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.

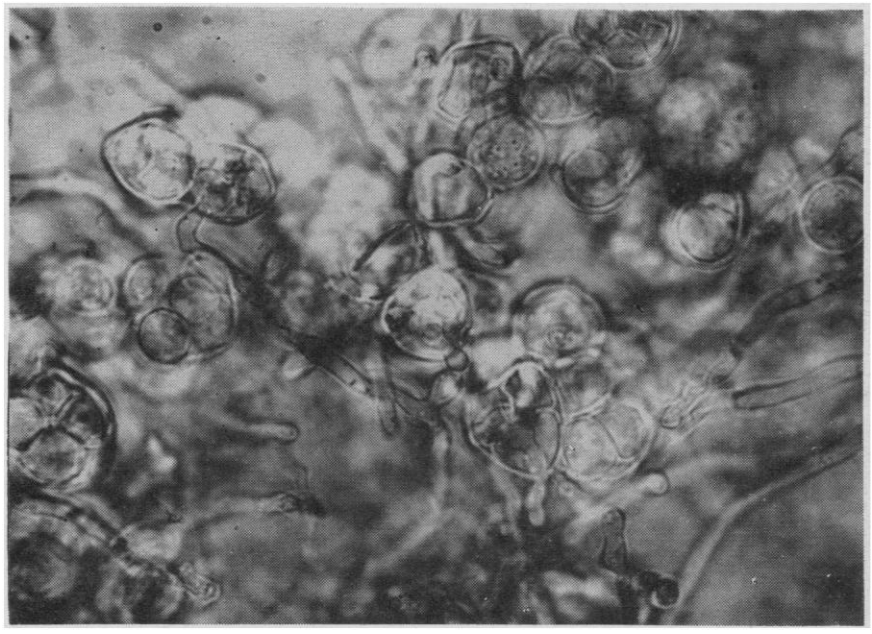

Fig. 1. Empty cells of the alga *Trebouxia impressa,* phycobiont of the lichen *Physcia stellaris,* penetrated by hyphal branches of the fungal component of *Collema tenax.* Culture on Bristol's inorganic medium. (× 1200)

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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References and Notes

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2. E. Henriksson, *Svensk Botan. Tidskr.* **52**, 391 (1958).
3. One of us (E. H.) was aided by a grant from the Swedish Natural Science Research council.
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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