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Ecological and physiological investigations on *Eichhornia crassipes* (MART.) SOLMS.
1. The effect of different environmental conditions on the development of root colour

by

Ursula-Brigitte Schlüter & Bodo Furch

Dipl.-Biol. Ursula-Brigitte Schlüter, Priv. Doz. Dr. Bodo Furch, Botanisches Institut der Universität Kiel, Olshausenstraße 40, 2300 Kiel (FRG).
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Abstract

The cell walls of *E. crassipes* roots show areas of strong coloration, which can range from light brown through brown, blue, violet to blue-black, while the plants appear generally healthy. This work describes the environmental factors which control the particularly noticeable blue colouration of the roots. From studies conducted under defined conditions in growth chambers, substrate nitrogen deficiency in the light produces an intense blue root colouration. Thus with nitrate or ammonium deficiency of the nutrient medium *E. crassipes* can be considered a nitrogen monitor.

Keywords: *Eichhornia crassipes*, ecological studies, development of root colour, *E. c.* as an indicator of N deficiency in nature.

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Introduction

Eichhornia crassipes (Pontederiaceae) is a tropical floating plant. It is endemic to the Neotropics and has, in this century in particular, spread throughout the Tropics and Subtropics through human influence (GOPAL & SHARMA 1981). Due to its enormous vegetative reproduction potential it is one of the most dreaded aquatic weeds of the 10 described *Eichhornia* species and has attained the widest distribution. Although the existence of several biotypes has been suspected, it has not been possible to show any genetic variation (WAIN & MARTIN 1980).

Over a hundred years ago a peculiarity, which is apparently not rare in the Pontederiaceae, although particularly pronounced in *E. crassipes*, was noted, e. g. HILDEBRAND (1883); the roots produce a coloured zone in their cell walls which can range from brown, via violet to black. The dependance of the root colouration on exogenous factors such as light, mineral and nutrient supply, pH, as well as oxygen, nitrogen and hydrogen sulphide concentrations was investigated in hydroculture.

Material and methods

The plant material originated from white waters of the Amazon river near Manaus, Brazil. The plants were cultivated in greenhouses or culture chambers in Kiel, where their proliferation was entirely vegetative (B. FURCH & ZIMMERMANN 1983). As far as possible the culture conditions conformed to conditions at the original site with respect to the following parameters: day length 13 hrs. relative humidity 85 %, day temperature 28 °C, night temperature 26 °C, water temperature 27 °C. Quantum emission of light in PAR bands at the plant surface was 110 μmol photons x m^{-2} x sec^{-1}, with maxima at 440, 560 and 660 nm. The various nutrient conditions were based on KNOP'S water culture medium; although the original concentration had to be reduced by 80 % in order to approximately conrespond to the maximum level at the central Amazonian location (K. FURCH 1984), so that aqueous ionic concentrations in the culture chambers would be tolerated (SCHLÜTER 1985).

Light-tight, black plastic containers were used to maintain individual daughter plants in 10 l nutrient solution, after several weeks pre-culture. Daughter plants are those individuals which are still relatively small, have produced no roots and must therefore initially receive their nutrients from the mother via stolons.

pH, conductivity and molarity of the nutrient solutions were identical at the start of most of the investigations (pH 5.5, conductivity 250 μS). The investigations lasted for 30 to 40 days. For technical reasons maintainance in a through-flow system was not feasible. Therefore, when necessary, nutrient solutions were renewed every 3 days to exclude the possibility that the results of the study were uncontrollably influenced by falling nutrient concentration and pH.

Bubbling compressed air, nitrogen or hydrogen sulphide through the culture medium allowed the substrate partial pressure to be varied. Shading the root region of the floating plants was achieved by placing a close fitting double collar of black pool plastic around the individual *Eichhornia* plants.

Hydrogen sulphide, oxygen, ammonium and nitrate were measured using standard colorimetric methods and other N-containing compounds were measured using an amino acid analyser.

Harvesting and preparation of the plant material, separation, drying and pulverisation of the roots and pigment extraction, as well as the photometric measurement of the relative soluble pigment content followed MATTERN & B. FURCH (1987).

The plants were so set up, under otherwise constant conditions, that deficiency of a single important nutrient or element was induced at any one time.

Tab. 1: Summary of the amounts of available nutrients in variously induced deficiency conditions in water culture. Total molarity, pH and conductivity are identical under all conditions.
Control (full medium) : 20 % KNOP. pH = 5,5 conductivity = 250 μS.

Condition	KCl	KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	Fe/EDTA	Substituting ions
20 % KNOP	K : 26222 Cl : 23775	K : 14366 PO_4 : 34892	Mg : 4932 SO_4 : 19486	Ca : 12590 NO_3 : 105030	Fe : 2010	--- ---
N-absence	K : 26222 Cl : 23775	K : 14366 PO_4 : 34892	Mg : 4932 SO_4 : 19486	--- ---	Fe : 2010	Ca : 33667 Cl : 29780
S-absence	K : 26222 Cl : 23775	K : 14366 PO_4 : 34892	--- ---	Ca : 12590 NO_3 : 105030	Fe : 2010	Mg : 4862 : 7090
P-absence	K : 26222 Cl : 23775	--- ---	Mg : 4932 SO_4 : 19486	Ca : 12590 NO_3 : 105030	Fe : 2010	K : 13924 NO_3 : 22078
K-absence	--- ---	--- ---	Mg : 4932 SO_4 : 19486	Ca : 12590 NO_3 : 105030	Fe : 2010	Na : 10796 PO_4 : 34189
Ca-absence	K : 26222 Cl : 23775	K : 14366 PO_4 : 34892	Mg : 4932 SO_4 : 19486	--- ---	Fe : 2010	K : 65737 NO_3 : 104215
Mg-absence	K : 26222 Cl : 23775	K : 14366 PO_4 : 34892	--- ---	Ca : 12590 NO_3 : 105030	Fe : 2010	Na : 9195 SO_4 : 19203
Fe-absence	K : 26222 Cl : 23775	K : 14366 PO_4 : 34892	Mg : 4932 SO_4 : 19486	Ca : 12590 NO_3 : 105030	---	EDTA : 13401

Results and Discussion

1. The influence of soluble plant nutrients on colour development

Table 2 shows the effect of the deficinecy studies on growth, health and root colour. The control plants were in very good, generally healthy, condition after 40 days. In terms of biomass they had a doubling time of 6 days, which represented a 115 % increase in fresh weight per week. Under optimal conditions plant doubling time is between 5 and 15 days. The growth rates are among the highest observed to date. Most of the roots produced under these conditions are colourless.

Tab. 2: Effect of the nutrient solutions in Tab. 1 on growth and plant condition and on root colour. Experiment duration: 40 days.

	biomass-production per week (% fresh-weight)	branch	leaves	roots
20 % KNOP	115	plants healthy	green colored no pathogenic symptoms	normal growth uncolored (young r.) light blue (elder r.)
N-absence	111	well developed floating leaves	green colored no pathogenic symptoms	normal growth when illuminated, enhanced growth and dark blue coloration when kept in dark
P-absence	47	plants healthy	partially necrotic	normal growth brown and blue colored
S-absence	43	growing point partly dead	yellow colored necrotic	normal growth brown and blue colored
Ca-absence	34	small, rotting	rotting	dead, brown colored
K-absence	22	necrotic	small and dead	normal growth brown colored
Mg-absence	15	bulbs necrotic	chlorotic stripes	normal growth brown colored
Fe-absence	4	plants die		

Surprisingly, with respect to growth and general condition, plants grown under N-deficiency over a 6 week period showed no difference from the controls. The plants are able to store nitrogen and, to a lesser extent, phosphate (BOYD & VICKERS 1971; TUCKER & DEBUSK 1983). From our experience classical deficiency symptoms first appear after 80 days in culture. All other deficiency conditions (from P- to Fe-deficiency) were increasingly deleterious. Growth rates decrease, shoots and leaves exhibit recognised deficiency symptoms and after only 40 days some have already died. Fe-deficiency caused complete death of all plant parts after only a week.

Fig. 2 (see below) also shows that during the study period, the particularly intense dark blue root colouration developed only in the root region under N-deficiency in the light. Under other growth conditions colouration is either slight or completely absent. For clarification of the deficiency effects described in Tab. 2, Fig. 1 gives the root colouration intensity using visual criteria; after a maximum 40 days, all new roots were blue-black if N had been previously removed. A low but measurable quantum flux (PAR) of more than 0,5 μmol x m^{-2} x sec^{-1} must be present in the root region to allow colour to develop in the root cell walls.

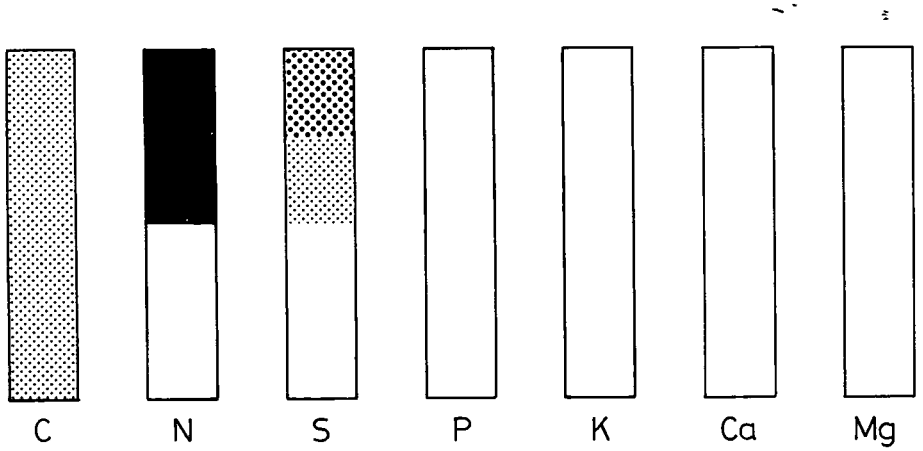

Fig. 1:
Diagram showing the effect of the separate deficiency cultures on the intensity of root colouration. C = control, N = without nitrogen, S = no sulphur, P = no phosphorus, K = no potassium, Ca = no calcium and Mg = no magnesium. Experiment duration = 40 days.

Since it could be concluded from the described investigations that, particularly in the absence of nitrate-N, strong root colouration was induced, nitrogen concentration and N-sources in the medium were subsequently varied (Tab. 3). In addition to nitrate, ammonium, a combination of nitrate and ammonium, urea, asparagine and glutamine were used; the enclosed area delimits the "sensible" concentrations, at which good growth of 75 % (nitrate), 86 % (ammonium), 65 % (nitrate + ammonium), 82 % (urea), 78 % (asparagine) and 83 % (glutamine) increase in fresh weight per week occurred. (Plant doubling time 8 - 9 days). Furthermore these concentrations had no inhibitory effect on plant development. From regular analyses it could be shown that all N-sources were very rapidly taken up, often within a week.

1. The relative pigment concentration i. e. the amount of extractable pigments, increased with decreasing nitrate or ammonium availability.

2. When a combination of both nitrate and ammonium were given the plants reacted to a reduction in ammonium by increasing pigment deposition in the roots.

3. Urea, and both amino acids asparagine and glutamine, were measurably taken up and stored by the plants, although any effect of their concentration on relative pigment concentration is not demonstrable (SCHLÜTER 1985).

Tab. 3: Variation in nitrogen concentration and carriers in the medium.
The enclosed values show the range of reasonable (= approximately natural) concentrations.

Substance ($\mu g\ N \cdot l^{-1}$)						
NO_3^-	NH_4^+	$NH_4^+ + NO_3^-$		Urea	Asparagine	Glutamine
237300	–	–	–	–	–	–
23730	11816	–	–	11191	–	–
–	5908	5908	11816	–	–	–
2373	1181,6	1181,6	21357	1119,1	2321	2415
–	590,8	590,8	22544	559,55	–	–
237,3	118,16	118,6	23493,7	111,91	–	–
–	–	59,08	23611,4	55,95	–	–
23,73	11,81	11,81	23706,3	11,19	–	–

2. The effect of dissolved gases on pigment production

E. crassipes often grows in waters which are not oxygen saturated where the water sometimes smells of hydrogen sulphide. For this reason we varied oxygen partial pressure (Fig. 2) in the nutrient (by bubbling nitrogen) or rendered the water oxygen-free (by bubbling hydrogen sulphide). Fig. 2 shows that varying the partial pressure between 2.4 and 6.2 mg oxygen/l by nitrogen bubbling produced no significant difference in the relative pigment concentration; similarly bubbling hydrogen sulphide (individual data not given) up to 4 mg hydrogen sulphide/l medium, had no effect. A slight effect only was shown as a result of oxygen reduction through hydrogen sulphide addition: biomass production and root colour intensity were reduced. Pigment synthesis in Amazonian water hyacinth roots is dependant on the pH of the medium to a certain extent: however this is unimportant as far as evaluation of the studies described here are concerned since the pH of the nutrient medium remained, or was held, constant (pH 5.5). It remained indisputable that oxygen content, within the cited limits (0 - 6.2 mg/l oxygen, 75 % saturation at 27 °C), had no influence on relative pigment levels in the roots.

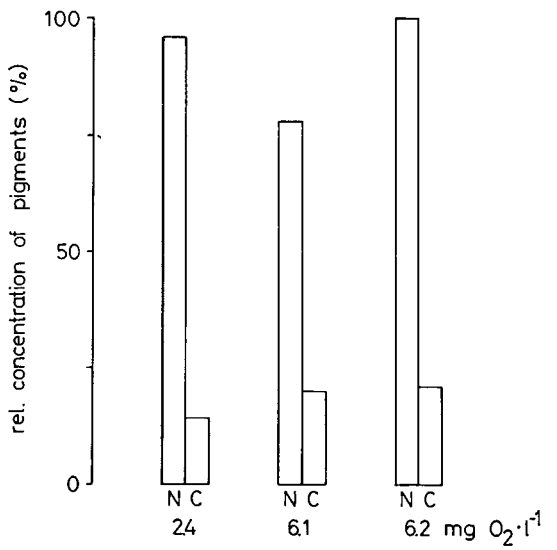

Fig. 2:
Influence of oxygen partial pressure in the medium on pigment development in the roots.
C = control, N = no nitrogen.

3. The influence of light on pigment development

Light is an important factor for normal plant growth. In the previously described growth cabinet studies a quantum flux (PAR) of 110 µmol x m^{-2} x sec^{-1} at leaf level and about 10 µmol x m^{-2} x sec^{-1} in root regions was achieved and maintained. In later studies only the roots of the floating plants were darkened at the water surface. Thus a reduction in the quantum flux in the root region (= in the medium in the containers below the floating plants) to 0.5 µmol x m^{-2} x sec^{-1} was achieved without influencing the other plant organs. The effect of light and dark under different nitrogen concentrations is shown in Fig. 3: these investigations and others, SCHLÜTER (1985) show that light is necessary for the induction of pigment synthesis and deposition. The induction of pigment synthesis by light, together with other features, reveals that anthocyanin or anthocyanin – like pigments are involved (B. FURCH & ZIMMERMANN 1983; MATTERN & B. FURCH 1987).

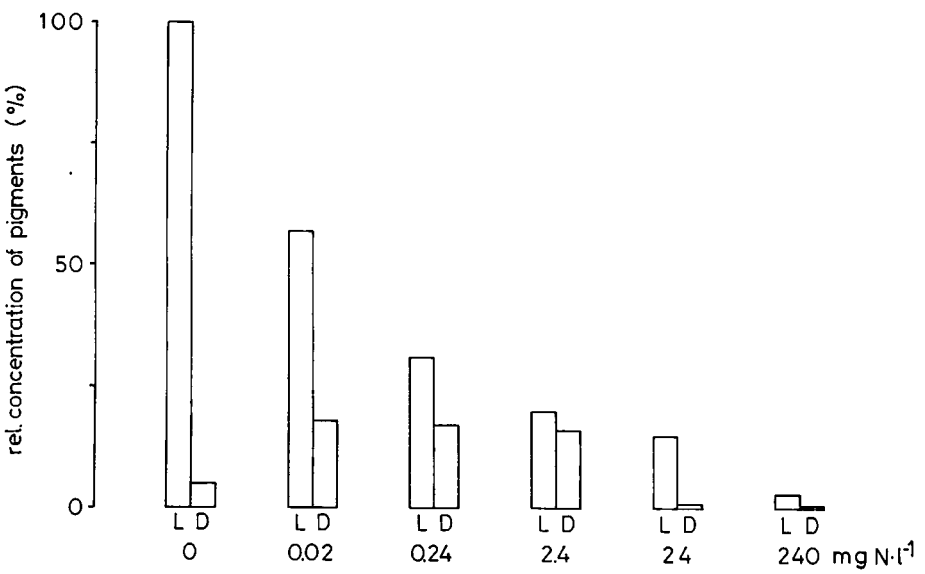

Fig. 3:
Influence of light (L = 10 µmol photons x m^{-2} x sec^{-1}) and dark (D = less than 0,5 µmol photons x m^{-2} x sec^{-1}) combined with different nitrogen concentrations (N as nitrate) on relative pigment development in the roots.

It is clear from our studies under defined conditions that the Neotropical biotype of *E. crassipes* can indicate nitrogen deficiency, as long as nitrate or ammonium are the nitrogen sources involved and the root region receives adequate illumination. With decreasing nitrogen levels in the medium, the visible and photometrically demonstrable relative pigment concentration increases. Whether this conclusion can be simply extrapolated throughout its usual occurrence range is at present unknown.

Zusammenfassung

Der in Zentralamazonien häufige, neotropische Biotyp von *Eichhornia crassipes*, eine Pontederiacee, zeigt eine Besonderheit, die sonst bei höheren Pflanzen selten ist: Die Wände ihrer Wurzeln sind gefärbt. Die Farbgebung ist unterschiedlich und die Skala reicht von weißlich über violett zu blau, braun und blauschwarz. Besonders eine Blaufärbung ist häufig in den Gewässern um Manaus/Amazonas anzutreffen. Die vorliegenden Untersuchungen zeigen die Abhängigkeit der Blaufärbung von Umwelt-Parametern auf:

1. Die relative Pigmentkonzentration in den Wurzeln (= der Umfang der extrahierbaren und photometrisch bestimmbaren Pigmente) nimmt zu mit fallendem Angebot von NO_3-N oder NH_4-N.
2. Werden beide Stoffe kombiniert gegeben, erweist sich besonders ein Ammoniummangel als Auslöser einer Pigmentsynthese.
3. Harnstoff, sowie Glutamin und Asparagin werden von den Pflanzen zwar aufgenommen, haben aber keinen signifikanten Einfluß auf die Farbigkeit der Wurzeln.
4. Licht, d. h. mehr als 0,5 μmol Photonen x m^{-2} x s^{-1} (scalar) im Wurzelraum der Eichhornien sind nötig, um die Pigmentbildung auszulösen.

Da einerseits Harnstoff, Glutamin und Asparagin den Pflanzen am natürlichen Standort selten und Nitrate und Ammoniumverbindungen andererseits häufig als N-Quelle zur Verfügung stehen dürften, kann man *Eichhornia crassipes* als Stickstoff-Mangel-Anzeiger bezeichnen.

Summary

E. crassipes, a member of the Pontederiaceae which is common in central Amazonia, exhibits a peculiarity for higher plants: the walls of the roots are coloured. The pigmentation is variable, ranging from whitish through violet to blue, brown and blue-black. Blue coloration is frequently encountered in water near Manaus/Amazonia.

These studies show the dependancy of the blue colour on environmental factors.

1. The relative root pigment concentration (= the extractable and photometrically determined pigments) increase with decreasing nitrate or ammonium nitrogen.
2. In the presence of both compounds, ammonium deficiency is the particular trigger for pigment synthesis.
3. Urea, glutamine and asparagine are taken up by the plants, but do not have a significant effect on root pigmentation.
4. Light, above 0,5 μmol photons x m^{-2} x sec^{-1} (scalar) in the root region of the *Eichhornia* plants is necessary for pigment development.

Since urea, glutamine and asparagine are rarely available to plants in their natural habitats, while nitrate and ammonium compounds are common as nitrogen sources, *E. crassipes* can be considered an indicator of nitrogen deficiency.

Resumo

O biótipo neotrópico de *Eichhornia crassipes*, uma Pontederiácea, freqüente na Amazônia Central, mostra uma peculiaridade aliás rara em plantas superiores: as paredes das raízes desta planta são coloridas. A tonalidade da coloração é diversa, e a escala das cores varia de esbranquicado via violáceo até azul, marron ou preto azulado. Especialmente a coloração azul encontra-se freqüentemente nos corpos d'água da redondeza de Manaus. Os presentes estudos demonstram a dependência da coloração azul de parâmetros do meio ambiente:

1. A concentração relativa de pigmentos nas raízes = a extensão dos pigmentos extraíveis e fotometricamente determináveis aumenta com a diminuição da oferta de NO_3-N ou NH_4-N.

2. Com a oferta combinada de ambas estas substâncias revela-se, é especialmente a carência de amônio como desencadeador de uma síntese de pigmento.
3. Uréia, como também glutamina e asparagina, são absorvidas pelas plantas, porém não têm nenhuma influência significativa sobre a coloração das raízes.
4. Luz, i. e. mais do que 0.5 μmol fótons x m^{-2} x s^{-1} (escalar) no espaço radicular das Eichhornias são necessários para desencadear a formação de pigmentos.

Uma vez que uréia, glutamina e asparagina somente raras vezes encontram-se disponíveis as plantas no habitat natural enquanto que nitrato e compostos de amônio se acham freqüentemente à disposição como fontes de N, *Eichhornia crassipes* pode ser apontada como indicador de carência de nitrogênio.

Acknowledgments

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