

## NOTES

# Some Effects of Nitrogen and Phosphorus Concentration on the Phenology of *Hydrilla verticillata* (L. f.) Royle

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### INTRODUCTION

It is generally assumed that the submersed weed *Hydrilla verticillata* (L.f.) Royle tolerates a wide range of nutrient conditions (2,4). However, detailed reports on the chemical characteristics of its habitat are relatively scarce, especially on a world-wide basis.

The survival strategy of plants can be changed under the influence of nutrients (1). In this context it is conceivable that growth and reproduction of hydrilla may be affected by different nutrient levels, a supposition which seems to be corroborated by the fact that hydrilla shows a high phenotypic plasticity (2). Variances in growth and multiplication strategy could have consequences for the management of the weed.

In the present study, a hydrilla clone from Indonesia was exposed to various nitrogen and phosphorus concentrations. Observations were made on growth, turion production, flowering and the number of spines at the lower side of the leaves.

### MATERIALS AND METHODS

A clone of hydrilla which originally had been collected in 1980 in the Curug lake in West Java, Indonesia (5), was cultivated in laboratory aquaria (50 x 50 x 30 cm) and 2-liter Erlenmeyer flasks. These stock cultures were kept in an environmentally controlled chamber (A) at  $25 \pm 2$  C. The light intensity at plant level was  $9000 \text{ erg cm}^{-2} \text{ sec}^{-1}$  (from Philips TL 65W/33 white fluorescent tubes) and the light regime was 8L:16D. This photoperiod had been shown to be optimal for flowering (6). The aquaria were filled with rain water and the bottoms were covered by a layer of clay (2 cm) and a layer of superficially washed sand (1 cm). The plants were cultured in Erlenmeyer flasks under axenic conditions. The flasks were filled with 20% Long Ashton medium (3) and the bottom was covered by a layer of sand (1 cm). The sand was washed twice with tap water and subsequently at least five times with demineralized water. Sterilization of the plant material was achieved by exposing young tops to a 3% sodium hypochlorite solution for 2-15 min (depending on size and thickness of the tops). After autoclaving, the nutrient medium was supplemented with

1.2 mM  $\text{NaHCO}_3$  and adjusted to a pH of 7.5 with diluted NaOH.

The experiments were carried out in:

- Aquaria (50 x 50 x 30 cm) in an environmental chamber B, which was kept at the same temperature as environmental chamber A. The plants were exposed to an illumination of  $10,000 \text{ erg cm}^{-2} \text{ sec}^{-1}$  from white fluorescent tubes (Philips TL 65W/55). The photoperiod was the same as in A. The aquaria contained a layer of sand (2cm) on the bottom which was washed in a similar way as the sand in the Erlenmeyer flasks in the stock cultures.
- Two-liter Erlenmeyer flasks in environmental chamber A under the same conditions as described for the stock cultures. Every two weeks the medium was changed.

Both the aquaria and flasks were filled with 20% Long Ashton medium containing different concentrations of  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ : 0.15, 1.50 and 15.00 mg/l of  $\text{NO}_3^-$  and 0.02, 0.20 and 2.00 mg/l of  $\text{PO}_4^{3-}$ . All the nine  $\text{NO}_3^-/\text{PO}_4^{3-}$  combinations were tested, as well as the combination of 148.8 mg/l  $\text{NO}_3^-$  and 22.60 mg/l  $\text{PO}_4^{3-}$ , which occurs in standard 20% Long Ashton medium. The experiments in the aquaria (one aquarium per concentration) were started with 20 detached shoots of approximately 12 cm long (from stock cultures). The experiments in the Erlenmeyer flasks were started with shoots which were approximately 7 cm long (from stock cultures in Erlenmeyer flasks). Detached shoots were used with an apical tip (two Erlenmeyer flasks per concentration), without an apical tip, i.e. the tip was cut off (one Erlenmeyer flask per concentration) and rooted shoots with an apical tip (one Erlenmeyer flask per concentration).

The aquaria and Erlenmeyer flasks were maintained in the growth chambers under experimental conditions for 8 weeks. In the aquaria axillary turions were counted and growth was measured as dry weight. Spines on the lower side of the leaves were assessed under a low power magnifying microscope in plants which had been grown in Erlenmeyer flasks. Young tops were used (at least three tops per Erlenmeyer flask) and the leaves between ten internodes counted from the second clearly visible internodium under the top, were taken into consideration.

### RESULTS AND DISCUSSION

The results of the experiments in aquaria suggest that the formation of axillary turions is stimulated when the

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TABLE 1. NUMBER OF AXILLARY TURIONS (EXPRESSED ON A BASIS OF 1 G DRY WEIGHT OF PLANT MATERIAL), AND DRY WEIGHT OF *Hydrilla verticillata* (L.f.) ROYLE AFTER CULTIVATION IN AQUARIA DURING A PERIOD OF 8 WEEKS ON 20% LONG ASHTON MEDIUM CONTAINING VARIOUS LEVELS OF  $\text{PO}_4^{3-}$  AND  $\text{NO}_3^-$ .

Nitrate (mg/l)	Phosphate (mg/l)							
	0.15		1.50		15.0		148.8 <sup>1</sup>	
	turions	dry wt. (g)	turions	dry wt. (g)	turions	dry wt. (g)	turions	dry wt. (g)
0.02	29.0	3.0	6.8	4.3	3.9	3.6	—	—
0.20	24.2	2.4	5.5	4.0	10.0	3.8	—	—
2.00	4.8	6.3	7.3	5.6	14.0	5.8	—	—
22.60 <sup>1</sup>	—	—	—	—	—	—	0.1	10.2

<sup>1</sup>Indicates the concentrations which normally occur in the medium.

nitrogen and phosphorus concentrations in the water are low (Table 1). The highest numbers (expressed on a basis of 1 g dry weight of the plant material) were found at the lowest concentration of phosphorus (0.15 mg/l  $\text{PO}_4$ ) in combination with the lowest and second lowest concentration tested of nitrogen (0.02 and 0.20 mg/l  $\text{NO}_3^-$ ). These numbers were 29.0 and 24.2. In standard 20% Long Ashton medium, the turions were virtually absent. With a few exceptions there was no production of subterranean turions.

From Table 1 it can be seen that growth (expressed as dry weight) was markedly inhibited at the lower concentrations of nitrogen (and that the effect of increased phosphorus concentration is small). Under these conditions the plants became chlorotic suggesting that nutrient values were critical for growth. The formation of axillary turions may be a means for hydrilla to survive conditions of nutrient stress.

The formation of flowers also seemed to be stimulated by decreases in nitrogen and phosphorus concentrations. The highest number of flowers was observed at the lowest concentrations of nitrogen and phosphorus (in combination) tested, i.e. 3.3 female flowers and 2.0 male flowers as expressed on the basis of 1 g dry weight of the plant material. Moreover, the female flowers were frequently (circa 30% of the flowers) formed on axillary turions. One turion even produced two flowers. On a cross section it could be seen that the flower on a turion is initiated in an axil of a modified leaf. As a turion is in fact a short, specialized shoot, this formation of female flowers is from a morphological point of view not very remarkable. However, the formation of a flower by a dormant structure is very unusual and has never been described in hydrilla, although it may not occur under natural growing conditions.

The results on flowering should be considered as preliminary. If the production of flowers in hydrilla is stimulated by a decrease of the nutrients in the water and the hydrosoil, this would imply that, apart from the formation of axillary turions, the survival strategy of the species under nutrient stress also includes the increased formation of seeds.

Plants did not produce turions or flowers in the Erlenmeyer flasks. This could be due to the relatively small size

of the plants and the small biomass (approximately 150 mg dry weight at the lowest concentrations of nitrogen and phosphorus).

Rooted shoots had more spines than shoots which were not rooted. In general, the number of spines in the rooted shoots was higher at the lower  $\text{PO}_4^{3-}$  concentrations (up to an average of 8.17 spines per leaf whereas in standard 20% Long Ashton medium the average number of spines was only 1.77). On the basis of these results it is suggested that the number of spines at the lower side of the leaves is environmentally regulated. As the effect was only brought about when the shoots were rooted at the beginning of the experiment, it is conceivable that plant growth regulators are involved. It could be hypothesized that spine formation is triggered via a plant growth regulator which is produced in the roots.

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