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A PRELIMINARY STUDY OF THE INTERNAL GAS COMPOSITION OF LEMNA GIBBA L.

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SUMMARY

The internal atmosphere of gibbous as well as flat modifications of *Lemna gibba* G3 was determined by gas chromatography after cultivation in vitro under conditions of continuous light. A similar analysis was done on a gibbous strain of *L. gibba* collected in the field. The gaseous constituents of the air chambers were found to be N_2, O_2 and CO_2. The CO_2 concentration was approx. 1 % in gibbous forms of strain G3 as well as the field strain and approx. 3 % in flat forms of strain G3. The O_2 concentrations tended to be slightly higher in the gibbous forms than in the flat. The CO_2 to O_2 ratio of the flat modification was much higher than in the gibbous forms. The remaining part of the internal atmosphere consisted of nitrogen. Methane and ethylene could not be detected.

1. INTRODUCTION

The internal structure of aquatic plants is generally characterized by large intercellular spaces which increase buoyancy and facilitate diffusion of gases into the cells. A general review of the physiological significance of this lacunar system has recently been published by HUTCHINSON (1975). Data on the composition of the gases which fill the air chambers are scarce as only a few studies have been conducted in this field. HARTMAN & BROWN (1967) reported that in the submerged species *Elodea canadensis* Michx. and *Ceratophyllum demersum* L. the main constituent of the internal atmosphere is nitrogen while the CO_2 to O_2 ratio depends on the rate of photosynthesis. Under anaerobic environmental conditions large quantities of methane were measured in the internal atmosphere of *E. canadensis* (HARTMAN & BROWN 1966). LAING (1940) reported a direct relationship between the CO_2 to O_2 ratio and photosynthesis in *Nuphar advenum* Aiton and some emergent species. This latter investigator suggested that in these plants anaerobic respiration occurs when the oxygen level is low. NYGAARD (1958) found a large variation in oxygen content in *Lobelia dortmanna* L., whereas the CO_2 content remained more or less constant.

In the duckweed species *Lemna gibba* L. the air chambers are arranged in two to three layers in the leaf-like structure (usually called frond) which together with the root forms a single plantlet. Under the influence of external conditions the air chambers of the lowest layer may expand considerably giving the plants a hemispherical shape, i.e. they become gibbous. This ex-

pansion is the result of an increase in cell division during early development of the frond, leading to more cells in the partition walls of the air chambers (PIETERSE 1975). In nature, gibbous modifications occur mainly during the summer season, with temperature, light and the chemical composition of the water probably being involved, although a genetic predisposition seems to affect the degree of gibbosity as well (DE LANGE & SEGAL 1968; DE LANGE & PIETERSE 1973; DE LANGE 1974; PIETERSE 1975, 1976). Under laboratory conditions gibbosity can be induced in flat plants by cultivating them in the presence of specific chelating agents like EDDHA and salicylic acid, or the ethylene-releasing compound ethrel (PIETERSE et al. 1970a, b; PIETERSE 1975, 1976).

In the present investigation the gas composition of the air chambers of *L. gibba* G3 was measured both in flat and gibbous plants after cultivation in the laboratory, and in a gibbous strain collected in the field. The main objective of this study was to compare the internal atmosphere of gibbous and flat plants in order to obtain information on the physiological background and a possible ecological significance of gibbosity.

2. MATERIALS AND METHODS

Plants of *Lemna gibba* L. strain G3, a strain which was originally collected in the botanical garden Catania on Sicily in 1953 (KANDELER 1969), were aseptically cultured in 125 ml Erlenmeyer flasks with 50 ml of the standard nutrient M-medium supplemented with 1 % sucrose (HILLMAN 1961) at a temperature of 25 ± 2 °C. Gibbous modifications were grown on M-medium enriched with 1 % sucrose and 10 mg.l^{-1} EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid) while flat forms were grown on the same medium without EDDHA. Each inoculum consisted of a colony of 4 flat fronds and the analysis was done after 3 weeks when the surface of the medium was completely covered with plants. The cultures were exposed to a continuous illumination of 12,000 erg. $cm^{-2}.s^{-1}$ from white fluorescent tubes.

The gibbous field strain was collected in a ditch near the village of Loenen in The Netherlands on June 29th, 1976. These plants were analyzed after 48 hours in ditch water in an environmental chamber under the same conditions of temperature and light as described for the growth of strain G3.

About 5–15 grams of plant material was rinsed several times with tap water and subsequently fresh weight was determined after removal of the adhering water with Kleenex tissues. The plants were placed in a glass funnel which was then inverted in a beaker filled with saturated $(NH_4)_2SO_4$ – solution. A glass capsule, closed at one end with a selfsealing rubber membrane, was fixed to the arm of the funnel with a rubber sleeve. The funnel was kept submerged in the beaker with a heavy metal ring. Before the analysis air was extracted from the funnel through the membrane using a syringe. The apparatus was then placed in a desiccator attached to an air pump and the internal pressure was reduced to 2700 $N.m^{-2}$, extracting the internal plant gases which rose into the capsule and collected under the membrane. After readjusting the pressure inside the

desiccator to normal, the gases were extracted with a syringe and analyzed. Gas was extracted separately for N_2, O_2 and CH_4 analysis, CO_2 analysis and ethylene analysis.

The analysis of the gas was carried out by gas chromatography. N_2 and O_2 were measured on molecular sieve type 5A (30–60 mesh Linde), packed in a stainless steel column, length 1.00 m, int. diam. 0.635 cm, at room temperature, which was also used for the detection of CH_4. CO_2 was determined on Porapak Q (50–80 mesh Waters assoc. inc.), packed in a stainless steel column, length 1.50 m, int. diam. 0.635 cm, at 50 °C. For the detection of ethylene a silica gel (60–80 mesh Perkin-Elmer) packed steel column was used, length 1.20 m, at 80 °C. The concentrations of the gases in the sample were calculated by comparison with pure gas samples. Total gas volume was expressed on basis of fresh weight. Analysis was done in three replicates, for which a fresh sample was extracted.

3. RESULTS AND DISCUSSION

The gas volume extracted ranged from 0.10 to 0.38 $ml.g^{-1}$ and from 0.90 to 0.95 $ml.g^{-1}$ fresh weight of flat and of gibbous G3 plants, respectively. Consequently, as could be expected, the internal gas volume was found to increase when the plants become gibbous. The gas volume of the (gibbous) plants from the field was about the same as the gibbous G3 plants (from 0.86 to 1.02 $ml.g^{-1}$ fresh weight).

The internal gas composition is shown in *table 1*. The CO_2 concentration was approx. 1 % in gibbous G3 plants as well as in the field strain, while this concentration was higher (approx. 3 %) in flat G3 plants. These values are more or less comparable with those found in other aquatic plants. NYGAARD (1958) found 1.01 to 1.18 % in *Lobelia dortmanna* after exposure to strong indirect daylight. LAING (1940) measured 2.3 to 4.9 % CO_2 in the petiole of *Nuphar*

Table 1. The composition of gases in the internal atmosphere in flat and gibbous forms of *Lemna gibba* G3 and the (gibbous) field strain.

	CO_2 (%)	O_2 (%)	N_2 (%)	$CO_2 : O_2$ × 100 (±σ)
Flat G3	3.26	22.9	73.8	14.2
	2.80	21.2	76.0	(±1.4)
	3.43	22.8	73.8	
Gibbous G3	1.08	18.7	80.2	5.03
	0.94	21.0	78.1	(±0.43)
	1.03	21.1	77.9	
Field strain	1.04	16.6	82.4	6.07
	1.06	17.4	81.6	(±0.19)
	1.01	17.3	81.7	

advenum in bright sunlight and 1.0 to 3.1 % in the upper parts of several emergent plant species. However, the CO_2 content was much higher in the dark and in the lower parts of these plants, i.e. rhizomes and stolons. On the other hand the CO_2 percentages reported for the totally submerged species *Elodea canadensis* and *Ceratophyllum demersum* were clearly lower, 0.3 and 0.6% respectively (HARTMAN & BROWN 1967).

The oxygen concentration was also lower in the gibbous forms: approx. 21 % in gibbous G3 and approx. 17 % in the field strain compared with approx. 22 % in the flat G3 plants. Consequently the $CO_2 : O_2$ ratio was considerably lower in gibbous G3 and in the gibbous field strain than in flat G3 *(table 1)*. In addition it was found that the remainder of the internal atmospheres consisted of nitrogen. Methane and ethylene could not be detected, these gases being either absent or possibly present in extreme low concentrations ($<$ 0.001 %).

It is a possibility that the lower $CO_2 : O_2$ ratio in gibbous plants than in flat plants suggests that gibbosity is correlated with a higher rate of photosynthesis, which would be in line with the findings of DE LANGE & SEGAL (1968), viz. the occurrence of the flat modification in sub-optimal conditions. On the other hand under optimal conditions in vitro, PIETERSE (1975, 1976) found that flat plants grow more vigorously than gibbous plants.

The present study should be considered as preliminary and further research is in progress.

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