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FURTHER INDICATIONS THAT ETHYLENE IS THE GIBBOSITY REGULATOR OF THE LEMNA GIBBA/LEMNA MINOR COMPLEX IN NATURAL WATERS

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SUMMARY

In the laboratory flat forms of the *Lemna gibba/Lemna minor* complex which are potentially gibbous, swell up in the presence of extremely low concentrations of ethylene (from 24 nl/l air upwards). In control cultures, where the ethylene concentration is 16 nl/l air or lower, the plants remain flat. In ditches in the Netherlands ethylene concentrations occur which are in the same range as the concentrations which induce gibbosity in the laboratory. These are further indications that ethylene is the gibbosity regulator of the *Lemna gibba/Lemna minor* complex in natural waters.

1. INTRODUCTION

A typical characteristic of the duckweed species *Lemna gibba* L. is that the fronds can be swollen at the lower side. This gibbosity, which is caused by an elongation of the vertical partition walls of the air chambers (PIETERSE 1975; EFRAT et al. 1977), makes the fronds hemispherical in appearance. In the field flat forms of *L. gibba* are difficult to distinguish from the related species *L. minor* L. (DE LANGE & PIETERSE 1973, DE LANGE 1975; KANDELER 1975; PIETERSE 1975; DE LANGE & WESTINGA 1979) and as a consequence it has been suggested to combine the two taxa in a species complex (DE LANGE & PIETERSE 1973).

In nature the gibbous plants are aestival forms which, as was already described in the early work of GUPPY (1895), produce flat plants in the autumn. It is generally assumed, however, that flat forms of *L. gibba* also occur during the summer season and it was supposed that they develop under less optimal circumstances, e.g. in less eutrophic and brackish waters (DE LANGE & SEGAL 1968). If gibbous plants are cultivated in the laboratory the thickness of the newly produced fronds decreases and after a few weeks only flat fronds are formed. This phenomenon was also observed when the plants were cultured on a nutrient medium containing high amounts of nitrates and phosphates irrespective of the season (PIETERSE et al. 1970a, b).

In 1970 it was discovered that gibbosity can be induced *in vitro* if flat specimens of *L. gibba* strain G3 are cultivated on a nutrient medium which is supplemented with EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid), a synthetic chelating agent (PIETERSE et al. 1970a). Subsequently it was proposed to use this

reaction to EDDHA as a general method to distinguish flat forms of *L. gibba* from *L. minor* (DE LANGE & PIETERSE 1973). EDDHA – more recently it was observed that salicylic acid brings about the same effect (PIETERSE 1976) – not only induces gibbosity but also induces or enhances flowering (PIETERSE et al. 1970b, c). In the light of these observation it was supposed that in nature gibbosity might be induced by natural chelating agents (PIETERSE 1975). On the other hand, in nature flowering of *L. gibba* is extremely rare and, in the light of the findings in the laboratory, this would not be expected if chelating agents regulate gibbosity in natural waters.

More recently it was observed that gibbosity can also be induced by adding the ethylene-releasing compound Ethephon to the nutrient medium (PIETERSE 1976) or by exposing the plants to ethylene gas (PIETERSE 1977, unpublished observation). Ethylene, contrary to EDDHA or salicyclic acid, does not induce or enhance flowering and in combination with EDDHA flowering even decreases whereas the fronds become excessively gibbous. In the light of these new findings it was then suggested that in nature ethylene might be the gibbosity regulator (PIETERSE 1976) as this gas is commonly produced in waterlogged soil under anaerobic conditions (SMITH & RUSSELL 1969).

In order to test this hypothesis ethylene was measured in water samples which were collected from ditches in the field covered by either partly gibbous or totally flat vegetations of the *L. gibba*/*L. minor* complex. In addition flat modifications of the potentially gibbous strain *L. gibba* G3 were exposed to extremely low ethylene concentrations in the laboratory.

2. MATERIALS AND METHODS

On August 23rd, 1979, and September 11th, 1979, water was sampled from various ditches in the Provinces of North Holland and Utrecht covered by a *L. gibba*/*L. minor* vegetation. The samples were collected at a depth of about 10 cm, in 250 ml bottles, which were closed under water, without leaving an air bubble. Data on the location of the different collection sites are presented in *table 1*. In addition it is shown in *table 1* whether the *Lemna* vegetation at these sites consisted of gibbous or/and flat fronds. The water samples were kept at $\pm$ 4°C and within 24 hr 50 ml were transferred by means of a syringe to a 125 ml septumstoppered bottle already filled with $(NH_4)_2SO_4$ to give a saturated solution. Subsequently 1 ml of the resulting gas was siphoned off and the ethylene content determined by means of a gas chromatograph (Varian aerograph series 1400 with flame ionization detector). The column used was alumina (60–80 mesh), length 75 cm, diameter 2 mm. The concentration of ethylene was calculated by comparison with a gas sample containing 10.000 nl/l ethylene. As a control a sample of tap water was used.

In order to test the effect of low concentrations of ethylene a very small amount of the gas was released into an environmental chamber (6 × 4 × 2.5 m) in which *L. gibba* G3 plants were cultured aseptically in 100 ml Erlenmeyer flasks under continuous illumination of 12,000 erg/cm^{-2}/sec^{-1} at plant level from

Table 1. Provenance and ethylene content of the various water samples which were collected from sites covered by plants of the *Lemna gibba/Lemna minor* complex.

Sample No.	Location	Date of collection	Morphology of the *Lemna* vegetation	Ethylene concentration nl/l water
1	Egmond, ditch north of Kromme Hoogedijk	23-8-1979	flat/gibbous	21.8
1*	Egmond, ditch north of Kromme Hoogedijk	11-9-1979	flat/gibbous	29.6
2	Egmond, Vennewatersweg naar Abdij	23.8.1979	flat/gibbous	19.7
3	Egmond, ditch south of Kromme Hoogedijk	23-8-1979	flat	16.4
4	Egmond, Zeeweg	23-8-1979	flat/gibbous	29.1
5	Heiloo, Mossellaan	11-9-1979	flat/gibbous	24.0
6	Loosdrecht, kromme Rade	11-9-1979	flat	14.7
7	Lage Vuursche	11-9-1979	flat	14.8
tap water		12-9-1979		7.3

white fluorescent tubes. The flasks, which were closed by aluminium foil, contained 50 ml of M-medium (HILLMAN 1961) supplemented with 1 % sucrose. The temperature in the environmental chamber was kept at 25°C ± 2°C. Controls were cultured in the same environmental chamber at a later date without additional ethylene. In addition, simultaneously with the control cultures, plants were grown on M-medium containing 10 mg/l of the chelating agent EDDHA. (For reasons of comparison flasks containing this medium, but without *L. gibba* G3 plants were also placed in the environmental chamber.) After a period of 14 days the gibbosity of the plants was assessed and the ethylene concentration in the air in the Erlenmeyer flasks measured by means of a gas chromatograph.

3. RESULTS AND DISCUSSION

The ethylene concentrations in the various samples from the field are shown in *table 1*. They varied from 14.7 to 29.6 nl/l while in the control (tap water) the concentration was 7.3 nl/l. In ditches where only flat *Lemna* plants occurred the ethylene concentrations were 16.4 nl/l or lower. In the ditches where gibbous as well as flat fronds occurred the ethylene concentrations varied from 19.7 to 29.6 nl/l.

In the laboratory all flat *L. gibba* G3 cultures which were exposed to ethylene gas became excessively gibbous. The ethylene concentrations which were measured in the Erlenmeyer flasks above the cultures in 4 replicates were 24, 28, 28, and 28 nl/air, respectively. In the control cultures where the plants remained flat these values were 12, 12, 13, and 16 nl/l air, respectively. As a consequence it may be assumed that low concentrations, i.e. as low as 24 nl of ethylene per liter air, induce gibbosity in flat forms which are potentially gibbous.

As the *Lemna* plants float on the surface of the water it may be expected that

ethylene can be absorbed both from the water and the air. In this regard the ethylene concentration in the air in closed Erlenmeyer flasks in the laboratory may be compared with the ethylene concentrations in the water in the field. The ethylene, which is probably produced in the ditch substrate (SMITH & RUSSEL 1969) may be expected to diffuse continuously via the water into the open air. In the light of these results it appears that an ethylene concentration around 20 nl/l water as well as around 24 nl/l air would be sufficiently high to induce gibbosity. Samples 1–4 were collected after heavy rains. When a sample was taken two and a half weeks later at the same site as sample 1 (1*) the ethylene concentration had risen from 21.8 nl/l to 29.6 nl/l which shows that the concentrations in the field may fluctuate.

When gibbosity was induced in the laboratory by means of EDDHA the ethylene concentration in the air in the Erlenmeyer flasks was generally higher than in the controls devoid of EDDHA, i.e. 18, 20, 19, 19, and 15 nl/l, respectively, whereas the values in the controls were 12, 12, 13 and 16 nl/l air. Flasks containing EDDHA without *Lemna* plants showed about the same values as the controls, 14, 12, 13, 13 and 14 nl/l air respectively. Although these results should be considered as preliminary it could be that EDDHA brings about its effect on gibbosity via the endogenous production of ethylene. If this assumption proves to be correct it should be taken into consideration that concentrations of endogenous ethylene within the plants are expected to be higher than in the air in the Erlenmeyer flasks above the plants.

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