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Improving floral initiation in potted *Picea abies* by supplemental light treatment

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Highlights

- Supplemental light treatment:
  - Increases the proportion of genotypes initiating reproductive buds.
  - Increases floral induction, especially of female floral buds.
  - Facilitates breeding programmes, and seed production of highly improved base material from new selections for vegetative production programmes, to be more efficient.

Abstract

Light is an important environmental factor for all green plants. Its intensity, spectral composition and photoperiod can affect the regulatory pathways in plants that lead to floral initiation. In this report, results are presented from three experiments in which supplemental light with metal halide lamps (250 µmol m^{-2} s^{-1}, 20 hours day^{-1}, approx. 6 weeks) was tested as a complement to other flowering stimulation treatments (elevated temperature, treatment with gibberellin A_4 and A_7 ($GA_{4/7}$), restricted water supply) applied to potted *Picea abies* (L.) Karst. in the greenhouse. Flower stimulation in a greenhouse resulted in more floral initiation compared to flower stimulation outdoors. Supplemental light treatment increased floral initiation further, and to a larger extent in female than in male flowers. It also increased the proportion of trees and genotypes that induced reproductive buds. In a practical application of the supplemental light treatment to potted *Picea abies* breeding material, 90.6% of the clones produced either female or male flowers, or both. A subset of the same material kept outdoors, and thus subjected to natural light and temperatures, produced no flowers despite being treated with $GA_{4/7}$ and receiving a restricted water supply. In conclusion, supplemental light treatment facilitates breeding programmes, and seed production of highly improved base material from new selections for vegetative production programmes, to be more efficient.

Keywords Norway spruce; flower stimulation; strobili initiation; indoor seed orchard; seed production
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1 Introduction

The Swedish forest sector currently suffers from a shortage of improved *Picea abies* (L.) Karst. seeds in most planting zones (Almqvist et al. 2010). This shortage has two major causes: the irregular flowering pattern of *P. abies* and damage caused by insects and fungi. The economic impact of improved plant material can be great. It has been estimated that the gain in Sweden could be 1.7 billion SEK per year in the second half of this century (Karlsson and Rosvall 2010). Lack of improved spruce seed from seed orchards therefore threatens the ability of the forestry sector to contribute to the transition to a bio-based economy. Climate change requires improved reforestation material to be available from seed orchards. The Swedish breeding programme has testing programmes that ensure that the reforestation material produced is well able to cope with the stress that the anticipated changes in the climate will cause. If the breeding programme is delayed, either by problems with flower initiation needed to produce the next generation of trees or by other issues, the seed orchards will fail to produce the necessary increase in adaptation to new climatic conditions, and consequently Swedish forests will be jeopardized by a shortage of climate-proofed regeneration material.

Picea abies has a wide geographic distribution from northern Italy and Greece to northern Norway (Lat: 41–69° N), and from the European West Coast to the Asian East Coast (Long: 5–155° E)(Schmidt-Vogt 1977). *P. abies* is a shade tolerant species with higher demands for nutrients and water than e.g. *Pinus sylvestris* L.. *Picea abies* is a wind-pollinated, predominantly outcrossing species with a capacity for long distance dispersal of viable pollen (Lindgren and Lindgren 1996). Bud development in *P. abies* starts with bud scale initiation in spring simultaneously with shoot elongation (Owens and Blake 1986; Hejnowicz and Obarska 1996). Differentiation into vegetative or reproductive buds occurs during a period of approximately two weeks, after the termination of bud scale initiation and at the end of the shoot elongation (Dunberg 1979; Owens and Blake 1986).

Bud morphogenesis in conifers is affected by environmental factors such as temperature, light intensity, photoperiod and soil moisture potential. For the initiation of vegetative bud formation, temperature is the most important factor, with the optimum for *Picea* species being at least 25 °C. Light intensity, photoperiod and soil moisture potential within the normal range of growth conditions have rather limited effects (Pollard and Logan 1977). Light quality affects the maintenance of growth at the end of the growing season in conifers, with far red light having a larger effect than red light. Blue light has been shown to be unable to prevent cessation of growth and bud set (Mølmann et al. 2006).

Conifer breeding and research face a major obstacle because of the very long generation times of spruces and pines. There is therefore a strong desire to control the transition from the vegetative to the reproductive growth phase, but this has proven difficult as yet (Flachowsky et al. 2009). There are at least six regulatory pathways involved in the decision to flower. Light (intensity and quality) and photoperiod affect at least one of these pathways (reviewed in Klintenäs 2012). The graft-transmissible signal that in *Arabidopsis thaliana* has been identified as the FLOWERING LOCUS T (FT) protein can induce early flowering and shorten the juvenile phase in both herbs and trees (Bohlenius et al. 2006). However, functional FT orthologues are not present in conifers (Karlgren et al. 2011), and it is still not known whether any molecule(s) analogous to 'florigen' are active in conifers.

Light is an important environmental factor affecting floral initiation in conifers, as in other plants. High light intensity increases initiation (Owens and Blake 1985 and references therein), and the lower amount of light available for sub-canopy trees reduces cone production (Marquard and Hanover 1984; Greene et al. 2002). For seedlings, accelerating growth treatments, including artificial light treatments, have been used to speed up ontogenetic ageing in order to decrease the time required to attain flowering competence (Rudolph 1979; Bolstad et al. 1992; Cecich et al.

1994). The photoperiod, the relative duration of the light and dark periods, does not have any clear direct effect on floral initiation, but it may affect the sex of the flower buds (Owens and Blake 1985 and references therein), although for *Picea* species the positive effect of latitudinal movement on reproductive initiation may be photoperiodic (Dunberg 1979). Crown transparency can also affect the level of cone production, with the highest coning occurring in conifer trees with the least transparent crowns (Innes 1994).

Although light is thus known to be an important environmental factor governing flower initiation, there is a lack of studies exploring the possibility of enhancing floral initiation by providing supplemental light to potted *P. abies* trees used for crossing in breeding programmes and in the production of improved material as the basis for vegetative propagation options such as the production of rooted cuttings or emblings derived from somatic embryogenesis.

The aim of this study was to explore the possibilities of increasing floral initiation in potted *P. abies* trees using supplemental light. Here I present results on floral initiation from three experiments and one practical implementation in which supplemental light was provided to potted *P. abies*.

2 Material and methods

The three experiments presented here were conducted between 2001 and 2013, and at two different locations. The extended time span was due to a combination of the closure of the research facility first used (in Brunsberg) and the availability of suitable experimental material. In experiment 1 the effect of flowering stimulation treatments in the greenhouse (elevated temperature, hormone treatment and restricted water supply) with or without supplemental light was compared. In the later experiments (nos. 2 and 3) the number of treatments was expanded to get more information on whether the effect observed was genuinely a light and not a temperature effect (no. 2) and on how photoperiod may alter the effect of light (no. 3).

2.1 Experiment 1

The experiment was performed in 2001 at Brunsberg Field station, Skogforsk, (Sweden, 59°37′N, 12°58′E, alt. 80 m. a.s.l.). Six clones originating from old plus trees (>100 years, selected between Lat 59–61° N) were used. The grafts had been growing in the pots for several years before the start of the experiment and had shown flowering competence. The pots were of three sizes (45, 160 and 350 litres). The grafts were grown outdoors except during the period of flower-stimulating treatment in the greenhouse.

The greenhouse used was covered with double air-filled layers of polyethylene plastic for heat insulation. The temperature regime was set at 25/15 °C during the day/night respectively, and the grafts were subjected to restricted irrigation (watering was done when elongating shoots were showing wilting symptoms) to ensure that they experienced minor drought stress throughout their time in the greenhouse.

In this experiment there were two treatments:

1. Flowering stimulation in the greenhouse ($GA_{4/7}$ treatment and restricted irrigation)
2. Flowering stimulation in the greenhouse with supplemental light

In each treatment each clone was represented by 1–2 grafts in each of the three sizes of pot. In total, the experiment consisted of 59 grafts.

The material was transferred into the greenhouse on June 15, and in treatment 2 the supplemental light started a few days later. The supplemental light treatment was provided by one 400 W

metal halide lamp for each tree. The lamps were installed above the trees at a distance that gave a supplemental light intensity of about 250 µmol m^{-2} s^{-1} at the top whorl of the tree. Supplemental light was given for 20 hours each day (03:15–23:15). In both treatments all grafts were treated with gibberellin $A_{4/7}$ ($GA_{4/7}$) on two occasions (July 2 and July 15). At the times of treatment the shoots on the upper whorl had elongated to, respectively, approx. 60% and 90 % of their final lengths. Because the size of pot (and subsequently tree) differed, the total $GA_{4/7}$ dose was 20, 30 or 40 mg $graft^{-1}$ for trees in pots of 40, 160 or 350 litres respectively. The flowering stimulation treatment, including the supplemental light in treatment 2, was continued until July 20. After that the grafts remained in the greenhouse until August 14 when they were transferred outside again. Between July 20 and August 15 the greenhouse was fully ventilated to keep the temperature as similar to the outside temperature as possible.

The number of female flowers and male flowers on each graft was counted in spring the year after treatment.

2.2 Experiment 2

The experiment was performed in 2006 at Ekebo Research station, Skogforsk, (Sweden, 55°57´N, 13°07´E, alt. 85 m. a.s.l.). Six clones originating from old plus trees (>100 years, selected between Lat 49–61° N) were used. The grafts in this study were made in 1997, and were transplanted into 110 litre pots in 1998. The grafts had shown flowering competence prior to the start of the experiment. The grafts were grown outdoors except during the period of flower-stimulating treatment in the greenhouse.

The greenhouse used was covered with polycarbonate panels. The temperature regime was set at 25/15 °C during the day/night respectively, and the grafts were subjected to restricted irrigation (watering was done when elongating shoots were showing wilting symptoms) to ensure that they suffered from light drought stress throughout their time in the greenhouse.

In this experiment there were five treatments:

1. Flowering stimulation in the greenhouse ($GA_{4/7}$ treatment and restricted irrigation)
2. Flowering stimulation in the greenhouse with supplemental light
3. Flowering stimulation in the greenhouse with infrared heating
4. Flowering stimulation outdoors ($GA_{4/7}$ treatment and restricted irrigation)
5. Control, natural conditions outdoors

In treatment 1 four of the clones were represented by 2–3 grafts per clone. In the other treatments all six clones were included, with 1–4 grafts per clone. In total the experiment consisted of 66 grafts.

The material was transferred into the greenhouse on June 20, and in treatment 2 and 3 the supplemental light and infrared heating treatments started two days later. The supplemental light treatment was provided by one 400 W metal halide lamp for each tree. The lamps were installed above the trees at a distance that gave a supplemental light intensity of about 250 µmol m^{-2} s^{-1} at the top whorl of the tree. The infrared heating source (1000 W) was installed above the grafts at a height giving the same temperature at the top whorl as the supplemental light. Supplemental light and infrared heating was given for 20 hours each day (03:15–23:15). During periods of the day with high sun insolation (over 450 W m^{-2} (PAR) outside) and high temperatures, the supplemental light and infrared heating were turned off to avoid overheating. Grafts were treated with $GA_{4/7}$ as stem injections on two occasions. At the times of treatment, the shoots on the upper whorl had elongated to, respectively, approximately 60% and 90 % of their final lengths. A total $GA_{4/7}$ dose of 30 mg $graft^{-1}$ was given each of the grafts in treatment 1–4. The flowering stimulation treat-

ment, including the supplemental light and infrared heating in treatment 2 and 3, was continued until July 28. After that the grafts remained in the greenhouse until August 14 when they were transferred outside again. Between July 28 and August 15, the greenhouse was fully ventilated to keep the temperature as similar to the outside temperature as possible.

The number of female flowers and male flowers on each graft was counted in spring the year after treatment.

2.3 Experiment 3

The experiment was performed in 2013 in the same facility at Ekebo research station as Experiment 2. The aim of the experiment was to study both the effect of the flower-stimulating treatment presented here, and the effect of different climatic conditions during flower and seed development on seedling performance (data not presented here). Eleven clones from the breeding population, of varying age at selection, were used in this study. The potted trees were cuttings (5 clones) and grafts (6 clones) and they were transplanted into 100 litre pots in 1998 (grafts) and 2002 (cuttings). Two of the grafted clones also had ramets in 45 litre pots. For these clones, the ramets in different pot sizes were distributed evenly among treatments. All clones used had shown flowering competence prior to the start of the experiment.

In this experiment there were four treatments:

A. Flowering stimulation outdoors at latitude 60.3° N
B. Flowering stimulation outdoors at latitude 55.9° N
C. Flowering stimulation in the greenhouse with temperature and day length corresponding to conditions at latitude 45° N. Supplemental lighting was used to increase light intensity.
D. Flowering stimulation in the greenhouse with supplemental light. Day length during flowering stimulation was 20 h. This treatment was similar to treatment 2 in Experiment 1 and 2.

In each treatment the 11 clones were represented by 2–4 trees per clone. In total, the experiment consisted of 165 potted trees.

In treatment C, the trees were transferred into the greenhouse on April 10, and temperature and day length regimes that should mimic the climate at latitude 45° N started on April 22. The supplemental light used to increase light intensity was provided by one 400 W metal halide lamps for each tree. The lamps were installed above the trees at a distance that gave a supplemental light intensity of about 250 µmol m^{-2} s^{-1} at the top whorl of the tree. Day length was adjusted by using blackout fabric. The trees in treatment C were grown in the greenhouse until October 2.

In treatment D, the trees were transferred into the greenhouse on June 10, when the supplemental light treatment was started. The treatment was identical to treatment 2 in Experiment 2. The supplemental light treatment lasted for 6 weeks. The trees remained in the greenhouse until August 14, when they were transferred outside again.

Grafts were treated with $GA_{4/7}$ as stem injections on two occasions. At the times of treatment the shoots on the upper whorl had elongated to, respectively, approximately 60% and 90% of their final lengths. A total $GA_{4/7}$ dose of 30 mg $graft^{-1}$ was given to each of the grafts in all treatments. Treatment dates in treatment A were June 25 and July 3, in treatment B June 25 and July 4, in treatment C June 12 and 19 and in treatment D June 19 and 25.

The number of female flowers and male flowers on each graft was counted in spring the year after treatment.

2.4 Practical implementation

In 2014 the spruce breeders decided to treat a part of the breeding population with the treatment described as Treatment D in Experiment 3 (this treatment is also very similar to treatment 2 in Experiment 1 and 2). The same facility at Ekebo Research station as in Experiment 2 and 3 was used.

The material was grafts from 32 clones selected for one breeding population aimed at central Sweden. Grafts of the clones had been grown both in 110 litre pots and in soil in a clonal archive for several years without showing any flowering competence. From each clone 1–2 potted grafts were transferred into the greenhouse, giving a total of 49 grafts. A further 1–21 grafts clone^{-1} (on average 3 grafts clone^{-1}), in total 95 grafts, were left outdoors or in the soil based archive. All the grafts were grown outdoors except during the period of flower-stimulating treatment in the greenhouse.

The grafts were transferred into the greenhouse on June 10–11 and the supplemental light treatment started on June 13. The supplemental light and heat treatment lasted for 6 weeks (ending July 22). The trees were then transferred outside again on July 23.

Grafts were treated with $GA_{4/7}$ as stem injections at two occasions. At the time of treatment the shoots on the upper whorl had elongated to, respectively, approximately 60% and 90% of their final lengths. A total $GA_{4/7}$ dose of 30 mg graft^{-1} was given to each of the grafts in all treatments. In the greenhouse the treatment dates were June 24 and July 5, and the period outside was between June 5 and July 9. Of the grafts outdoors, approximately 60% were treated with $GA_{4/7}$.

The number of female flowers and male flowers on each graft was counted in spring the year after treatment.

2.5 Statistical analyzes

Statistical analyses of the frequency of trees with flowers were performed using the SAS (version 9.4) software package and the Proc GLM module and Eq. 1, and for the number of female/male flowers per tree analyses were performed using the Proc Genmod (Experiment 1 and 2) and Proc Glimmix (Experiment 3) models and Eq. 2 with a Poisson distribution and a logarithmic link function. Tukey-Kramer adjusted significance levels were used for multiple comparisons.

$$y_{ijk} = \mu + b_i + c_j + d_k + e_{ijk}, \quad (1)$$

$$\log(y_{ijk)} = \mu + b_i + c_j + d_k + e_{ijk}, \quad (2)$$

where:
y_{ijk} = dependent variable. 0, 1-variable, tree has/has not female/male flowers.
μ = overall mean
b_i = fixed effect of clone, random in Experiment 3 for number of female/male flowers
c_j = fixed effect of treatment
d_k = fixed effect of pot size (in Experiment 1 only)
e_{ijk} = residual, $(N(0, \sigma_e^2))$

Table 1. Percentage of trees with female and male flowers in Experiments 1–3.

Treatment	Experiment 1 % trees with		Experiment 2 % trees with		Experiment 3 % trees with	
	Female	Male	Female	Male	Female	Male
Control, natural conditions outdoors, Lat 55.9° N	--	--	11.5	67.2	--	--
Flowering stimulation, outdoors, Lat 55.9° N	--	--	27.4	59.8	8.5	19.4
Flowering stimulation, outdoors, Lat 60.3° N	--	--	--	--	15.9	22.7
Flowering stimulation, greenhouse, simulated Lat 45° N	--	--	--	--	15.3	12.3
Flowering stimulation, greenhouse	95.4	97.6	40.9	72.7	--	--
Flowering stimulation, greenhouse, infrared heating	--	--	55.4	51.7	--	--
Flowering stimulation, greenhouse, supplemental light	83.9	93.3	74.4	84.4	75.8	63.7

3 Results

Both natural flower induction and artificial flower induction outdoors resulted in a low proportion of trees with flowers in all cases (experiments). The treatment that simulated Lat 45° N did not increase the proportion of trees flowering compared to flowering stimulation in outdoor conditions. Flower induction in the greenhouse both with and without infrared heating had a similarly positive effect on the proportion of trees producing flowers, whereas induction in the greenhouse with supplemental light resulted in the highest overall proportion of trees with flowers (Table 1).

In Experiment 1 the supplemental light treatment produced significantly more female flowers than the flowering stimulation treatment in the greenhouse without supplemental light (Table 2). Compared to flowering stimulation outdoors, all treatments in the greenhouse produced significantly more female flowers in Experiment 2, and the treatment with supplemental light produced significantly more female flowers than flowering stimulation without supplemental light or with infrared light. In Experiment 3 the treatment with supplemental light in the greenhouse produced significantly more female flowers than the other treatments (Table 2).

Table 2. Mean numbers of female flowers per tree in Experiments 1–3. For tests of significant differences, LsMeans of transformed data (Transf) are presented for each experiment. Treatments with significant differences ($p < 0.05$) are indicated by different letters (significance test within experiment). Mean values calculated as LsMeans of untransformed data (Untransf) are presented to indicate the level of flowering and the magnitude of differences between treatments.

Treatment	Experiment 1 No. female flowers		Experiment 2 No. female flowers		Experiment 3 No. female flowers	
	Transf	Untransf	Transf	Untransf	Transf	Untransf
Control, natural conditions outdoor, Lat 55.9° N	--	--	–2.426 (d)	0.3	--	--
Flowering stimulation, outdoors, Lat 55.9° N	--	--	0.279 (bc)	2.9	0.506 (b)	5.9
Flowering stimulation, outdoors, Lat 60.3° N	--	--	--	--	0.254 (b)	3.8
Flowering stimulation, greenhouse, simulated Lat 45° N	--	--	--	--	0.445 (b)	4.4
Flowering stimulation, greenhouse	2.969 (b)	23.2	–0.595 (b)	3.6	--	--
Flowering stimulation, greenhouse, infrared heating	--	--	0.714 (c)	5.3	--	--
Flowering stimulation, greenhouse, supplemental light	3.498 (a)	36.9	2.286 (a)	19.0	2.524 (a)	35.9

Table 3. Mean numbers of male flowers per tree in Experiment 1–3. For tests of significant differences, LsMeans of transformed data (Transf) are presented for each experiment. Treatments with significant differences ($p < 0.05$) are indicated by different letters (significance test within experiment). Mean values calculated as LsMeans of untransformed data (Untransf) are presented to indicate the level of flowering and the magnitude of differences between treatments.

	Experiment 1 No. male flowers		Experiment 2 No. male flowers		Experiment 3 No. male flowers	
Treatment	Transf	Untransf	Transf	Untransf	Transf	Untransf
Control, natural conditions outdoors, Lat 55.9° N	--	--	–0.707 (c)	32.8	--	--
Flowering stimulation, outdoors, Lat 55.9° N	--	--	–1.100 (e)	20.1	1.069 (c)	15.0
Flowering stimulation, outdoors, Lat 60.3° N	--	--	--	--	0.958 (c)	14.2
Flowering stimulation, greenhouse, simulated Lat 45° N	--	--	--	--	1.440 (b)	23.3
Flowering stimulation, greenhouse	5.405 (b)	247	–1.563 (d)	25.3	--	--
Flowering stimulation, greenhouse, infrared heating	--	--	0.1648 (a)	72.6	--	--
Flowering stimulation, greenhouse, supplemental light	5.491 (a)	268	–0.044 (b)	60.5	2.869 (a)	94.7

In Experiment 1 the supplemental light treatment produced significantly more male flowers than the flowering stimulation treatment in the greenhouse without supplemental light (Table 3). Compared to flowering stimulation outdoors, all treatments in greenhouse produced significantly more female flowers in Experiment 2, and the treatment with infrared heat produced the largest number of male flowers. In Experiment 3 the treatment with supplemental light in the greenhouse produced significantly more male flowers than the other treatments (Table 3).

In the practical implementation of the light stimulation treatment, using a regime similar to treatment D in Experiment 3, female flowers were produced on 84.4% of the clones and male flowers on 71.9% of the clones. In total 90.6% of the clones had ramets with either female or male flowers, or both, leaving only 3 of the 32 clones without any flowers. On the ramets of the clones that were left outside there was no female or male flowering at all.

4 Discussion

4.1 Percentage of trees with flowers

Picea abies is a species that has a long juvenile phase and that continues to be very reluctant to induce flowers buds even after entering the adult, flowering-competent phase (Tirén 1935; Lindgren et al. 1977). Results from the experiments presented here confirm this. In natural conditions outdoors, and with flower stimulation outdoors, only a small proportion of the trees produced female flowers. Male flowers were produced on a larger proportion of the trees, but the best result, in Experiment 2, was that about two third of the trees had male flowers, indicating that induction of male flowers may be less dependent than female flower induction on the right mix of environmental stimuli. The largest proportion of trees with female and male flowers was obtained in the treatment with supplemental light in the greenhouse in Experiment 2 and 3. In Experiment 1 the supplemental light did not increase the proportion of trees with flowers compared to flower stimulation in the greenhouse without supplemental light. In the year in which this experiment was carried out, the natural sunlight conditions in the experimental area were better than normal, with 15% more sunlight hours than normal (SMHI 2001), resulting in very successful flower initiation in the greenhouse even without supplemental light.

4.2 Female and male flowering in the experiments

The simulated southward transfer in Experiment 3 did not result in any increase in flower induction, although real southward transfer is known to have this effect in *P. abies*. Southward transfer changes the time at which shoots start to elongate because of the earlier increase in temperature, and the time when floral bud initiation takes place is also altered. Such a transfer also changes the photoperiod, as the maximal day length decreases with southward transfer and at the same time the intensity of solar insolation increases due to the sun's higher position in the sky during the day. In the simulated transfer, the day length was shortened and the light intensity was increased by supplemental light but it is probable that the supplemental light was insufficient to mimic the increased light intensity that would be obtained by real southward transfer. The lack of any positive response in terms of flower initiation in Experiment 3 therefore supports the view that it is the increase in solar insolation intensity that is the driver of the positive effect brought about by southward latitudinal transfer, rather than the change in photoperiod as suggested by Dunberg (1979).

The most striking results in the experiments reported here are the pronounced effect, on both female and male flower induction, of the supplemental light treatment in the greenhouse. The light treatment was performed with metal halide lamps creating a day length of 20 hours, which at Lat 55.9° N corresponds to a day 2.5 hours longer than that naturally occurring at the summer solstice, and the 20 h day length was used for a period of six weeks. The light spectrum given by a metal halide lamp differs from natural daylight (Bergstrand 2015). In the photoperiodic light network, it is primarily the relationship between red and far red light, which control the phytochrome system, but also the effect of blue light on the expression of the gene encoding CONSTANS, that are of greatest importance in the control of flower initiation, at least in Arabidopsis (Klintenäs 2012). It is not yet known whether this is also the case in conifers like *P. abies* . Whether the positive effect of the supplemental light treatment is due to the altered day length, the altered light spectrum or a combination of them is not a question that the results presented here can answer. Further experiments are therefore needed.

4.3 Practical implementation of light stimulation treatment

From a breeder's point of view, it is of utmost importance to get flowers on as many clones as possible in breeding material in order to avoid loss of genetic diversity in this material during production of the next generation. Whether a specific clone produces female or male flowers is not important, as long as there are enough clones with flowers of both types in the population as a whole. In the practical implementation reported here there were female flowers on 27 and male flowers on 23 clones; only three clones had no flowers at all out of the 32 in the breeding material, even though only, on average, 1.5 ramet clone^{-1} was treated. Among the 27 clones with female flowers there were on average 27 female flowers ramet^{-1}, enough to complete the desired crosses to produce the next generation in the breeding population. A treatment effect of this magnitude increases the effectiveness of a breeding programme, as the breeder can do the crosses according to the concept of positive assortative mating (Rosvall and Mullin 2003).

5 Conclusions

Treating potted *Picea abies* with supplemental light (metal halide lamps, supplemental light intensity of about 250 µmol m^{-2} s^{-1} at the top whorl of the tree, 20 hours day for six weeks) together with other flower stimulating treatments ($GA_{4/7}$-treatment and drought stress) in the greenhouse:

increases the proportion of genotypes that initiate reproductive buds;
increases floral induction, especially of female flower buds;
facilitates breeding programmes, and seed production of highly improved base material from new selections for vegetative propagation programmes, to be more efficient.

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