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VIABLE SOIL ALGAE FROM THE HERBARIUM OF THE MISSOURI BOTANICAL GARDEN

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ABSTRACT

Soil particles from herbarium sheets of aquatic, bog and terrestrial plants were used as innoculum on Bold's modified Bristol's medium. Forty-six of the 124 innoculations were successful, and algae were obtained from specimens up to 60 years old. Green algae were the most frequent survivors from plants collected within the last 30 years, and blue-greens from the period before that.

INTRODUCTION

Although many have recognized the importance of desiccation resistence to survival of microorganisms (Evans, 1959; Hortobagyi, 1960; Lund, 1962), few investigators have approached this question through attempts to culture algae from soil samples kept dry in excess of a few years.

Bristol (1919) identified 11 genera (7 blue-green, 3 green, 1 diatom) which grew from 16 samples of partially air-dried English soils sealed in lead-capped bottles for 23-70 years. Algae and fungi developed even in the 70-year-dry soil when moistened with Bristol's inorganic salt solution and placed in light for some months.

Lipman (1941) attempted to grow algae from fragments of numerous herbarium sheets of green and blue-green algae. The only culture which developed was a specimen of *Nostoc commune* collected in 1853. Lipman repeated this experiment twice, establishing a record viability of 86 and 87 years for this species.

Becquerel (1942) took dry soil from 10 non-disinfected herbarium specimens of various mosses and fern allies spanning 56-98 years in age. When placed in sterile inorganic salts medium (pH 5) of unreported composition in window light, 14 different genera ultimately developed (4 blue-green, 9 green, 1 diatom). All

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soils yielded viable algae including the 98-year-old sample from which the diatom *Nitzschia palea* grew.

In contrast to these reports, Parker (unpublished) failed to obtain viable algae from numerous herbarium specimens of lichens 35 to more than 100 years old following mild surface sterilization of the plant fragments.

We know of no efforts besides these to treat directly the longevity of algae or population viability under drought conditions. Therefore, we set out during 1967-'69 to examine more extensively the questions treated by Bristol (1919), Lipman (1941), and Becquerel (1942).

Methods

Using the herbarium of the Missouri Botanical Garden (MO), we obtained, after much searching, 50 specimens of bog or aquatic vascular plants dating from 1831 to 1966 on which small crumbs of dry soil remained. A second series comprising 74 specimens of *Gramineae* (mostly terrestrial) dating from 1885 to 1966.

Unlike the herbaria used by Lipman (1941) and Becquerel (1942), all our source material except the 1966 *Gramineae* had received some kind of disinfection. At least back to 1955, all plants at MO were fumigated with carbon tetrachloride, naphthalene and paradichlorobenzene before mounting. Before this time formaldehyde or mercuric chloride were used. After filing, the specimens are continuously subject to the vapors of the same chemicals used for the initial fumigation.

We picked dry soil crumbs no more than a few millimeters in diameter from roots with ethanol-flamed forceps and placed them aseptically into 50-ml cotton-plugged Erlenmeyer flasks or Pyrex screw-cap test tubes, both approximately half full of sterile Bold's (1949) modified Bristol's medium. Cultures were developed in approximately 3000 lux of constant, cool-white fluorescent light at 22-23° C, then placed in subdued light. After approximately two months, containers with visible growth were examined microscopically to determine the taxonomic affiliation of the viable algae. We made no attempts to identify all algae to species or genus level, a task now requiring elaborate sub-culturing and specialized skills for many groups (Bold & Parker, 1962; Chantanachat & Bold, 1962; Bischoff & Bold, 1963; Brown & Bold, 1964; Cox & Bold, 1966; Koster, 1966; Smith & Bold, 1966; Pearson & Kingsbury, 1966; Allen & Stanier, 1968). As new cultures developed, they too were observed, allowing in most cases, nearly two years for the appearance of visible growth.

Results

Although visible growth appeared as late as one year in a few cultures, most algae grew to maximum proportions within a few months. In all, 46 of the 124 soil crumbs yielded viable algae contrasting strikingly with results of Bristol (1919) and Becquerel (1942) who obtained viable algae from all their soil samples. Fungi developed in a greater number of cultures than did algae. Table 1 lists the algae which grew from the bog and aquatic plant series and from the predominantly terrestrial *Gramineae*.

TABLE 1. CHRONOLOGICAL LIST OF HERBARIUM SOURCE MATERIAL YIELDING VIABLE ALGAE. AQUATIC PLANTS (FIRST SERIES) AND GRAMINEAE (SECOND SERIES)

Date Collected	*Source Plant*	*Algae Identified*
12-18-66	*Limnocharis flava*	*Oscillatoria,* chlorococcacean, *Ulothrix (Uronema?)*
12-18-66	*Limnocharis flava*	*Oscillatoria,* chloroccacean, *Navicula*
5-27-62	*Alisma gramineum*	*Oscillatoria, Aulosira*
5- 5-55	*Isoetes melanopoda*	2 chlorococcacean spp.
3-11-51	*Equisetum hyemale*	chlorococcacean sp.
9-26-46	*Triglochin* sp.	*Phormidium?*
4-24-43	*Equisetum arvense*	2 chlorococcacean spp.
9-27-40	*Equisetum hyemale*	2 chlorococcacean spp.
5-26-38	*Equisetum ferrissii*	chlorococcacean sp.
7-23-35	*Typha latifolia*	*Nostoc*
4-14-35	*Equisetum hyemale*	*Nostoc*
7- 9-31	*Saggittaria umeata*	chlorococcacean sp.
1910	*Triglochin maritima*	*Nostoc*
12-24-66	Unidentified, unsterilized	*Lyngbya, Gleocystis* (or palmelloid chlorococcalean)
12-19-66	Unidentified, unsterilized	chlorococcacean, *Protosiphon*
12-14-66	Unidentified, unsterilized	chlorococcalean, *Protosiphon* and *Botrydium?*
12-14-66	Unidentified, unsterilized	*Oscillatoria,* chlorococcacean, *Scenedesmus*
12-13-66	Unidentified, unsterilized	chlorococcalean, chaetophoracean
12-12-66	Unidentified, unsterilized	chlorosphaeralean
12- 7-66	Unidentified, unsterilized	chlorosphaeralean, *Cylindrocapsa*
12- 7-66	Unidentified, unsterilized	*Anabaena, Lyngbya*
9-28-62	*Uniola laxa*	*Stigonema,* chlorococcalean, chlorosphaeralean
9- 4-62	*Panicum agrostoides*	*Protosiphon*
9- 2-62	*Manisaria rugosa*	*Protosiphon*
7-13-62	*Paspalum pubescens*	*Nostoc, Gloeocystis* (or palmelloid chlorococcalean), *Scenedesmus?*
9-30-61	*Echinochloa frunentacea*	chlorococcacean
9- 2-61	*Aristida wrightii*	chlorococcacean
9-17-60	*Trioda* sp.	2 chlorococcacean spp.
6-17-59	*Avena fatua*	chlorococcalean
6-24-57	*Stipa comata*	*Anabaena,* chlorococcacean
6- 6-57	*Bouteloua curtipendula*	*Anabaena*
6- 5-57	*Aristida wrightii*	chlorococcacean
8-10-55	*Panicum agrostoides*	chlorococcalean
8-22-53	*Echinochloa frumentacea*	chlorococcalean
4-28-53	*Splenophalis* sp.	*Trebouxia,* chlorococcalean, *Gloeocytis*
8- 6-51	*Calamogrostis epigejos*	chlorosphaeralean
8-23-49	*Calamogrostis canadensis*	chlorococcalean
5-29-48	*Panicum polyanthes*	*Hapalosiphon,* chlorococcalean
8-19-46	*Stipa columbiana*	chlorococcacean
7-13-46	*Andropogon virginicus*	*Nostoc*
7-11-45	*Stipa comata*	chlorococcacean
10- 3-43	*Bouteloua curtipendula*	*Chroococcus?,* chlorococcacean
8- 7-42	*Paspalum pubescens*	*Anabaena*
9-12-41	*Muhlenbergia setifolia*	chlorococcacean
6-27-40	*Panicum agrostoides*	chlorococcacean, chlorosphaeralean
9- 3-33	*Paspalum ciliatifolium*	*Anabaena*

These data illustrate that no alga survived more than 60 years. Green algae dominated in frequency of appearance during the most recent 30 years, while blue-green algae dominated the preceding 30-year period. Only one diatom grew and this from the most recent material. Our results show further that similar viabilities occurred for the aquatic plants as for the predominantly terrestrial ones. Although we did not identify species, it was clear that recent collections (1966) yielded a greater diversity of cytological strains (probably species) than older collections, especially the non-preserved *Gramineae*. Figure 1 illustrates the steady loss in viability of algal populations with increasing age of dried soil sample.

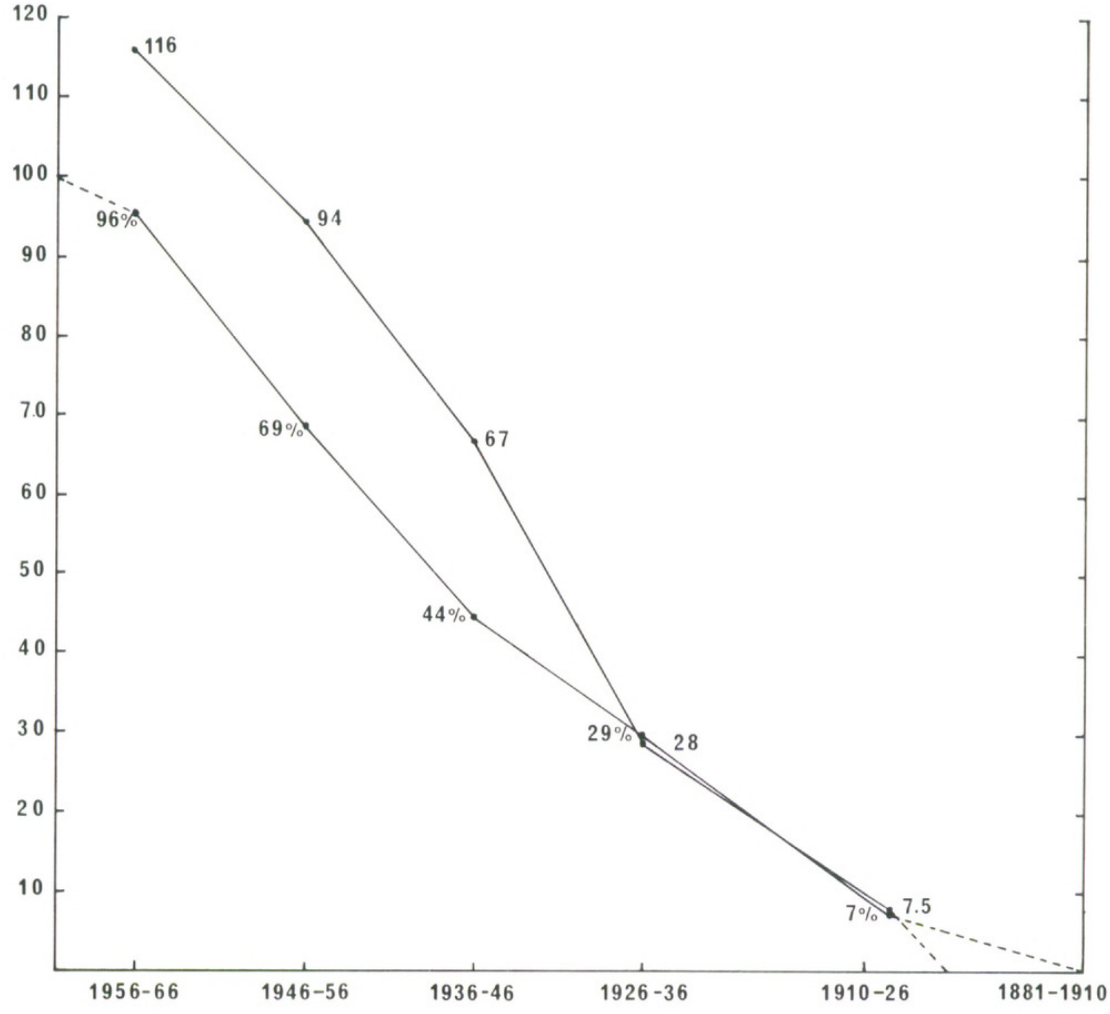

Fig. 1. Graph showing loss in algal viability, expressed in two ways: (1) as % of cultures developing algae; (2) as (Minimum No. Species Populations/No. Cultures) × 100. For convenience, calculations were based on approximately 10-year periods as shown on abcissa.

Noteworthy among the genera not reported by earlier workers but found in our cultures were a chaetophoracean alga, *Cylindrocapsa, Scenedesmus, Ulothrix,* and *Navicula,* all from recent specimens, and the genus *Protosiphon* in somewhat older source material.

Among the blue-green algae, filamentous forms dominated in our cultures as with those of Bristol (1919) and Becquerel (1942), the *Nostocaceae* appearing most frequently. Among the green algae, unicellular chlorococcalean forms dominated in all these studies. Diatoms were of rare occurrence.

Discussion

We have confirmed the findings of Bristol (1919) and Becquerel (1942) that a variety of soil algae, mainly belonging to the Chlorophycota and Cyanophycota, remain viable in dry soil for several decades in sufficient numbers to regenerate a visible population in response to favorable growing conditions. Table 2 illustrates this fact and other details we shall discuss. Our data also agree with that of Lipman (1941) which suggested that blue-green algae, especially *Nostoc* and *Anabaena,* possess greater survival capacities than other algae.

That only one diatom grew in our cultures as well as those of Bristol (1919) and Becquerel (1942), may reflect partially the media employed. Neither Bristol's

Table 2. Frequency of Occurrence of Viable Algae (by genus) in Dry Soil Reported by Bristol (1919), Becquerel (1942) and Us.

Genus and Major Group	*Bristol*	*Becquerel*	*Parker et al.*
Cyanophycota:			
Anabaena	7	0	5
Aulosira	0	0	1
Chroococcus?	0	0	1
Cylindrospermum	2	0	0
Hapalosiphon	4	0	1
Lyngbya	0	0	2
Nodularia	1	0	0
*Nostoc**	11	1	5
Oscillatoria	—	1	4
Phormidium	1	0	1?
Plectonema	4	0	0
Siphononema	0	1	0
Stigonema	0	2	1
Chlorophycota:			
Botrydina	0	1	0
chaetophoracean sp.	0	0	1
chlorococcacean spp.	0	0	23
chlorococcalean spp.	0	0	9
Chlorococcum	11	1	0
chlorosphaeralean spp.	0	0	5
Coccomyxa	0	1	0
Cylindrocapsa	0	0	1
Dactylothece	0	1	0
Dictyococcus	0	1	0
Gloeocystis	0	0	3
Microcystis? (a green)**	0	1	0
Oocystis	0	1	0
Protosiphon	0	0	4
Scenedesmus	0	0	2
Stichococcus	4	3	0
Trebouxia (= *Cystococcus*)	0	4	1
Trochiscia	4	0	0
Ulothrix (Uronema?)	0	0	1
Chrysophycota:			
Botrydium?	0	0	1
Navicula	0	0	1
Nitzschia	1	1	0

* See also Lipman (1941) for record longevity.

** Becquerel's reference to this blue-green algal genus as a green alga renders its identity questionable.

(1919) medium nor Bold's (1949) modified Bristol's medium favors diatoms, and Becquerel (1942) did not describe the composition of his medium. Xanthophycean algae, however, grow satisfactorily in these media, so their absence suggests a low viability for this group in air-dried soil.

The pattern of reduced viability with increasing age of soil samples terminating at 1910 in our study lends credence to our assumption that our experimental technique was entirely sterile and that algal contamination of soil crumbs in this herbarium is infrequent. Bristol (1919) also took precautions to avoid contamination, but we are not as certain that Becquerel (1942) was equally careful to maintain sterile conditions. If the algae which developed in Becquerel's soil samples were of the same age as the source material (65-98 years), then we might conclude that some factor(s) brought about reduced maximum longevity of algae in our soil samples. For example, treatment of our herbaria with preservatives or differences in soil moisture contents might explain our apparent higher mortality. Indeed, Bristol's soil samples possessed moisture contents somewhat higher than most fully air-dried soils, and neither her soils nor those used by Becquerel were pretreated with carbon tetrachloride or continuous vapors from paradichlorobenzene and naphthalene.

Another possible explanation for the older soils of Bristol (1919) and Becquerel (1942) yielding viable algae is that they used larger amounts of soil in their inocula. Our soil crumb inocula, which were unusually small (1-3 mm) as a result of meticulous cleaning of herbarium mounted plants, may have contained significantly fewer viable algal cells initially. This feature would tend to produce a more steeply sloping mortality curve.

Bristol (1919) encountered blue-green algae more frequently than green algae, while we and Becquerel (1942) obtained the opposite result. This difference between our data and that of Bristol could have resulted from differences in media or soils. The salts in Bristol's medium are 4× the concentration of Bold's (1949) modified Bristol's medium, while the ratios of salts (e.g., sodium nitrate/potassium phosphate) are the same. One might postulate that, over a long enough period of time, moist soil will experience some dentrification via the mixed microbial community present, and the N/P ratio will drop. This ratio should drop more in Bristol's solution than in Bold's modified medium, because the phosphate level will remain essentially constant. Such a lower N/P ratio may have favored the development of nitrogen-fixing blue-green algae which dominated Bristol's cultures, especially following the appearance of green algae.

Our data demonstrates the remarkable ability of soil algal populations to survive for many years in an air-dry condition. We cannot conclude that individual algal cells live this long, because cell division might have occurred during the long holding period. At least, given sufficient moisture, some soil algae can reproduce in absolute darkness (Parker, 1961). However, we have assumed that algal cell division does not occur in dry soil and that the mortality of individual cells within a population may be considerable with but a few cells surviving many years. The importance of this feature to mechanisms of algal dispersal and survival over periods of drought can not be ignored.

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