Parasitic Relationship between Two Culturally Isolated and Unrelated Lichen Components

Abstract. The presence of numerous haustoria, with accompanying death of the algal cells, was noted in a mixed cuture of the fungal symbiont (mycobiont) of *Collema tenax* (Sw.) Ach., em. Degel. and *Trebouxia impressa* Ahm., the algal partner (phycobiont) of *Physcia stellaris* (L.) Nyl. The parasitic action was noted even on media which would optimally sustain the independent growth of the individual symbionts.

The nature of the lichen association is often a difficult one to define. Frequently described in textbooks as the perfect example of a symbiotic partnership, it is becoming increasingly evident (*1*) that, in many lichens, the relationship is not so idyllic. In species of *Collema,* a genus of lichen with *Nostoc* as the phycobiont, the fungal component does not form typical haustoria, but, rather, a loose association exists between the hyphae and the algal cells. It has been shown, however, that the previously isolated and cultivated mycobiont of *Collema tenax* has a lethal effect on its *Nostoc* partner if the two are grown together under cultural conditions (*2*). We thought it would be of interest to see whether this lethal action occurred with another type of lichen phycobiont—namely, *Trebouxia impressa,* a unicellular green alga earlier isolated from *Physcia stellaris* (*3*). The former lichen was collected from Knivsta, Sweden; the latter, from Bedford, Mass.

The medium used was that of Bristol (*4*), slightly modified, as follows: K_2HPO_4, 0.5 gm; $NaNO_3$, 0.5 gm; $MgSO_4 \cdot 7H_2O$, 0.15 gm; $CaCl \cdot 2H_2O$, 0.05 gm; NaCl, 0.05 gm; ferric citrate, 0.01 gm; citric acid, 0.01 gm; $Na_2MoO_4 \cdot 2H_2O$, 0.25 mg; agar, 15 gm; redistilled H_2O, 1000 ml. Comparative series of similar cultures were also performed. The first was with the addition of 20 gm of saccharose to the above medium, the second with addition of 20 gm of glucose, and the third, with addition of 20 gm of malt extract. The *p*H's of the media were about 7.5. Cultures were maintained in test tubes.

The alga, taken from a pure clonal culture, was inoculated as a thin line along the slanted agar surface. Ten days later, after visible growth of the alga, the fungus, taken from a pure polyspore culture, was inoculated in the middle of this algal string. Before inoculation, the fungus was washed in sterile water to eliminate, or at least make negligible, any accompanying transfer of nutrient material. Control sets of cultures were made with the phycobiont alone on all types of media utilized. The tubes were kept at 17°C at a light intensity of 200 lux and with a light duration of 16 hr/day. Illumination was provided by a ramp of varicolored fluorescent lamps (designed to simulate natural light), made at the Swedish factory of Philips. The cultures were examined after 3 months' growth.

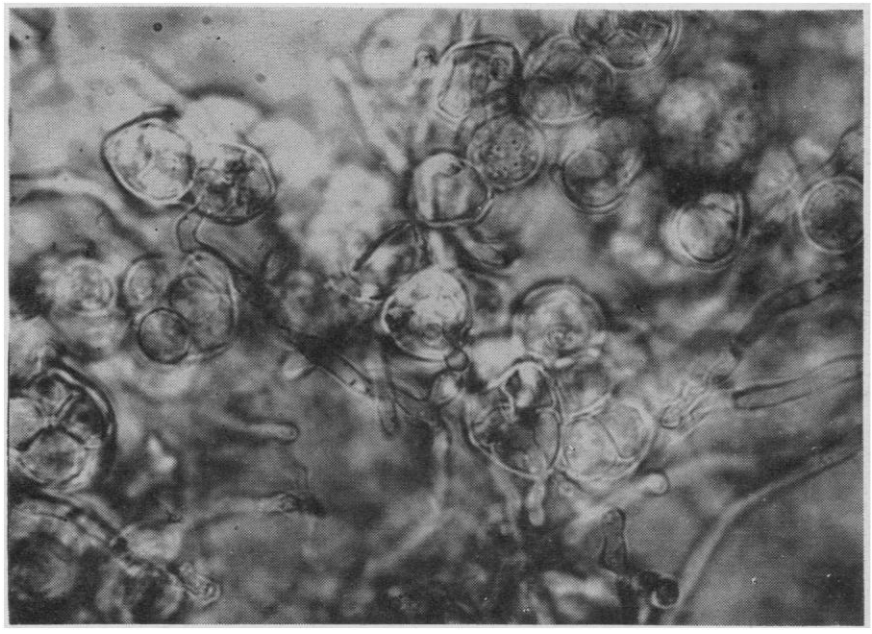

Fig. 1. Empty cells of the alga *Trebouxia impressa,* phycobiont of the lichen *Physcia stellaris,* penetrated by hyphal branches of the fungal component of *Collema tenax.* Culture on Bristol's inorganic medium. (× 1200)

In the mixed cultures growing on inorganic medium, scattered spots of dead algal cells were noted, a condition which might have resulted from a lethal action (via some diffusible substance) of the mycobiont. If this was the case, however, the lethal effect was very weak and erratic, as evidenced by the small size and the isolated and unrelated nature of these necrotic spots. Microscopic examination of these spots showed many dead and empty algal cells, a large percentage of them being filled with fungal hyphae. Although the percentage was quite low, algal cells with a healthy appearance were seen, with penetrating fungal haustoria. The good growth of the mycobiont on this inorganic medium can only attest to its direct or indirect utilization of the algal cells for its organic substances. The algae in the control tubes of inorganic medium appeared healthy in all respects, both macroscopically and microscopically. The other cultures on nutrient media, although lacking visible necrotic zones (because of the rapid growth of the algae) did show, microscopically, the same numerous dead algal cells filled with hyphal branches. Here again, healthy-looking algal cells were seen with haustoria.

It is hoped that this experiment will provide further insight into the highly enigmatic problem of lichenization. It is through continued investigations on the independent symbionts that a clearer understanding of the composite plant will be obtained.

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* John G. Bergquist fellow of the American Scandinavian Foundation for 1958.

6 July 1959

Another Meteorite Crater Studied

A recent expedition from the American Meteorite Museum (January 1959) for the study of meteorite craters in Australia discovered a gross error in the reported size of the little Dalgaranga crater in Western Australia. In the literature this crater is credited with a diameter of 225 feet and a depth of 15 feet. Consequently, the museum expedition personnel (consisting of Mrs. H. H. Nininger, Allan O. Kelly, a geologist from Carlsbad, Calif., and myself) were

quite surprised to find that the crater has a diameter of only 70 feet and a depth of only 10½ feet.

The crater had been reported originally by E. S. Simpson in 1938. Simpson's report was based upon a description given him by a former manager of the Dalgaranga sheep station, who reported finding several meteorite fragments around the crater in 1923. Simpson did not go to see the crater. His erroneous report was the basis of the incorrect measurements which were incorporated in the recent British Museum Catalog of Meteorites and which have been given in numerous other publications.

A survey, including a description of the meteorite fragments that were recovered at the site during the recent visit to the crater, is being prepared by the museum.

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15 September 1959

Effect of Chlorine Dioxide on Lignin Content and Cellulose Digestibility of Forages

A significant negative correlation exists between the percentage of lignin in a forage and the digestibility of the dry matter and the crude fiber. Lignin is susceptible to decomposition by chlorine and its oxides. The removal of lignin from plant material by sodium chlorite in aqueous acid solutions is part of the procedure for the preparation of holocellulose. Holocelluloses prepared from wood have been found to have digestion coefficients of 80 to 90 (*1*), which are higher than those of cellulose in untreated wood. The treatment of forages to remove lignin should lead to an improved utilization of the fibrous constituents of forages. Various treatments of forages or low-grade feeds have been carried out with delignifying agents; an example is the work of Prianishnikov and Tomme (*2*). They treated straw with aqueous ClO_2 and then with aqueous sulfite; however, a loss in soluble constituents resulted, even though the digestibility of the crude fiber was increased. So far as we know, no observations on the effect of ClO_2 gas in the dry state on the digestibility of forage have been made.

Experiments were carried out on a laboratory scale to degrade the lignin without loss of soluble constituents of forage. Three forages were treated as follows: Air was bubbled through a solution of sodium chlorite, sodium acetate, and acetic anhydride and then passed through a glass tube containing 5 to 10 gm of finely ground roughage in an air-dry state for 4 to 24 hours. The sample, still apparently air-dry, had an acid odor which could be removed by exposure to air, by vacuum treatment, or by a short aeration with air containing ammonia. The product was analyzed for acid-insoluble lignin (*3*), and the digestion coefficient of its cellulose was determined by an artificial-rumen technique developed at the Pennsylvania Agricultural Experiment Station (*4*). The results appear in Table 1. The dry treatment of dried and ground grass and straw with ClO_2 resulted in a marked decrease in the acid-insoluble lignin content as determined by chemical analysis and in a significant increase in the digestibility of the cellulose as indicated by the artificial-rumen technique. The changes were related to the amount of sodium chlorite that was used (*5*).

Table 1. Lignin content and cellulose digestibility of forages treated with ClO_2 gas.

Treatment	Hygroscopic moisture (%)	Acid-insoluble lignin (%)	Digestion coefficient of cellulose
Orchard grass, flowering, treated in 10-gm lots			
No treatment	5.6	5.3	36.9
0.2 gm $NaClO_2$	6.2	5.3	39.2
2.4 gm $NaClO_2$	5.7	3.9	48.0
3.0 gm $NaClO_2$	6.2	3.6	46.3
Reed canary grass, late dough, sample previously extracted with benzene-alcohol, treated in 5-gm lots			
No treatment	5.2	5.5*	30.9
1.2 gm $NaClO_2$	6.2	3.7*	40.0
3.0 gm $NaClO_2$	7.2	2.0*	46.9
Wheat straw, treated in 8-gm lots			
No treatment		6.7	22.8
2.5 gm $NaClO_2$†		2.8	36.2
3.0 gm $NaClO_2$		3.9	24.0
3.2 gm $NaClO_2$		1.85	52.2

* Percentage of original grass. † Final aeration with vapor from ammonium carbonate solution.

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5. This report is contribution No. 173 of the U.S. Regional Pasture Research Laboratory, Crops Research Division, U.S. Agricultural Research Service, in cooperation with the 12 northeastern states and authorized for publication 10 July 1959, as paper No. 2385, Journal Series, Pennsylvania Agricultural Experiment Station.

15 July 1959

Sodium- and Potassium-Sensitive Glass Electrodes for Biological Use

Abstract. Continuous, accurate recording in circulating fluids from a sodium and a potassium electrode is described. The Na electrode is capable of discriminating $\triangle[Na^+]$ of less than 1 meq/lit. in 140 meq/lit., and the K electrode is capable of discriminating $\triangle[K^+]$ of less than 1 meq/lit. in the range of 1 to 10 meq/lit. with good reproducibility. The electrodes may be used singly or in pairs with a common reference calomel electrode for simultaneous monitoring of $\triangle[Na^+]$ and $\triangle[K^+]$ in mixed solutions. Problems of streaming potential dependent on flow rate and electrode shape, as well as transient K^+ response by the Na electrode, are discussed.

Eisenman, Rudin, and Casby have elegantly demonstrated that the ternary glass system, Na_2O-Al_2O_3-SiO_2, may be systematically varied to produce electrodes with high selective affinity for individual cations (*1*). They reported that NAS_{11-18} glass (Na_2O, 11 moles percent; Al_2O_3, 18 moles percent; SiO_2, 71 moles percent) was a particularly effective Na electrode and that effective K electrodes might also be prepared. Since then, working in cooperation with Eisenman *et al.*, we have been able to adapt NAS_{11-18} glass for practical use in biological systems by using metal-connected electrodes to overcome problems inherent in the nature of the glass (*2*). The present report (*3*) is concerned with the limits of precision of the Na electrode operating alone or paired in a two-electrode system with a K-selective electrode.

Tests of precision and reproducibility were carried out with a continuous flow system at constant rate. The electrode was mounted in a shielded cage, and the inflow and outflow tubes were interrupted by an air gap to reduce stray electrical interference. In such a system, solutions flowing past the electrode membrane can be changed only gradually and, theoretically, never completely. Pockets of solution in the line may also produce erratic mixing. The reproducibility of potentials is, nevertheless, limited only by the drift rate of the electrometer which, for short intervals, is negligible (Fig. 1*A*). Precision is limited mainly by the accuracy of the standards and the ± 20 μv noise level of the Cary electrometer (equivalent to less than 0.2 meq/lit. on a base of 140). Recovery of Na added to plasma can be equally good.

As in the case of the H^+ electrode the streaming potential of the Na^+ electrode is affected by flow rate, particularly in unbuffered solutions. The response is a function peculiar to each electrode but, in general, for electrodes with a capacity of less than 1 ml, the