

## Growth and proliferation *in vitro* of *Vaccinium corymbosum* under different irradiance and radiation spectral composition

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### Abstract

Plantlets of highbush blueberry (*Vaccinium corymbosum*) cvs. Atlantic, Berkeley and Elizabeth, were exposed *in vitro* to radiation of different spectral compositions obtained by filtering the cool-white light with either 2 types of polymethylmethacrylate (PMMA) layers or glass and different photosynthetic photon flux density (PPFD, ranging from 10 to 180  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Red colour of leaves was the first response to the light treatments: after 14 d under unfiltered light, the shoots exposed to higher PPFD showed dramatic reddening of leaves and sprouts, especially in cv. Atlantic; cutting wavelengths shorter than 520 nm (no-B-PMMA filter) prevented those effects. On average, cv. Atlantic yielded the highest number of shoots per explant (10.4), followed by cv. Elizabeth (9.1) and cv. Berkeley (6.5). No-B-PMMA increased the proliferation rate in all the 3 genotypes, especially in cv. Atlantic. On the other hand, cutting wavelengths between 650 and 760 nm (no-R-PMMA filter) generally depressed the proliferation rate. No-B-PMMA induced remarkable changes in the morphology of the shoots - more elongate leaves and longer internodes - especially in cv. Atlantic.

*Additional key words:* highbush blueberry, light quality, micropropagation, photomorphogenesis.

### Introduction

Irradiance influences the activity of the axillary buds, and thus the proliferation of *in vitro* cultures, possibly by inducing variation in cytokinin-like substances

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**Abbreviations:** PMMA - polymethylmethacrylate; no-B-PMMA - filter cutting wavelengths shorter than 520 nm; no-R-PMMA - filter cutting wavelengths between 650 and 760 nm; PPFD - photosynthetic photon flux density;  $P_r$  - red-absorbing phytochrome;  $P_{fr}$  - far-red-absorbing phytochrome.

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(Villalobos *et al.* 1984, Baraldi *et al.* 1988); exogenous cytokinins added to the cultivation medium control the proliferation rate and are able to surrogate light-mediated responses (Lercari *et al.* 1986).

Culture response to PPFD has been reported also in *Vaccinium*. In rabbiteye blueberry (*Vaccinium ashei*), shoot elongation varied depending on the photoperiod (Nickerson 1978) and PPFD between 2 500 and 4 000 lux (about 30 and 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) determined a regular formation of secondary shoots (Young and Cameron 1985). In highbush blueberry (*Vaccinium corymbosum*) cv. Bluetta, variations in PPFD influenced shoot proliferation and growth which were highest at 55  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and decreased at higher PPFD (Noë and Eccher 1994).

However, the photomorphogenic responses are not related only to PPFD and duration of exposure (photoperiod) but also to spectral composition (Economou and Read 1987). In tobacco, *e.g.*, Weiss and Jaffe (1969) observed that blue radiation enhances proliferation while red radiation (660 nm) has no effect; Seibert *et al.* (1975) found that the most effective wavelength was 467 nm, followed by the purple radiation (419 nm) although in some cases the axillary bud formation occurred only in contemporary presence of blue and red radiation.

The aim of the research was to investigate 1) the effects of light of various spectral composition and different PPFD during *in vitro* cultivation on highbush blueberry growth and morphology, and 2) the interactions between light treatments and highbush blueberry genotypes.

## Materials and methods

**Plants:** Shoots of *Vaccinium corymbosum* L. cvs. Atlantic, Berkeley and Elizabeth, were grown *in vitro* in plastic boxes on PMN medium with the addition of 7.5 mg dm<sup>-3</sup> N<sup>6</sup>(2-isopentenyl)adenine (2iP) and subcultured every 45 d (Eccher *et al.* 1986, Eccher and Noë 1989). Growth chamber temperature was 24  $\pm$  1 °C, PPFD of 55  $\mu\text{mol m}^{-2} \text{s}^{-1}$  supplied by Philips TLD 36W/33 fluorescent tubes and photoperiod 16 h. When the shoots were 10 - 15 mm long (4 nodes on average) the plantlets were exposed to light - 4 different spectral compositions, 3 levels of PPFD - and after 14, 30 and 60 d the number and length of secondary shoots were recorded, and the colour (in a scale from 0 = green to 2 = red) and morphology of the leaves and sprouts were observed.

**Light treatments:** The cool-white light (spectral composition the same as described in Noë and Eccher 1994) was modified by filtering through glass (which strongly reduces UV-A and UV-B radiation) or through layers of PMMA (a plastic material produced by Elf-Atohaas and marketed as ALTUGLAS<sup>TM</sup>). Clear PMMA cuts the radiation of wavelength less than 360 nm. In the present experiments 2 types of coloured PMMA layers were used: 1) no-B-PMMA, capable of cutting wavelengths shorter than 520 nm (Fig. 1) in order to avoid UV-A, UV-B and blue radiation, where UV-B/blue photoreceptors are active, and 2) no-R-PMMA capable of cutting wavelengths shorter than 400 nm and between 650 and 760 nm (Fig. 1), eliminating

UV-A, UV-B, red and far-red radiation, where phytochrome is active with both the primary ( $P_r$  666 nm and  $P_{fr}$  730 nm) and the secondary (380 nm) peaks of absorbance. No-B-PMMA layers are commonly available and marketed as *ALTUGLAS*<sup>TM</sup> 100 - 15 000; no-R-PMMA layers have been specially produced for this research by the *Elf Atochem Italia* laboratories in Rho (Milan, Italy) using a selected mixture of pigments.

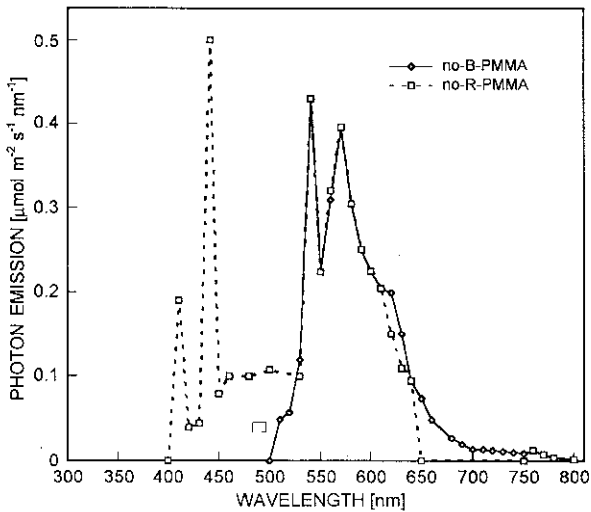


Fig. 1. Emission curves of cool-white light transmitted through the filters no-B-PMMA and no-R-PMMA.

The three levels of PPFD for each spectral composition were obtained by either increasing the number of lamps over the growth shelter or varying the distance to the plants. In strong light, the temperature in the tubes was controlled by dipping their bottom in circulating water at ambient temperature. Very high PPFD with various spectral composition were tested in order to investigate whether cutting of selected wavelengths may reduce photoinhibition.

The 16-h photoperiod was used. The emission curve was recorded with a *LICOR 1800* (Lincoln, USA) spectroradiometer. The PPFD was measured with a *LICOR 1100* quantum meter.

**Experimental design and statistical analysis:** The experimental design required 3 blocks per treatment, with 8 shoots per block, replicated 3 times, for a total of 864 shoots. The proliferation rate (number of shoots), the mean length of the secondary shoots and the leaf colour were statistically analysed by the analysis of variance, with cultivar, PPFD, spectral composition and their interactions as the treatment factors. In the tables, values in lines without a common letter are significantly different at  $P < 0.05$ , according to Duncan's Multiple Range Test.

## Results

Shoot proliferation and growth and leaf reddening were significantly influenced by spectral composition, PPFD, cultivar and their interactions according to Fisher's Test. After 60 d under cool-white unfiltered light (Table 1), cv. Elizabeth showed the highest proliferation rate and mean length of the shoots at PPFD of  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The total length of the shoots was more than double in cv. Elizabeth (108 mm) than in cv. Berkeley (50 mm) and also low was in cv. Atlantic (68 mm). On the other hand, at PPFD of  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$  cvs. Berkeley and Atlantic significantly increased the proliferation rate and their total shoot length was 89 and 90 mm, respectively, while it was only 41 mm in cv. Elizabeth. At  $125 \mu\text{mol m}^{-2} \text{s}^{-1}$  the total shoot length was 54.6 mm in Elizabeth, 37.1 mm in Atlantic, and 36 mm in Berkeley and very high reddening of leaves and sprouts appeared.

Table 1. Secondary shoot number, mean shoot length [mm], and leaf colour (0 = green, 2 = red) in *Vaccinium corymbosum* grown *in vitro* for 60 d under unfiltered white light at PPFD 40, 60 and  $125 \mu\text{mol m}^{-2} \text{s}^{-1}$  or under glass filter at PPFD 30, 75 and  $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Parameter	Cultivar	Unfiltered			Glass filter		
		40	60	125	30	75	180
Shoot number	Atlantic	8.5 a	9.0 a	5.3 b	6.4 b	13.7 a	8.5 b
	Berkeley	5.0 b	8.9 a	4.5 b	6.8 ab	9.4 a	5.5 b
	Elizabeth	13.5 a	8.2 b	9.1 b	8.1 a	9.0 a	7.3 a
Shoot length	Atlantic	8 ab	10 a	7 b	7 c	13 a	10 b
	Berkeley	10 a	10 a	8 a	8 b	12 a	10 b
	Elizabeth	8 a	5 b	6 b	6 a	6 a	6 a
Leaf colour	Atlantic	0.2 b	0 b	1.7 a	0.2 c	0.5 b	1.0 a
	Berkeley	0.1 b	0 b	0.9 a	0 b	0 b	0.4 a
	Elizabeth	0.2 c	0.3 b	1.1 a	0 b	0.5 b	1.4 a

Under the glass layer at PPFD of  $75 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 1), Atlantic showed high proliferation rate (13.7), mean secondary shoot length (13 mm) and total length (178.1 mm); also Berkeley performed best at the same PPFD. At the highest PPFD ( $180 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), Berkeley showed little leaf reddening and Atlantic was able to produce a high total shoot length (85 mm). At PPFD of  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ , formation and growth of the secondary shoots were the least.

No-R-PMMA layers resulted in a very low PPFD (Table 2). However, Atlantic and Berkeley showed a vigorous growth at  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$  with total shoot length of 169 and 93 mm, respectively. At PPFD of  $55 \mu\text{mol m}^{-2} \text{s}^{-1}$  Elizabeth had a high proliferation rate - but very short shoots. At any PPFD level, the shoots were normally coloured.

When light was transmitted through no-B-PMMA layer, the shoot growth was strongly promoted (Table 2). At PPFD of  $45 \mu\text{mol m}^{-2} \text{s}^{-1}$  the best total shoot length was obtained for the 3 cultivars (337.6 mm for Atlantic, 193.5 mm for Berkeley and

142.2 mm for Elizabeth) as well as the best proliferation rate and mean shoot length. Atlantic benefited particularly from this light spectral composition with exceptionally high proliferation rates and good mean shoot length also at very low ( $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and high ( $115 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) PPFD (Fig. 2). No-B-PPMA prevented leaf reddening even at high PPFD. In Atlantic, it also caused remarkable changes in the morphology of the shoots (more elongate leaves, longer internodes), and an exceptional number of secondary shoots.

Table 2. Secondary shoot number, mean shoot length [mm], and leaf colour (0 = green, 2 = red) in *Vaccinium corymbosum* grown *in vitro* for 60 d under filter no-R-PMMA at PPFD 10, 20 and  $55 \mu\text{mol m}^{-2} \text{s}^{-1}$  or under filter no-B-PMMA at PPFD 20, 45 and  $115 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Parameter	Cultivar	No-R-PMMA			No-B-PMMA		
		10	20	55	20	45	115
Shoot number	Atlantic	6.6 b	13.0 a	6.3 b	10.8 c	21.1 a	15.1 b
	Berkeley	3.9 b	6.2 a	6.3 a	3.5 b	12.9 a	5.2 b
	Elizabeth	4.6 b	4.4 b	11.2 a	7.9 c	15.8 a	13.2 b
Shoot length	Atlantic	8 b	13 a	9 b	10 b	16 a	12 b
	Berkeley	10 b	15 a	10 b	9 b	15 a	12 ab
	Elizabeth	4 b	4 b	5 a	7 b	9 a	10 a
Leaf colour	Atlantic	0 a	0 a	0 a	0 a	0.1 a	0 a
	Berkeley	0 a	0 a	0.1 a	0 a	0 a	0 a
	Elizabeth	0 b	0 b	0.4 a	0 a	0.1 a	0 a



Fig. 2. Shoot proliferation and growth of *Vaccinium corymbosum* under no-B-PMMA filter, after 60 d at PPFD (from left to right) 20, 45, and  $115 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

Red colour of leaves and sprouts was the first evident response to the different PPFD and spectral composition. It varied in individual cultivars and according to the time of exposure. In general, the lower the PPFD the lower the red colour, and *vice versa*. In Elizabeth and Atlantic in particular, unfiltered light of PPFD higher than  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  caused sharp increases in leaf reddening, while shoots remained

completely green with no-B-PMMA, also at PPFD of 45 and 115  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Berkeley leaf colour was not so strongly influenced by spectral composition; initially it responded to incremental PPFD but later it gradually recovered from leaf red colour; after 60 d it was almost green up to PPFD of 115  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and very small reddening was observed at higher PPFD. In Atlantic and Berkeley the glass layer greatly protected the shoots from red colour; at PPFD of 125  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under unfiltered light the shoots were redder than at PPFD of 180  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under the glass filter.

## Discussion

Use of no-B-PMMA - filtering the UV-B and blue light - caused remarkable changes in growth and development of *Vaccinium corymbosum* in *in vitro* cultures. The blue light is regarded as the photomorphogenetically active region of the spectrum (Björn 1986). High blue/red ratio reduces stem elongation in many plants especially at higher PPFDs (Cosgrove 1986, Mortensen and Strømme 1987) and blue light is supposed to release the apical dominance and thus promoting axillary shoot production (Chee 1986, Chee and Pool 1989). Under no-B-PMMA, where there was no transmission at wavelengths shorter than 520 nm and thus no UV-A, UV-B and blue irradiation, the opposite was observed, as Appelgren (1991) already noticed on *Pelargonium*. In fact, the very low blue/red ratio strongly promoted axillary shoot production and elongation in *Vaccinium corymbosum*. Baraldi (1988) also indicated that low blue/red ratio promoted cytokinin synthesis and thus increased axillary shoot activity. In the present experiment, we also determined changes in leaf morphology very similar to those observed when high cytokinins are added to the medium. Nevertheless, we observed at the same time elongation of the shoots, indicating that light treatments possibly interfered with other growth regulators.

Although in our research we did not concentrate on the red/far-red ratio, under the no-R-PMMA treatment we completely eliminated red and far-red light. Very low red/far-red ratio often promoted stem elongation and high ratios inhibited it (Smith and Morgan 1983, Mortensen and Strømme 1987), however, some authors found the opposite (Appelgren 1991, Barro *et al.* 1989). In the present research, in the absence of red and far-red light, no effects were observed on shoot elongation.

When red colour of leaves and sprouts occurred, it appeared a short time (14 d) after light exposure; leaf reddening, especially in Berkeley, decreased afterwards, possibly due to the ability of the cultures to adapt to light conditions. Less UV-A, UV-B and blue radiation strongly reduced the incidence of leaf reddening even at very high PPFD (115  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ).

## Conclusions

In *Vaccinium corymbosum*, each cultivar responded differently to PPFD and spectral composition indicating that it is difficult to generalize even with reference to the

same species. Nevertheless, the best proliferation and secondary shoot growth were achieved with PPFD from 20 to 70  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; higher or lower PPFD strongly depressed axillary shoot formation and growth. The avoidance of UV-B and, mainly, blue radiation enhanced secondary shoot formation and growth and preserved from leaf red colour in all the 3 genotypes.

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