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The role of petioles in light acquisition by *Hydrocotyle vulgaris* L. in a vertical light gradient

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Abstract In natural herbaceous vegetation plants are exposed to a vertical light gradient. In experiments, however, morphogenetic responses of stoloniferous plants to shade have nearly always been tested under homogeneous shade conditions. In this study we simulated a vertical light gradient and found that the response of *Hydrocotyle vulgaris* in this gradient differed considerably from the responses to homogeneous shade. Petioles grew longer while at the same time the specific weight of petioles increased. The elongated petioles raised leaf-blades into better-lit places resulting in higher biomass. Though leaves in the light gradient started their growth under low-light conditions, the size of the leaf-blade was the same as in high light. Internodes were longer than in homogeneous shade conditions but specific weight decreased, probably due to increased allocation to the fast-growing petioles.

Keywords Vertical light gradient · Morphological plasticity · Clonal growth · Petiole · Internode

Introduction

Until recently responses of stoloniferous plants to shade have been tested in shade cages casting homogeneous shade (Solangaraachchi and Harper 1987; Methy et al. 1990; Evans 1992; Huber 1996). This differs from the natural situation and constrains the growing conditions of the plants in a different way. In homogeneous shade PAR (photosynthetically active radiation) and R:Fr (red: far-red ratio) are low, resembling the light environment low in the vegetation. This creates conditions that will induce morphogenetic responses such as petiole elongation (Solangaraachchi and Harper 1987; Huber 1996), but in

homogeneous shade the plant can never succeed in positioning the leaf-blades in better-lit environments.

In natural stands of vegetation the amount of light decreases gradually due to reflection and absorption of light by the plants (Fliervoet 1984; Hirose and Werger 1995). Here, elongating petioles will raise leaf blades along a vertical gradient (Ballaré 1994). The exposure of leaf-blades to higher light levels may increase light harvesting and increase carbohydrate production. Since petioles have to bear the weight of the leaves, petiole elongation may not occur by etiolation alone and additional biomass investment may be necessary. Biomass investment in petioles of plants growing in homogeneous shade only involves extra costs to the plant, while in a vertical gradient positioning of the leaf blades in better-lit places might compensate for the additional biomass needed for petiole growth.

Just as plasticity in petiole length allows the plant to position its leaf blades in better-lit places, elongation of internodes may be profitable in foraging for better-lit patches. In an inventory of foraging responses in clonal plants, De Kroon and Hutchings (1995) found plasticity in internode length to be around 30%. Based on a model study (Sutherland and Stillman 1988) they concluded that such plasticity was insufficient to result in a significant accumulation of ramets in “good” patches. Again, the experiments cited contrasted plant responses in homogeneous shade and full daylight. Internode plasticity might actually be greater if plants can exploit a vertical light gradient and have a higher carbon gain, which might increase their potential to place ramets in gaps selectively.

The other parameter that is considered to influence ramet accumulation in good patches is branching (Sutherland and Stillman 1988; Hutchings and De Kroon 1994). Low branching frequency of ramets in shade might, however, be a consequence of a low growth rate (Huber and Stuefer 1997) rather than inhibition of the outgrowth of axillary buds by low R:Fr ratio (Robin et al. 1994; Lötscher and Nösberger 1997; but see Thompson 1993).

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In this study we grew *Hydrocotyle vulgaris*, a stoloniferous plant with petiolate leaves, in a vertical light gradient and compared this with growth in homogeneous high-light and shade treatments. We hypothesise that:

1. *Hydrocotyle* is able to exploit the vertical light gradient by elongating its petioles and positioning its leaves in better-lit places.
2. Elongation of petioles requires the petioles to be stronger, as reflected in a higher mass per unit length. Plants in the vertical light gradient will be better able to construct heavier petioles than plants in the full shade treatment.
3. Response to the vertical light gradient will not only increase plasticity in vertical spacers (petioles) of the plant but will also increase plasticity in the horizontal spacers (internodes) in comparison to plants under homogeneous shade.
4. Due to a higher growth rate, branching frequency will be higher in the vertical light gradient than in homogeneous shade.

Materials and methods

The experiment was performed with *H. vulgaris* L., a species with plagiotropic stems that can grow either below- or above-ground. The latter was the case in this experiment and therefore they will be referred to as stolons. Ramets of *Hydrocotyle* only have one petiolate leaf and can potentially form a flower head and a branch (Dong 1995). At the beginning of August 1996 apical parts with two small ramets were planted in a $1.00 \times 0.17 \times 0.14$ m tray. Cuttings were derived from three parent plants that originated from different sites and were considered to be different genotypes. Every genotype was replicated three times. Trays were filled with a mixture of sieved potting compost and sand (1:1). Slow-release fertilizer (Osmocote Plus, Grace Sierra International, Heerlen, The Netherlands) was added at a rate of $10 \text{ kg N ha}^{-1} \text{ week}^{-1}$. The experiment was performed in a plastic greenhouse with open sides. Plants were subjected to three light treatments:

1. Homogeneous high light (H) which consisted of a cage covered with transparent plastic (PAR = 80%; R:Fr = 1.1)
2. A vertical light gradient (G; Fig. 1) which was established by hanging double layers of sheets of green filter (LEE Colortran International, Andover, UK) (no. 122, fern green), vertically from mesh wires fastened at a height of 0.20 m and 0.025 m apart; the edges were also covered with double layers (PAR = 15%; R:Fr = 0.5)
3. Homogeneous low light (L) which was created in a cage covered with similar green filter and shade cloth (PAR = 15%; R:Fr = 0.3)

Plants were grouped per treatment: environmental conditions in the greenhouse, except for the experimentally

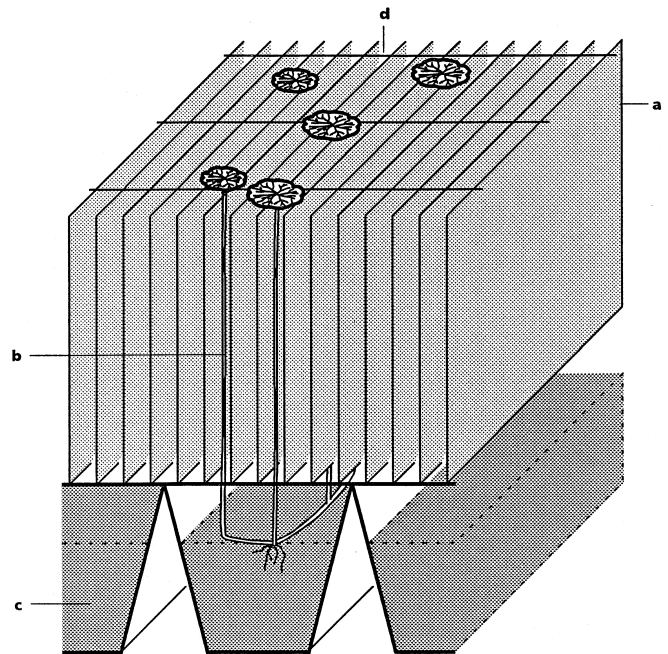


Fig. 1 The vertical light gradient treatment. Only one tray of *Hydrocotyle* is shown and the side covering is left out of the figure (a sheets of green filter hanging from d mesh wire, b *Hydrocotyle*, c trays)

manipulated light conditions, did not differ between treatments. Light measurements (PAR and R:Fr) were made with probes similar to the ones used and described by Pons and van der Toorn (1988) and Huber and Wiggerman (1997). On overcast days light intensity at ground level was the same for treatments G and L. R:Fr differed between treatments, but it was not possible to change R:Fr without changing PAR. However, on sunny days direct radiation passing through several layers of filter in treatment G resulted in a lower light intensity and R:Fr ratio at ground level than in treatment L. During the course of the experiment weather conditions were predominantly overcast and radiation in the greenhouse was therefore diffuse.

Plants were harvested after 5 weeks. Internode length, petiole length, leaf area and dry mass of these structures were measured on three successive ramets (the fifth, sixth and seventh, measured from the apex) which had been formed on the primary stolon during the experiment. Treatment effects for these parameters were tested with a two-way nested ANOVA with genotypes and treatments as factors and data for individual ramets nested under genotype.

Number of ramets and number of branches on the main stolon were counted and dry mass of stolon, petioles and leaf blades was measured after drying plants in an oven for 72 h at 70°C . Differences in total biomass, allocation, and branching frequencies between treatments were tested in a two-way ANOVA. Biomass data were log transformed, while percentages biomass and branching frequencies were arcsine transformed (GLM procedure in SAS; SAS 1988).

Results

Hydrocotyle grown under a vertical light gradient had longer petioles and internodes than in homogeneous shade. Specific mass of petioles was higher, while specific mass of internodes was lower than under both homogeneous light conditions (Fig. 2).

Leaf sizes were the same under high-light and gradient conditions while they were much smaller under homogeneous low-light conditions. Specific leaf mass did not differ between treatments (Table 1).

Above-ground plant mass in *Hydrocotyle* was higher in the vertical light gradient than in the homogeneous shade treatment but this did not translate into differences in allocation pattern between plants in the two treatments (Table 1). Plants growing in the light gradient invested less biomass in stolon and leaf-blades while more was allocated to petioles than in the high-light treatment. Ramet mass was higher in the light gradient than in the high-light treatment because of the long petioles, but the difference was not significant (Table 1).

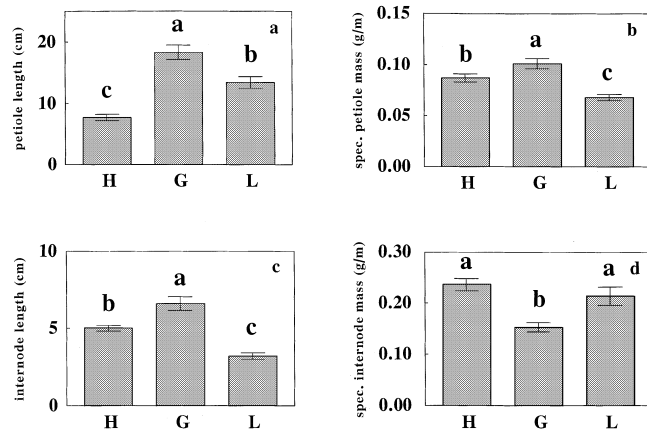


Fig. 2 a Petiole length, b specific petiole mass, c internode length and d specific internode mass in *Hydrocotyle*. Error bars give ± 1 SE. Different letters indicate significant differences between treatments at $P < 0.05$

Table 1 Mean values for biomass parameters, branching frequencies and leaf size. Branching frequency is calculated as the number of branches on the main stolon divided by the number of leaves on the main stolon. Mass ratios are calculated by dividing

	H	G	L
Above-ground plant mass (g)	7.075 \pm 1.602 (a)	1.353 \pm 0.333 (b)	0.315 \pm 0.046 (c)
Stolon mass ratio	0.310 \pm 0.015 (a)	0.234 \pm 0.018 (b)	0.261 \pm 0.016(ab)
Petiole mass ratio	0.213 \pm 0.008 (b)	0.375 \pm 0.103 (a)	0.345 \pm 0.091 (a)
Leaf blade mass ratio	0.476 \pm 0.015 (a)	0.390 \pm 0.019 (b)	0.391 \pm 0.018 (b)
Inflorescence mass ratio	0.001 \pm 0.000	0.001 \pm 0.001	0.002 \pm 0.002
Ramet mass (g)	0.041 \pm 0.004(ab)	0.051 \pm 0.006 (a)	0.026 \pm 0.003 (b)
No. of ramets primary stolon	16 \pm 0.7 (a)	13 \pm 0.7 (b)	11 \pm 0.8 (b)
No. of unbranched apical ramets	2 \pm 0.4 (a)	4 \pm 0.6 (b)	5 \pm 0.3 (b)
Branching frequency (%)	83 \pm 13 (a)	55 \pm 13 (b)	37 \pm 14 (b)
Lamina			
Leaf size (cm ²)	10.12 \pm 0.81 (a)	8.95 \pm 0.93 (a)	5.13 \pm 0.50 (b)
Specific leaf mass (g/m ²)	23.83 \pm 1.81	24.24 \pm 1.37	20.30 \pm 0.88

Branching frequencies were significantly lower in the shade treatments than in the high-light treatment; while branching was higher in the vertical light gradient than in the homogeneous shade treatment the difference was not significant (Table 1). All three genotypes exhibited the same responses for all parameters measured.

Discussion

As in other experiments, petioles were found to be longer under shaded conditions than in high light (Sol-angaarachchi and Harper 1987; Thompson and Harper 1988; Methy et al. 1990; Evans 1992; Thompson 1993a,b; Huber 1996; Price and Hutchings 1996). Petioles in the vertical light gradient grew even longer than those under homogeneous shade. The elongation response is thought to be regulated via blue (B) light and R:Fr ratio. Because sites of perception for B and Fr seem to be in the leaf blade, petiole elongation will be inhibited once leaf blades are exposed to high light (Vince-Prue et al. 1976; Casal and Smith 1988; Lötscher and Nösberger 1997). This was the case in the vertical gradient, where petioles stopped elongating as soon as they reached the top of the 0.2-m-high sheets. If the sheets in our experiment had been higher, elongation might not have stopped until leaves reached high light. Field observations showed that petioles of *Hydrocotyle* can be as long as 0.4 m (Dong 1995), twice as long as found in our vertical gradient. Petioles of *Trifolium fragiferum*, however, did not reach the top of a natural vegetation stand; elongation of petioles stopped when leaf blades reached an average light level of 30% (Huber and Wiggerman 1997). It is not clear what inhibited petiole elongation in the homogeneous shade treatment. The impossibility of reaching better-lit places under homogeneous shade conditions and the consequent limitation of carbohydrates might have made it impossible for the plant to further elongate their petioles.

As was reasoned in our second hypothesis the specific mass of petioles in the light gradient was higher than that in high-light conditions. This was also found for

the mass of the respective structures by total above ground plant mass. Average ramet mass is measured from three individual ramets on the primary stolon. Different letters in parentheses indicate significant differences at $P < 0.05$

Trifolium fragiferum in a natural situation (Huber and Wiggerman 1997). Raising leaves along the gradient to better-lit places resulted in a higher biomass than in the homogeneous shade. However, biomass production in high-light conditions was more than 5 times higher than in the vertical light gradient. This might be due to the costs associated with the longer petioles and the time lag between leaves in the vertical light gradient and high-light treatment reaching the same productivity levels.

Although internode length of *Hydrocotyle* was longer in the vertical light gradient than in high light, instead of shorter as was the case in the homogeneous shade treatment, plasticity was not higher than that found in other studies (see De Kroon and Hutchings 1995; Evans and Cain 1995). This leads to rejection of our third hypothesis. Our results contrast with the higher plasticity in internodes found in some natural patchy vegetations (Thompson 1993b; Waite 1994; Huber and Wiggerman 1997). While internodes grew longer in the vertical light gradient than under homogenous shade, their specific mass was considerably less. This is possibly explained by the rapidly growing petioles in the vertical gradient being strong sinks that reduced allocation to the stolon (Ballaré et al. 1991) and resulted in lower specific mass of internodes in the vertical light gradient than in either homogeneous light condition.

Our hypothesis that there would be increased branching with a faster growth rate was only partially confirmed. Plants in high-light formed more ramets on the primary stolon and had significantly higher branching frequency, and apical dominance was lower than in the shade treatments. No significant differences were found, however, between the two shade treatments. In a comparable experiment, branching in *Trifolium repens* was not influenced by selective shading of the stolon, but in this experiment petioles could reach high light by only a little elongation, resulting in a biomass that was even higher than in high light (Lötscher and Nösberger 1997).

In conclusion, exposing *Hydrocotyle* to a vertical light gradient instead of homogeneous shade resulted in a strongly different response to shading. In the vertical light gradient elongating petioles raised leaves to higher light levels resulting in increased biomass production than in homogeneous shade. Internodes grew longer than in homogenous shade but specific weight was lower.

Valid experimental testing of responses of stoloniferous plants to the effects of surrounding vegetation thus requires the creation of a vertical light gradient. Comparison of responses in high light and homogeneous shading provides only a poor comparison with natural growth conditions.

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