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Effect of photoperiod and nitrogen supply on basal shoots development in *Rhododendron catawbiense*

J. FUSTEC* and F. BEAUJARD**

*Faculté des Sciences, UMR SAGAH, 2 Boulevard Lavoisier, F-49045 Angers Cedex, France**
*INRA, UMR SAGAH, 42 rue Georges Morel, BP 57, F-49071 Beaucouzé Cedex, France***

Abstract

In order to control young plant form by modifying culture conditions, plants of *Rhododendron catawbiense* from *in vitro* culture were grown in a greenhouse under different photoperiodic treatments (long or short days) combined or not with a several-week nitrogen starvation. After 12 weeks of culture under long days (16 h) with nitrogen supply, plants showed a rhythmic acrotonous development. When long days were combined with a six-week nitrogen starvation, the apical growth pause was extended leading to an increase of the number of acrotonous lateral ramifications. Short-day (8 h) treatment affected distal burst potential and moreover when a concomitant nitrogen starvation was applied. This lack of distal development allowed basal buds swelling, leading to basitonous plants. When plants were returned back to long days after 2, 4 or 6 weeks under short days, distal buds resumption competed with basal shoots development. Durable basitonous plants were obtained by a 12-week short days treatment combined with a 6-week nitrogen starvation.

Additional key words: day length, morphogenesis, nitrogen starvation, woody plant.

Introduction

The influence of photoperiodism on floral induction, floral stem growth, and also on vegetative development has been studied in many herbaceous (Vince-Prue 1997, Jouve *et al.* 1998, Blažková *et al.* 2000) and woody species (Nitsch 1957, Longman 1991). Concerning morphogenesis of temperate woody plants, experiments and observations have often been carried out using deciduous species (Crabbé 1981, Champagnat 1989, Barnola and Crabbé 1991).

Rhododendron catawbiense (*Ericaceae*), is a temperate evergreen woody plant. Following a rhythmic endogenous development, the vegetative system architecture depends on growth units location (Beaujard *et al.* 1993). Development of ramifications in one-year *R. catawbiense* grown in a greenhouse can be affected by day length and mineral supply. Long days (LD) with complete nutrient solution are favourable to rhythmic acrotonous flushes sprouting up. In contrast, short-day treatments (SD) combined with a several-week nitrogen starvation allow basal shoots development (Beaujard *et al.* 1993, Fustec *et al.* 2000).

The present study was carried out toward *1*) an early control of vegetative development of *R. catawbiense* by modifying culture conditions, *2*) a better understanding of the effects of day length and nitrogen supply on burst potential of buds and the subsequent plant shape.

Materials and methods

In February, plants of *Rhododendron catawbiense* Michaux. cv. Album made up of a single axis, were obtained from head-cuttings of *in vitro* propagated plants transferred *ex vitro* (Fustec *et al.* 2000). In May, plants were pricked out in 600 cm^3 plastic containers filled with moist perlite (covered with waterproofed flint), and placed on culture tables in a greenhouse (temperature 21 ± 2 °C, relative humidity > 80 %). They were maintained under 16-h photoperiod (long days - LD, irradiance 800 μmol m^{-2} s^{-1}, with natural day length

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*Corresponding author; fax: (+33 2) 41735352, e-mail: joelle.fustec@univ-angers.fr

extended by using low pressure Na vapour lamps) and received a complete mineral solution by sub-irrigation 30 min per 12 h: (macroelements in mmol dm^{-3}) 10 NO_3, 4.82 P, 8 S, 1 Cl, 4 K, 2 Na, 10 Ca, 6 Mg, 3 NH_4, (microelements in µmol dm^{-3}) 82.4 B, 1.88 Cu, 43 EDTA-Fe, 43.6 Mn, 1.12 Mo, 12.96 Zn, pH 6.5. When they have reached the second growth pause, 112 plants were divided in 14 groups ($n = 8$). In order to modify the form of the plants, a different treatment was applied to each group:

1) L treatments: Plants were maintained under LD for 12 weeks. In L0, they received the complete nutrient solution during all the experiment. In L2-, L4- and L6- plants received a nutrient solution without nitrogen for two, four and six weeks: (macroelements in mmol dm^{-3}) 4.82 P, 6.5 S, 3.75 Cl, 3 K, 7.5 Ca, 4.5 Mg (same microelements and sub-irrigation frequency).

2) S treatments: Four groups of plants were placed under 8-h photoperiod (short days - SD) for 12 weeks. In S0 treatment, plants received the complete nutrient solution during all the experiment. In S2-, S4- and S6-, just after transfer to SD, nitrogen supply was interrupted for two, four and six weeks.

3) SL treatments: Six groups of eight plants were transferred to SD for two, four or six weeks, before returning back to LD. In SL2+, SL4+ and SL6+ treatments, plants continuously received the complete nutrient solution. In SL2-, SL4- and SL6-, nitrogen supply was interrupted concomitantly to SD period.

Buds were regularly observed. After 12 weeks, acrotonous and basal shoots formed during the treatments were counted and measured. Non parametric Mann-Whitney test was used for data treatment (*Instat* software).

Results

Plant form: In L0 and L2- treatments, about six or seven plants followed a monopodial development but only one or two in L6- and L4- groups (Fig. 1). Thus, a four or six-week nitrogen suppression was favourable to a sympodial development with two or three acrotonous lateral ramifications. Under LD, very few basal shoots were produced (L4-).

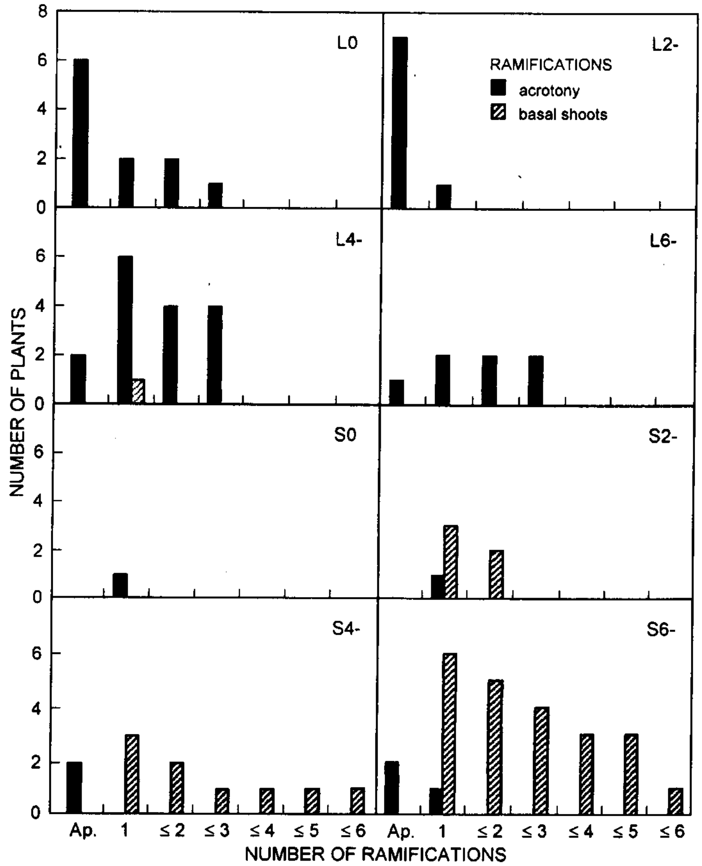

Fig. 1. Number of ramifications developed in plants grown under LD or SD without nitrogen starvation (*L0*, *S0*), under 2-week nitrogen starvation (*L2-*, *S2-*), 4-week nitrogen starvation (*L4-*, *S4-*), or 6-week nitrogen starvation (*L6-*, *S6-*); Ap.- developed apical shoot; 1, ≤ 2, ≤ 3… - maximum number of lateral axes; 8 plants per treatment.

In plants grown for 12 weeks under SD (Fig. 1), acrotonous development was limited and the apical bud burst only in two plants of S4- and S6- treatments. In S0 group, only one distal bud burst was observed and no basal shoot developed. When nitrogen was removed for several weeks, the number of basal shoots produced by the plants increased markedly with the length of nitrogen starvation. In S4- and S6- treatments, plants could bear until six basal shoots.

A 2 or 4-week SD treatment (SL2+, SL4+; Fig. 2) allowed the development of about two basal shoots in five to seven plants. However, the return to LD led to the growth resumption of three or four acrotonous buds in all plants. Apical bud burst occurred only in SL2 treatments. In SL2 and SL4 treatments, nitrogen starvation seemed not to affect significantly the morphogenesis of plants in comparison with day length: SL2+ and SL4+ were respectively similar to SL2- and SL4-. More differences existed between SL6+ and SL6-. Less than four SL6+ plants produced acrotonous and/or basitonous shoots while SL6- allowed basal shoots development in seven plants. After return to LD, acrotonous resumption was limited to few plants compared with SL2 and SL4 treatments.

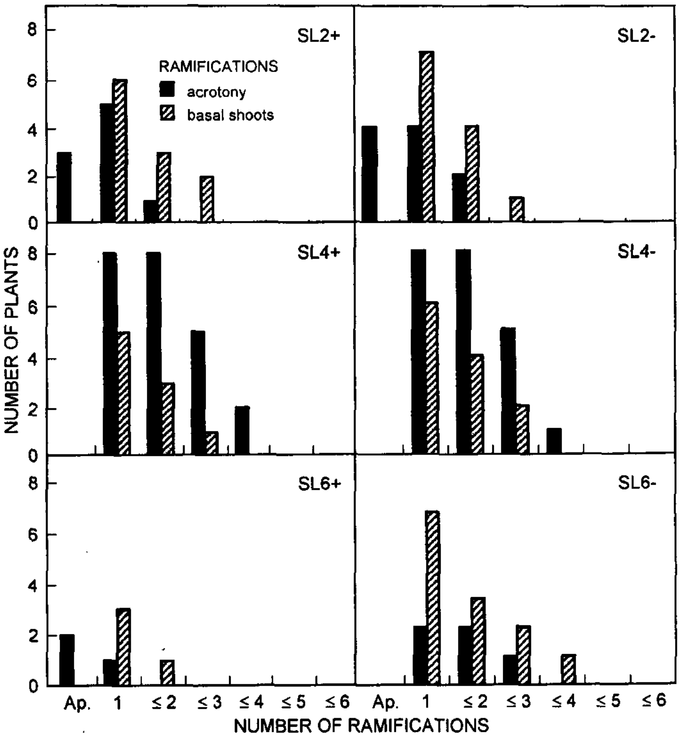

Fig. 2. Number of ramifications developed in plants grown under LD after been placed under SD for two weeks (*SL2*), four weeks (*SL4*), or six weeks (*SL6*), without nitrogen starvation (+) or with nitrogen starvation (-) during SD phase; Ap. - developed apical shoot; 1, ≤ 2, ≤ 3... - maximum number of lateral axes; 8 plants per treatment.

Apical bud-burst delay: In L0 (monopodial plants), apical resting pauses lasted about 17 d (Table 1). Six-week nitrogen starvation increased significantly distal bud-burst delay. In SL plants, no apical bud-burst was observed. When they were returned to LD, apical bud burst occurred after 23 to 25 d. Considering plants grown under SD apical bud-burst delay was always longer than 40 d.

Acrotonous growth units morphology: Acrotonous units grown in L0 conditions showed 5.7 cm axes, bearing about seven leaves in their distal part (Table 1). When nitrogen was temporary removed, axes length tended to increase (L0 was significantly different from L4- and L6-). In plants temporary placed under SD, growth units length was markedly longer in SL6 than in other SL treatments (Table 1). Nitrogen starvation did not modify growth units morphology except in SL6- plants in which they bore about 10 leaves (Table 1).

Axes grown under SD were smaller than those developed under LD (< 4 cm; Table 1). These values tended to decrease when the length of nitrogen starvation was extended (< 1.5 cm in S6-) and the leaf number dropped in the same way (one or two leaves in S6-).

Basal shoots morphology: Basal shoots began to grow about 8 weeks after transfer to SD. In SL6-, S4- and S6- treatments, plants produced longer axes than in other culture conditions and were bearing more leaves (six to seven) than in SL2, SL4 and SL6+ (Table 1).

Table 1. Bud-burst delay, axis length and leaf number of acrotonous and basal shoots after 12 weeks culture. Means ± SD; $n = 8$; * - $P < 0.05$; ** - $P < 0.01$; means differ significantly when followed by different letters; (t) - after return to LD.

Treatment	Acrotonous growth units bud-burst delay [d]	leaf number	axis length [cm]	Basal shoots leaf number	axis length [cm]
L0	16.4 ± 1.4 a	6.9 ± 1.3 a	5.7 ± 0.8 a	-	-
L2-	16.8 ± 1.8 a	7.1 ± 0.8 a	5.5 ± 1.4 ab	-	-
L4-	17.3 ± 0.6 a	7.1 ± 1.5 a	6.7 ± 1.1 bc	-	-
L6-	19.5 ± 1.6 b	7.1 ± 0.5 a	7.2 ± 1.9 c	-	-
SL2+	23.5 ± 3.2 b (t)	6.4 ± 1.5 a	7.9 ± 1.2 cd	4.6 ± 1.6 a	5.0 ± 1.2 a
SL2-	23.5 ± 3.2 b (t)	5.8 ± 0.9 b	7.1 ± 2.3 c	3.4 ± 1.8 ab	4.3 ± 1.2 abc
SL4+	23.0 ± 0.6 b (t)	5.2 ± 0.6 b	8.7 ± 1.6 de	-	-
SL4-	23.0 ± 0.6 b (t)	5.8 ± 0.7 b	7.0 ± 1.2 ce	3.0 ± 2.3 ab	3.3 ± 1.5 b
SL6+	25.4 ± 1.9 b (t)	7.2 ± 1.9 a	10.4 ± 0.9 f	1.9 ± 0.7 b	3.4 ± 1.5 bc
SL6-	25.4 ± 1.9 b (t)	10.1 ± 0.4 c	10.1 ± 0.4 f	6.2 ± 1.2 c	6.7 ± 1.7 de
S0	> 40	3.1 ± 1.3 d	3.2 ± 1.7 g	-	-
S2-	> 40	4.2 ± 1.9 d	2.3 ± 1.7 g	-	-
S4-	> 40	4.0 ± 2.2 d	1.3 ± 1.2 h	6.9 ± 1.8 d	7.8 ± 2.0 ef
S6-	> 40	2.0 ± 1.3 e	0.8 ± 0.3 h	6.7 ± 2.0 cd	9.0 ± 2.3 f
	**	*	*	*	*

Discussion

In this experiment, both endogenous growth rhythm and day length modulated *R. catawbiense* development. Under LD, 20-d growth pauses were favourable to acrotony. Under SD, the extended distal resting period, comparable with a dormancy, may involve a decrease of apical dominance allowing organogenesis in basal buds. A similar influence of day length on apical dominance and morphogenesis has been described in other plants like in *Arabidopsis thaliana* (Jouve *et al.* 1998). On the other hand, this kind of relationships between distal and proximal buds have been studied in deciduous shrubs like *Corylus avellana* in relation to temperature (Crabbé 1981, Suzuki 1990, Barnola and Crabbé 1991). In *R. catawbiense* grown under SD, like in dormant buds of other woody species, that lack of apical burst can be linked to a loss of length growth ability due to a decreasing capacity to diversify non-adenylic nucleotides pool in the apical bud (Fustec *et al.* 2000). This poor shoot elongation capacity could explain the morphology of the shorter acrotonous growth units produced under SD.

When plants were transferred back under LD after several weeks under SD, two to four distal buds sprouted, competing with basal swollen buds development. The number of acrotonous lateral axes increased with the SD period length and thus, with the weakening apical dominance. However, after a 6-week SD phase, acrotonous growth resumption concerned only few plants, and the resultant units were longer than in other treatments, as if they showed etiolation signs. Such growth units competed more strongly with basal swollen buds than short ones produced under SD. In consequence of this distal growth resumption, basal shoots evolve toward thin plagiotropic axes bearing few leaves.

Shoot development of woody plants needs important quantities of nitrogen: a large part of this element comes from storage compounds quickly translocated to growing leaves competing or not with roots absorption (Nambiar and Fife 1991, Moing and Gaudillère 1992, Roubelakis-Angelakis and Kliwer 1992, Millard 1994). Under LD, starvation phase could decrease nitrogen amount supplying the apical bud and involve an extension of the burst delay favourable to the development of axillary buds. It has been shown in other woody species that under SD, nitrogen is used for synthesis of storage proteins and is not available for shoot growth (Arora *et al.* 1992, Coleman *et al.* 1993). A lack of nitrogen combined with SD treatment could be favourable to basal buds organogenesis while strongly preventing distal growth resumption. When nitrogen was supplied again, the swollen basal buds located near the roots could use it to evolve in vigorous orthotropic shoots that could become taller than the main axis in about six months. At this stage, the weakening main axis died and basitonous shape could be maintained after transfer to LD.

Results showed clearly the important sensibility of *R. catawbiense* to day length and the subsequent modifications in buds relationships leading to a large pattern of architectures from acrotonous to strictly basitonous individuals. Nitrogen starvation was not so efficient as day length on buds activity. Nevertheless, the combination of these two parameters allows a good control of plant shape and would be interesting to study physiological mechanisms responsible of growth pauses, dormancy and shoot elongation.

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