The Water Relations of the Alga *Cyanidium caldarium* in Soil

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SUMMARY

The effects of water potential on the photosynthesis, growth and viability of natural populations and laboratory cultures of *Cyanidium caldarium* were measured. *C. caldarium* was very sensitive to reduced water potential, matric reduction being more harmful than osmotic. The distribution of this alga in different soil areas appeared to be related to the sensitivity to water stress, larger populations being found in areas with higher water potential.

There are very steep gradients of water potential in the soils where *Cyanidium caldarium* is found. The water potential of the surface crust is much too low to allow growth and *C. caldarium* is found in a subsurface layer. The reduced light intensity below the soil surface interacts with the increased water potential to define the position of the algal layer.

INTRODUCTION

Algae are often thought of as aquatic organisms, and almost all algal studies have been of aquatic forms. It is nonetheless true that there are many terrestrial algae which live in diverse types of soil throughout the world (Shields & Durrell, 1964). Most discussions of soil algae have been very general and largely confined to descriptions of the taxa present in a certain type of soil. An excellent summary of these taxonomic investigations is presented by Bold (1970). Although the need for ecological studies of soil algae is often discussed (Shields & Durrell, 1964; Lund, 1962; Lund, 1967; Bold, 1970), the classical studies of Fritsch (Fritsch, 1922; Fritsch & Haines, 1923) and of Stokes (1940) remain as the most direct ecological examinations of these organisms. One of the main conclusions from these latter studies was that the water status of the soil was of paramount importance for the growth of the algae and for their survival under conditions of desiccation.

At the beginning of this investigation, there existed no adequate method for quantitatively measuring the activity of soil algae in their natural environment. The development of a technique for measuring $^{14}\text{CO}_2$ uptake in soil (Smith, Fliermans & Brock, 1972) made possible a study of the effects of water stress on algae directly in their natural environments.

The general importance of water in biological systems is well established and much is known of the specific water relations of micro-organisms in laboratory culture (Scott, 1957). There have been a number of studies on the effects of varied availability of soil water on fungi (Griffin, 1969; Adebayo & Harris, 1971; Sommers, Harris, Dalton & Gardner, 1970) and bacteria (Clark, 1967), but no similar detailed examination has been made of any soil algal population. The work described here bears upon the problem of water availability for populations of the alga, *Cyanidium caldarium*, in hot, acid soils of Yellowstone National Park, Wyoming, U.S.A. The distribution of *C. caldarium* in soils of different water content

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was surveyed, and the effects of experimentally varying water availability on the activity and viability of the organism both in soil and in culture were measured. The investigation was aided greatly by the nature of the areas studied and by the fact that the detailed physiological ecology of *C. caldarium* was known (Doemel & Brock, 1970, 1971*b*). The extreme conditions of pH and temperature in these soil areas greatly limit the species diversity so that the habitats contain essentially pure cultures of *C. caldarium*.

The availability of water to organisms may be defined in terms of the activity or potential of water under the given conditions. Water activity and water potential are discussed in detail by Scott (1957). Water activity, a_w , is defined as the ratio of the vapour pressure of a solution to that of pure water and is expressed on a scale from 0 (no water) to 1 (pure water). Water potential is proportional to the natural logarithm of the water activity and may alternatively be defined in terms of the free energy difference between a solution and pure water. If the energy of a solution is expressed in terms of volume, the units are dimensionally the same as pressure. Therefore, water potentials are usually expressed as $-bars$, indicating that the energy of any solution is always negative with respect to pure water.

Water potential may be altered in two ways, which are termed osmotic and matric (Griffin, 1969). Osmotic contributions to water potential occur in solutions as the result of interactions between solute molecules and water molecules. Matric water potential results from the adsorption of water molecules to surfaces at interfaces in solid substrates, such as soil. When the water potential of a solution is varied by the addition of some solute, the only effects are osmotic. Variation of matric water potential involves the addition or removal of bulk water to or from a solid sample. This variation not only alters interface adsorption phenomena, it also changes the concentration of dissolved solutes and therefore creates osmotic changes in addition. In the present work, the effects of both matric and osmotic water stress were measured.

METHODS

Soil water content. Soil samples were placed in tared airtight vessels immediately after their collection. The vessels were weighed upon return to the laboratory, opened, and dried at 110 °C for 18 h. When the drying was completed, the containers were resealed, cooled in a desiccator and weighed. Water content was expressed as % of water/100 g of dry soil.

Soil pH. The pH of the soil water was measured by the dilution technique of Doemel & Brock (1971*a*). One gram of soil whose water content had been determined was mixed with 0.5 ml of distilled water, and the pH of the resulting slurry measured with a glass combination electrode (Broadley-James) and a Corning model 12 pH meter. Successive dilutions were made and the pH measured until the soil water had been diluted 50-fold. The pH values obtained at the various dilutions were plotted semi-logarithmically as a function of the soil-water dilution and the resultant curve was extrapolated to zero dilution. This extrapolated value was taken as the pH of the native soil water.

Conductivity of soil water. A known amount of soil was diluted with distilled water and the conductivity read with a conductivity meter (Yellow Springs Instruments Co., Yellow Springs, Ohio). The soil was diluted further and conductivity readings were made until the conductivity was less than 500 μ mhos. The conductivity readings were plotted as a function of the dilution of the soil water and extrapolated to zero dilution. This extrapolated value was taken as the conductivity of the native soil water. The conductivity values were expressed as NaCl equivalents and the water potentials calculated from Lang (1967).

Chlorophyll extraction. All samples were extracted with both acetone and *N,N*-dimethyl

formamide (DMFA). As reported by Doemel & Brock (1971*b*), complete extraction of chlorophyll from *Cyanidium caldarium* was possible only with DMFA. It was determined that no chlorophyll whatsoever was extractable with acetone from laboratory cultures of *C. caldarium*. Therefore the extraction of each soil sample with both solvents allows determination of the proportion of the total chlorophyll present in a sample which was from *C. caldarium*. Extractions were done with small (0.1 to 0.3 g) amounts of soil to minimize interference from suspended soil particles. The addition of 0.1 ml of 1 % MgCO_3 ensured that the extracted chlorophyll would not be converted to pheophytin. All extractions were with 5 ml of solvent in 15 ml glass centrifuge tubes overnight in the dark at 4 °C. The absorbance of the samples was read at 667 nm (DMFA) and 665 nm (acetone) with a DB-G spectrophotometer (Beckman). Chlorophyll was quantified by use of the appropriate extinction coefficients in acetone (Seely & Jensen, 1965) and DMFA (Volk & Bishop, 1968).

Culture conditions. Strain 206 of *Cyanidium caldarium* was obtained from W. N. Doemel. The alga was cultured in Allen's salts medium (Allen, 1959) adjusted to pH 3 (with 10 N- H_2SO_4) at 45 °C with an incident illumination of about 900 foot-candles. Algal cultures were bubbled continuously at a slow rate with 5 % CO_2 in air. The cultures were transferred to fresh medium approximately every ten days.

Most-probable-number determinations. Soil samples were suspended in 10 ml of Allen's medium. Serial tenfold dilutions were made from this suspension and three tubes containing 9 ml of Allen's medium were inoculated from each of six consecutive dilutions. The tubes were vigorously mixed with a vortex mixer at every step to ensure uniform distribution of the soil particles in the dilutions. The inoculated tubes were incubated at 45 °C in the light for three weeks. Each tube was agitated with pure CO_2 for 20 s at the beginning of the incubations and every second day thereafter. At the end of three weeks, all tubes were examined for the presence of *Cyanidium caldarium*. The most probable number (m.p.n.) of cells in each sample was calculated from the appropriate tables (American Public Health Association, 1965).

Matric variation of soil potential. Matric water potential of soil samples was varied by isopiestic (equal vapour pressure) equilibration. Sodium chloride solutions of the proper molalities for the water potentials desired were solidified with 2 % (w/v) agar (Difco) in 100 mm plastic Petri dishes (Falcon). The agar discs created in this way were used to control the vapour pressure of solid samples by equilibration in the agar dish system of Harris, Gardner, Adebayo & Sommers (1970). It was necessary to allow the samples to remain in the dark for a total of four days and to replace the agar after two days in order to reach equilibrium. The precaution of equilibration in the dark reduced the chance of the establishment of temperature gradients between the agar and the soil within the Petri dish. Such gradients must be avoided if the desired equilibrium is to be reached (Scott, 1957). It was found necessary to replace the NaCl-agar disc after two days because the equilibration altered the water potential of the agar as well as of the soil. One such replacement was sufficient.

Membrane-filter experiments. All Millipore filters used were 25 mm in diameter and had a pore size of 0.45 μm . A 3 ml sample of a *Cyanidium caldarium* culture was washed three times with distilled water by centrifugation at 7500 rev./min in a table-top centrifuge (Ivan Sorvall Inc., Norwalk, Connecticut, U.S.A.) and then resuspended in 200 ml of distilled water. The algal number in the suspension was determined with a Petroff-Hausser counting chamber. Filters were prepared by the application of 10 ml of the diluted suspension to the filter on a fritted-glass support under a slight vacuum. The number of organisms on each filter was between 2×10^7 and 5×10^7 .

Filters were placed in serum bottles for $^{14}\text{CO}_2$ incorporation assay. Dark controls were bottles wrapped with aluminium foil. The bottles were stoppered and pre-incubated for 10 min at 45 °C in the light; 0.2 ml of air was then withdrawn from each bottle, and 0.2 ml of $^{14}\text{CO}_2$ -air mixture added. The $^{14}\text{CO}_2$ was generated as described below (see *Photosynthesis experiments*). The uptake of radioisotope was terminated after the desired incubation time by the removal of the cap and the release of unincorporated $^{14}\text{CO}_2$. The amount of $^{14}\text{CO}_2$ incorporation was determined by liquid scintillation counting of the filters. Filters were dried and then placed in scintillation vials containing 10 ml of fluid consisting of 0.375 g of 2,5-diphenyloxazole (PPO, Beckman Instruments Co.) and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP, Packard Instrument Co.) per litre of toluene.

Viable counts of filters. Suspensions were diluted in sterile 9 ml volumes of Allen's medium until fewer than 100 viable organisms remained per 10 ml. The entire 10 ml contents of the appropriate dilution tubes were applied to 0.45 μm membrane filters on fritted-glass supports under a slight vacuum.

The filters containing algae were placed on agar medium in 100 mm plastic Petri dishes. The agar medium was prepared as follows: double-strength Allen's medium (pH 3) and 2.8 % (w/v) ionagar (Oxoid, Colab Laboratories, Chicago, Illinois, U.S.A.) were autoclaved separately, cooled to 50 °C and then mixed. The final mixture had a pH of 3.1, an agar concentration of 1.4 % and the same salts compositions as Allen's medium.

The Petri dishes containing the filters were incubated within a transparent plastic box at 45 °C at a light intensity of 1800 foot-candles provided by fluorescent lights in a plant-growing chamber (Controlled Environments, Winnipeg, Canada). Light intensity was measured with a Gossen Pilot light meter. The atmosphere of the box containing the plates was enriched to 10 % CO_2 (v/v) by the addition of dry ice every second day.

Growth-rate experiments. Tubes containing Allen's medium and various amounts of either sucrose or NaCl were inoculated with *Cyanidium caldarium* and incubated with continuous bubbling with 5 % CO_2 in air at 45 °C in the light. At various times samples were withdrawn, and the pH, OD_{580} , and algal number were determined.

Photosynthesis experiments. An exponentially growing culture of *Cyanidium caldarium* was diluted into Allen's medium containing various amounts of either sucrose or NaCl. Immediately after this dilution, 4 ml samples were pipetted into 5 ml vials which were closed with screw caps and placed at 45 °C to pre-incubate. After 10 min 1.0 μCi (0.1 ml) $\text{NaH}^{14}\text{CO}_3$ (New England Nuclear, Boston, Massachusetts, U.S.A.) (1 $\mu\text{Ci}/10 \mu\text{g}$) was added to each vial to begin the incubations. After various times replicate vials were removed and uptake stopped by the addition of formaldehyde to a final concentration of 3.7 % (v/v). Dark controls were vials wrapped with aluminium foil.

The incorporation of radioisotope was determined by filtering 0.5 ml samples of the suspension through 0.45 μm membrane filters. The filters were washed with 5 ml of 10^{-3} N-sulphuric acid to remove any unincorporated $^{14}\text{CO}_3^-$, dried for 1 h, and counted as above.

Incorporation of $^{14}\text{CO}_2$ by soil samples. Photosynthesis of *Cyanidium caldarium* was measured directly in natural soil by a modification of the procedure described by Smith *et al.* (1972). Soil samples were placed in sterile plastic bags (Whirl-Pak, Nasco) and homogenized by thorough agitation of the bag. Small portions (0.2 to 0.5 g) of the homogenized sample were then placed in tared 20 ml ampoules (Kimble). Dark controls were ampoules wrapped with aluminium foil. Each ampoule was sealed by placing the ampoule inside the bottom of a 5 ml sleeve-type serum cap. All pre-incubations and incubations of natural samples were done in a thermal stream at Roaring Mountain in Yellowstone National Park which provided a range of temperatures from 25 to 90 °C. All incubations described here were for

1 h at 45 °C, the temperature optimum for *C. caldarium* (Doemel & Brock, 1970), and pre-incubation time was 10 min. $^{14}\text{CO}_2$ was generated as follows: Two ml of 10 N- H_2SO_4 were pipetted into a 60 ml serum bottle (Wheaton) which was then closed with a sleeve-type rubber stopper. After 1 ml of air was removed from the bottle with a syringe, 1 ml (40 μCi) of $\text{NaH}^{14}\text{CO}_3$ (New England Nuclear) (1 $\mu\text{Ci}/10 \mu\text{g}$) was injected through the cap into the bottle and was converted to $^{14}\text{CO}_2$ by reaction with the acid.

After the pre-incubation period, 0.3 ml of air was removed from each ampoule. Incubations were begun by the injection of 0.3 ml of the $^{14}\text{CO}_2$ -air mixture into each ampoule with a Hamilton gas-tight syringe fitted with a Chaney adapter. The syringe was filled by first injecting n ml of air into the $^{14}\text{CO}_2$ -generating serum bottle and then withdrawing n ml of $^{14}\text{CO}_2$ -air mixture. This procedure left the pressure inside the bottle equal to that of the atmosphere and also caused an easily calculable reduction in the $^{14}\text{CO}_2$ concentration within the bottle. Introduction of the radioisotope in the gaseous state allows photosynthesis to be measured without any change in the water status of the soil sample. Incubations were terminated by the removal of the serum cap to release unincorporated $^{14}\text{CO}_2$. Ampoules were then weighed to determine the mass of soil. Samples were processed and $^{14}\text{CO}_2$ incorporation measured by the wet-oxidation method described previously (Smith *et al.* 1972).

Measurement of injected $^{14}\text{CO}_2$ concentration. After all samples in a given incubation series were injected, six 5-ml serum bottles (Wheaton) were stoppered and injected with 0.3 ml of $^{14}\text{CO}_2$ -air mixture each. Upon return to the laboratory these bottles were evacuated into phenethylamine scintillation fluid with a modification of the apparatus described by Smith *et al.* (1972).

Controls for dried samples. All isopiastically-dried filters and soil samples were checked for the effect of simple passage of time during the equilibration. The equilibration durations of 12 h for the filters and four days for the soil samples could conceivably have harmful effects aside from water-potential change. In all cases, a parallel equilibration was run in which a replicate of the solid substrate was equilibrated at its native water potential, that is, the water potential which caused no change in the weight of the substrate. In all cases the values obtained for those control samples were almost the same as those obtained for the untreated or 'zero-time' samples.

Standardization of $^{14}\text{CO}_2$ uptake by soil samples. The results of $^{14}\text{CO}_2$ incubations were normalized on the basis of the mass of soil incubated. However, this normalization required modification for samples whose water potential was varied matrically. Correction factors were therefore calculated to convert the mass of dried soil to equivalent mass of untreated natural sample. The bases for these calculations were the water-content values of the soil at the various water potentials.

RESULTS

*Soil habitat of *Cyanidium caldarium**

Cyanidium caldarium was found in several areas (solfataras) in Yellowstone National Park. The areas which were sampled were: Amphitheater Springs (two sites), a fumarole at Amphitheater Springs, Norris Geyser Basin (two sites), and Roaring Mountain. The pH of all sites was between 1.7 and 2.3, water contents ranged from 20 % to 67 % and temperatures ranged from 26 to 45 °C. The number of these algae/g of soil and their ability to photosynthesize were correlated with the water status of the soils, populations being larger and photosynthesis greater at the wetter areas.

All the areas sampled except the fumarole at Amphitheater Springs contained the algae

in a layer slightly below the surface. They were at the surface in the fumarole. At all of the sites where *Cyanidium caldarium* occurred in a subsurface layer, the surface material was noticeably drier than that from the algal layer. Quantitative data were obtained only at Amphitheater Springs. At this site the surface material, which formed a layer about 3 mm thick, had a water content of 11 %. The 2 mm thick algae-containing layer had a water content of about 43 %. There was no chlorophyll in the surface layer and microscopic examination revealed no organisms. X-ray diffraction analyses of thin sections of the surface and algal layers were carried out by Dr M. Walter at Yale University, and showed that the soil in both layers was composed of opaline silica and quartz, although the granule size of the surface material was considerably larger. Since the silica was translucent, it probably permitted enough light through to allow photosynthesis to occur. *C. caldarium* is capable of heterotrophic growth (Doemel & Brock, 1971*b*), but the concentration of organic matter in these soils (1.05 %, w/w, at the Amphitheater Springs site, most of which was *C. caldarium* as revealed by protein assay) was probably not sufficient to support the observed algal populations.

Chemical analysis of Amphitheater Springs soil

A sample of soil from the Amphitheater Springs area in Yellowstone National Park was analysed by the Soil Analysis Laboratory of the University of Wisconsin, and the following results obtained (mg/kg soil): NH_4^+ , 163.5; NO_3^- , 1.0; Ca^{2+} , 10.0; Mg^{2+} , 4.5; K^+ , 50.0; Na^+ , 21.5. The ammonium concentration reported here is similar to values reported for solfatara regions in Italy (Rigano & Conforti, 1966–1967). The soil water of this soil had a pH of 2.1.

The conductivity of the total soluble salts from the Amphitheater Springs soil was 2050 μmhos . The conductivity dilution technique also showed that a 0.0125 molal solution of NaCl had a conductivity of 2100 μmhos . The water potential of such a NaCl solution is -0.5 bars (Lang, 1967) and may be taken as an approximation of the osmotic water potential of Amphitheater Springs soil.

The low osmotic water potential is important in interpreting the results of experiments in which soil water potential was varied by isopiestic equilibration. Isopiestic equilibration allows direct control of the total water potential of a given soil sample. The water potential attained, however, is the sum of the matric and osmotic forces within the soil. As explained above, the osmotic contribution to water potential in Amphitheater Springs soil is very slight. Therefore isopiestic equilibration of this soil directly controls matric water potential.

Water-holding characteristics of natural soils

Samples of algae-containing soil from Amphitheater Springs, Roaring Mountain and a fumarole in the Amphitheater Springs area were divided into subsamples of about 2 g each, and these were equilibrated to different relative humidities. After equilibration, the water content of each sample was measured and the relationship between water potential and water content is presented in Fig. 1. The shape of these curves is the same as that reported for some standard soils (Harris *et al.* 1970), although the soils examined here have much greater water-holding ability. Also included in Fig. 1 is a curve resulting from the rehydration of dried fumarole soil (dotted line). These values were obtained by equilibration of soil with an atmosphere of low water potential and subsequent equilibration of the same soil with a series of higher water potentials. This curve demonstrates that the phenomenon of hysteresis occurred in these samples, i.e. the wetting of the soil lagged behind the drying.

Fig. 1

Fig. 2

Fig. 1. Water-retention characteristics of soils from Yellowstone National Park. Samples were isopiastically equilibrated to the indicated water potentials and water contents were measured. ○—○, Amphitheater Springs soil; ■—■, fumarole soil; ●—●, Roaring Mountain soil; △—△, rehydrated soil from fumarole. Arrows indicate the native water potentials of the soils.

Fig. 2. Effect of water potential on photosynthesis by *Cyanidium caldarium* populations in Amphitheater Springs soil. Points are the averages of five replicates.

Therefore the water content of a soil sample at a given water potential will depend on the wetting history of that sample.

Matric regulation of water potential

Replicate subsamples of soil from Amphitheater Springs were equilibrated to different water potentials and photosynthesis measured by $^{14}\text{CO}_2$ uptake. Reduction in water potential resulted in a sharp decrease in photosynthesis (Fig. 2). The ability to incorporate $^{14}\text{CO}_2$ was reduced to 50% of the initial level at -12 bars, and was removed completely at -28 bars. When samples were rehydrated, some reactivation of photosynthesis was observed. The extent of reactivation depended on both the level to which the water potential had been lowered and the level to which it was returned. The lower the water potential was before rehydration, the less extensive was the recovery of uptake ability. Similarly, the higher the water potential was after rehydration, the more extensive was the recovery. Although some recovery of photosynthetic ability was observed in all cases, the fact that complete reactivation never occurred suggests that there was some permanent damage to the organisms.

In addition, soil samples from Roaring Mountain and the Amphitheater Springs fumarole were isopiastically equilibrated to different water potentials and then incubated with $^{14}\text{CO}_2$ for 1 h. Reduction of water potential was harmful to the *Cyanidium caldarium* populations at these sites, just as it was for the cells at Amphitheater Springs. Photosynthesis was

Fig. 3

Fig. 4

Fig. 3. Effect of water potential on viability of *Cyanidium caldarium* populations in Amphitheater Springs soil.

Fig. 4. Effect of water potential on photosynthesis by a liquid culture of *Cyanidium caldarium*. Points are the averages of four replicates. ●—●, NaCl; □—□, sucrose.

reduced to 50 % of the initial level at water potentials of -29 bars and -18 bars for samples from Roaring Mountain and the Amphitheater Springs fumarole, respectively. Rehydrated samples regained the ability to photosynthesize in the same manner as rehydrated samples from Amphitheater Springs.

To study cellular damage from drying, viability was measured. Fig. 3 shows that the m.p.n./g of Amphitheater Springs soil decreased in direct proportion to the decrease in water potential at water-potential values less than -10 bars. Viability was reduced by 50 % at -12 bars, but, since the viability was not completely removed even at the lowest water potential tested (-61 bars), it was not possible to define an extinction value quantitatively. Isopiestic rehydration of replicate dried samples did not result in any increase in the viable count.

Osmotic regulation of water potential

¹⁴CO₂ incorporation by pure cultures of *Cyanidium caldarium* was also sensitive to decreased osmotic water potential (Fig. 4). A 50 % reduction in ¹⁴CO₂ uptake occurred at -21 bars and extinction occurred at -32 bars. Since similar results were obtained when the water

Fig. 5. Growth curves for cultures of *Cyanidium caldarium* in the presence of various concentrations of sucrose. ●—●, No sucrose (control); □—□, 0.2 M-sucrose, ■—■, 0.5 M-sucrose, △—△, 1.0 M-sucrose; ○—○, 1.75 M-sucrose.

Fig. 6. Effect of water potential on growth rate of cultures of *Cyanidium caldarium*. ●—●, NaCl; □, sucrose.

Table 1. *Critical water potentials and water activities for Cyanidium caldarium*

Substrate	Measurement	50 % Inhibition	
		Water potential* (bars)	Water activity*
		Matric variation	
Soil	¹⁴ CO ₂ uptake	- 12	0.993
Soil	Viability	- 12	0.993
Filter	¹⁴ CO ₂ uptake	- 18	0.987
Filter	Viability	- 26	0.983
		Osmotic variation	
Liquid	Growth rate	- 42	0.969
Liquid	¹⁴ CO ₂ uptake	- 21	0.985

* The water potential values were obtained from the figures previously presented and the corresponding water activities were calculated as described by Lang (1967).

potential was decreased with either sucrose or NaCl, the effect is one of water potential and is not the result of specific solute toxicity.

Fig. 5 shows the effect of sucrose on growth of *Cyanidium caldarium*. Similar data were obtained with NaCl. Lowered water potential increased the doubling time, lowered the final algal density and increased the lag. Growth rates calculated from this Figure are expressed as a function of water potential in Fig. 6. As with $^{14}\text{CO}_2$ incorporation, the observed reduction in growth rate is clearly related to water potential rather than to specific solute. Reduction to 50 % occurred at -42 bars while extinction was at -80 bars.

DISCUSSION

The sensitivity of *Cyanidium caldarium* to water stress is summarized in Table 1. For comparison, the water potentials at which 50 % inhibition occurred are presented. *C. caldarium* is somewhat more sensitive to water stress in soil than it is in culture, showing a 50 % reduction in soil of -12 bars for both photosynthesis and viability, whereas with a pure culture on membrane filters the 50 % reduction values are -18 bars for photosynthesis and -26 bars for viability. Such differences between soil and culture might be expected. Possibly, algae in natural soil are less vigorous than those in a culture, and damage from water stress is more likely.

These studies were done in detail only in one soil in the Amphitheater Springs area, but the general effect of water potential on photosynthesis was confirmed in Roaring Mountain soil and a fumarole soil, 50 % inhibition occurring at -29 bars and -18 bars, respectively. The native water potential of the Roaring Mountain soil (-4 bars) was significantly lower than that of the Amphitheater Springs soil and that of the fumarole soil (-1 bar to -2 bars). The fact that the most desiccation-resistant population of *Cyanidium caldarium* was found in the soil with the lowest natural water potential suggests that this alga can adapt to the water conditions of its environment.

Photosynthesis of *Cyanidium caldarium* is more sensitive to matric than to osmotic water stress. This conclusion is supported by Table 1 and by a comparison between Fig. 2 (matric stress) and 4 (osmotic stress). Cells under matric stress have lost 30 % of their photosynthetic ability at -10 bars, while osmotically stressed cells have suffered no reduction whatsoever at -10 bars. As discussed by Griffin (1969), matric reduction of water potential has indirect

effects not directly related to water potential, the most important being that the removal of bulk water reduces solute diffusion as a result of the interruption of previously continuous water pathways. A greater effect of matric than of osmotic stress on growth has been found by Adebayo & Harris (1971) in *Phytophthora cinnamomi* and *Alternaria tenuis* although Cook, Papendick & Griffin (1972) found virtually identical responses to both kinds of water stresses in the fungus *Ophiobolus graminis*. As discussed by Adebayo, Harris & Gardner (1971), organisms can compensate for osmotic water stress by accumulating solute so that its concentration inside the cell is greater than outside, thus allowing for inward diffusion of water. When the stress is matric, there is no solute available for accumulation.

The growth experiments probably provide the most favourable means of determining the true lower limit of water potential tolerance for *Cyanidium caldarium*, since the organism is placed in a nutrient medium with adequate energy in the form of light. Growth ceased at about -80 bars (a_w 0.943), suggesting that this is the lowest limit at which *C. caldarium* might develop in nature. However, in nature the lowest water potential at which *C. caldarium* was found was -4 bars (a_w 0.997), suggesting that conditions in nature are rarely favourable enough for the full adaptation potential of the alga to be realized. *C. caldarium* is more sensitive to lowered water potential than other micro-organisms. Many fungi are capable of growth at -491 bars (a_w 0.700), while bacteria have water potential tolerance intermediate between fungi and *C. caldarium*, the lowest value known being -207 bars (a_w 0.860) (Scott, 1953).

The susceptibility of *Cyanidium caldarium* to water stress probably explains why it lives in a layer 3 to 5 mm below the surface of the soil. Water potential in this layer ranged from -2 to -4 bars, yet the water potential of the surface crust was less than -50 bars, much too low for development of the alga. Presumably *C. caldarium* lives no deeper in the soil because of decreased availability of light, thus forming a thin layer where both water potential and light are adequate.

The marked sensitivity to drying of *Cyanidium caldarium* raises problems regarding the dispersal of the alga. It is world-wide in distribution (Doemel & Brock, 1970; Brock, unpublished) yet its hot, acid habitats are extremely small in extent. No specialized structures resistant to drying are known, and, because the alga does not grow at neutral pH, aquatic dispersal is unlikely. Conceivably the alga is transmitted through the air inside small soil particles which have a water-impermeable exterior so that the water potential remains high.

Detailed water-potential requirements have not been reported for any other soil algae. It has been observed that some algal species are able to survive laboratory storage in their native air-dried soils for over 70 years (Bristol, 1919; Parker, Schanen & Renner, 1969; Trainor, 1970). Some of these organisms survived because they form distinct inactive resting stages. In all of these instances it was clear that the numbers of viable organisms were declining as storage proceeded. Although complete killing of *Cyanidium caldarium* was not obtained, the large and rapid reduction in viability (10000-fold in four days) as a function of decreased water potential suggests that this alga is more sensitive to desiccation than the green and blue-green algae reported in soil by other workers (see above). Fritsch & Haines (1923) suggested that desiccation tolerance was a real characteristic of soil algae. They examined several soil and aquatic algae and found that the soil organisms were much better able to withstand both desiccation and exposure to hypertonic solutions than were the aquatic forms.

As mentioned previously, *Cyanidium caldarium* lives in a soil horizon where the water potential remains fairly constant. Algae which live on the surface of soils are exposed to considerable variation in water availability. It appears that the seasonal variation seen for

many such algae is directly correlated with the degree of desiccation of the soil (Shields & Durrell, 1964). The blue-green algae which form crusts in the desert appear to be special cases. Most of these organisms are found to have mucilaginous sheaths, and the considerable imbibition powers of these sheaths probably provide the organisms with sufficient water to allow the crust populations to maintain their relative constancy and resistance to seasonal variation (Shields, Mitchell & Drouet, 1957).

Lichens and mosses are well known for their ability to grow on dry substrates. The distributions of the lichen symbiotic association (Rogers, 1971) and of mosses (Lee & Stewart, 1971) have been correlated in at least some cases with desiccation tolerance. In lichens, it may be the fungal partner which is responsible for the desiccation resistance of the thallus, since fungi are able to tolerate lower water potentials than any other organisms (Scott, 1957).

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