Plant Physiology and Biochemistry 134 (2019) 31–39

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Interannual variation in UV-B and temperature effects on bud phenology and growth in *Populus tremula*☆

C. Strømme[a,b,*], U. Sivadasan[c], K. Nissinen[c], A. Lavola[c], T. Randriamanana[c], R. Julkunen-Tiitto[c], L. Nybakken[b]

[a] Department of Forestry and Wildlife Management, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, P. O. Box 400, NO-2418 Elverum, Norway

[b] Faculty of Environmental Sciences and Natural Resource Management, P.O. Box 5003, Norwegian University of Life Sciences, NO-1432 Ås, Norway

[c] Natural Products Research Laboratories, Department of Biology, University of Eastern Finland, P.O. Box, 111, FI-80101 Joensuu, Finland

ARTICLE INFO

Keywords:
Climate manipulation
Tree growth
Acclimation

ABSTRACT

Warming affects phenological processes such as spring bud break and autumnal bud set, and also growth rates of trees. Recently, it has been shown that these physiological processes also may be influenced by the ultraviolet-B (UV-B) part of the solar spectrum, and there are reasons to expect that the two environmental factors induce interactive effects when acting in concert. In this study, our aim was to elucidate how experimental enhancements in temperature and UV-B, alone and in combination, affect growth and seasonal phenology of Eurasian aspen (*Populus tremula*) over several growing seasons (three years). Moreover, we tested how environmentally induced changes in phenology affect the growth achieved over each season, that is, the importance of a prolonged growing season for growth yield. The plants grew in an outdoor experiment with modulated enhancements of temperature and UV-B during the growing season. Both UV-B and temperature enhancement affected bud set dates, while bud break dates were only affected by temperature enhancement. Temperature delayed bud set in all years, but gradually less over years, while UV-B yielded earlier bud set the first year but showed a delayed response the following years. Bud break was always earlier under temperature enhancement. The experimentally induced extension of the growing season in both ends had a positive effect of growth throughout the three-year period. However, the reduced responsiveness of bud set to both enhancement treatments suggest that the plants gradually acclimated to the modified climate, a finding that should also be investigated for other tree species.

1. Introduction

In environments characterised by seasonal climatic shifts, survival of different tree species requires appropriate timing of growth related processes to the part of the year cycle that has favourable climate. By sensing seasonal shifts in temperature and properties of solar light, trees are provided with a range of environmental cues that control the timing of phenological events (Olsen and Lee, 2011). Sensing day length, temperature and light quality allows tree species to couple phenological transitions to seasonal shifts as they occur. As boreal and temperate tree species have wide distributions across latitudes and elevations, the adaptations to climatic seasonal patterns have resulted in different provenances.

For several tree species in temperate climates, an increasing amount of evidence points towards an interplay of light and temperature parameters in driving the yearly growth cycle (Hänninen and Tanino, 2011). In relation to phenological shifts in autumn, light quality has been shown to affect growth cessation and the formation of winter buds in boreal tree species. In this respect, effects of far-red (FR) light have been shown in *Salix pentandra* (Junttila and Kaurin, 1985), hybrid aspen (*Populus tremua* x *Populus tremuloides* (Olsen et al. 1997); Norway spruce (*Picea abies*) (Clapham et al. 1998; Mølmann et al. 2006) and silver birch (*Betula pendula*) (Tsegay et al. 2005). In addition, effects blue light in relation to autumn phenology have been shown in Norway spruce (Mølmann et al. 2006; Opseth et al., 2016), and for bud burst in

☆ This article is part of the Special Issue "Interactive effects of UV-B radiation in a complex environment" published at the journal Plant Physiology and Biochemistry 134, 2019.

* Corresponding author. Department of Forestry and Wildlife Management, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, P.O. Box 400, NO-2418, Elverum, Norway.

E-mail address: christian.stromme@inn.no (C. Strømme).

https://doi.org/10.1016/j.plaphy.2018.08.029
Received 18 April 2018; Received in revised form 22 August 2018; Accepted 23 August 2018
Available online 28 August 2018
0981-9428/

spring for birch (*Betula pendula*), black alder (*Alnus glutinosa*) and oak (*Quercus robur*) (Brelsford and Robson, 2018). Moreover, ultraviolet-B (UV-B) light has been shown to interact with temperature in relation to bud formation (Strømme *el al.* 2015 & 2018). Evidence of UV-B as a growth regulator for plants is of recent origin, and a possible role in day-length sensing has been shown in *Arabidopsis thaliana* (Fehér et al. 2011).

During the last decades, increased growth has been observed for tree species from high latitudes of the northern hemisphere (Jacoby & D'Arrigo, 1995; Hember et al., 2012; Kauppi et al. 2014; Schaphoff et al. 2016). This growth increase has been related to climatic warming, which has been reported to be particularly strong in northern geographical areas (Serreze et al. 2000; Hartman et al. 2013). A survey of 63 studies investigating temperature effects on tree species suggests that direct effects of warming are generally beneficial to tree growth in non-tropical areas (Way and Oren, 2010). In particular, it was shown that photosynthetic rates increased more strongly than respiration. In addition, plant growth is generally considered to occur at temperatures above 5 °C, possibly due to low-temperature constraints on biochemical processes in the plant cells (Körner, 2016). As boreal and temperate tree species are more limited by temperature than tree species in warmer climates (Way and Oren, 2010), warming probably results in more days over this critical temperature. Moreover, higher temperatures stimulate tissue growth by shortening the length of the plant cell cycle (Francis and Barlow, 1988), and may also favour tree growth by extending the yearly growth period. Widespread observations of advanced spring phenology have been related to global warming (Menzel et al. 2006; Bertin, 2008). For most temperate and boreal tree species, bud break in spring is driven by accumulating heat sums and is largely, with a few exceptions, a temperature-driven process (Sarvas, 1972; Körner and Basler, 2010). This process also requires a degree of chilling to occur, which may differ substantially between species (Hänninen and Tanino, 2011).

Experimental warming has been shown to positively affect growth in field conditions (Nybakken et al. 2012; Randriamanana et al. 2015; Strømme et al. 2018). In addition to earlier growth onset in spring, evidence from field studies shows that temperature yields a prolonged growing season, also through delayed bud set in autumn (Rohde et al. 2011; Strømme et al. 2015, 2018). As for spring phenology, there is concern for insufficient fulfilment of chilling requirement in relation to warming effects on autumn phenology, and that the response may be carried over to bud break in spring (Hänninen and Tanino, 2011). As most studies of climatic effects on trees have been performed in controlled or semi-controlled environments, it is not yet clear whether such carry-over effects of warming occur in field conditions.

To sum up, both from large-scale observation studies and from environmental manipulation with small plants we know that both UV-B and enhanced temperatures affect several physiological processes in trees. However, we know little about to what degree the different processes contribute to the total growth increment through a growing season. Can the same plant make use of both an early start and a late ending of the season, or is there a limitation to growth during one season? As most warming studies have lasted only one growing season, there is also little knowledge on the effect of prolonged exposure of the same individuals.

In this study, we compiled growth and phenology data of *Populus tremula* subjected to enhanced levels of UV-B and temperature in a modulated field experiment spanning over three years. Our main aim was to detect how climate change influences achieved growth through effects on the length of the growing season. Earlier results on the same plants showed that temperature enhancements increase growth (Randriamanana et al. 2015; Nissinen et al. 2017; Sivadasan et al. 2018), and result in earlier bud break in spring and delayed bud set in autumn (Strømme et al., 2015; Sivadasan et al. 2017). It was also shown that enhanced UV-B forced bud set in the autumn as well as bud break in the spring after the first growing season (Strømme et al., 2015). In this study, we hypothesized that 1) a prolonged growing season would be beneficial to plant growth in terms of height and diameter increase across all three study years. We further hypothesized that 2) enhanced levels of UV-B would yield earlier bud set in autumn and earlier bud break in spring. Also, we hypothesized 3) that temperature enhancement would yield delayed bud set in autumn as well as earlier bud break in spring, adding significantly to the positive effect of temperature on growth. Furthermore, we hypothesized 4) that a combined UV-B and temperature treatment would yield dissimilar effects from single treatments on bud set and bud break. We also tested whether any of the tested relationships would differ between plant sexes and across experimental years.

2. Materials and methods

2.1. Plant material

Plants used in the field experiment originated from six female and six male aspens located in Southern and Eastern Finland. For a thorough description of sampling locations, micropropagation of individuals and growth conditions, see Strømme et al. (2015). The *in vitro* propagated plantlets were potted into 1-L pots filled with 70% nonfertilised peat and 30% vermiculite and kept in a greenhouse between 2 May and 7 June 2012 prior to planting in the field.

2.2. Experimental set-up

The field experiment was situated in Joensuu, Eastern Finland (62°60′ N, 29°75′ E). The experimental set-up included 36 plots in a 6 × 6 matrix with 3 m between the plots in all directions, as explained in details by Nybakken et al. (2012), originally containing 60 female and male plants in each (five plants of each clone). Each plot was added a 10 cm layer of 0.8% limed mineral soil. A metal net fence of 1.5 m was structured around the experimental field to prevent intrusion of large mammals, and a metal sheet shelter was implanted 60 cm into the soil and 60 cm above the soil level to exclude voles. The plants received a combination of UV-B radiation and temperature enhancement that were obtained through continuous modulation to +30% and +2 °C of ambient UV-B and temperature levels, respectively. The achieved levels were +28.0 ± 0.4% and +1.35 ± 0.042 °C of ambient UV-B and temperature levels, respectively. The possible treatment combinations were one of the following six treatments or treatment combinations: enhanced temperature (T), enhanced UV-B (UV-B), UV-B + T, enhanced UV-A (UV-A), UV-A + T, and control with ambient temperature and UV radiation (C). Enhancement treatments involved UV-lamps and IR-heaters mounted above experimental plots and held by adjustable aluminum frames bolted on metallic posts. Each aluminum frame was appended by six 40 W UV fluorescent lamps (1.2 m long, UVB-313, Q-Panel Co., Cleveland, OH, USA) following a cosine distribution (Björn, 1990). The emission spectrum was measured with an Optronic OL-756 portable UV-VIS spectroradiometer (Optronic Laboratories, Orlando, FL, USA), and cellulose diacetate filters were wrapped around each lamp to screen out radiation below 290 nm in the UV-B treatment plots. As the UV-B tubes also emit some UV-A radiation, UV-A controls were also included in the experimental set-up (UV-A and UV-A + T): in twelve plots, the UV tubes were wrapped with polyester film in order to remove UV-B, so that only the enhanced levels of UV-A were achieved. The temperature treatment was provided by two infrared (IR) heaters (CIR 110, FRICO, Partille, Sweden), bolted along the middle axis of the aluminum frames. The frames were lifted every third week to maintain a 60 cm distance between the highest shoot tip and the radiators/UV lamps.

The enhancement system was run between 1 June (day 151) and 1 October 2012 (day 275), between 5 June (day 156) and 13 September 2013 (day 256), and between 8 May (day 128) and 28 July (day 209) 2014. In 2013, there was no modulated enhancement of temperature

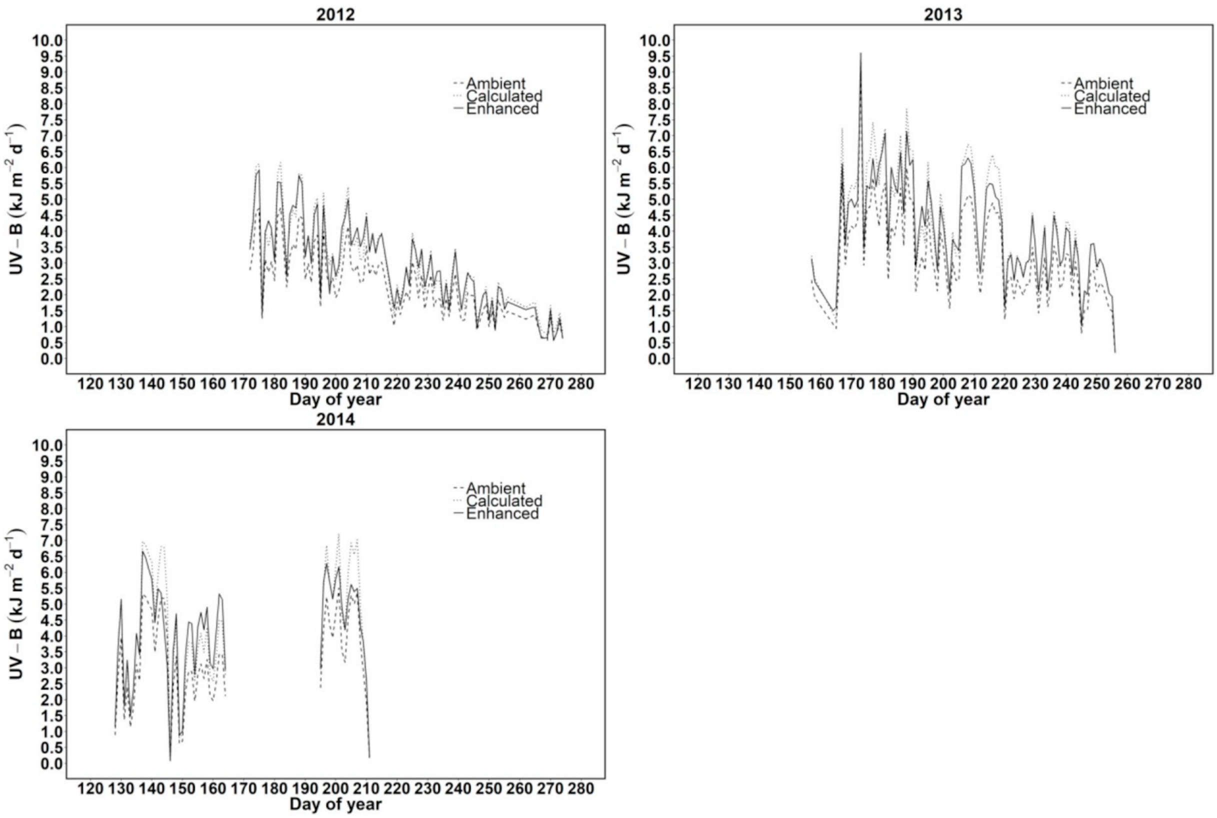

Fig. 1. Performance of ultraviolet (UV)-B enhancement at the experimental site during 2012 (top left), 2013 (top right) and 2014 (bottom) measured by four broadband UV-B sensors. Calculated set-point values are 30% higher than ambient levels, while enhanced values show the performance of the modulated UV-B enhancement.

and UV-B between 13 and 24 July due to a thunderstorm. In 2014, there was no climate data recorded between 14 June and 13 July due to a technical error. Furthermore, the system was switched off on July 28th in the same year, since the aluminum frames could be raised no further with increasing plant height.

2.3. Environmental data

Four ThiesClima sensors (Thies, Göttingen, Germany) were used for measuring the UV-B radiation (250–325 nm with a peak of 300 nm). Two sensors were placed above the control frames for ambient UV-B levels, and two under the frames of UV-B enhancement plots for set-point values. Temperature enhancement modulation was achieved using self-made linear temperature sensors with four PT1000 probe elements fabricated with four connection cables. The set point values were achieved by placing two probe elements above the control frames and two under the temperature enhancement frames. Calculations of set point values and control of enhancement of UV lamps and IR radiators were implemented by a modulator software (IPC100 configuration program and e-console measuring and data saving program, Gantner Instruments GmbH, Darmstadt, Germany). Both UV-B and T was registered and logged every 10 min during the periods the system was running (Figs. 1 and 2). Monthly precipitation measurements were obtained from a meteorological station at Linnunlahti that is situated less than 200 m away from the experimental site (Fig. 3).

2.4. Phenology registrations

We used the scoring system for autumn phenology described in Strømme et al. (2015), which is a simplified version of the scoring system developed by Rohde et al. (2011). The three-stage system used for scoring apices during autumnal bud formation discerns between three stages; growing apex (1), green bud having closed bud scales (0.5), and brown/red mature bud (0). The apical stages for each plant were determined throughout the growing season by observing the terminal end of the primary shoot. As the apices were located on the primary shoots, branches were not considered. In situations where green closed buds broke in autumn and apices resumed growth, apices were scored as growing. Some plants were affected by *Venturia* shoot blight, grazed upon by intruding herbivores or broken by mechanical damage and therefore not included in the apical scoring. Thus, apical stages were recorded only for healthy plants introduced during the same growing season, and the number of plants scored were 672 females and 680 males in 2012, 317 females and 291 males in 2013, and 98 females and 91 males in 2014. In 2012, the first apical scoring was performed on 15 August, while for 2013 and 2014 the first apical scoring occurred on 20 and 12 August, respectively.

The scoring system for spring bud-break stages was based on Fu et al. (2012). The registered stages were as follows: a closed bud (0), a swollen bud or elongated bud with green scales (1), green leaf tips out of the bud with leaf bases hidden (2), broken bud with at least one petiole (3), and an unfolded leaf with visible leaf blade and stalk (4). In 2013, registrations for spring bud break started as soon as the first stage transitions (from stage 0–1) were observed, being on 6 May (day 126), in 2014 on 22 April (day 112), and in 2015 on 4 May (day 124). Spring bud-break stages were recorded every four days for the years 2013–2014 and every second day in 2015.

2.5. Growth registrations

The basal diameter and height of the plants were registered approximately every third week during the growing season. In 2012, the last measurement was done on 25 September (day 269), while the first and last day of measurement were 21 May (day 141) and 3 September

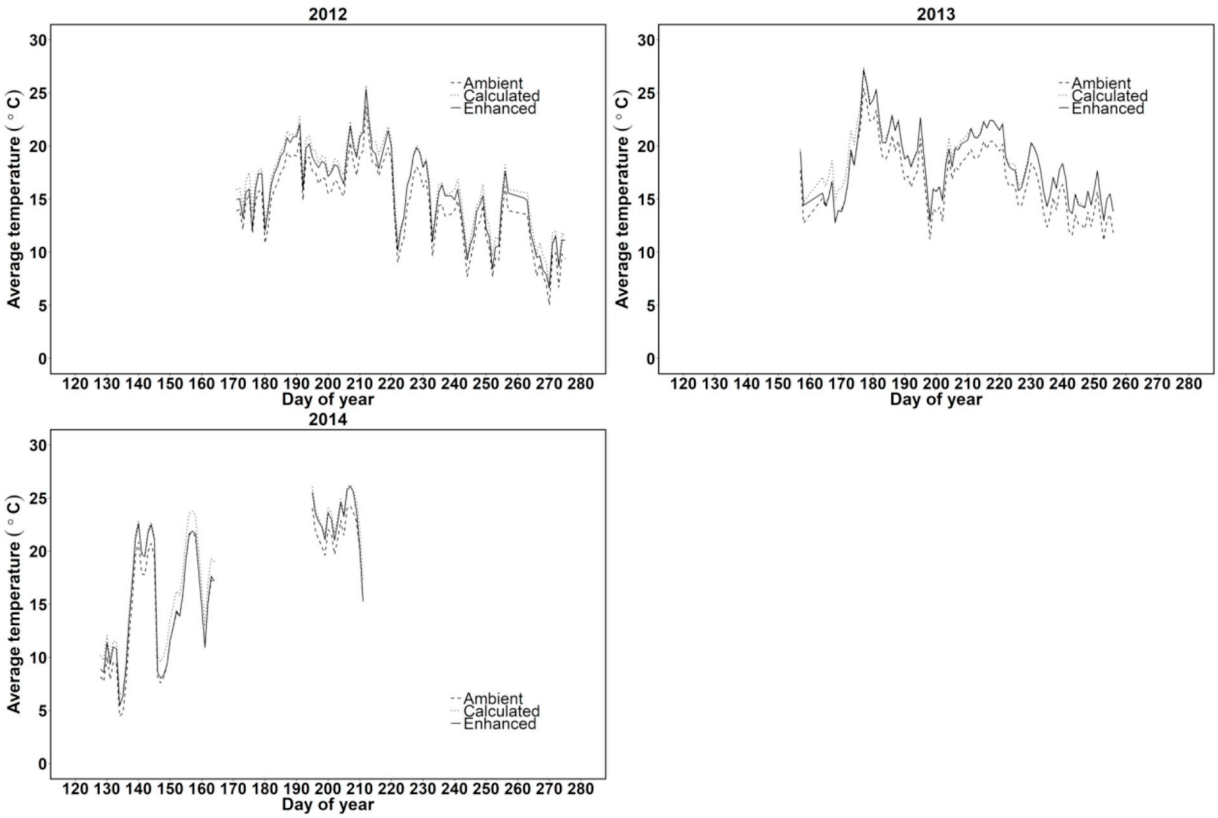

Fig. 2. Performance of temperature enhancement at the experimental site during 2012 (top left), 2013 (top right) and 2014 (bottom) measured by four temperature sensors. Calculated set-point values are 2 °C higher than ambient levels, while enhanced values show the performance of the modulated temperature enhancement.

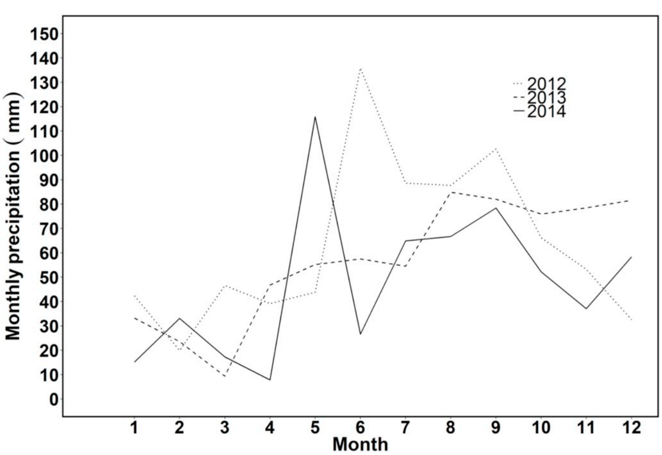

Fig. 3. Total monthly precipitation measured at the Linnunlahti meteorological station in years 2012–2014.

(day 246) in 2013 and 6 May (day 126) and 9 September (day 252) in 2014. To investigate a possible relationship between bud set dates and plant growth, we only used the total growth achieved from spring to autumn during each growing season In order to test a possible relationship between bud break dates and growth, we used growth parameters measured when all plants had completed bud break.

2.6. Statistical analyses

First, we tested the effect of UV-treatment (three levels), temperature treatment (two levels), plantlet sex (two levels) and year (three levels) on bud set dates (day of year) in autumn and date of completed bud break (day of year) in spring using the R software for statistical computing (R Core Team, 2014). Using the same covariates, we also tested for effects on plant height and basal diameter measured at the end of the growing season for the years 2012–2014. In order to investigate whether the duration of the growing season affected plant growth, we also tested for possible relationships between bud set date in autumn and final yearly measurements of height and basal diameter. Furthermore, we also tested for possible relationships between spring bud break and growth in terms of height and basal diameter measured on 10 June (day 161) in 2013 and on 17 June (day 168) in 2014 when most plants had fully broken buds. The selection of appropriate statistical tests and models were partly based on procedures described in Zuur et al. (2009). For each statistical test, the final model was selected based on a global model that included all relevant covariates and their interactions. We applied the dredge function in the MuMIN-package (Barton, 2015) to the global model, and thus obtained a model-selection table where all possible models were ranked based on their respective AIC-values. Thus, each model selection table provided us with the most parsimonious model for each analysis. In the model selection process, we included plant clone (random term) and plot (random term) by using the lmer function in the lme4 package (Bates et al. 2015) as their inclusion yielded improved models based on AIC comparison.

3. Results

Plants grown under temperature enhancement finished bud formation later, but the significant interaction with year reveals that plants under this treatment set buds earlier in 2013 by 6.6 days ($P < 0.001$) and by 8.3 days in 2014 ($P < 0.001$) (Table 1) than in 2012. Plants grown under UV-B enhancement had earlier bud set dates in 2012, but the significant interaction between UV-B enhancement and year as well as the term coefficients reveals that plants receiving this treatment delayed bud set in 2014 by 3.5 days ($P = 0.032$). Male plants grown under UV-B enhancement set buds 1.8 days earlier than females

Table 1
Parameter estimates, SE and t-values for covariates in the linear mixed models used to investigate the effects of elevated autumn temperature, elevated autumn ultraviolet (UV)-A and UV-Bon bud set dates in females and males of *Populus tremula* in three consecutive years (2012, 2013, 2014) and bud break dates during the following spring (2013, 2014, 2015).

	Fixed effects terms	Coefficient	SE	t-value
Bud set date	Intercept***	247.0	2.36	104.9
	Male	0.2	3.04	0.05
	Year 2013***	−5.7	0.88	−6.46
	Year 2014***	−11.0	1.41	−7.73
	UVA enhancement	−0.1	1.36	−0.11
	UVB enhancement	−0.5	1.35	−0.37
	Temperature enhancement***	9.5	1.10	8.65
	Male x UVA	−0.7	0.89	−0.82
	Male x UVB*	−1.8	0.88	−2.07
	Male x Temp*	1.5	0.73	2.04
	Year 2013 × UVA enhancement	−1.3	1.00	−1.26
	Year 2014 × UVA enhancement	2.2	1.61	1.40
	Year 2013 × UVB enhancement	1.9	1.01	1.91
	Year 2014 × UVB enhancement*	3.5	1.61	2.15
	Year 2013 × Temperature enhancement***	−6.6	0.83	−8.00
	Year 2014 × Temperature enhancement***	−8.3	1.32	−6.31
	Year 2013 × Male*	1.8	0.82	2.23
	Year 2014 × Male	0.6	1.31	0.47
Bud break date	Intercept***	142.6	0.17	840.0
	Temperature enhancement*	−0.33	0.14	−2.3
	Year 2014***	0.8	0.10	7.8
	Year 2015***	8.9	0.11	83.5

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

($P = 0.039$), as seen from the significant interaction between plant sex and UV-B enhancement (Table 1). Male plants were also more responsive to temperature enhancement, and finished bud set 1.5 days later than females under this treatment ($P = 0.041$), as shown by the significant interaction between plant sex and temperature enhancement (Table 1). Males had in general later bud set 1.8 days later than females in 2013, but not in 2014 ($P = 0.026$), as shown by the significant interaction between plant sex and year (Table 1).

Bud break in spring occurred slightly earlier (0.3 days) for plants grown under autumn temperature enhancement ($P = 0.026$) (Table 1). Overall, bud break occurred 0.8 days later in 2014 ($P < 0.001$) and 8.9 days later in 2015 ($P < 0.001$) than in 2013. For bud break, there were no significant effects of UV-B enhancement, and there were no significant differences between females and males.

Temperature enhancement yielded more pronounced plant growth both in terms of height and diameter (Table 2). The significant interaction with year shows that this effect was stronger in 2013 ($P < 0.001$) and 2014 ($P < 0.001$) for height increase, and similarly for diameter increase in 2013 ($P < 0.001$) and 2014 ($P < 0.001$) (Table 2).

There was a significant positive relationship between bud set date and plant growth, both in terms of height and diameter (Table 3; Fig. 4). This means that the prolonged growing season affected growth positively. In terms of height increase, there was a significant interaction between bud set date and year, indicating a stronger positive impact of bud set dates on height growth in 2013 ($P < 0.001$) and 2014 ($P < 0.001$), as compared to 2012. There was also a similar interaction for diameter growth, being significantly higher in 2013 ($P < 0.001$) and 2014 ($P < 0.001$).

An earlier start of the growing season was also positive for growth, as there was a positive relationship between early bud break and growth both in terms of height ($P = 0.002$) and basal diameter ($P = 0.001$). This effect was stronger in 2014, as shown by the significant interaction between bud break date and year for both growth responses (Table 4; Fig. 5).

Table 2
Parameter estimates, SE and t-values for covariates in the linear mixed models used to investigate the effect of elevated autumn temperature on plant size in females and males of *Populus tremula* in three consecutive years (2012, 2013, 2014).

	Fixed effects terms	Coefficient	SE	t-value
Height	Intercept***	56.9	8.45	6.7
	Temperature enhancement***	42.5	8.08	5.3
	Year 2013***	59.3	2.57	23.1
	Year 2014***	123.5	4.14	29.8
	Temperature enhancement x Year 2013***	44.8	3.91	11.5
	Temperature enhancement x Year 2014***	63.0	6.23	10.1
Basal diameter	Intercept***	6.4	0.45	14.4
	Bud set date***	2.4	0.48	5.0
	Year 2013***	2.9	0.16	18.1
	Year 2014***	6.4	0.26	24.8
	Temperature enhancement x Year 2013***	2.9	0.25	12.0
	Temperature enhancement x Year 2014***	4.7	0.39	12.1

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

Table 3
Parameter estimates, SE and t-values for covariates in the linear mixed models used to investigate the effects of bud set dates on plant size in females and males of *Populus tremula* in three consecutive years (2012, 2013, 2014).

	Fixed effects terms	Coefficient	SE	t-value
Height	Intercept	24.1	30.36	0.79
	Bud set date	0.2	0.12	1.85
	Year 2013***	−225.7	50.04	−4.51
	Year 2014	−85.1	67.48	−1.26
	Bud set date x Year 2013***	1.3	0.21	6.17
	Bud set date x Year 2014***	1.0	0.28	3.60
Basal diameter	Intercept***	9.6	1.92	5.04
	Bud set date	$-8.1*10^{-3}$	$7.36*10^{-3}$	−1.11
	Year 2013***	−16.0	3.19	−5.03
	Year 2014*	−9.2	4.30	−2.14
	Bud set date x Year 2013***	$8.3*10^{-2}$	$1.30*10^{-2}$	6.38
	Bud set date x Year 2014***	$7.5*10^{-2}$	$1.81*10^{-2}$	4.15

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

In the model selection process for all statistical tests, the interaction term between UV-B enhancement and temperature enhancement was included in the global model. Still, the term was not present in any of the most parsimonious (and thus final) models.

4. Discussion

Our models showed clearly that the climate change mediated lengthening of the growing season was positive for plant growth, both in terms of later bud set dates in autumn, as well as earlier bud break dates in spring. In turn, both UV-B and temperature enhancement affected bud set dates, while bud break dates were only slightly affected by temperature enhancement in autumn. Still, significant interactions between year and enhancement treatments indicate that the plants gradually acclimated to the altered climate, resulting in weaker responses in later years. Considering that most enhancement studies involve exposure lasting a single growing season, it may be questioned whether results of those studies are representative of how young trees interact with climate in nature.

The observed advancement of bud set dates under UV-B enhancement during the first growing season reflects the positive effect of UV-B on the process of bud formation reported in Strømme et al. (2015), where UV-B was found to interact with temperature enhancement and

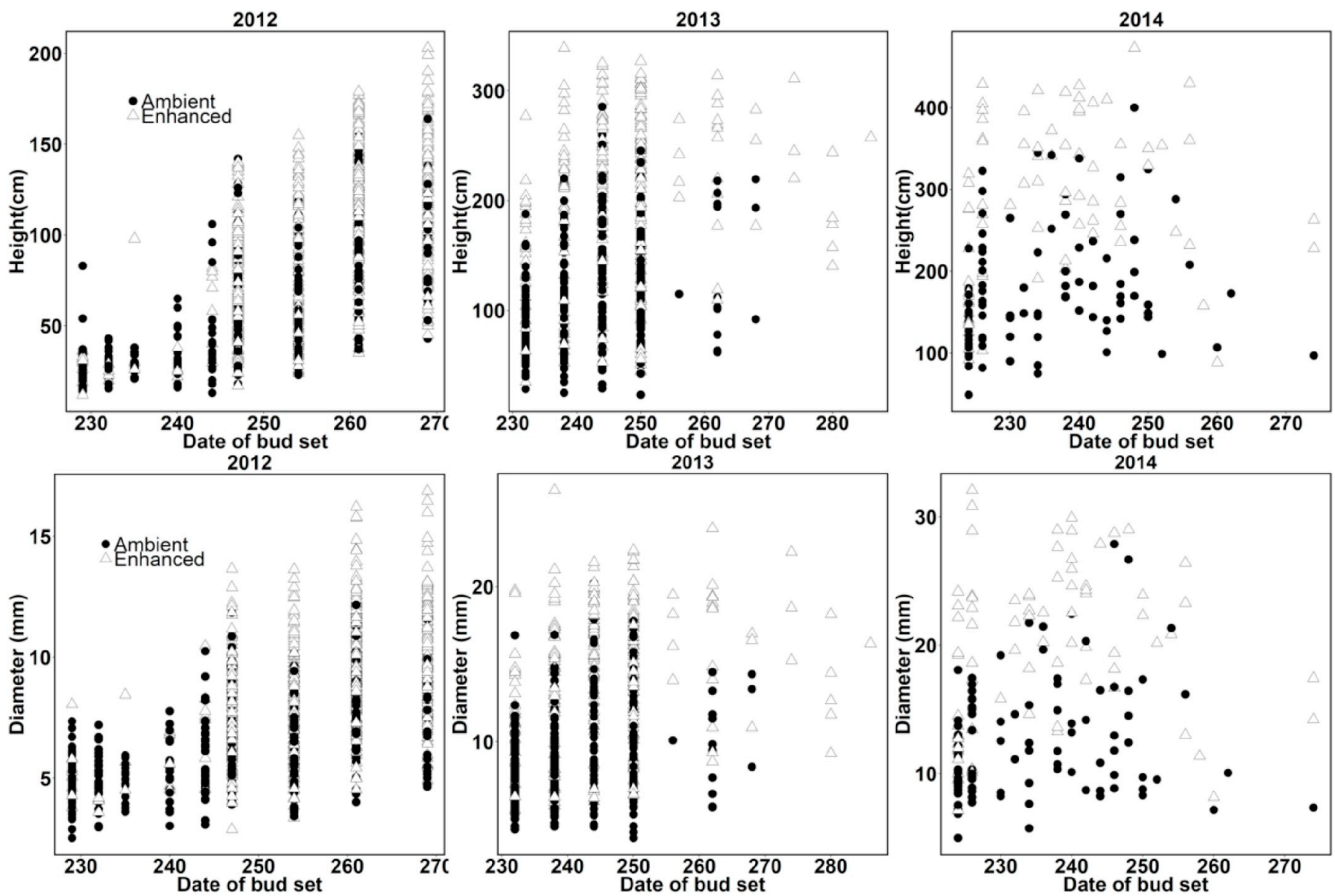

Fig. 4. The relationship between dates of completed bud set and plant growth properties of *Populus tremula* clones grown under ambient temperature and enhanced temperature measured on 25 September (Day 269) for 2012, 3 September (Day 246) for 2013 and 9 September (Day 252) for 2014.

Table 4
Parameter estimates, SE and t-values for covariates in the linear mixed models used to investigate the effects of bud break dates on plant size in females and males of *Populus tremula* in two consecutive years (2013, 2014).

	Fixed effects terms	Coefficient	SE	t-value
Height	Intercept*	362.7	175.38	2.07
	Bud break date	−1.8	1.23	−1.43
	Year 2014**	778.3	236.79	3.29
	Bud break date x Year 2014**	−5.1	1.66	−3.05
Basal diameter	Intercept	6.2	12.60	0.50
	Bud break date	0.02	0.09	0.19
	Year 2014***	58.2	17.02	3.42
	Bud break date x Year 2014**	−0.4	0.12	−3.18

Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.

plant sex in driving the transitions between phenotypic stages. As UV-B has been shown to inhibit thermomorphogenesis in *A. thaliana* (Hayes et al., 2017), it is relevant to further investigate whether similar interactions are found in relation to growth and developmental processes in *P. tremula*. The bud set dates in this study correspond to the final stage of the bud formation process and do not indicate any interactions between UV-B and temperature in this respect. Instead, the data suggest that further studies of autumn phenology in *P. tremula* should consider that responsiveness to higher levels of UV-B or temperature may decrease with plant age.

Although enhanced temperature delayed bud set dates, also this effect varied between years. Available evidence shows that warming delays the process of bud formation (Rohde et al. 2011; Strømme et al. 2017), and it can be argued that earlier bud set dates in 2013 and 2014 may have occurred as a result of colder autumn temperatures. However, temperature data for Joensuu shows that autumn temperatures were similar across years in the period of bud formation (until October 1, day 274) (Fig. 4; Appendices Table 1). Moreover, the significant factor Year in the statistical tests accounted for inter-annual differences in bud set timing for all plants. Thus, the significant interaction between temperature enhancement and year clearly show that plants responded progressively less to temperature enhancement in terms of bud set timing. The explanation may be that the *P. tremula* plants gradually change from a free growth pattern to fixed or predetermined growth at later developmental stages. In woody plants with fixed growth, the bud contains all leaf primordia for the annual shoot, while free growth is characterised by a simultaneous formation of leaf initials (nodes) and elongation of internodes (Olsen, 2010). Under fixed growth, autumnal bud set in plants are little affected by photoperiod, for example (Junttila, 2007). This phenomenon is scarcely described, and the mechanisms behind little studied, but probably deserves more attention in the future if we want to improve tree growth models by including seasonal phenology.

As most studies of climatic responses in tree species have been conducted on juvenile individuals, it remains unclear whether such effects also occur in adult trees. Phase-dependent responses have been shown in relation to spring phenology for some tree species (Hänninen. 2007; Vitasse, 2013), but information related to autumn phenology is scarce. Moreover, there is little evidence of responses to climatic manipulations occurring over several years, but within the same ontogenic phase. Even though our data were obtained using clones originating from adults through micropropagation, the plants grown in the field

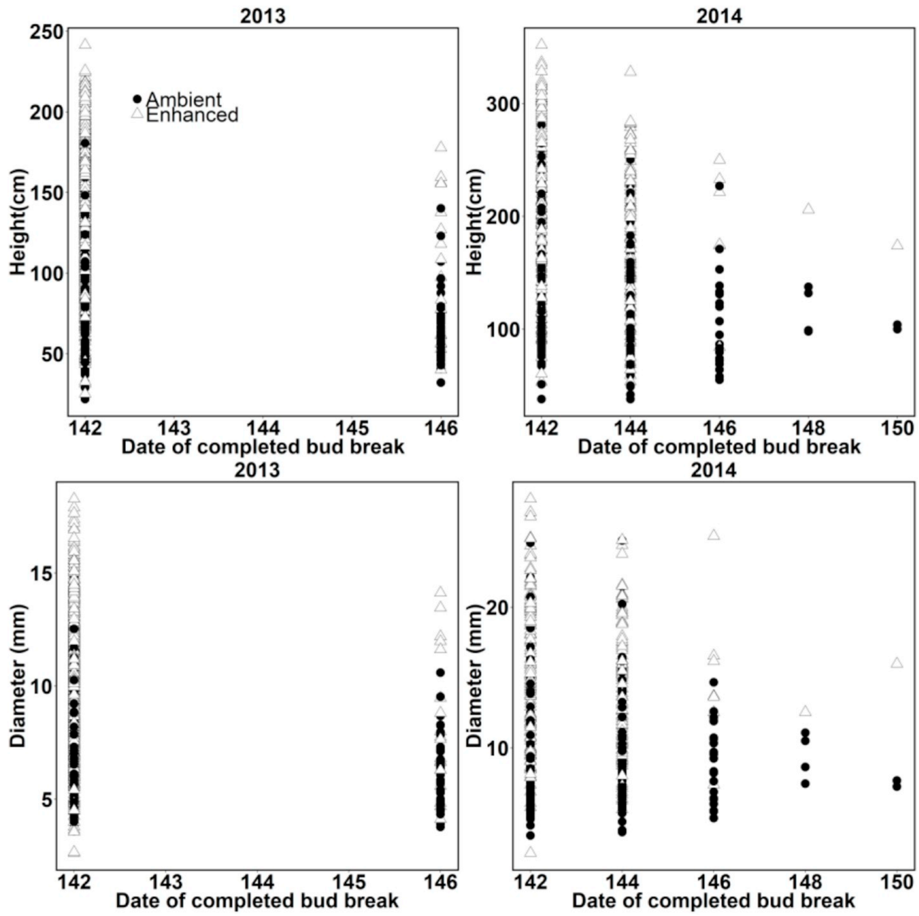

Fig. 5. The relationship between dates of completed bud break and plant growth properties of *Populus tremula* clones grown under ambient temperature and enhanced temperature measured on 10 June (Day 161) for 2013 and 17 June (Day 168) for 2014.

were at a juvenile stage. Thus, our results indicate that bud set in juvenile trees of *P. tremula* become increasingly less susceptible to temperature modulation in the years following planting. In this respect, there is need for further research that spans more than three years in order to verify whether individuals become progressively less susceptible to such warming effects.

Insufficient chilling has been suggested to yield delayed bud break the following spring (Hänninen and Tanino, 2011), and possibly be a carry-over effect from high temperatures during the previous autumn. Bud break in spring was positively affected by autumn warming, and this effect did not differ across the year (no significant interaction between temperature treatment and Year). Thus, our data does not suggest any such carry-over effect across seasons, as buds most likely received sufficient chilling in winter. However, the possibility of warming yielding insufficient chilling should be tested for *P. tremula* in field conditions where winter climates are substantially warmer in order to verify whether such effects may occur in nature.

The growth responses across years show that temperature effects on height and basal diameter increase were progressively higher with each year. This could be an indication of cumulative effects of warming on plant growth, where positive effects of warming in one year add to warming effects in the next. Indeed, warming yielded increased specific leaf area and leaf nitrogen content and assimilation rates in 2012 (Randriamanana et al. 2015), which may have resulted in higher nutrient storage in stems to be mobilized in spring. Since we did not measure root growth, we cannot account for any warming effects on gross water and nutrient uptake. As root growth occurs above a minimum temperature (Schenker et al. 2014), it could be argued that warming had a positive effect in this respect. However, this is contradicted by an analysis based on 63 different studies that shows no increase in root growth under increased temperature, and instead shows decreased biomass allocation to roots (Way and Oren, 2010).

In addition, the duration of the growing season was clearly positive for growth in terms of both height and basal diameter. On the one hand, the positive relationship between bud set date and growth shows that delaying bud set was positive for plant growth. On the other hand, the negative relationship between bud break date and growth shows that earlier bud break was beneficial to plant growth. The significant interaction between date and year for both processes shows that plants benefitted more from an extension of the growth period over time both in terms of height and basal diameter growth. This may be due to bud set occurring progressively earlier, and bud break progressively later, with each year, indicating that an extension of the growing season would occur in a warmer period than in 2012. In this respect, our data indicate that *P. tremula* benefits from an extended growing season if temperatures in the extended period are sufficiently warm to sustain growth.

Winter buds represent a vital physiological adaptation for plants to survive freezing temperature (Welling and Palva, 2006; Gusta and Wisniewski, 2012), and there is evidence of earlier bud break resulting in frost damage for a number of deciduous species (Augspurger, 2009). Moreover, a higher susceptibility of juvenile trees to frost damage than adults has been shown for some species (Vitasse et al. 2014). We found no evidence of frost damage on shoot tips, neither in late autumn nor in spring, throughout our study.

In conclusion, our data show that growth in the deciduous tree *P. tremula* benefits from an extended growing season, both due to delayed bud set and earlier bud break. In terms of bud set timing under experimental UV-B and temperature increase, the analysis covering three growing season shows that plants became less responsive to the treatments with increasing age, which is possibly a consequence of acclimation. Moreover, plants showed a gradual shift in timing of bud set and bud break dates with increasing age irrespective of treatments, showing that timing of these processes in plants introduced the first year may differ substantially from observations in the following years. Considering that most tree species have lifespans covering decades and in many cases centuries, their capacity for short- and long term acclimation to shifting climatic conditions is far from understood. Thus, our understanding of tree responses to light and temperature shifts should clearly benefit from further field studies that span over several years.

Appendices

Table A1
Monthly mean temperatures (provided as ˚ C) in Joensuu obtained from the Finnish Meteorological Institute.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2012	−8.8	−13.3	−3.4	0.6	9.7	13.5	17.4	14.1	10.5	4.1	0.6	−11.0
2013	−8.4	−4.4	−10.4	1.1	12.0	17.4	16.9	16.2	10.6	4.5	1.6	−1.8
2014	−10.2	−1.7	−0.1	2.8	9.7	13.2	19.1	16.7	10.5	1.8	−0.1	−4.0
2015	−7.8	−3.2	−0.8	2.5	9.2	13.6	18.1	15.1	11.6	4.1	1.5	−0.9

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