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Nitrogen Fixation by *Gunnera–Nostoc* Symbiosis

THE genus *Gunnera* (Haloragaceae) contains forty species, all herbaceous, ten being endemic to New Zealand. Glands occurring at the bases of leaves become invaded by the blue-green alga *Nostoc puntiforme*[1,2] which becomes intracellular[3] and is capable of nitrogen fixation in culture[2,4]. Algal glands have been described for several New Zealand species[5,6].

Blue-green algae enter into symbiosis with a variety of plant groups[7,8] and for some of these, the phycobiont has been shown to fix nitrogen making it available to the host. It has been assumed[8] that the same holds true for *Gunnera–Nostoc*, although Scott[9] speculates that any contribution of fixed nitrogen from *Nostoc* to *Gunnera* must be negligible. This communication reports several experiments which establish the nitrogen fixing ability of the *Gunnera–Nostoc* symbiosis.

Growth experiment. *G. dentata* Kirk plants were established in sand culture, supplied with minus-nitrogen nutrient, and grown for ten weeks in a controlled growth room. Plants thrived, showing no deficiency symptoms, while radish seedlings in the same conditions developed severe nitrogen deficiency symptoms fourteen days after germination. Mean relative growth rate for *Gunnera* was 2·9 g/100 g/day while mean nitrogen increased from 0·783 mg/plant to 6·84 mg/plant.

Acetylene reduction experiment. Sliced stem tissue of *G. arenaria* Cheesem containing *Nostoc* glands was placed in serum bottles and gassed with an acetylene mixture. Gas samples were assayed at intervals for ethylene by gas chromatography. Results (Table 1) indicate a linear production of ethylene with time and inhibition of ethylene production by hydrogen, two features common to nitrogen fixing organisms.

Table 1. PRODUCTION OF ETHYLENE FROM TISSUE SLICES OF *G. arenaria**

Exposure time (h)	mμmoles C_2H_4/mg algal protein $-H_2$	$+0·1$ atmospheres H_2	Per cent inhibition
1	30	14	53
2	54	21	61
4	83	26	69
8	145	65	55

* Gas mixture: 0·1 atmosphere C_2H_2, 0·2 atmosphere O_2, 0·7 atmosphere Ar, or with 0·1 atmosphere H_2 replacing 0·1 atmosphere Ar.

Table 2. ATOMS PER CENT EXCESS ^{15}N IN VARIOUS PARTS OF *G. arenaria* PLANTS AFTER EXPOSURE TO $^{15}N_2$*

Exposure time (h)	Plant part: Node cluster	Leaf	Internode	Root
1·5	0·400	0·010	0·006	0·004
3	0·613	0·022	0·018	0·016
5	1·184	0·080	0·144	0·039
9	2·231	0·463	0·182	0·184

* Gas mixture: 0·1 atmosphere N_2 (95 atoms per cent excess ^{15}N), 0·2 atmosphere O_2, 0·7 atmosphere Ar, 0·001 atmosphere CO_2. Means of two plants.

$^{15}N_2$ experiment. Rooted rosettes of *G. arenaria* were incubated in the light in 200 ml. flasks containing $^{15}N_2$. Single flasks containing two plants were harvested at intervals and the 70 per cent ethanol soluble nitrogen assayed for ^{15}N, all values being measured against standard plant nitrogen from unexposed plants.

The node cluster (containing *Nostoc* glands) was rapidly and heavily labelled while the slower incorporation of ^{15}N into other parts of the host verifies that fixed nitrogen is readily transferred to the host. After nine hours, a small but measurable quantity of ^{15}N was detectable in the ethanol insoluble fraction indicating that fixed nitrogen had been incorporated into host protein.

Gunnera–Nostoc therefore represents an effective nitrogen fixing symbiosis in which the host may rely completely on the alga for combined nitrogen. Because *Gunnera* in New Zealand occupies pioneer situations in wet areas the symbiosis is of considerable ecological significance. From the growth experiment described it is estimated that up to 72 g N/m²/annum (64 lb N acre^{-1} year^{-1}) may be added to the ecosystem by this symbiosis.

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Phosphatase Release of Inorganic Phosphorus in Lake Kinneret

ALTHOUGH the activity of enzymes mediating the release of soluble inorganic phosphorus (P_i) in lakes has received some attention[1], it is still difficult to estimate their ecological role. Some results for *in situ* activities of alkaline phosphatases have been obtained by measuring the release of P_i from water samples saturated with chloroform. This compound, routinely used for P_i assays[2,3], seems to have no effect on phosphatase activity.

The experiments reported here were carried out on Lake Kinneret (Lake Tiberias) in northern Israel (area 174 km², maximum depth 42 m, epilimnion *p*H range 7·9–9·0). Samples from 1 metre depth were taken at a central lake station from April to July 1969. This period covers the end of homothermy and stabilization of the thermocline and is characterized by heavy algal blooms, usually of the dinoflagellate *Peridinium westii*.

Glass jars, thoroughly washed with 0·5 N HCl, were filled with 4 litres of lake water; 50 ml. of chloroform was added and the jars were stored at 22° C in darkness. Samples, taken immediately and at intervals, were assayed for total phosphorus[4], dissolved inorganic phosphorus[5] and bacterial count by standard plate methods[6]. Phosphatase activities of unfiltered sub-samples were also determined with *p*-nitrophenyl phosphate[1] with the addition of chloroform to the tubes. The activities of alkaline phosphatases extracted from plankton or obtained commercially, assayed with *p*-nitrophenyl phosphate, were unaffected by chloroform. Results of a typical experiment are presented in Fig. 1 and a summary of seven experiments is given in Table 1.