THE ROLE OF GIBBERELLINS IN THE THERMO- AND PHOTOCONTROL OF STEM ELONGATION IN *FUCHSIA*

Frank M. Maas and Joke van Hattum
Research Institute for Agrobiology and Soil Fertility (AB-DLO), P.O. Box 14, 6700 AA Wageningen, The Netherlands

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Abstract

Stem elongation in *Fuchsia x hybrida* was influenced by cultivation at different day and night temperature or in different light qualities. Internode elongation of plants grown at a day (25°C) night (15°C) temperature difference (DIF+10) in white light was almost twofold of that of plants grown at the opposite temperature regime (DIF-10). Orange light resulted in a threefold stimulation of internode elongation as compared to white light DIF-10. Surprisingly, internode elongation in orange light was similar for plants grown at DIF-10 and DIF+10. In white light an increase in irradiance level did not affect shoot length at either DIF treatment. Flower development was accelerated at DIF-10 as compared to DIF+10 in both white and orange light. To examine whether DIF- and light quality-induced changes in shoot elongation were related to changes in gibberellin metabolism or plant sensitivity to gibberellins, the stem elongation responses of paclobutrazol-treated plants to exogenously applied GAs were determined. In the absence of applied gibberellins, paclobutrazol (> 0.32 μmol plant^{-1}) strongly retarded shoot elongation. This inhibition was nullified by the application of GA_1, GA_4, GA_9, GA_{15}, GA_{19}, GA_{20}, GA_{24} and GA_{44} of about 10 to 32 nmol plant^{-1}, but not by GA_{12} which only slightly increased stem elongation at an amount of 100 nmol plant^{-1}. The results are discussed in relation to possible effects of DIF and light quality on endogenous gibberellin levels and gibberellin sensitivity of fuchsia, and their effect on stem elongation.

1. Introduction

Stem length and the extent of lateral branching are important morphogenetic characteristics contributing to the ornamental value of pot and bedding plants. Low irradiance levels and mutual plant shading often result in enhanced stem elongation and diminished lateral shoot development, a growth response known as the 'shade avoidance syndrome' (Smith, 1994). Blue light and the red to far-red ratio of the light perceived by a blue-light photoreceptor and by phytochrome, respectively, are the two most important light quality parameters that mediate this shade avoidance response. Several studies, reviewed recently by Erwin & Heins (1995) and by Myster & Moe (1995), have demonstrated that the extent of stem elongation may be controlled by the difference between day and night temperature (DIF) and also by temperature drops of a few hours at particular times during day or night. The use of DIF as an environmentally more friendly alternative for the chemical control of stem elongation has been examined by

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various research groups and for a wide range of horticultural plant species (Myster & Moe, 1995; Erwin & Heins, 1995).

The most widely used chemicals to regulate stem elongation (ancymidol, tetcyclasis. unicazole, triapenthenol and paclobutrazol, but not CCC) act at least in part by inhibiting the endogenous synthesis of gibberelllins by the plants (Graebe, 1987). In recent years a number of studies have been published demonstrating the importance of GA_1 in stem elongation and its synthesis being affected by environmental factors like temperature (Hazebroek & Metzger, 1990; Hazebroek et al., 1993) and light (Garciá-Martínez et al., 1987; Reid et al., 1990; Olsen et al., 1995; Gawronska et al., 1995). The aim of our study is to examine whether changes in stem elongation of pot and bedding plants by differences in light quality and DIF are mediated by changes in endogenous GA-levels or by changes in plant responsiveness towards GAs. In the present study fuchsia, a bedding plant species of which stem elongation has been shown to respond to both DIF (Tangarås, 1979; Vogelezang et al., 1992) and light (Vince-Prue, 1977; Aphalo et al., 1991; Erwin et al., 1991) was used as a model plant.

2. Materials, Methods, Experiments

2.1 Plant material and growth conditions

Fuchsia x hybrida 'Dollarprinzessin' cuttings were purchased from a nursery. The cuttings were planted in plastic 0.08 litre pots in a soil mixture (60% peat soil, 30% peat, 10% river sand) containing 0.75 kg m^{-3} PG-mix and 3 kg m^{-3} Dolokal. At the start of the experiments all plants were pruned by cutting through the internode just above the most basal pair of leaves. The growth of the two lateral shoots emerging from the axillary buds of this pair of leaves was studied during the experiments. Plants were grown at day/night temperatures of 25/15°C (day-night temperature difference = 10°C (DIF+10)) or the reverse (15/25°C = DIF-10). The photoperiod was 12 h day^{-1} and the photosynthetic photon flux (PPF) at plant level was 200 µmol m^{-2} s^{-1} or 120 µmol m^{-2} s^{-1} (cool white fluorescent lamps). Blue-deficient orange-coloured light was obtained by filtering the light of the white fluorescent tubes through one layer of orange acetate filter which transmitted only wavelengths above 500 nm (for the spectral characteristics, see Maas & Bakx, 1995). Plants were well watered with tap water supplied to the irrigation mat on which the pots were placed. Relative humidity of the air in the growth cabinets was kept at 75 ± 0.5 % at 25°C and 54 ± 0.5% at 15°C in order to maintain a constant vapour pressure deficit of 0.79 kPa.

2.2 Application of growth regulators

2.2.1 Paclobutrazol

At the start of the first experiment, just after pruning, the plants were given 0 (control plants), 0.01, 0.032, 0.1, 0.32, 1, 3.2 or 10 µmol of paclobutrazol (Bonzi, ICI Holland BV, Rotterdam) as a 10 ml drench to the soil at the basis of the plant (10 plants $treatment^{-1}$). In all other experiments plants received either 0 or 1.0 µmol paclobutrazol.

2.2.2. Gibberellins

Gibberellins (purchased from Prof. L.N. Mander, Univ. Canberra, Australia), were dissolved in 50 or 75% v/v ethanol and applied to the plants as a 5 µl droplet to the cut surface of the internode that was cut through at the beginning of the experiment. Control plants received only 5 µl of 50 or 75% ethanol. These amounts of ethanol alone did not significantly affect lateral shoot development.

2.3 Growth measurements

Plants were harvested 4 weeks after the start of the treatments. At harvest both lateral shoots were divided into leaves and stems. The length of the stems and the fresh and dry weight (after 24h at 80°C) of stems and leaves was determined. Specific leaf area was calculated from the area and fresh weight of discs punched out of the leaves.

3. Results

3.1 Effects of light and temperature on shoot growth

Various aspects of shoot development of fuchsia were analysed for effects of light and DIF (Table 1). After a period of 4 weeks in white light the length of the lateral shoots grown at DIF-10 was about 43% less than at DIF+10, independent of the PPF at which the plants were grown. However, at a PPF of 120 µmol m^{-2} s^{-1} the difference in light quality significantly affected stem elongation. Shoots grown in orange light at DIF-10 and DIF+10 were about 150 and 50% taller than those grown in white light at DIF-10 and DIF+10, respectively. Shoot lengths in orange light were similar for plants grown at DIF-10 and DIF+10. Similar differences in shoot length were observed for plants grown for 8 weeks at the same temperature and light conditions (Figure 1). Flower development was enhanced at DIF-10 compared to DIF+10 in both white and orange light. After 8 weeks at DIF-10 flower development had already advanced to the stage of flower bud opening, whereas the size of the buds at DIF+10 was less than a few millimetres (Figure 1). Shoot dry weight was significantly less at DIF-10 than at DIF+10, but it was not affected by PPF or light quality (Table 1). The enhanced shoot elongation in plants grown at DIF+10 as compared to DIF-10 in white light and in plants grown in orange as compared to white light was accompanied by a decrease in the leaf to stem dry weight ratio of the shoots. The dry weight per unit shoot length (specific stem weight) increased slightly as a response to DIF+10 in 200 µmol m^{-2} s^{-1} white light and in 120 µmol m^{-2} s^{-1} orange light as compared to DIF-10, but was not affected in plants grown in 120 µmol m^{-2} s^{-1} white light. Specific leaf area (SLA) of plants grown at DIF-10 was ca. 17% less than at DIF+10. At a PPF of 120 µmol m^{-2} s^{-1} SLA was larger than at 200 µmol m^{-2} s^{-1}, but it was not influenced by DIF or by light quality.

3.2 Inhibition of shoot development by paclobutrazol

Shoot elongation was inhibited by amounts of paclobutrazol > 0.1 µmol added to the soil at the onset of the experiment (Figure 2). Application of 0.32 to 3.2 µmol strongly reduced shoot elongation in fuchsia. Plants given these amounts of paclobutrazol no

longer showed a difference in shoot elongation when grown at DIF-10 or DIF+10. Apart from a decrease in shoot elongation, paclobutrazol also reduced total shoot growth. After a growth period of 4 weeks the dry weight of a lateral shoot of plants grown at DIF+10 or DIF-10 and given 1.0 µmol of paclobutrazol was only 20% of the weight of the accompanying control plants. Paclobutrazol did not significantly affect the number of leaves formed over the 4-week growth period, but it slightly accelerated flower bud formation at both DIF treatments (results not shown).

3.3 Effects of applied gibberellins on shoot elongation

Application of GA_1 could nullify the inhibitory effect of paclobutrazol on stem elongation (Figure 3A). Irrespective of whether plants were grown in white or orange light or at DIF-10 or DIF+10 the amount of GA_1 needed to restore stem elongation to the level of the control plants was 10 to 32 nmol. Application of similar amounts of GA_1 to plants not treated with paclobutrazol showed that the differences in shoot length as a response to light quality and DIF could be almost completely abolished (Figure 3B). Further, the latter treatments demonstrated that the length of the plants grown in orange light could only be slightly increased above that of the control plant by GA_1 application, indicating that orange light at both DIF-10 and DIF+10 already resulted in maximum stem elongation. Contrary to the control plants grown in orange light, shoot elongation of orange-light grown plants treated with paclobutrazol and given suboptimal amounts (< 32 nmol) of GA_1 was influenced by the DIF treatments to about the same extent as the white-light grown plants (Figure 3A).

In a second and third experiment, plants were given either GA1 or similar amounts of other type of gibberellins (GA_4, GA_9, GA_{12}, GA_{15}, GA_{19}, GA_{20}, GA_{24} or GA_{44}). With the exception of GA_{12} all these other GAs could nullify the inhibition of stem elongation caused by paclobutrazol (Figures 4 and 5), although there were some slight differences in the maximum lengths obtained and the amounts of GAs at which these maximum lengths were reached. For example, addition of GA_{15} abolished the difference in shoot length of white-light grown plants between the DIF-10 and DIF+10 treatment. At both temperature treatments the maximum length obtained at an amount of $GA_{15} \geq$ 32 nmol was similar to that of the white light control plants grown at DIF+10. Contrary to GA_1, GA_{15} application did not restore shoot elongation of orange-light grown plants to that of the controls but only to that of the white light grown plants at DIF+10. A similar response was found for GA_4. On the other hand, application of GA_{19}, GA_{20} and GA_{44} restored shoot elongation of both white and orange-light grown plants at DIF-10 and DIF+10 to that of the orange-light grown control plants, i.e. the maximum length of the white light GA-treated plants was higher than that of the white-light controls.

4. Discussion

Gibberellins and stem elongation

Stem elongation in fuchsia could be reduced both by DIF-10 and by a single application of paclobutrazol to the roots. The reduction in stem elongation by DIF-10 as compared to the length at DIF+10 was equivalent to that of an amount of paclobutrazol

of about 0.1 to 0.32 µmol applied to the plants grown at DIF+10, demonstrating the possibility to reduce the amount of chemical growth retardants needed to control stem elongation in fuchsia by growing the plants at a lower day than night temperature. Our results obtained with plants grown in growth chambers under artificial lighting are in agreement with those obtained in greenhouse experiments (Tangarås, 1979; Vogelezang et al., 1992). However, a lack of blue light resulted in a stimulation of internode elongation and made the plants insensitive to DIF. Similar increases in internode elongation by orange (blue-deficient) light were also observed in *Rosa x hybrida* (Maas & Bakx, 1995) and *Phaseolus vulgaris* (Maas et al., 1995). Based on its spectral distribution the orange-light stimulation of stem elongation was considered to be a photomorphogenetic response mediated by a blue-light photoreceptor and not by phytochrome. The lack of response to DIF in blue-deficient light may be of horticultural significance in circumstances in which greenhouse-grown plants are exposed to long-term supplementary lighting from high pressure sodium lamps emitting very small amounts of blue light at relatively low levels of sunlight and short natural day lengths. Application of GA_1 could enhance internode elongation of white-light grown plants to the same extent as that of orange-light grown plants and abolish the effect of DIF. Therefore, one explanation for the stimulation of stem elongation and the disappearance of the DIF response by orange light could be an increase in the endogenous levels of GA_1.

Paclobutrazol inhibits the synthesis of gibberellins by inhibiting the oxidation of the gibberellin precursor entkaurene to ent-kaurenoic acid, whereas is has no effect on later steps in the gibberellin biosynthesis pathway (Graebe, 1987). The fact that application of gibberellins to paclobutrazol treated plants could restore shoot elongation to that of the control plants suggest that the mechanism by which paclobutrazol reduces shoot elongation in fuchsia is indeed by reducing the endogenous synthesis of gibberellins. Thus far, the levels of endogenous gibberellins in control and paclobutrazol-treated fuchsia's have not been determined to prove this hypothesis. Mutants of pea showing dwarf phenotypes have been shown to have reduced levels of GA_1 and growth can be restored to that of the wild type plants by application of GA_1. Stimulation of shoot elongation in *Thlaspi arvense* as a response to a cold period was found to be related to an increased hydroxylation of the GA-precursor kaurenoic acid into 7-OH kaurenoic acid (Hazebroek et al., 1993). Photoperiodic control of stem elongation in *Silene armeria* (Talon & Zeevaart, 1992) and *Salix pentandra* (Olsen et al., 1995) was related to the synthesis and levels of GA_1. Far-red light induced stimulation of shoot elongation in *Vigna sinensis* was found to be related to increased levels of GA_1 due to stimulation of the 3-ß hydroxylation GA_{20} to GA_1 (Garciá-Martínez et al., 1987). Stem elongation in pea on the other hand was shown not to be dependent on the endogenous level of GA_1, but on the responsiveness of the plant tissue towards GA_1 (Reid et al., 1990). The data reported in the literature indicate that GA_1 is the type of gibberellin involved in the control of stem elongation in plants. Therefore, we assume that the differences observed in stem elongation in fuchsia as a response to DIF and light quality in our experiments resulted from either changes in endogenous synthesis of GA_1 or changes in plant sensitivity toward GA_1. The response of paclobutrazol-treated fuchsia's to increasing amounts of exogenously applied GA_1 when grown at DIF-10 or DIF+10 and in white or orange light was quite similar, i.e. at each temperature and light quality approx. the same amount of applied GA_1 was needed to restore stem elongation to that of the

accompanying control plants. Assuming endogenous GA_1 levels were negligible in the paclobutrazol-treated plants and that at all these growth conditions the same amount of exogenously applied GA_1 yielded similar endogenous GA_1 levels, this result suggests that the difference in stem elongation at DIF-10 and DIF+10 and in white and in orange light were not caused by differences in endogenous GA1 levels in the control plants, but from differences in plant responsiveness to GA_1 at these temperature and light quality conditions. However, determination of endogenous GA_1 levels are needed to verify this assumption. If in fuchsia GA_1 is also the gibberellin species controlling the extent of stem elongation all other types of GAs that were added and capable of nullifying the growth retardation by paclobutrazol must have been converted into GA1. With the exception of GA_{12} and to a lesser extent also GA_{15}, all applied GAs restored shoot elongation to that of the controls and at approx. the same amount as GA_1. Therefore, the rate of metabolic conversion of these GAs to GA_1 was not limiting and not involved in the control of stem elongation by DIF or light quality. The lesser response to GA_{12} and GA_{15} could be due to differences in either uptake, transportation, or metabolic conversion by the plants. To fully explain the role of the gibberellins in the control of stem elongation by temperature and light, not only the levels of GA_1 but also the rates of its synthesis from precursor GAs and its deactivation should be studied.

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Table 1. Effects of a day-night temperature difference of +10°C (DIF+10) or -10°C (DIF-10) on the length, dry weight (DW), dry matter content (DMC), specific stem weight and specific leaf area (SLA) of shoots of *Fuchsia x hybrida* grown for 4 weeks in white or orange light. Data represent the averages of 6 (length) or 3 measurements ± standard deviation.

	Light quality and photosynthetic photon flux (μmol m^{-2} s^{-1})		
	White 200	White 120	Orange 120
Shoot length (mm)			
DIF+10	148 ± 35	149 ± 26	232 ± 25
DIF-10	86 ± 12	82 ± 8	208 ± 13
Stem dry weight (mg)			
DIF+10	98 ± 36	81 ± 24	159 ± 6
DIF-10	46 ± 12	42 ± 5	108 ± 14
Leaf dry weight (mg)			
DIF+10	433 ± 74	304 ± 70	349 ± 11
DIF-10	284 ± 53	236 ± 39	234 ± 19
Shoot dry weight (mg)			
DIF+10	531 ±104	385 ± 94	508 ± 10
DIF-10	330 ± 65	278 ± 43	341 ± 32
Leaf:stem DW ratio			
DIF+10	4.7 ±1.4	3.8 ±0.3	2.2 ±0.1
DIF-10	6.3 ±0.6	5.6 ±0.6	2.2 ±0.1
Specific stem DW (mg mm^{-1})			
DIF+10	1.06 ±0.42	0.54 ±0.08	0.69 ±0.08
DIF-10	0.53 ±0.05	0.51 ±0.02	0.52 ±0.05
Stem dry matter content (%)			
DIF+10	7.9 ±0.8	6.9 ±0.1	6.9 ±0.2
DIF-10	6.8 ±0.2	6.7 ±0.2	6.8 ±0.7
Leaf dry matter content (%)			
DIF+10	11.2 ±0.9	9.4 ±0.1	9.9 ±0.3
DIF-10	10.2 ±0.1	9.7 ±0.6	9.1 ±0.5
SLA (cm^2 g^{-1} fresh weight)			
DIF+10	47 ± 0	53 ± 4	54 ± 2
DIF-10	39 ± 1	50 ± 2	57 ± 3

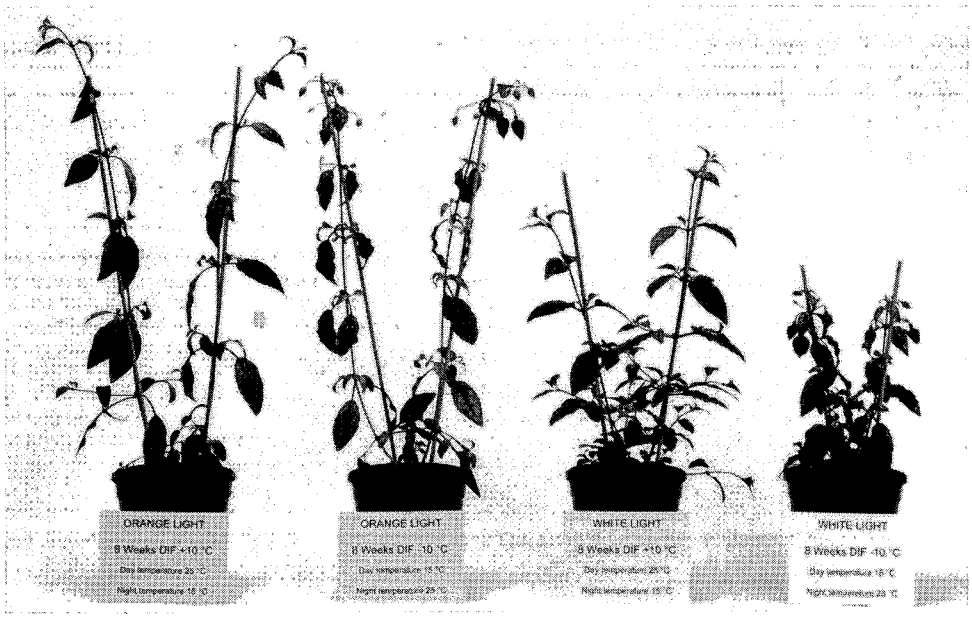

Figure 1. *Fuchsia x hybrida* 'Dollarprinzessin' after 8 weeks cultivation in orange or white light at DIF-10 or DIF+10.

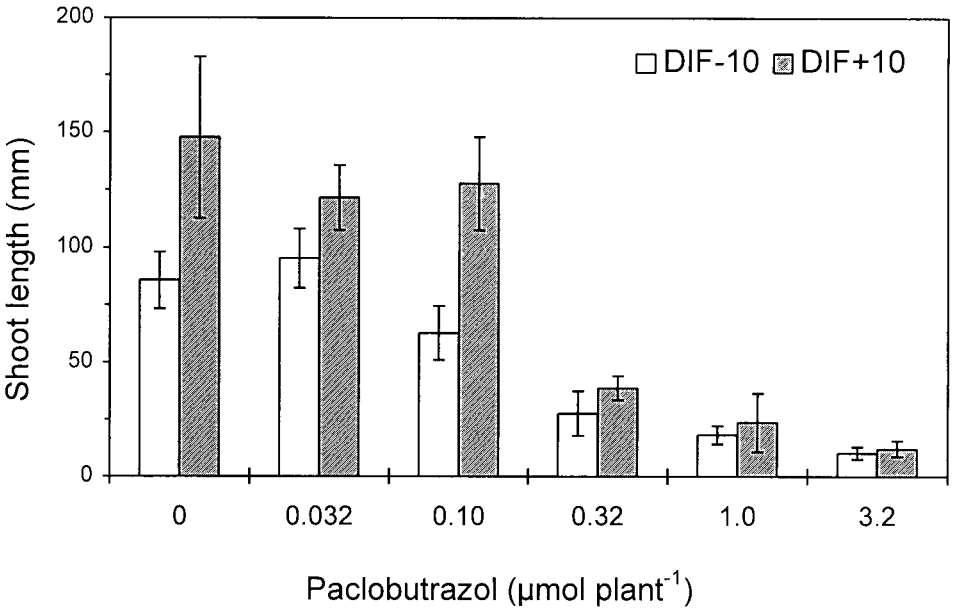

Figure 2. Effect of paclobutrazol on the length of lateral shoots of *Fuchsia x hybrida* grown for 4 weeks in white light at DIF-10 or DIF+10. The data represent the average length of 6 lateral shoots from 3 plants per treatment ± standard deviation.

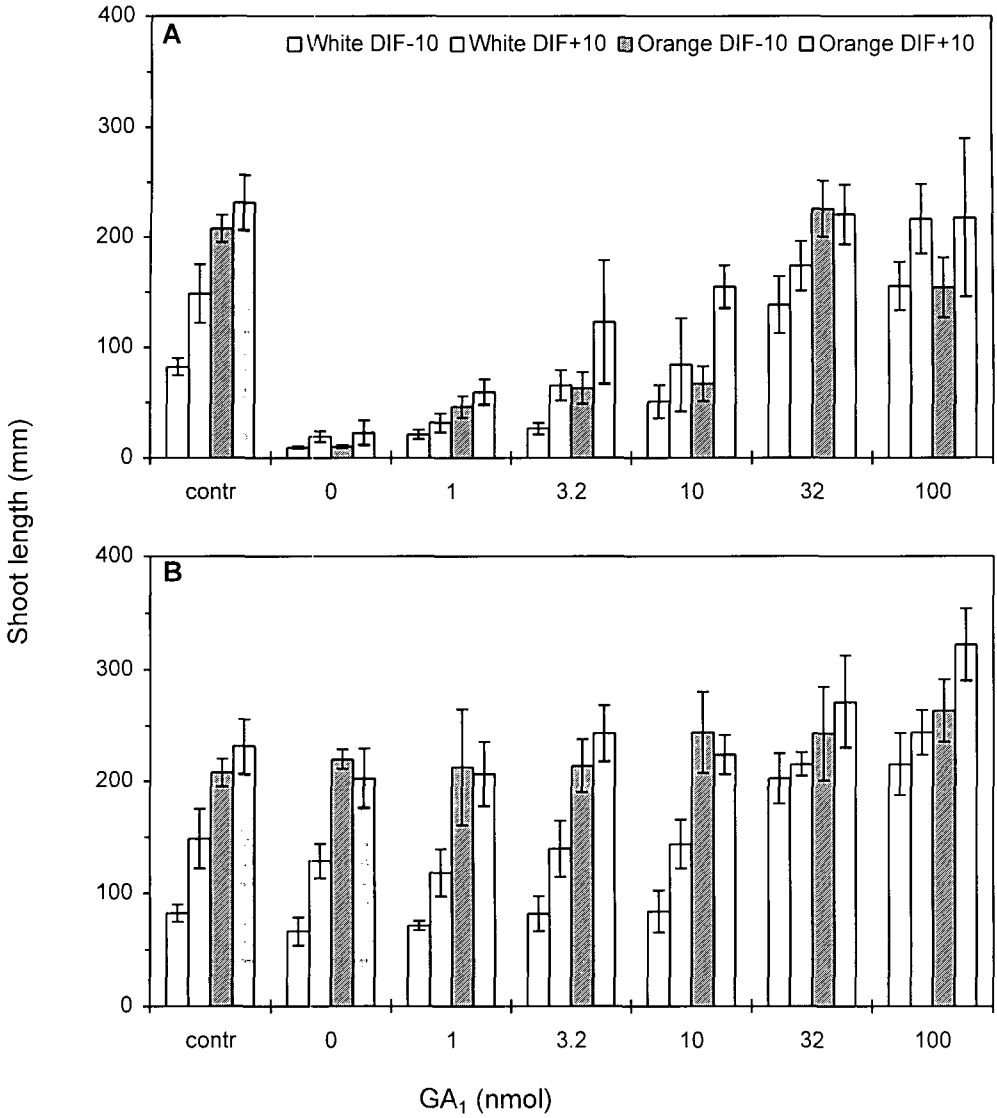

Figure 3. Effect of GA_1 application on the length of lateral shoots of *Fuchsia x hybrida* grown for 4 weeks in white light or orange light and at DIF-10 or DIF+10. At the start of the treatment plants were given 1 µmol (A) or 0 µmol paclobutrazol (B) to the roots. Otherwise as for Fig. 2.

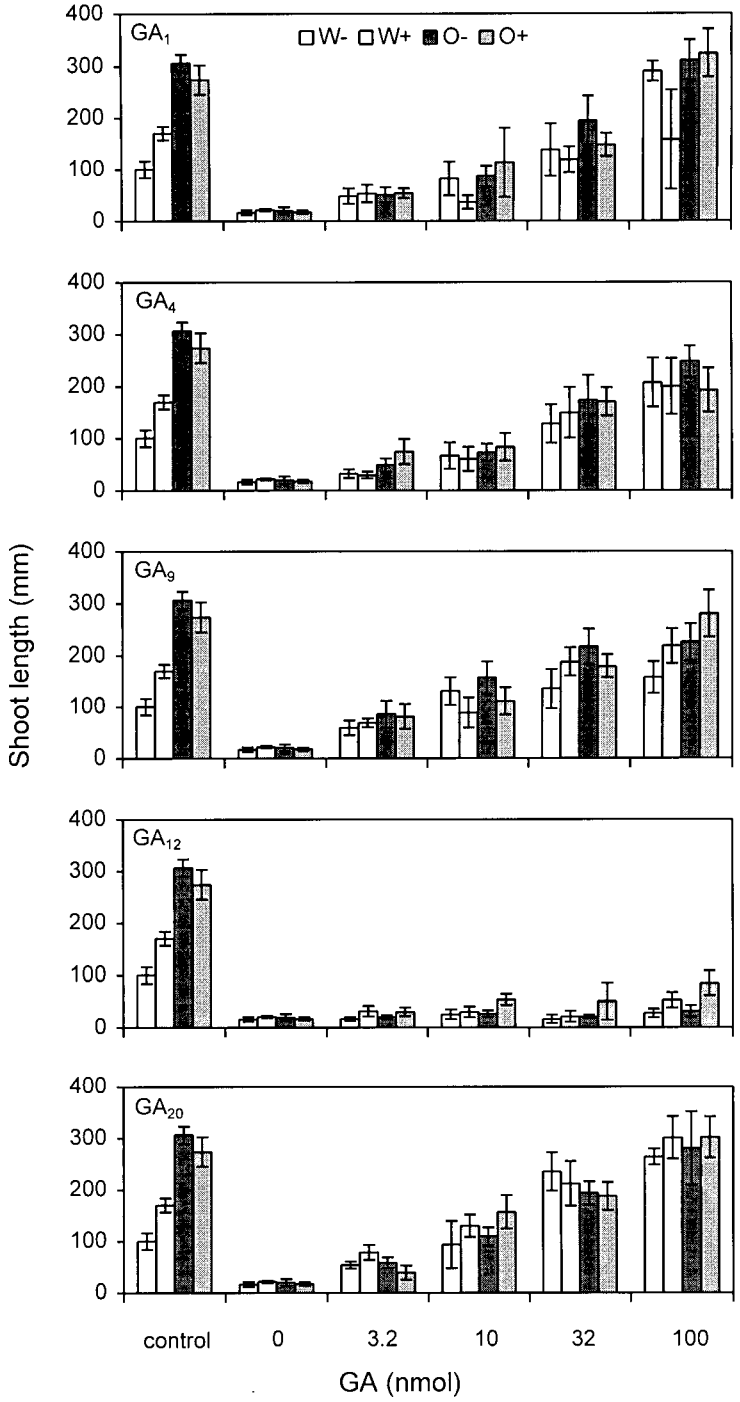

Figure 4. Effect of application of GA_1, GA_4, GA_9, GA_{12} and GA_{20} on the length of lateral shoots of *Fuchsia x hybrida* treated with 1 µmol paclobutrazol and grown for 4 weeks in white light or orange light at DIF-10 or DIF+10. Otherwise as for Fig. 2.

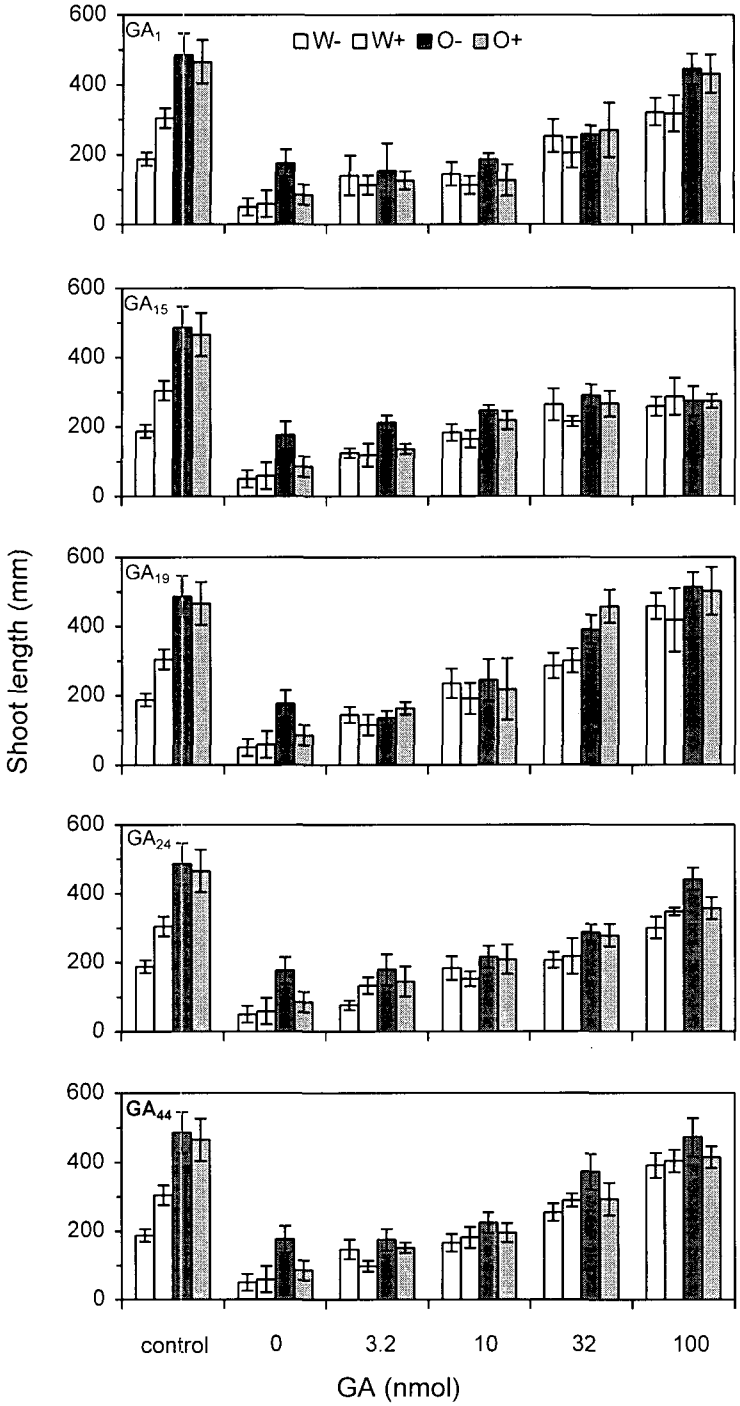

Figure 5. Effect of application of GA_1, GA_{15}, GA_{19}, GA_{24} and GA_{44} on the length of lateral shoots of *Fuchsia x hybrida* treated with 1 µmol paclobutrazol and grown for 4 weeks in white light or orange light at DIF-10 or DIF+10. Otherwise as for Fig. 2.