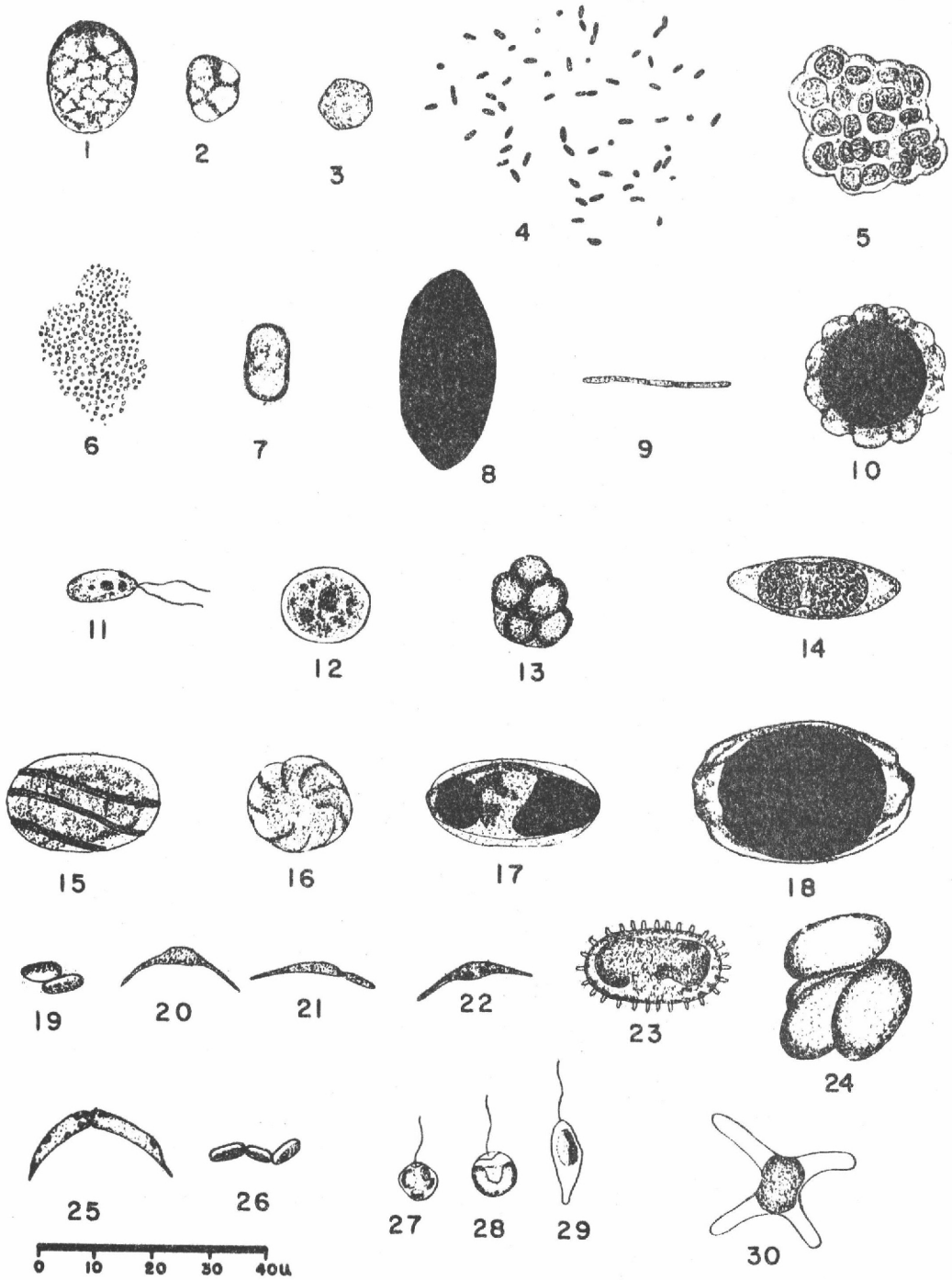

SPECIES
Sisters Wilderness Area
Mt. Jefferson
Mt. St. Helens
Mt. Rainier
Glacier National Park
Sample Number
2 6 9 17 19 29 30 37 42 43 44 45 46 48 49 50 51 53 56 57 22 11 24 25 27 28 39 40
Pilgeria Brasiliensis Schmidle
Aphanothece saxicola Nägeli
Chionaster nivalis (Bohlin) Wille
Chodatella brevispina Fritsch
Chlamydomonas nivalis (Bauer) Wille
Chlamydomonas sanguinea Lagerh.
Chlamydomonas yellowstonensis Kol
Coccomyxa dispar Schmidle
Chromulina chionophila Stein
Chroococcopsis nivalis n. sp.
Gloeocapsa alpina (Näg.) Brand
Microcystis parasitica Kütz
Myxosarcina amethystina Copeland
Ourococcus cascadensis n. sp.
Raphidonema nivale Lagerh.
Romeria elegans Woloszynska var. nivicola Kol
Scotiella cryophila Chodat
Scotiella gigantea n. sp.
Scotiella nivalis (Chodat) Fritsch
Sphaerellopsis rubra Stein
Stichococcus bacillaris Nägeli
Synechococcus aeruginosus Nägeli
Trebouxia cladoniae (Chod.) G.M. Smith
Predominant
Frequent
Scarce
C – Culture

Plate 2.


1
2
3
4
5
6
7
8
9
10
11
12
13
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21
22
23
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25
26
27
28
29
30
0 10 20 30 40u

Plate 3.

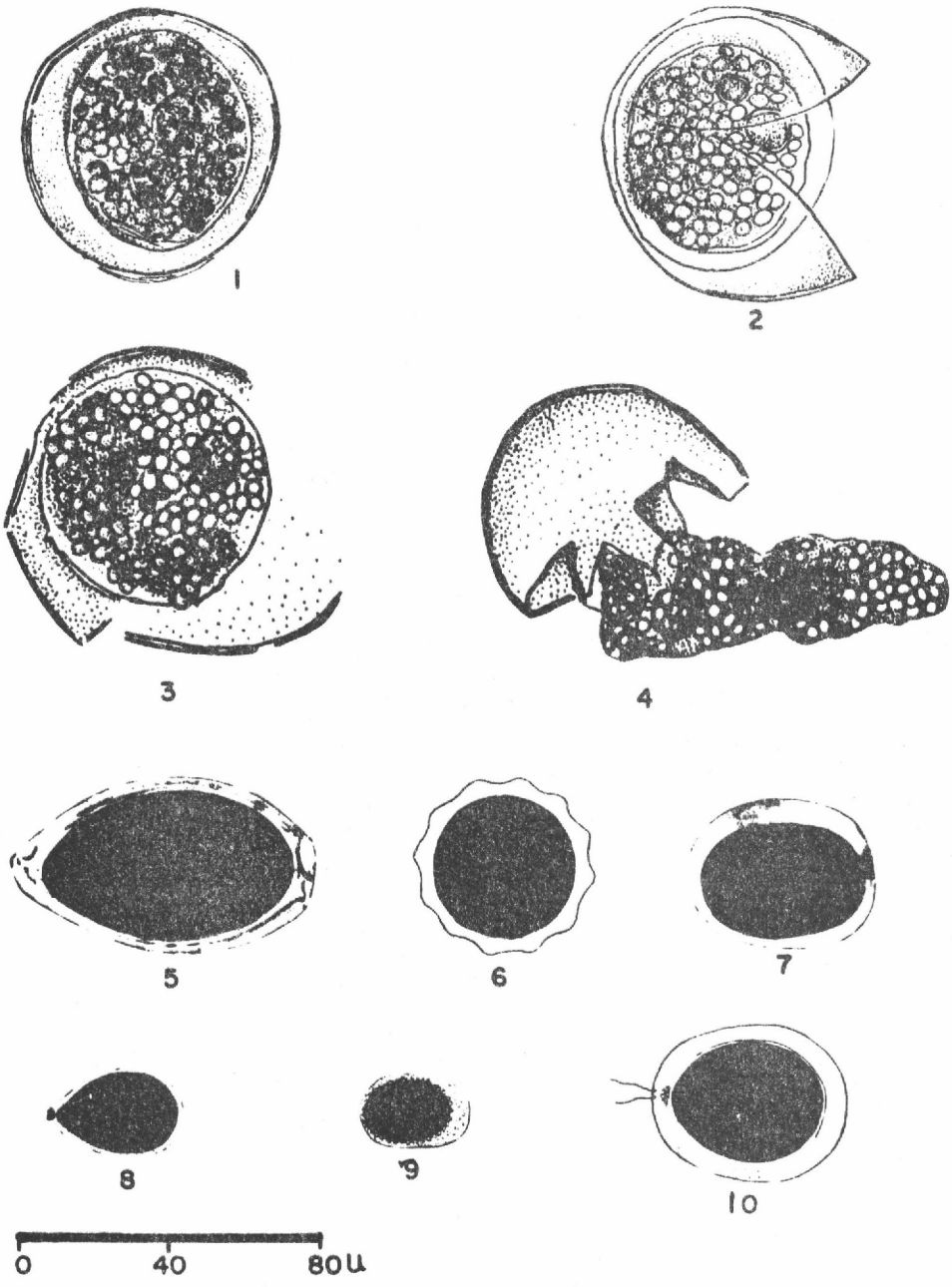

Plate 4.

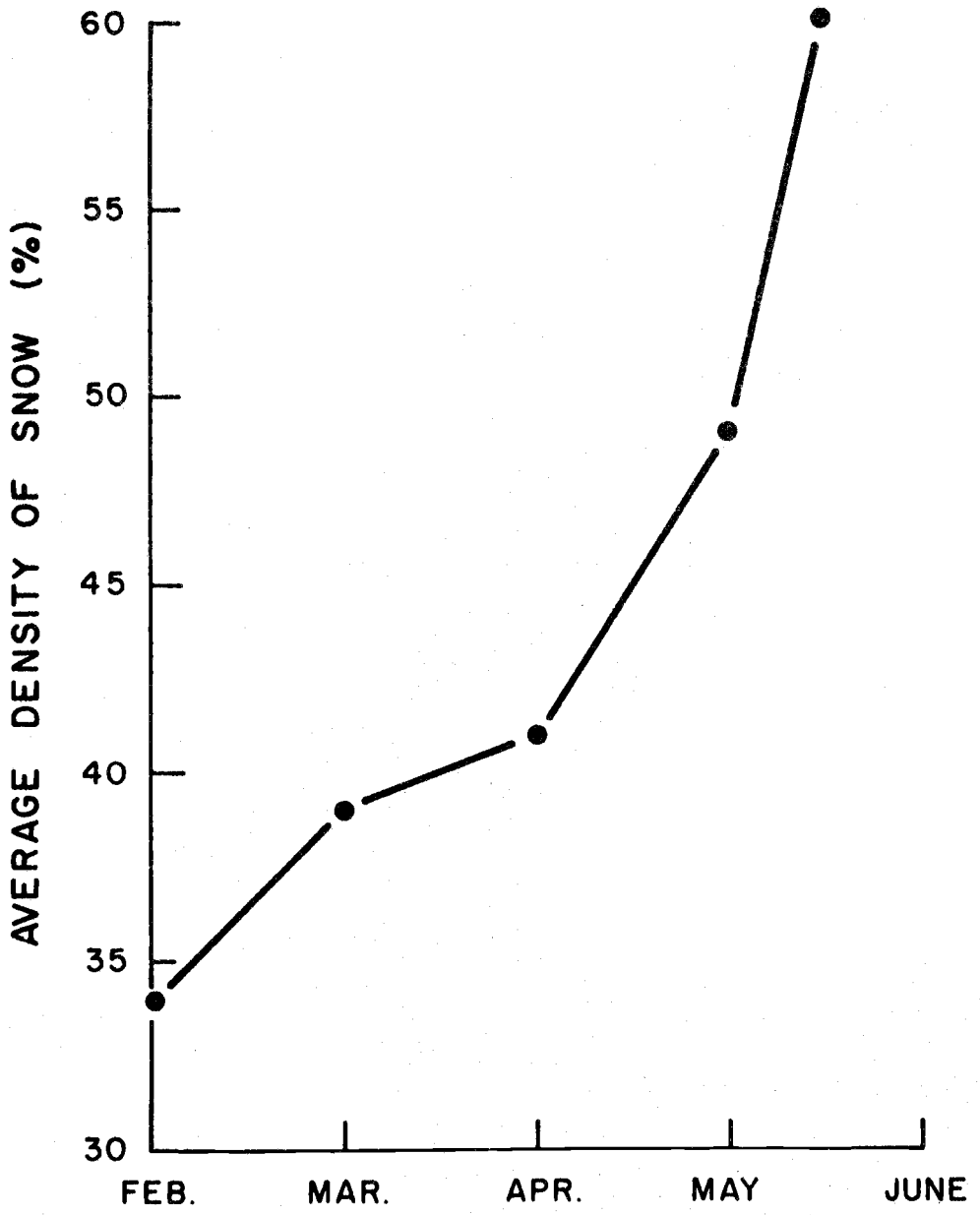
AVERAGE DENSITY OF SNOW (%)
60
55
50
45
40
35
30
FEB.
MAR.
APR.
MAY
JUNE

Plate 5.

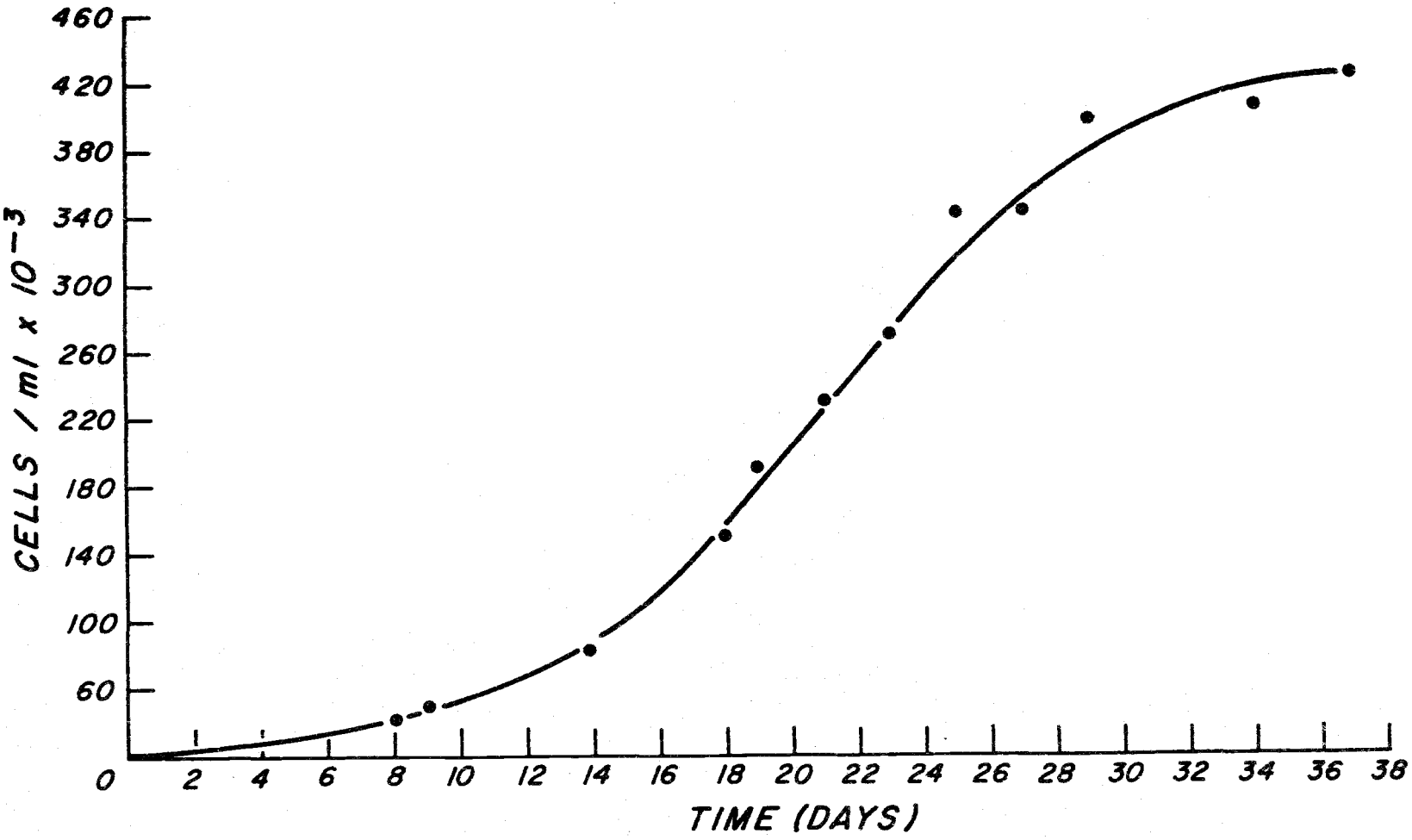
460
420
380
340
300
260
220
180
140
100
60
0
CELLS / ml x 10−3
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
TIME (DAYS)

Plate 6.

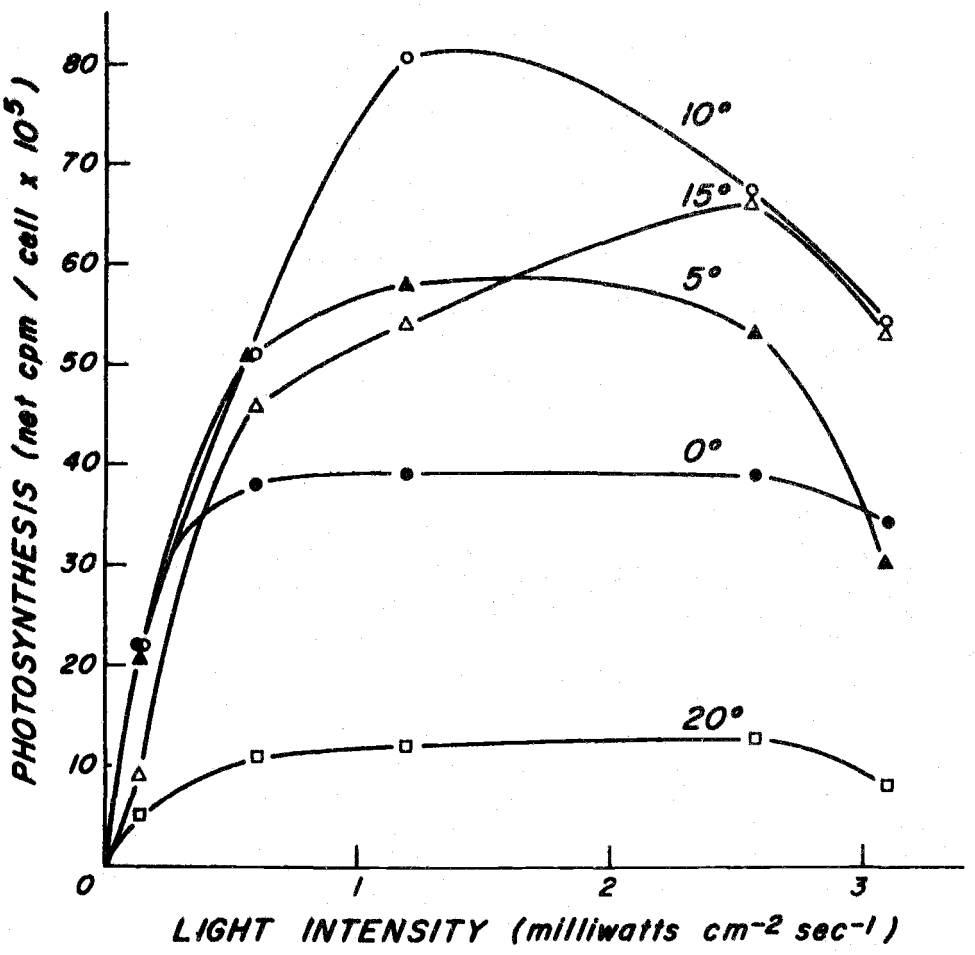
PHOTOSYNTHESIS (net cpm / cell x 10^5)
80
70
60
50
40
30
20
10
0
1
2
3
LIGHT INTENSITY (milliwatts cm^{-2} sec^{-1})
10°
15°
5°
0°
20°

Plate 7.

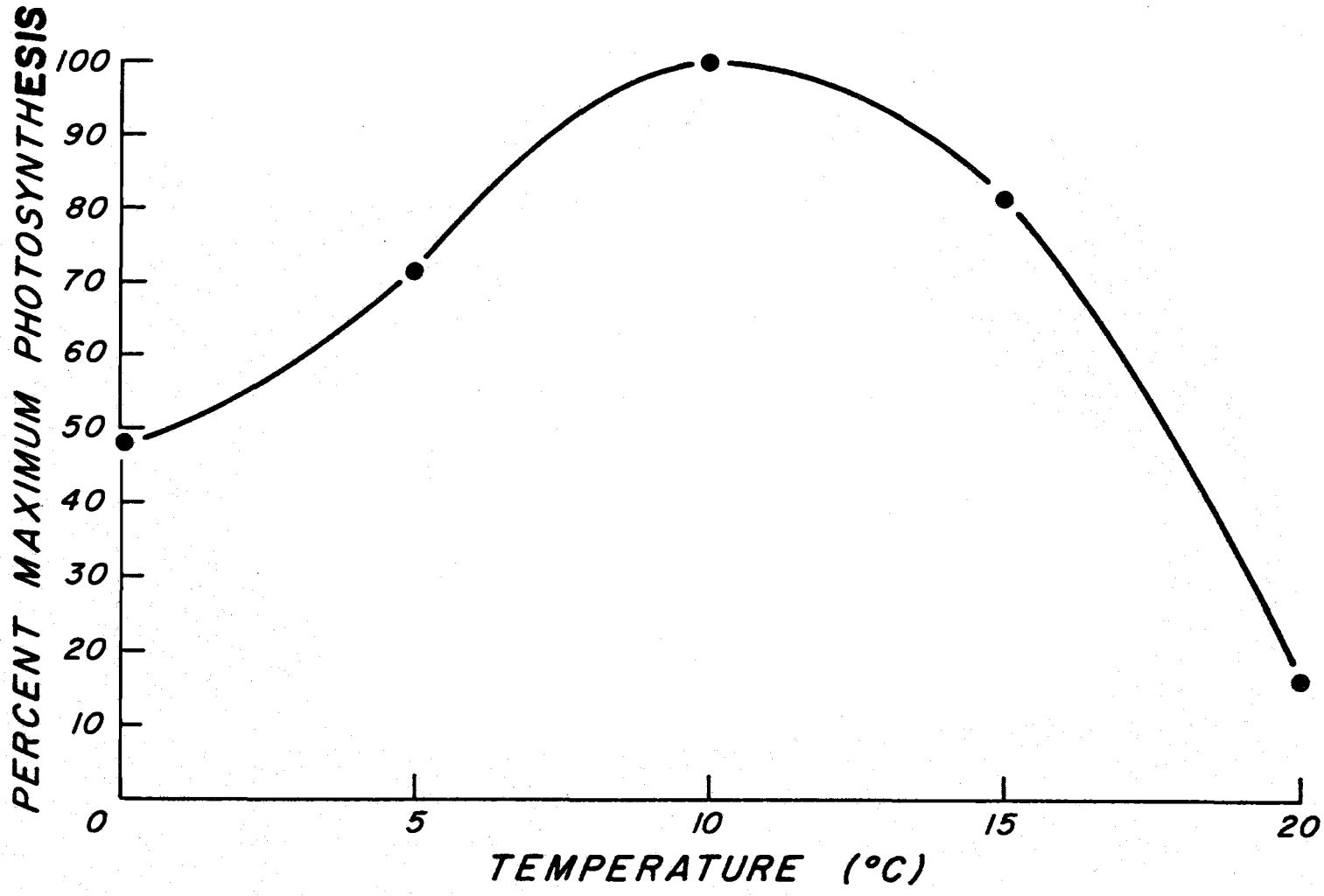
PERCENT MAXIMUM PHOTOSYNTHESIS
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
TEMPERATURE (°C)

Plate 8.