ACTA BOT. ISL. 4: 10-15, 1976.

Associations between the blue-green algae Anabaena variabilis and Nostoc muscorum and the moss Funaria hygrometrica with reference to the colonization of Surtsey

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ABSTRACT: Nitrogen fixation rates of *Anabaena* and *Nostoc* grown on lava sand in association with *Funaria* were not affected by the presence of the moss. Growth and total nitrogen contents of *Funaria* and growth of the algae were increased by association. The possible importance of such associations in the colonization of Surtsey is discussed.

Algae-moss associations have been observed on the volcanic island of Surtsey. SCHWABE and BEHRE (1972) found that 6 years after the island's formation all moss locations studied were populated by algae, especially *Anabaena variabilis*. BROCK (1973) observed *Oscillatoria* and *Anabaena* associated with mosses growing in lava caves. HENRIKSSON and HENRIKSSON (1974) recorded nitrogen fixation by *Nostoc muscorum* and *Anabaena variabilis* in soils inhabited by mosses, including *Funaria*. Further studies have confirmed these reports (SCHWABE 1974).

Heterocystous blue-green algae are an important source of combined nitrogen in soils due to their capacity for atmospheric nitrogen fixation (reviewed by STEWART 1973). Nitrogen fixed by the algae may be rapidly transferred to the associated moss as demonstrated by STEWART (1967) using the stable isotope N^{15}, and recording uptake by the moss *Bryum* of nitrogen fixed by *Nostoc* in dune-slack soil. Mosses can also have an active role in associations by increasing nitrogen fixation rates in soil crusts containing blue-green algae (REDDY & GIDDENS 1975).

Production of growth substances by blue-green algae which complements their role as nitrogen fixers, has been recorded (VENKA-

TARAMAN & NEELAKANTAN 1967). Growth substances liberated can also affect the growth of higher plants (DADHICH *et al.* 1969, MISHUTIN & SHIL'NIKOVA 1971).

Because blue-green algae and mosses are colonizers of Surtsey, interactions between these organisms could play an important role in the further development of the ecosystem by affecting algal nitrogen fixation and growth of both algae and mosses. The aim of our laboratory study has been to compare growth and nitrogen fixation rates in associations between *Funaria* and *Anabaena* and *Nostoc*.

MATERIAL AND METHODS

Funaria hygrometrica (Hedw.) was isolated from soil collected on Surtsey in summer 1970 and routinely grown in 100 ml Erlenmeyer flasks in the laboratory on the inorganic salt medium of GORHAM *et al.* (1964) supplemented with 200 mg/l $NaNO_3$. Trace elements were added according to CLENDENNING *et al.* (1956) and the medium adjusted to pH 7 and solidified with agar. Flasks were incubated at 20°C and under continuous fluorescent light of approximately 1000 lux intensity. Axenic cultures were obtained by repeated subculturing and contamination tested for by plating material onto agar containing 0.5 % dextrose, 0.2% yeast extract and 0.2% bacteriological peptone followed by incubation of the plates at 25°C and 2000 lux.

Axenic cultures of *Anabaena variabilis* (Kütz.) (No. 1403/12) and *Nostoc muscorum* (Ag.) (No. 1453/12) were obtained from the Culture Centre of Algae and Protozoa, Cambridge, England. These algae were grown in 100 ml Erlenmeyer flasks containing 25 ml of nitrogen-free medium of ALLEN and ARNON (1955) at pH 7.5. All flasks were incubated at 25°C and 2000 lux continuous fluorescent light.

Lava sand was collected on Surtsey and stored in a polythene bag until use. For an analysis of the lava sand see HENRIKSSON and HENRIKSSON (1974). 25 g portions of sand were put into 9 cm diameter glass Petri dishes and moistened with sterile distilled water either alone or containing 200 mg/l $NaNO_3$. Algal cultures were harvested by centrifugation at 1000 x g for 10 minutes and resuspended in sterile distilled water. Algae (equivalent to 1 mg dry wt./dish) or small pieces of moss protonemata were added to the relevant dishes which were then incubated at 20°C and 2000 lux under an 18 hour day. For determinations of nitrogen-fixing capacity some material was also grown under the same conditions on sand previously sterilized by dry heat (110 for 2 days).

After 3 weeks growth, sand cores 5 mm deep x 8 mm diameter were collected with a cork borer and assayed for their nitrogen-fixing capacities. Assays were performed using the acetylene reduction technique (STEWART *et al.* 1967) as modified by HENRIKSSON *et al.* (1972) and STEWART *et al.* (1971). Growth of *Funaria* was estimated by counting the numbers of gametophytes per dish after 7 weeks growth. Algal biomass in each dish was found by determination of chlorophyll *a* (SMITH & BENITEZ 1955). After removal of chlorophyllous moss protonemata and gametophytes, the sand from each dish was extracted for 6 hours in 80% acetone and absorption at 663 nm measured in a Zeiss PMQ II spectrophotometer. Total ni-

trogen contents of moss gametophytes were found using the method of BOISONNAS and HASELBACH (1953). Gametophytes were harvested, washed thoroughly in distilled water and dried overnight at 105°C prior to weighing and subsequent analysis for total nitrogen.

RESULTS

Both *Anabaena variabilis* and *Nostoc muscorum* exhibited nitrogenase activity when grown on lava sand (Table 1). No activity was detected in either of the controls or sand containing *Funaria* only. Activities were rather variable but were generally higher in sterile sand, and in sand inoculated with *Nostoc*. The presence of *Funaria* did not significantly affect activities except in sterile sand containing *Anabaena* and *Nostoc* together where activity was reduced when *Funaria* was present.

TABLE 1. Effect of different inocula on nitrogenase activity in lava sand after 3 weeks growth, and total nitrogen contents of *Funaria* gametophytes after 7 weeks growth. All incubations were at 20°C and 2000 lux 18 hour day.

Inocula	n moles C_2H_4 hr^{-1} cm^{-2}. Means of triplicate samples ± standard error		% nitrogen contents of moss gametophytes grown in unsterilized sand
	Sterilized sand	Unsterilized sand	
Control (sand only)	0.0	0.0	-
Nostoc	4.24 ± 1.74	2.40 ± 0.28	-
Anabaena	0.74 ± 0.58	0.38 ± 0.15	-
Nostoc, Anabaena	4.53 ± 1.35	2.19 ± 1.51	-
Funaria		0.0	2.7
Nostoc, Funaria	4.22 ± 2.06	2.23 ± 0.87	3.9
Anabaena, Funaria	0.73 ± 0.73	0.61 ± 0.35	4.1
Nostoc, Anabaena, Funaria	1.12 ± 0.23	1.25 ± 0.44	3.8

Total nitrogen contents of *Funaria* gametophytes grown in sand inoculated with *Anabaena* and/or *Nostoc* were higher than in gametophytes grown in uninoculated sand. Gametophytes grown on unamended sand or sand amended with $NaNO_3$ increased in number when either or both algae were present (Table 2). Algal growth as measured by chlorophyll *a* content increased when *Funaria* was present.

DISCUSSION

Both algae are capable of growing and fixing nitrogen on lava sand under the laboratory conditions used. The algal species used were obtained from laboratory cultures so it is probable that if diffe-

TABLE 2. Effect of different inocula on growth of *Anabaena*, *Nostoc* and *Funaria* in lava sand after 7 weeks incubation at 20°C and 2000 lux 18 hour day.

Inocula	Numbers of gametophytes/dish*)		Algal growth µg chlorophyll *a*/dish*) (unamended sand)
	sand	sand amended with $NaNO_3$	
Control (sand only)	-	-	0.0
Nostoc	-	-	17.3 ± 3.4
Anabaena	-	-	17.8 ± 2.7
Nostoc, *Anabaena*	-	-	22.0 ± 3.8
Funaria	24 ± 8	16 ± 3	0.0
Nostoc, *Funaria*	51 ± 6	65 ± 13	27.8 ± 1.5
Anabaena, *Funaria*	74 ± 12	42 ± 10	19.7 ± 1.1
Nostoc, *Anabaena*, *Funaria*	97 ± 15	88 ± 12	30.4 ± 2.8

rent ecological races of these species exist, such algae colonizing Surtsey will have come from more closely related habitats and be even better adapted for growth on Surtsey. As nitrogenase activity was not found in uninoculated sand or sand inoculated with *Funaria* only, activity was due to the two algae tested and not to other micro-organisms in the sand. But fungi and bacteria may have been responsible for the lower rates recorded in unsterilized sand, by inhibiting the algae.

Blue-green algae liberate substantial amounts of fixed nitrogen (see FOGG *et al.* 1973 for references) and our findings also indicate this as nitrogen contents of *Funaria* gametophytes were higher when the sand was inoculated with either alga. As the lava sand is deficient in combined nitrogen (HENRIKSSON & HENRIKSSON 1974) association with blue-green algae can provide a source of combined nitrogen for the moss. However the nitrogen requirement of *Funaria* is quite low as it could grow on lava sand alone.

Funaria can also benefit from association with either alga by receiving compounds other than fixed nitrogen from the algae, because gametophyte numbers were greater in sand inoculated with algae than in uninoculated sand, even if a supply of combined nitrogen was provided. This effect is apparently due to the production of growth substances by the algae and their assimilation by *Funaria*. Conversely, *Funaria* had a growth-promoting effect on both algae. Exudates of various plants are known to stimulate the growth of blue-green algae (SHTINA 1972) and exudates of *Funaria* may therefore be responsible for its effect on the algae.

*) Results are the means of triplicate samples ± standard error.

The synergistic effects observed between *Funaria* and *Anabaena* or *Nostoc* are beneficial to the growth of both partners. As these associations occur on Surtsey the effects may be of importance to the island´s colonization in two ways. Directly, by increasing the establishment and dispersal of both moss and algae. Indirectly, by increasing soil organic matter thus augmenting the combined nitrogen content of the soil, reducing erosion and aiding in the retention of soil moisture.

Even though both algal species and *Funaria* are capable of independent growth, further work is required to determine if in certain habitats associations are obligatory and not just fortuitous.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the Surtsey Research Society and the Swedish National Research Council. G.A. RODGERS is grateful for the receipt of a Royal Society Overseas Fellowship.

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Received March 1976