

PHENOLOGICAL DEVELOPMENT OF ASPEN (*Populus tremula* L.) UNDER
CLIMATE CHANGE

UNNIKRISHNAN SIVADASAN

Master's thesis
University of Eastern Finland
Department of Biology
2016

UNIVERSITY OF EASTERN FINLAND

Department of Biology

UNNIKRISHNAN SIVADASAN: Phenological development of Aspen (*Populus tremula* L.) under climate change.

M.Sc. Thesis, pp.39

August 2016

ABSTRACT

The climate change, which is predicted to occur over this century, will increase the temperature and UV radiation. We examined how the single and combined treatments of elevated temperature and UV affect bud phenology of *Populus tremula* in a multifactorial outdoor experiment. This study mainly focuses on the impact of treatments on the bud set of *Populus* for the year 2013 and bud burst for the year 2014. Aspen plantlets for the experiment were originated from Eastern and Southern Finland. The aspen plantlets were planted in the field site in Joensuu, Finland (62° 60' N, 29° 75' E) in 11th June 2012. During 2013, 290 females and 319 male plants were scored for bud set and in 2014, 273 females and 309 males were scored for bud break. Female and male clones were distributed among 36 plots having 6 replicate plots of different treatments. UV-A and UV-B forced bud set in female clones in the year 2013. In 2014 bud break was delayed under UV-A and UV-B treatments. Elevated temperature had no significant effect on both bud break and bud set. No impact of counter action of UV-B and T was seen in the bud development. UV-A+T did not force or delay the bud break and bud set. Gender specific variations were observed in the bud phenology of *P. tremula* plants. Responses of *P. tremula* to different treatments altered as the plants aged.

TABLE OF CONTENTS

1 INTRODUCTION.....	4
2 PHENOLOGY AND INFLUENCING FACTORS.....	6
2.1 Phenology.....	6
2.2 Temperature and Photoperiod.....	7
2.3 UV radiation.....	10
3 OBJECTIVES AND HYPOTHESES.....	12
4 MATERIALS AND METHODS.....	13
4.1 Plant material.....	13
4.2 Experimental setup.....	14
4.3 Recording of Bud stages.....	16
4.4 Statistical analyses.....	17
5 RESULTS.....	17
6 DISCUSSION.....	22
7 CONCLUSIONS.....	25
ACKNOWLEDGEMENTS.....	25
REFERENCES.....	25

1 INTRODUCTION

Global mean temperature (T) is predicted to rise by 3 to 4.5°C by the end of this century due to the man-made greenhouse effects (Jacob et al. 2013, IPCC 2013). Temperature affects both plant growth and phenology. Recent researches show that temperature also affects growth cessation and bud set in different boreal tree species (Westergaard & Eriksen 1997, Heide 2003, Sjøgaard et al. 2008). From the recently conducted experiments it is clear that the temperature influences the flushing dates of plants (Fu et al. 2012). In some studied *Populus* species higher autumn temperatures delayed growth cessation and bud set (Kalcits et al. 2009, Rohde et al. 2011a).

Long-term depletion of the stratospheric ozone layer contributes to an increase in terrestrial solar ultraviolet-B radiation. This can result in wide variety of morphological and physiological responses in plants, which could be regarded mainly as acclimation (e.g. Mpoloka 2008). UV radiation is also identified as an important environmental signal modulating the growth and development in plants (Rozema et al. 1997, Caldwell et al. 2007, Ballaré et al. 2011, Jansen & Bornman 2012). Although the greenhouse gas emissions are controlled it will take decades to recover the ozone layer which is also dependant on several other external factors. Female and male plants respond divergently to increased UV-B radiation (Xu et al. 2010, Nybakken et al. 2012, Randriamanana et al. 2015a). In addition elevated temperature and UV-B radiation as combined can result in numerous responses (e.g. Ballaré et al. 2011, Nybakken et al. 2012, Randriamanana et al. 2015a). Even though less harmful than UV-B, UV-A penetrate deeper into leaves producing reactive oxygen species (Wilson et al. 2001). UV-A radiation also has impacts on the photosynthesis of plants (Sicora et al. 2006).

Populus species are widely used as model organisms among woody plants in experimental botany. It is currently a main model system for genetic, genomic and physiological research in trees. A representative of the genus, *P.tremula* is widely distributed in Finland. Poplars and aspens are of great ecological importance as large number of organisms including several endangered species is found in association (e.g. Lindroth 2008). Aspen species along with its hybrids are of great economic importance as they are used as resources for pulpwood, logs and energy wood. Numerous studies on climate change are conducted with aspen as it has a circum-boreal range largely overlaps with areas where drastic climate change is predicted to happen (IPCC 2007). Climate influences the structure and

function of forest ecosystems and plays an essential role in forest health. Along with adaptations to climatic conditions *Populus* also exhibits sex related responses to changing environmental factors (Rohde et al. 2011a). There is also a concern regarding the vulnerability of *Populus* populations following climate change due to the differences in performance and survival between females and males (Tognetti 2012).

Phenology is the study of the timing of individual processes in relation to environmental fluctuations. The timing of phenological events can be quite sensitive to different environmental conditions. Understanding the phenological responses of a plant is a key aspect to unravel impacts of climate warming (Lechowicz 1995). These include flushing or budburst, flowering, fruiting and autumn leaf-fall. Today, this well-established “science of the seasons” is also used by scientists to track the effects of global warming and climate change on organisms and to make predictions about the future health and function of natural and managed ecosystems. Bud burst phenology is one of the key drivers of ecosystem structure and functioning, and it is sensitive to global change. Phenological observations are therefore integrative measures of the condition of the physical, chemical, and biological environment (Haggerty & Mazer, 2008). Phenological responses of the trees could vary with the expected increase in autumn and spring temperatures due to climate change (Körner & Basler, 2010, Tanino et al. 2010, Hänninen & Tanino, 2011, Vitasse & Basler, 2013).

Plants harmonize their physiological process to seasonal changes through accurate sensing of environmental cues. Among these day length, temperature and light quality are the major trait moderators in plants. Plant performances during environmental change still stay unpredictable as they depend on their functional timings to different seasons (Ibáñez et al. 2010). The timing of bud burst relative to local climate conditions is very important for perennial plants, since if the buds are released too early they may suffer frost damage, which would mean a significant loss of resources or even death. On the other hand, if the buds are released too late they might find themselves at a competitive disadvantage to better timed individuals (Pellis et al. 2004). All over the world, the timing of the phenological events is shifting, and these shifts have been linked to recent global warming (Parmesan & Yohe, 2003, Root et al. 2003, Menzel et al. 2006). The disparities between advanced spring phenology and delayed autumn phenology could lead to very different responses in community dynamics and ecosystem processes. As climate-driven changes in phenology are becoming more apparent, the need to quantify these changes is becoming important nowadays. The initiation and the

progress of acclimation and the onset of growth are mediated by temperature and photoperiod (Polgar & Primack, 2011). In Europe, particularly during the onset of spring, global warming is altering the phenology of temperate trees (Cleland et al. 2007).

2 PHENOLOGY AND INFLUENCING FACTORS

Phenology

Phenology is a division in plant ecology that has been largely overlooked by plant ecologists (Cleland et al. 2007). Bud burst phenology is a fundamental phenomenon of tree survival and growth, a key driver of ecosystem structure and functioning, and it is sensitive to global change (Sakai & Larcher, 1987). Growth and dormancy are some of the environmental adaptations of plants to seasonal changes (Rohde et al. 2011b, Vitasse et al. 2014).

Life cycle processes in trees are dependent on the local climate they grow in. Early bud burst results in the early expansion of vegetative tissue and is advantageous to produce biomass but this will increase the risk of frost damage from late-spring freezing temperatures (Heide 2003). Plants have mechanisms to use photoperiod and temperature cues to balance the benefits of early bud burst (Murray et al. 1989, Hänninen 1995, Guak et al. 1998). But the changes in microclimate or prevailing landscape climate will also alter the balance within and among species (IPCC 2001).

Forest leaf coloration obtained from remote sensing data between 35°N and 70°N in northern hemisphere show that the length of the growing season has increased by 5 days on average per degree Celsius temperature rise between 1981 and 1991 (Zhang et al. 2004). Trees will be damaged if buds are not setting soon enough and a required amount of frost hardiness develops before the first frost of autumn. Late frost could kill the growing tissues too. In the case if bud set occurs too early in the fall or bud burst occurs too late in the spring, trees will have a shortened growing season, which reduces its competitive ability and growth potential. So the timing of bud set and bud burst has many implications for adaptation of trees to their natural environment and for tree breeding. Thus alteration in the temperature levels due to climate change will have drastic effects on the forests.

Certain evidences for the irregularity of bud set and bud break have already been visualized. In late 1930's more than 490,000 km² of white and yellow birch forest in eastern Canada and northeastern USA were damaged as a result of late winter thaws followed by spring frost is a dramatic example (Balch 1953, Braathe 1995). Earlier spring bud burst and later autumn senescence have lengthened the growing season by ~11 days since the 1960s (Menzel & Fabian, 1999). In boreal climates, the frequency and probability of such incidences are likely to increase with climatic warming.

Variation in plant phenology can be a major determinant of the distribution of herbivores on their host plants (Hunter 1992, Feder et al. 1993, Mopper & Simberloff, 1995). Differences in bud break phenology could also shift into season long differences in foliage quality (Kleiner 1989). Variation in the phenology of leaf availability can also be critical for insects that have a limited window of time for their complete development (e.g. Koski & Sievänen, 1985, Quiring 1994, Moore et al. 2000, Tikkanen & Julkunen-Tiitto, 2003).

There are even differences seen in plants from different regions in a common environment. Thus the correlation of latitude and place of origin in timing of bud set makes the climate change effects more regional and multitudinous. Bud break and bud set, show also strong genetic differentiation along latitudinal and altitudinal clines, typically resulting in locally adapted ecotypes (Howe et al. 2003, Savolainen et al. 2007, Aitken et al. 2008). If the growing seasons are increasing it could result in the carbon sequestration thereby increasing the terrestrial carbon sink and cutting down the atmospheric carbon dioxide concentrations (Way 2011).

Temperature and Photoperiod

As an impact of climatic warming temperature is predicted to increase in the coming decades (IPCC 2007). Temperature can affect the bud set and bud break in numerous ways (Olsen 2010, Tanino et al. 2010, Junttila & Hänninen, 2012). Different species rely on temperature and day length cues for their growth cessation (Pauley & Perry, 1954, Wareing 1956, Howe et al. 1996). Elevated temperature modifies the sensitivity to photoperiod signals for growth cessation and bud formation in *Populus* (Rohde et al. 2011a). Temperature is also a key driver of inter-annual variability in growth onset. Most temperate deciduous trees only initiate bud break after a considerable number of thermal hours (Lechowicz 1984).

Late season phenological events, like senescence and dormancy are also influenced by temperature. Higher temperatures did slow down the speed of chlorophyll degradation during leaf senescence in *P. tremula* (Fracheboud et al. 2009). In hybrid poplar, warm nights and low day temperature differences promoted deeper winter dormancy and cold hardiness (Kalcsits et al. 2009), implying that the temperature changes predicted by global climate models might enhance dormancy. The current scenario of global warming due to climate change is characterized not only by increasing mean temperatures, but also by decreasing differences between day and night temperatures, particularly in northern areas (Karl et al. 1993, Easterling et al. 1997, Beaubien & Hamann, 2011).

Climate warming is expected to have a contrasting effect on cold winter temperatures (chilling) and spring warming (forcing) which could result in alterations in bud set and bud break. The upper temperature limit for breaking of bud dormancy has been found to be as high as 12 °C both in Norway spruce (Hänninen 1990) and two species of birch (Myking & Heide, 1995).

In several deciduous species such as white birch, downy birch, black alder, Norway maple and *Populus* as well as in conifer species like Norway spruce high temperature under exposure to short days has been reported to result in accelerated winter bud formation and dormancy acquisition as well as deeper dormancy compared to relatively low temperatures (Westergaard & Eriksen, 1997, Heide 2003, Junttila et al. 2003, Søggaard et al. 2008, Kalcsits et al. 2009).

Dormancy is defined as the temporary suspension of the visible growth of any plant structure containing a meristem (Lang 1987). Endodormancy is a genetically controlled set state of inactivity while eco dormancy is a state of inactivity imposed by unfavorable environmental conditions. Endodormancy is also called the true dormancy as the factors within the meristem prevent the growth. For e.g. failure in the bud burst due to insufficient chilling even if it is exposed to warm condition. Ecodormancy is also known as the standby mode where the plant awaits the exact environmental stimuli. Temperature extremes and lack of water are two unfavorable environmental conditions. Global warming will result in negative effects in endodormancy by delaying the dormancy break, as the low temperature periods will become less. In another way as a positive effect on ecodormancy there will be promotion of growth and increase in growing season (Ueno et al. 2011). Kalcsits et al. (2009) reported that high temperatures during endodormancy induction in poplar lead to deeper endodormancy and

delayed bud burst in the spring. Specific temperatures are also capable of inhibiting or causing the break of the dormancy, by acting on endo- or ecodormancy (Sugiura & Honjo, 1997). It has been also suggested that night temperature might affect growth cessation, bud set and dormancy more than day temperature (Tanino et al. 2010). Variation in the influence of temperature can be seen according to the level, intensity, and stage of bud development (Couvillon & Erez, 1985).

Boreal and temperate forests are already inherited with the annual variations in temperature but even more is expected in near future (Menzel & Fabian, 1999, Parmesan & Yohe, 2003, Cleland et al. 2007, Hänninen & Kramer, 2007, Morin et al. 2009). Deciduous trees in arid regions depend more on precipitation events for optimizing phenology (e.g. Jolly & Running, 2004). Adaptive capabilities and geographical distribution of boreal trees have already been challenged by temperature. Compared to the normal events, earlier bud break, leafing, flowering, and fruiting in the spring or summer, and delayed autumn leaf coloration have been seen across Europe, Asia, and North America (Chuine & Beaubien, 2001, Zhang et al. 2004, Menzel et al. 2006, Delbart et al. 2008, Nordli et al. 2008, Gordo & Sanz, 2010). Urban areas where temperatures are commonly 1°–3°C higher had already visualized bud burst occurring 7 and 9 days earlier and dormancy onset 2.5 and 4 days later both in Europe and North America (Zhang et al. 2004).

Upon continued warming, extensive migration of plant species to higher latitudes and elevations is also predicted. Certain alpine tree line changes have been observed over the last century (Harsch et al. 2009). Compared to plants in lower temperature, higher temperature plants form earlier winter buds, show better bud development and deeper dormancy during exposure to short days (Junttila et al. 2003, Olsen et al. 2014). How and to which degree warming influence the timing and depth of winter dormancy in trees is still unclear, and may differ between species and ecotypes (Way 2011).

Photoperiod dependent species could be in trouble as they will not be able to increase their growing season when the temperatures gradually increase and day length stays unchanged (Way 2011). In European beech it is claimed that photoperiod can have a negligible effect on bud burst (Falusi & Calamassi, 1990) and in contradictory it was also found that the photoperiod might influence the accumulated forcing temperature for bud burst (Wareing 1953, Heide 1993). Short photoperiod delayed bud burst in *Abies alba*, *Picea abies*, *Quercus petraea* and *Tilia cordata* (Basler & Körner, 2012). Numerous responses like growth cessation,

terminal bud set and development of bud dormancy are exhibited in woody trees and shrubs as response to decreasing photoperiod (Nitsch, 1957, Vince-Prue, 1975). Elevated temperature could delay the autumn senescence too (Rosenthal & Camm, 1996). Rohde et al. (2011a) described that temperature affected the time between growth cessation and bud set, with warmer temperatures hastening the process of bud development. So rising temperature will result a delay in growth cessation and accelerate bud development in poplar. Heide (2003) had found out that the elevated temperature during short day dormancy induction delayed bud burst the following spring, which indicated that warmer temperatures had increased the depth of dormancy in *Betula pendula*, *Betula pubescens* and *Alnus glutinosa*. The same phenomenon was also seen in *Picea abies* and *Acer platanoides* (Heide 1974, Westergaard & Eriksen, 1997, Granhus et al. 2009).

UV radiation

UV radiation plays its role as a stressor, developmental signal and could cause physiological changes in plants. UV radiation sensitivity can result in a decrease in leaf area and biomass, and changes to flowering, reproduction and competition (e.g. Caldwell et al. 1995). Responsiveness to UV dose decrease with age in leaves of plants (Hunt & McSeveny, 2000). Studies reveal that UV-B light is not only detrimental to plants but also constitutes an important developmental signal (Jenkins 2009). In *Populus*, reduced elongation growth, leaf expansion, and biomass production was observed in response to increased UV-B levels (Ren et al. 2006, Xu et al. 2010). UV-B effects also vary latitudinally and species from higher altitudes are more tolerant to UV-B. For instance *Populus*-species originating from an altitude of 3500 m (*P. kangdingensis*) accumulated twice more methanol extractable UV-B absorbing compounds than a species originating from 1500 m (*P. cathayana*) (Ren et al. 2006).

Developmental changes such as thicker leaves, shorter petioles, leaf curling, alterations in leaf shape and width, decrease in stem elongation, increased axillary branching and altered root: shoot ratio may result as an effect of UV-B (Robson et al. 2014). Biosynthesis of flavonoids and other UV-B-absorbing phenolic components are stimulated by UV-B as a result of protective responses (Jansen 2002, Jansen & Bornman, 2012, Jenkins 2014). The content of certain flavonoids and a range of other antioxidants were up regulated in Norway spruce during short day exposure to UV-B (Lee et al. 2014). Strømme et al. (2015) had found that the bud break and bud set in *P.tremula* was accelerated by UV-B. Newsham et al. (1999) in a 30% UV-B elevation study had also visualized a transient effect of retardment in bud burst of *Quercus*

rober. Moreover UV-B had shown to increase flowering duration in *Phacelia campanularia* and *Phacelia purshii* (Conner & Neumeier, 2002) and delayed flowering time in *Limnanthes alba* (Sampson & Cane, 1999) and delayed or no change in flowering phenology (Kakani et al. 2003).

So as a whole forthcoming climatic change could result in fluctuations in temperature, light and UV radiation levels. This can severely affect the plant phenology, as they are mostly dependent on the environmental signals for their growth and development. This thesis is trying to seek how both bud break and bud burst of *P. tremula* are affected under temperature, UV-B, UV-A and their combined treatments.

3 OBJECTIVES AND HYPOTHESES

The main aim of the study was to measure the bud set and bud burst variations in *P. tremula* under enhanced temperature and UV-B treatments. Interactive effects of both temperature and UV-B were also assessed. Our hypotheses were:

1. Elevated temperature will delay the bud set.
2. Elevated temperature and UV-B will counteract on the bud development (bud set and bud break).
3. UV-A may influence the bud phenology.
4. Bud phenology may vary between the genders.

4 MATERIALS AND METHODS

4.1 Plant Materials

Aspen plantlets used in this experiment originated from Eastern and Southern Finland. They were from six male and female aspen individuals about 30-40 years old. Each genotype was collected from the locations: Kaavi 62°43' N, 28°42' E, Liperi 62° 41'N, 29° 33'E, Loppi 60° 43'N, 24°27'E, Pieksämäki 62°18' N, 27°07' E, Polvijärvi 62°52' N, 29°19' E and 62° 49'N, 29°20'E, Kontiolahti 62°38' N, 29° 41' E. Geographical distance was maintained in order to have the most variation among aspen genotypes. The growth medium used for micro propagation was a woody plant medium with 8.5 g L⁻¹ Agar and 5mg L⁻¹ indole butyric acid. Fluorescent tubes (Gro- Lux F36W, Havells Sylvania, Germany) of photon flux density, 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 400-750 nm were used to provide light at 23 \pm 0.1⁰ C and 18h photoperiod.

Before transferring to the green house on 2nd May 2012, plantlets were potted up with 70% commercial peat and 30% vermiculite and acclimated to relative air humidity 60%. High-pressure sodium lamps (GE Lighting, Cleveland, OH, USA) of 400W were used for enriching the light conditions. Temperature was set at 20⁰ C and the photoperiod was 18h. Due to additional warming of the lamps, there were temperature fluctuations between 20 and 23⁰ C, depending on the time of day. The plantlets were transferred to the field site in Joensuu, Finland (62°60' N, 29°75' E) on 7th June and planted on 11th June. Mortalities in plants occurred due to *Venturia* shoot blight and mechanical and herbivore damages. During 2012, 814 females and 838 males were recorded for bud set. During 2013, 667 females and 671 males were scored for bud break, and 290 females and 319 males for bud set. Reductions in the individual numbers are due to the harvesting of one individual per clone for different analyses every year.

4.2 Experimental setup

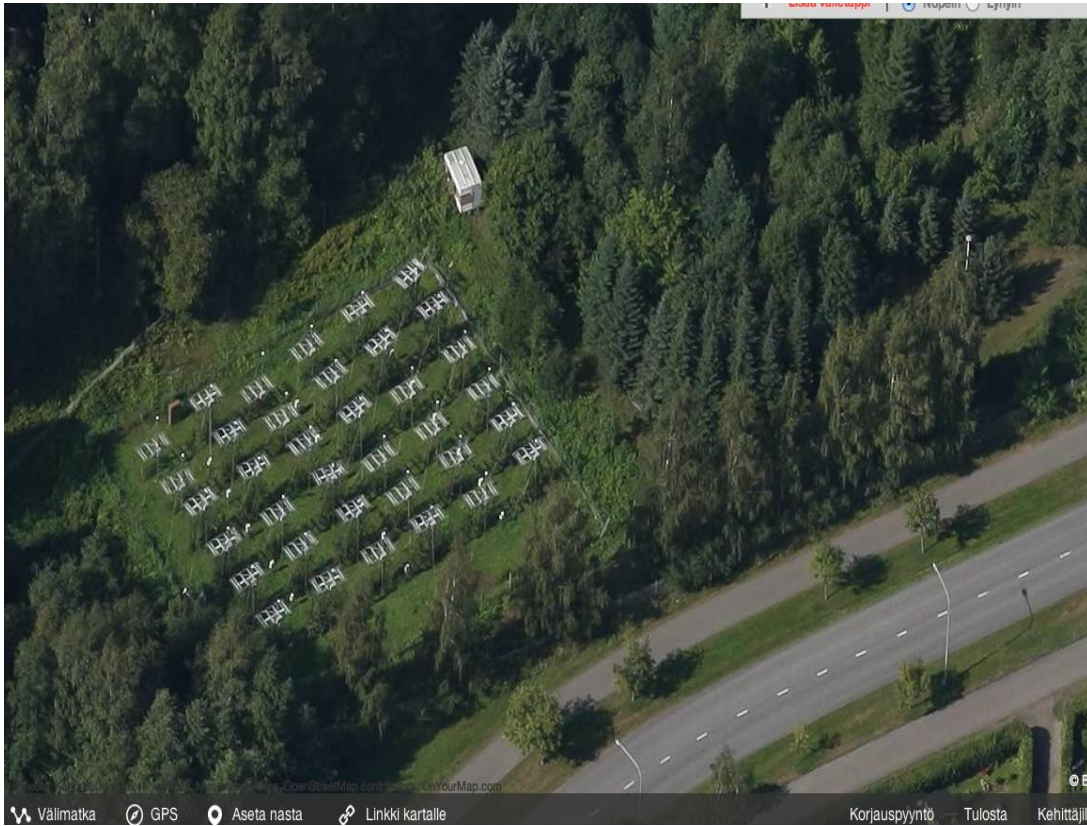


Fig.1. Satellite view of the experimental field in Botania, Joensuu.

P. tremula male and female clones were distributed among 36 plots in a 6×6 matrix. Each plot was added with a 10 cm layer of 0.8% limed mineral soil. Designing of the plots were in such a way that the spacing between them were 3m in all directions. Adjustable aluminum frames (1.5× 2.0 m) were bolted on metallic posts. Metal sheet shelter was implanted 60 cm into the soil and 60 cm above the soil level to prevent vole intrusion. A metal net fence of 1.5 m was structured around the experimental plot to prevent the large mammal invasions. Plants within each plot received one of the six different treatments and treatment combinations. They were increased temperature T, enhanced UV-B, and enhanced UV-A, UV-B+ T, UV-A+T and control with ambient temperature and UV radiation. The enhanced levels of T and UV were continuously regulated to +2°C and 30% increase, respectively.

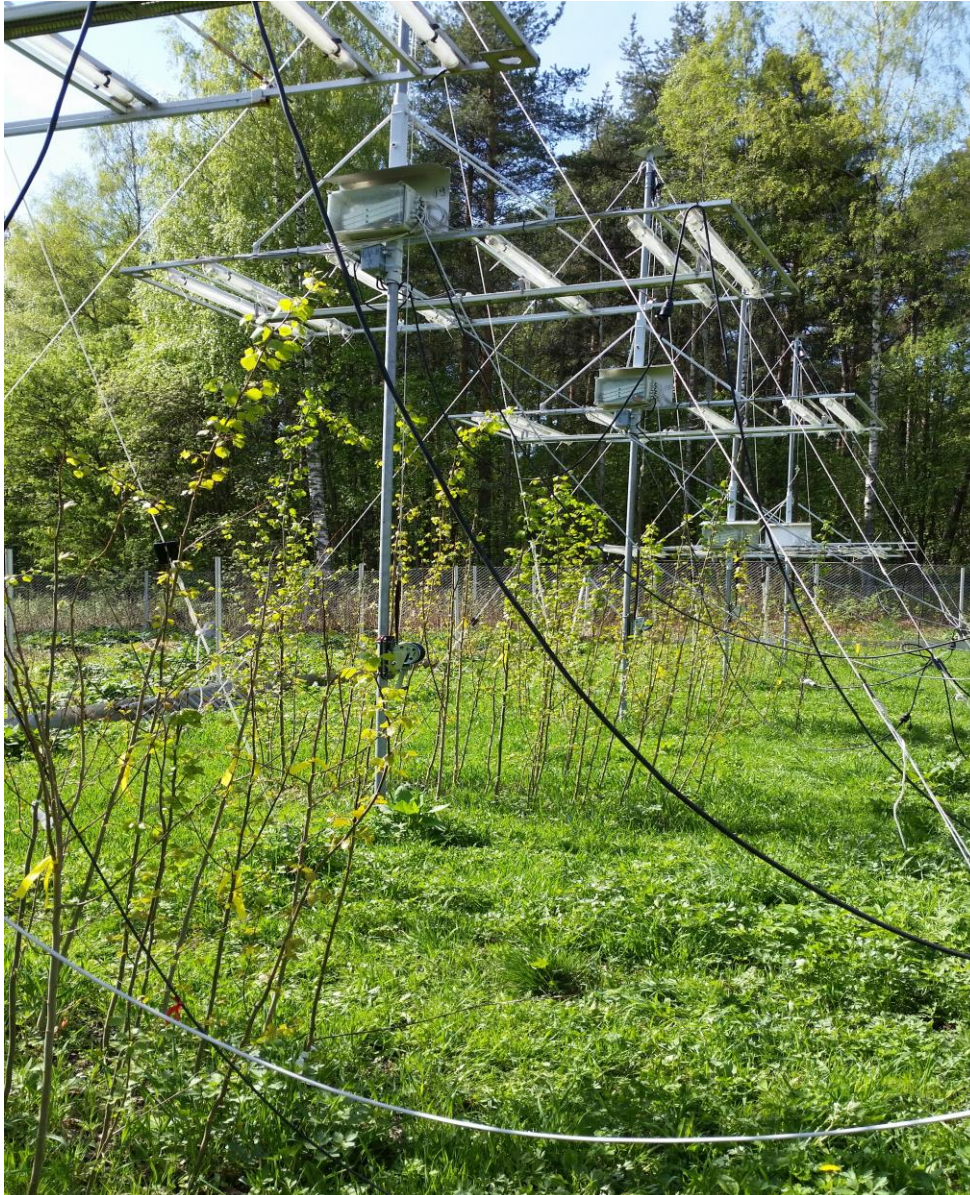


Fig.2. *P. tremula* plants under different treatments.

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) and kept at minimum 60 cm above the plant shoot tips. An Optronic OL-756 portable UV-VIS spectroradiometer (Optronic Laboratories, Orlando, FL, USA) was used to obtain the emission spectrum. Cellulose diacetate filters were wrapped around each lamp to debilitate the radiation below 290 nm in UV-B enhanced treatment plots. In six plots the UV tubes were wrapped with polyester film in order to remove UV-B, so that only the enhanced levels of UV-A are achieved. In unenhanced UV plots lamps were un-energized to procure the same level of shading as in enhanced plots. Two infrared (IR) heaters (CIR 105, FRICO, Partille, Sweden) were bolted along the middle axis of the aluminum frames for continuous temperature

enhancement. In unenhanced temperature plots IR radiators were replaced by wooden boards to attain the same shading levels. The frames were lifted every third week to maintain the 60 cm distance between the highest shoot tip and the radiators. The whole system started working in June 2013 to October 2013.

Four Thies Clima sensors (Thies, Göttingen, Germany) were used for measuring the UV-B radiation. These sensors measured the radiation between 250 and 325 nm with a peak of 300 nm. Two sensors were placed above the control frames ambient UV-B levels, and two under the frames of UV-B enhancement plots for set-point values. Temperature enhancement modulation was done using the self-made linear temperature sensors with four PT1000 probe elements with four connection cables. Set point values were gained 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 a using modulator software (IPC100 configuration program and e-console measuring and data saving program, Gantner Instruments GmbH, Darmstadt, Germany).

4. 3 Registration of bud set and bud break

The autumnal bud set was recorded from August 20th until October 19th 2013 at five days interval. Bud set was followed based on the instructions of Rhode et al. (2011b). First stage includes apices between full, active growth to apices with an open bud (1), closed green bud (0.5), and brown/red closed bud (0) (Fig.3).

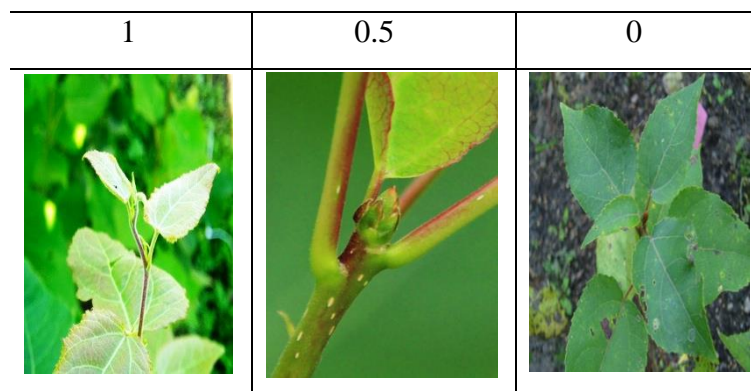


Fig.3. Different bud set stages

The spring bud break was scored from April 22nd to June 25th with an interval of two days, and was based on Fu et al. (2012). Bud break was classified into four stages: closed bud (0), closed bud with visible green leaf tip (1), green leaf diverging from bud axis but no visible petiole (2), broken bud with at least one visible petiole (3), at least one fully expanded leaf (4), (Fig.4.).

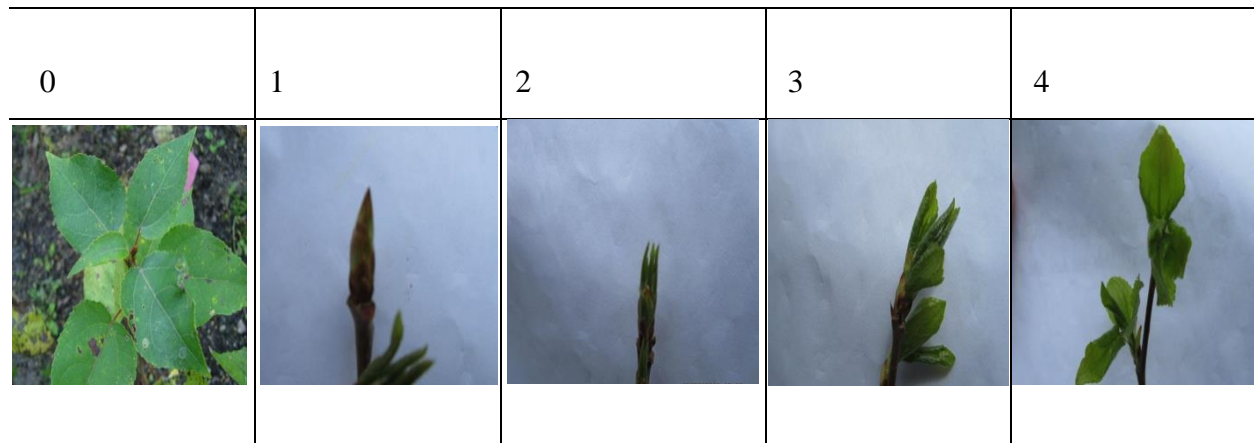


Fig.4. Different bud burst stages

4.4 Statistical Analyses

The effects of bud burst and bud set for the following years 2013, 2014, were tested using generalized linear mixed model (GLMM) in IBM®SPSS®Statistics Version 22. Bud stages were used as the target variable. Two levels of temperature (ambient and elevated), three levels of UV treatment (ambient, UV-A, UV-B), two levels of sex (male and female) and the day of year were fixed factors. Random factors were plot and clone identity. Along with these interactions between sex, day, temperature and UV treatments were also analyzed.

5 RESULTS

Bud set in 2013

According to the hypothesis there were variations in male and female responses. Female clones under UV-A treatment showed bud set six days earlier compared to the females clones in control plots ($P=0.043$) (Fig.5.). This was revealed by a significant interaction between UV-A and female. In the previous growing season (2012) UV-B forced bud set in male clones (Strømme et al. 2015) while in this growing season (2013) UV-B forced bud set in female clones compared to male clones ($P=0.017$) as there was a significant interaction between UVB and female (Fig.5.). Male clones under UV-A treatment significantly delayed in bud set

compared to females ($P=0.035$). This was explained by a significant interaction between UV-A and male. Bud break of female clones for the 2013 spring was earlier in all treatments (Strømme et al. 2015) which could have resulted a delay in bud set of female clones during autumn 2013 in control plots ($P=0.045$) (Fig.5.), revealed by a significant interaction between female and control treatment (Table.1). Elevated temperature was not having any effect on the bud set. Combined treatment effects UVB+T and UVA+T did not play any role in delaying or forcing the bud set.

Table.1. Parameter estimates, SE and t -values for covariates in the generalised linear mixed model run to investigate the effects of temperature, UV-A and UV-B on bud set in females and males of *P. tremula* during autumn 2013.

Fixed effect Terms	Coefficient	SE	t	P
Day***	-0.273	0.023	-11.961	≤ 0.001
Control \times Female*	0.088	0.044	2.008	0.045
UVA \times Female*	0.092	0.046	2.023	0.043
UVA \times Male*	0.096	0.045	2.107	0.035
UVB \times Female*	0.104	0.044	2.379	0.017

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

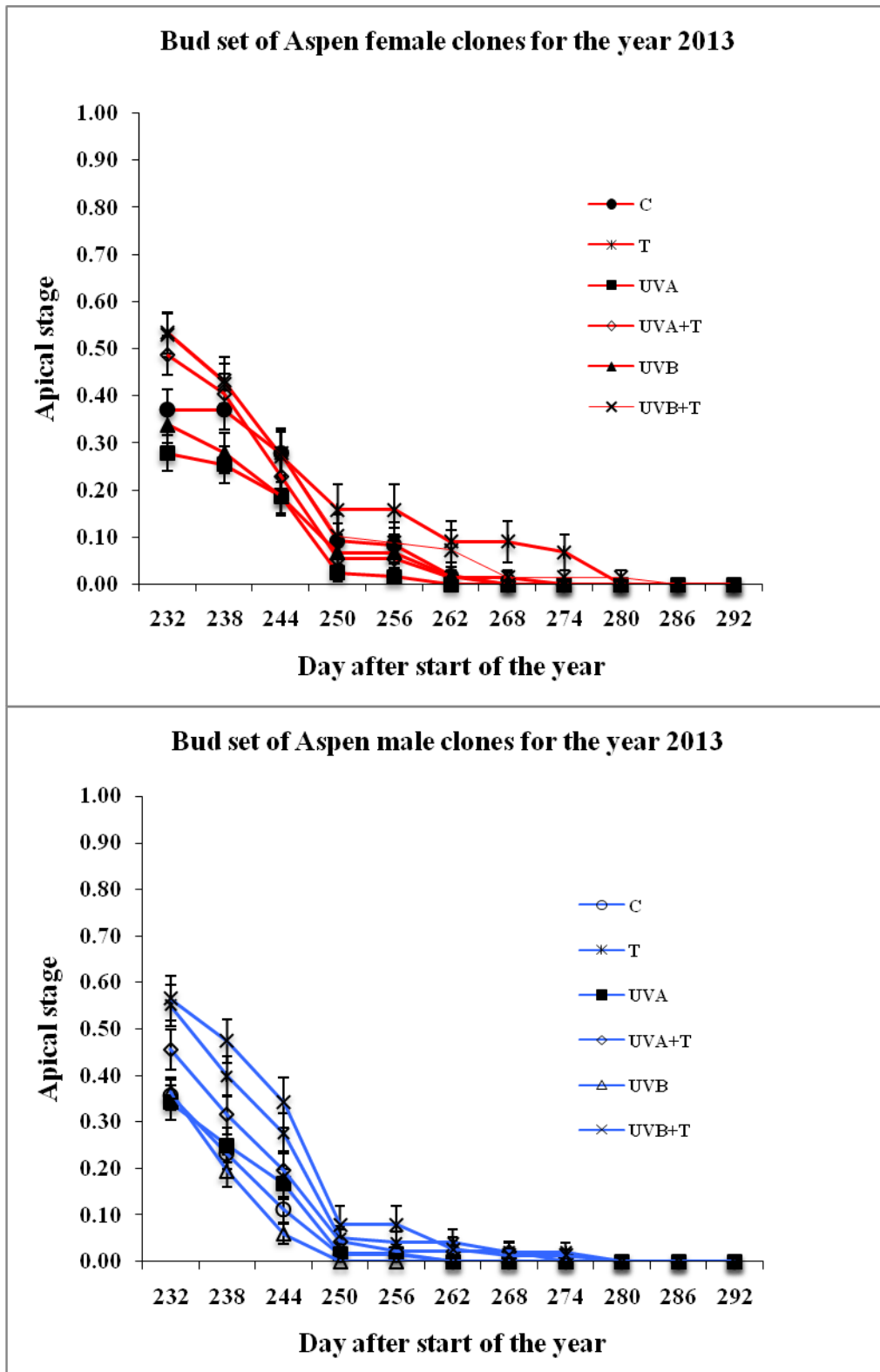


Fig.5. Bud set stages for male and female aspen plants under different treatments measured in 5 days interval for the year 2013 (Significant treatment effects are labeled with solid filled markers).

Bud break in 2014

For the bud break in spring 2014, enhanced UV-B significantly delayed bud break in female clones compared to male clones with a difference of four days ($P=0.033$) (Fig.6.). This was shown by a significant UV-B and female interaction. UV-A also played a role in delaying the bud break in females by four days ($P\leq 0.001$) with a significant interaction between female and UV-A (Fig.6.). Like for the autumnal bud set there was no influence of enhanced temperature on the bud break in spring. Even though UV-A and UV-B individually manifested their effect, when combined with enhanced temperature they were ineffectual.

Table.2. Parameter estimates, SE and t -values for covariates in the generalised linear mixed model run to investigate the effects of temperature, UV-A and UV-B on bud set in females and males of *P. tremula* during spring 2014.

Fixed effect Terms	Coefficient	SE	t	P
UVA*	13.522	4.406	3.069	0.002
Female***	20.724	4.498	4.607	≤ 0.001
Day***	0.698	0.028	25.279	≤ 0.001
UVA \times Female***	0.288	0.072	4.010	≤ 0.001
UVB \times Female*	0.109	0.051	2.130	0.033

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

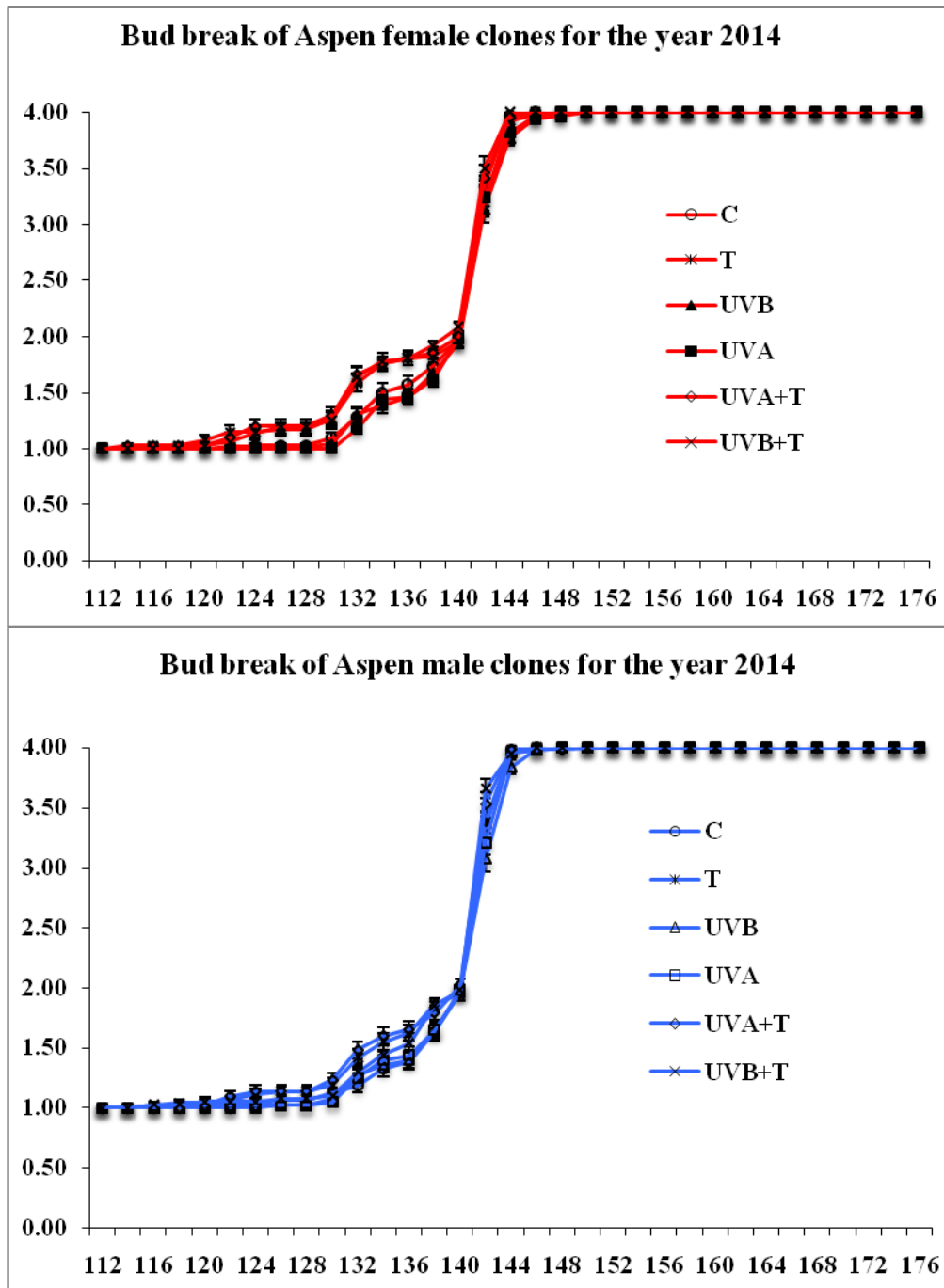


Fig.8. Bud break stages of male and female aspen plants under different treatments measured in two days interval during the year 2014 (Significant treatment effects are labeled with solid filled markers).

6 DISCUSSION

In my experiment the females were more responsive than males in all treatments. Often there have been differences between males and females in terms of plant phenology (e.g. Delph 1999). In a review comprising of 62 woody species females have been found outperformed by males (Obeso 2002). Some previous studies also show that poplar females are more sensitive to abiotic stress than males (Zhao et al. 2009, Chen et al. 2010a, 2010b). This is in contrast with the results of Strømme et al. (2015) where the male aspens were more responsive in the way that bud set and bud break were forced under UV-B treatment. Age of aspen plants might have been affected. Strømme et al. (2015) studied aspens after the first growing season while my studies were done after second growing season. So for the survival males might have had better defensive (e.g. antioxidants) system, which made them less responsive.

During 2013, bud set was forced for six days in females under UV-A treatment. Accordingly, Randriamanana et al. (2015b) reported shoot biomass and photosynthesis, amount of cinnamoyl salicortin and its derivatives and concentration of tremulacin were higher in female leaves compared to males under UV-A plots. Moreover, Maja et al. (2016) showed during the years 2013 and 2014 the females under UV-A plots had higher emissions of cis-3-hexenyl acetate, cis-3-hexenol and trans- β -caryophyllene. Cis-3-hexenyl acetate is a volatile compound involved in jasmonic acid defense signaling (Frost et al. 2008). The studies of Randriamanana et al. (2015b) and Maja et al. (2016) in the same experimental field showed that the females under UV-A plots were more responsive. Forcing effect of UV-B was observed in female clones when compared to the males. In a study of *Populus cathayana* under UV-B females significantly increased the UV-B absorbing compounds while males were not showing any significant effect (Xu et al. 2010). This could be because males have a more effective self-protection system (Jiang et al. 2013). Moreover, Xu et al. (2010) found higher amounts of anthocyanins in males than in females under ambient and enhanced UV-B treatments, which might give them a higher ability to repair damages caused by UV-B. Anthocyanins also esterify with cinnamic acids to perform protective tasks by absorbing UV-B radiation (Wood wall & Stewart, 1998, Chalker-Scott 1999).

Main hormones that play a role in regulation of changes in vegetative growth during plant maturity are auxins, cytokinins and abscisic acid (Marta & Sergi, 2009). Abscisic acid is an important signal involved in bud set during short days in *Populus* (Ruttink et al. 2007). Abscisic acid had been found to be involved in regulation of vegetative to reproductive phase transition in several woody perennials (Finkelstein et al. 2002). On the other hand, Tossi et al. (2009), Zheng et al. (2015) and Xu et al. (2010) have found that females normally have higher amount of abscisic acid, which could increase the production of nitric oxide for cell homeostasis and attenuate cell damage caused by UV-B radiation. If these has been the case in my studies higher amounts of abscisic acid could also have made the females more responsive. In my study, in autumn 2013 bud set was delayed in female clones in control plots which might be explained by the results of Zawaski et al. (2011) with *Populus* trees where lower levels of active gibberellic acid were shown to cause earlier bud set and late bud burst.

In the second (2013) and third (2014) growing season there were no effects of elevated temperature on the bud set and bud break of the aspen plants. This was in contradictory to the previous season (2012) results were temperature delayed the bud set in both male and female clones (Strømme et al. 2015). Vegetative phase change (based on age difference) in *Populus* × *canadensis* is demonstrated by changes in leaf shape and internode length (Wang et al. 2011). Change in the shape of the leaves of aspen plants in summer 2013 indicated that they are on the path of transition from the juvenile phase to the adult phase. The phase transition of the aspen plantlets from juvenile stage to vegetative stage would have made them less responsive to elevated temperature. Long-term exposure of more than one and half years would have already prepared the plants to start an acclimation process. This may explain contrasting results from the first growing season of aspens done by Strømme et al. (2015) when temperature delayed the bud set. Average temperature difference during the scoring of bud stages in 2013 was two degrees higher than the year 2012, which would have also partly made the results contradictory to Strømme et al. (2015). Autumnal bud development processes subsist of several intertwined and simultaneously occurring processes (Ruttink et al. 2007). Studies show that among some northern ecotypes including *Populus*, low temperatures result in growth cessation and bud set even under long days (Mølmann et al. 2005, Tanino et al. 2010, Olsen & Lee, 2011). Numerous studies examining frost dates, growing season length, growing degree days (heat index that can be used to predict when a crop will reach maturity) etc. have found that the changes are consistent with climate warming (Scheifinger et al. 2003, Kunkel et al. 2004).

Bud break in 2014 was influenced by UV-A and UV-B, but only in female clones. Both UV-A and UV-B delayed bud break by four days. Forced buds set in the autumn have influenced the delaying effect in bud burst under UV-A and UV-B. This indicates that there is a gender difference in response to enhanced UV-B radiation. Female bud development would have been slower if they are approaching the reproductive phase (Braatne et al. 1996). However in my case that cannot be the reason, because aspen is reaching the maturity for sexual reproduction after four to five years. Moreover, females of dioecious plants would have to allocate more carbon to reproduction than males (Hancock & Bringham, 1980, Korpelainen, 1992, Cipollini & Whigham, 1994). In addition, for example in *Populus tomentosa* it was identified that the female floral buds were developing more slowly than males along with other differences (Song et al. 2014). Acclimation responses of plants to UV-B include often induction of flavonoids and related phenolic compounds (Searles et al. 2001) that uses the available energy produced previous year and may be seen also in delay of bud break in next spring. Flavonoids are considered to have multiple functions and necessary in photo protection of plants (Agati & Tattini, 2010). In nature, the UV exposure to plants decreases towards winter and slowly increases towards summer (Hunt & McSeveny, 2000). During the first growing season (2012) in the study by Strømme et al. (2015) bud break had a strong forcing effect of UV-B in male clones. UV-A also helps to mitigate the effects of UV-B by activating DNA repair mechanisms through the enzyme photolyase (Ibdah et al. 2002). Plants ability to endure the damaging effects of UV-B depends on its ability to repair and replace the damaged molecules and by its ability to reduce exposure. In cases UV-B affects the expression of genes involved in UV protection thereby promoting plant survival (Jenkins 2009).

Although everything was planned carefully and conducted as planned there might have been different events that cause errors to the results obtained. Even though omitted from the result analysis there were numerous unopened buds in all plots during the scoring period that would have increased the time period of certain bud stages that can affect the result. Reasons for unopened buds may be many like *Venturia* infection, insufficient chilling, egg deposition by insects and physical damage. The bud set in 2013 was scored at an interval of five days while the bud break in 2014 was scored at an interval of two days in order to have a clear picture of bud stage changes. This interval difference will also have influenced the results. The temperature effect was not shown during the bud burst which could be because as the scoring started in May by the time many buds have already turned to stage 1 (closed bud with visible green leaf tip). It is possible that the initial stage 0 (closed bud) would have been influenced by the temperature sum required for bud burst that the plants would have already attained.

7 CONCLUSIONS

In our experiment we found that in successive years when plants are ageing the responses to enhanced UV and temperature can be different. We found that the male clones were more tolerant to UV radiation compared to the female clones. UV-B influenced the bud set and bud break as in year 2012. UV-A radiation that was not having any effect on *P. tremula* during the year 2012 while it affected the bud set and bud break in the years 2013 and 2014 when plants get older. *P. tremula*'s acclimation to enhanced UV-B and temperature could only be revealed by further long-term field experiments. Bud development is a key factor in shaping biological communities and a forecaster of the climate change phenomena in many populations. This depicts the importance of further research in investigating the signaling pathways and hormonal level changes to learn more about the responses of plants to temperature and UV-B. Delineation of the plant responses; whether they are specific or environmental factor dependent could also be a future perspective.

ACKNOWLEDGEMENTS

I would thank the Department of Biology of the University of Eastern Finland for its excellent facilities and support for conducting the thesis work. I am also glad to thank my supervisor, Professor Riitta Julkunen-Tiitto for all her moral support and guidance throughout my thesis work. I am also very grateful to my co-supervisor, Christian Bianchi Strømme who helped me to give the guidelines for the work. I am also expressing my gratitude for Teemu Tahvanainen for his help with the statistics. I am also thankful to Chenhao Cao for all his support and help during the data collection.

REFERENCES

- Agati, G., Tattini, M. 2010: Multiple functional roles of flavonoids in photo protection. – *New Phytologist* 186:786-793.
- Aitken, S.N., S. Yeaman, J.A., Holliday, T., Wang. Curtis-McLane, S. 2008: Adaptation, migration or extirpation: climate change outcomes for tree populations. –*Evolutionary Applications* 1: 95-111.
- Balch, R.E. 1953: The birch die back problem. Report of symposium on birch dieback. –Part 1. Canada Department of Agriculture, Forest Biology Division, Ottawa, ON, pp.1-5.

- Ballaré, C. L., Caldwell, M. M., Flint, S. D., Robinson, S. A., Bornman, J. F. 2011: Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. –*Photochemistry and Photobiology*, Science 10: 226-241.
- Basler, D., Körner, C. 2012: Photoperiod sensitivity of bud burst in 14 temperate forest tree species. –*Agricultural and Forest Meteorology* 165:73-81.
- Beaubien, E., Hamann, A. 2011: Spring flowering response to climate change between 1936 and 2006 in Alberta, Canada. – *Bioscience* 61: 514-524.
- Björn L.O. 1990: *Photobiology*. –Vol. 2. University of Lund, Lund, pp. 55-59.
- Blair J. Sampson., James, H. Cane 1999: Impact of enhanced ultraviolet-B radiation on flower, pollen, and nectar production. – *American Journal of Botany* 86:108-114.
- Braathe, P. 1995: Birch dieback caused by prolonged early spring thaws and subsequent frost.– *Norwegian Journal of Agricultural Sciences*. 20: 1-59.
- Braatne, J.H., Rood, S.B., and Heilman, P.E. 1996: Life history, ecology, and conservation of riparian cottonwoods in North America. In *Biology of Populus*, R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman, and T.M. Hincley, eds (Ottawa, Canada: NRC Research Press) 57–85.
- Brian, P. Haggerty., Susan, J. Mazer. 2008: *The Phenology Handbook A guide to phenological monitoring for students, teachers, families, and nature enthusiasts*. –University of California, Santa Barbara.
- Caldwell, M. M., Teramura, A. H., Tevini, M., Bornman, J. F., Björn, L. O., and Kulandaivelu, G. 1995: Effects of increased solar ultraviolet radiation on terrestrial plants. –*Ambio* 24: 166-173.
- Caldwell, M.M., Bornman J.F., Ballaré C.L., Flint S.D., Kulandaivelu G. 2007: Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. – *Photochemical and Photo biological Sciences* 6: 252-266.
- Chalker-Scott, L. 1999: Environmental significance of anthocyanins in plant stress responses. – *Photochemistry and Photobiology* 70:1-9.

- Chen, F.G., Chen, L.H., Zhao, H.X., Korpelainen, H., Li, C.Y. 2010a: Sex-specific responses and tolerances of *Populus cathayana* to salinity. – *Physiologia Plantarum* 140: 163-173.
- Chen, L.H., Zhang, S., Zhao, H.X., Korpelainen, H., Li, C.Y. 2010b: Sex-related adaptive responses to interaction of drought and salinity in *Populus yunnanensis*. – *Plant, Cell & Environment* 33: 1767-1778.
- Chuine, I., Beaubien, E. 2001: Phenology is a major determinant of tree species range. – *Ecology Letters* 4: 500-510.
- Cipollini, M. L., Whigham, D.F. 1994: Sexual dimorphism and cost of reproduction in the dioecious shrub *Lindera benzoin* (Lauraceae). – *American Journal of Botany* 81:65-75.
- Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A., Schwartz, M .D. 2007: Shifting plant phenology in response to global change. – *Trends in Ecology and Evolution* 22: 357-365.
- Couvillon, G.A., Jerez, A. 1985: Influence of prolonged exposure to chilling temperatures on bud break and heat requirement for bloom of several fruit species. – *Journal of the American Society for Horticultural Science* 110: 47-50.
- Delph, L.F., Geber, M.A., Dawson, T.E., Delph, L.F., (eds) 1999: Sexual dimorphism in life history. Gender and dimorphism of flowering plants. In Berlin: Springer, 149–173.
- Delbart, N., Picard, G., Toan, T.L., Kergoat, L., Quegan, S., Woodward, I., Dye D. 2008: Spring phenology in boreal Eurasia over a nearly century time scale. – *Global Change Biology*, 14: 603-614.
- Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E. et al. 1997: Maximum and minimum temperature trends for the globe. – *Science* 277: 364-367.
- Falusi, M., Calamassi, R. 1990: Bud dormancy in beech (*Fagus sylvatica* L.). Effect of chilling and photoperiod on dormancy release of beech seedlings. – *Tree Physiology* 6(4):429-438.

- Feder, J.L., T.A. Hunt., L. Bush, 1993: The effects of climate, host plant phenology and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella*. –Entomologia Experimentalis et Applicata 69: 117-135.
- Finkelstein, R.R., Gampala, S.S., Rock, C.D. 2002: Abscisic acid signaling in seeds and seedlings. –Plant Cell. 14 Suppl: S15-45.
- Fracheboud, Y., V. Luquez, L. Bjorken, A. Sjodin, H. Tuominen and S. Jansson. 2009: The control of autumn senescence in European aspen. –Plant Physiology. 149: 1982-1991.
- Frost, C.J, Mescher, M.C., Dervinis, C., Davis, J.M., Carlson, J. E., De Moraes C.M. 2008: Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. –New Phytologist 180:722-734.
- Fu, Y. H., Piao, S., Op de Beeck, M., Cong, N., Zhao, H., Zhang, Y., Menzel, A. and Janssens, I. A. 2014: Recent spring phenology shifts in western Central Europe based on multiscale observations. –Global Ecology and Biogeography 23: 1255-1263.
- Fu, Y.H, Campioli, M., Deckmyn, G., Janssens, I. A. 2012: The Impact of Winter and Spring Temperatures on Temperate Tree Budburst Dates: Results from an Experimental Climate Manipulation. –PLoS ONE 7(10): e47324.
- Gordo, O., Sanz, J. J. 2010: Impact of climate change on plant phenology in Mediterranean ecosystems. –Global Change Biology 16: 1082 -1106.
- Granhus, A., I.S. Fløisted, G. Søgaard. 2009: Bud burst timing in *Picea abies* seedlings as affected by temperature during dormancy induction and mild spells during chilling. – Tree Physiology 29:497-503.
- Guak, S., D.M. Olszyk, L.H. Fuchigami and D.T. Tingey. 1998: Effects of elevated CO₂ and temperature on cold hardiness and spring bud burst and growth in Douglas-fir (*Pseudotsu gamenziesii*). –Tree Physiology 18: 671-679.
- Hancock, J.F, Bringhurst, R.S. 1980: Sexual dimorphism in the strawberry *Fragaria chiloensis*. – Evolution 34:762-768.
- Hänninen, H. 1990: Modeling bud dormancy release in trees from cool and temperate regions. – Acta Forestalia Fennica 213: 1-47.

- Hänninen, H. 1995: Effects of climatic change on trees from cool and temperate regions: an ecophysiological approach to modelling of bud burst phenology. – *Canadian Journal of Botany* 73:183-199.
- Hänninen, H., Kramer, K. 2007: A framework for modelling the annual cycle of trees in boreal and temperate regions. – *Silva Fennica* 41: 167-205.
- Hänninen, H., Tanino, K.K. 2011: Tree seasonality in a warming climate. – *Trends in Plant Science* 16: 412-416.
- Harsch, M.A., Hulme, P.E., McGlone, M.S. and Duncan, R.P. 2009: Are treelines advancing? A global meta-analysis of treeline response to climate warming. – *Ecology Letters*, 12: 1040-1049.
- Heide, O.M. 1974: Growth and dormancy in Norway spruce ecotypes. – *Physiologia Plantarum* 31:131-139.
- Heide, O.M. 1993: Dormancy release in beech buds (*Fagus sylvatica*) requires both chilling and long days. *Physiologia Plantarum* 89(1): 187-191.
- Heide, O.M. 2003: High autumn temperature delays spring bud burst in boreal trees, counterbalancing the effect of climatic warming. – *Tree Physiology* 23: 931-936.
- Howe, G.T., Gardner, W.P. Hackett and G.R. Furnier. 1996: Phytochrome control of short-day-induced bud set in black cotton-wood. – *Physiologia Plantarum* 97: 95-103.
- Howe, G.T., Aitken, D.B. Neale, K.D. Jermstad, N.C. Wheeler and T.H.H. Chen. 2003: From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. – *Canadian Journal of Botany* 81:1247-1266.
- Hunt, J., McSeveny, T. 2000: Seasonal changes of UV-absorbing compounds in the leaves of two Southern Hemisphere temperate tree species. – *Ecological Society of America, ESA Abstracts*, oral presentation session 69. August 10, 2000.
- Hunter, M.D., 1992: A variable insect-plant interaction: The relationship between tree budburst phenology and population levels of insect herbivores among trees. – *Ecological Entomology* 17: 91-95.

- Ibáñez, C., Kozarewa, I., Johansson, M., Ogren, E., Rohde, A. Eriksson, M.E. 2010: Circadian clock components regulate entry and affect exit of seasonal dormancy as well as winter hardiness in *Populus* trees. *Plant Physiology* 153: 1823-1833.
- Ibdah, M., Krins, A., Seidlitz, H.K., Heller, W., Strack, D., Vogt, T. 2002: Spectral dependence of flavonol and betacyanin accumulation in *Mesembryanthemum crystallinum* under enhanced ultraviolet radiation. –*Plant Cell & Environment* 25:1145-1154.
- IPCC. 2001: Climate change 2001: the scientific basis. Contribution of Working Group 1 to the third assessment report of the IPCC. Cambridge University Press, Cambridge, 786.
- IPCC. 2007: Climate Change 2007: The Physical Science Basis. Cambridge: Cambridge University Press. 996.
- IPCC. 2013: Summary for policymakers. In: Stocker, T., Qin, D., Plattner, G.K., Tignor, M., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.
- Jacob, D., Petersen, J., Eggert, B., Alias A. and others 2013: EURO-CORDEX: new high-resolution climate change projections for European impact research. – *Regional Environmental Change* 14: 563-578.
- Jansen, M. A. K. 2002: Ultraviolet-B radiation effects on plants: induction of morphogenic responses. –*Physiologia Plantarum*, 116: 423- 429.
- Jansen, M.A.K., Bornman J.F. 2012: UV-B radiation: from generic stressor to specific regulator. –*Physiologia Plantarum* 145: 501-504.
- Jeffrey, K., Conner, Neumeier, R. 2002: The effects of ultraviolet-B radiation and intraspecific competition on growth, pollination success, and lifetime female fitness in *Phacelia campanularia* and *P. purshii* (Hydrophyllaceae) *American Journal of Botany*– 89:103-110.
- Jenkins, G. 2009: Signal transduction in responses to UV-B radiation. –*Annual Review of Plant Biology*, 60: 407-43.

- Jenkins, G. I. 2014: The UV-B Photoreceptor UVR8: From Structure to Physiology. –*The Plant Cell* 26: 21-37.
- Jiang, H., Korpelainen, H., Li, C., *Populus yunnanensis* males adopt more efficient protective strategies than females to cope with excess zinc and acid rain. – *Chemosphere*. 2013: 91: 1213-1220.
- Jolly, W.M., Running, S. W. 2004: Effects of precipitation and soil water potential on drought deciduous phenology in the Kalahari. – *Global Change Biology*, 10: 303-308.
- Junttila O., Hänninen H. 2012: The minimum temperature for budburst in *Betula* depends on the state of dormancy. –*Tree Physiology* 32:337-345.
- Junttila, O., J. Nilsen and B. Igeland. 2003: Effect of temperature on the induction of bud dormancy in ecotypes of *Betula pubescens* and *Betula pendula*. –*Scandinavian Journal of Forest Research*, 18: 208-217.
- Kakani, V.G., Reddy, K.R., Zhao, D., Sailaja, K. 2003: Field crop responses to ultraviolet-B radiation: A review. *Agricultural and Forest Meteorology* 120:191-218.
- Kalcsits, L, Silim, S. and Tanino, K. 2009: Warm temperature accelerates short-photoperiod induced growth cessation and dormancy induction in hybrid poplar (*Populus x spp*). – *Trees* 23: 971-979.
- Karl, T. R., Knight, R. W., Gallo, K. P., Peterson, T. C., Jones, P. D., Kukla, G., et al. 1993: A new perspective on recent global warming: asymmetric trends of daily maximum and minimum temperature. –*Bulletin of the American Meteorological Society* 74: 1007-1023.
- Kleiner, K.W., 1989: Source of variation in oak leaf quality as food for a gypsy moth: Implications for forest susceptibility. – Ph.D. Thesis, Pennsylvania State University, Pennsylvania.
- Körner, C., Basler, D. 2010: Phenology under global warming. –*Science* 327: 1461-1462.
- Korpelainen, H. 1992. Patterns of resource allocation in male and female plants of *Rumex acetosa* and *R. acetosella*. –*Oecologia*. 89:133-139.

- Koski, V. and R. Sievänen. 1985: Timing of growth cessation in relation to variations in the growing season. –In *Crop Physiology of Forest Trees*. Eds. P.M.A. Tigerstedt, P. Puttonen and V. Koski. Helsinki University Press, Helsinki, pp 167-193.
- Kunkel, K.E. et al. 2004: Temporal variations in frost-free season in the United States: 1895-2000. – *Geophysical Research Letters*, 31, L03201.
- Langkamp, P. 1987: *Germination of Australian Native Plant Seed*. –Inkata: Melbourne.
- Lechowicz, M.J. 1984: Why do temperate deciduous trees leaf out at different times? – *Adaptation and ecology of forest communities. The American Naturalist* 124: 821-842.
- Lechowicz, M.J. 1995: Seasonality of flowering and fruiting in temperate forest trees. –*Canadian Journal of Botany* 73: 175-182.
- Lee, Y. K., Alexander, D., Wulff, J. & Olsen, J. E. 2014: Changes in metabolite profiles in Norway spruce shoot tips during short-day induced winter bud development and long-day induced bud flush. –*Metabolomics*, 10: 842-858.
- Lindroth, R. L. 2008: *Chemical Ecology of Aspen: Herbivory and Ecosystem Consequences. Restoring the West 2008: Frontiers in Aspen Restoration*, Utah State University, Logan, UT.
- Maja, M.M. Kasurinen, A., Holopainen, T., Julkunen-Tiitto, R., Holopainen, J.K., 2016: The effect of warming and enhanced ultraviolet radiation on gender-specific emissions of volatile organic compounds from European aspen. –*Science of the Total Environment*. 15; 547:39-47.
- Menzel, A. and P. Fabian. 1999: Growing season extended in Europe. –*Nature* 397, 659.
- Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., et al. 2006: European phenological response to climate change matches the warming pattern. –*Global Change Biology* 12: 1969-1976.
- Mølmann, J.A., Asante, D.K., Jensen, J.B., Krane, M.N., Ernstsén, A., Junttila, O. & Olsen, J.E. 2005: Low night temperature and inhibition of GA biosynthesis overrides phytochrome action and induce bud set and cold acclimation, but not dormancy in *PHYA* over expressors and wild-type of hybrid aspen. –*Plant, Cell & Environment* 28: 1579-1588.

- Moore, N.P., J.D. Hart, P.F. Kelly and S.D. Langton. 2000: Browsing by fallow deer (*Dama dama*) in young broadleaved plantations: seasonality, and the effects of previous browsing and bud eruption. – *Forestry* 73:437-445.
- Mopper, S & D. Simberloff, 1995: Differential herbivory in an oak population: The role of insect phenology and insect performance. – *Ecology* 76: 1233-1241.
- Morin. X., Lechowicz, M.J., Augspurger, C.O', Keefe, J., Viner, D., et al. 2009: Leaf phenology in 22 North American tree species during the 21st century. – *Global Change Biology* 15: 961-975.
- Mpoloka, S.W. 2008: Effects of prolonged UV-B exposure in plants. – *African Journal of Biotechnology* Vol. 7 (25), pp. 4874-4883.
- Murray, M.B., M.G.R. Cannell, R.I. Smith. 1989: Date of budburst of fifteen tree species in Britain following climatic warming. – *Journal of Applied Ecology* 26: 693-700.
- Myking, T. and O.M. Heide. 1995: Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. – *Tree Physiology* 15: 697-704.
- Newsham, K. K., Greenslade, P. D. and Mcleod, A. R. 1999: Effects of elevated ultraviolet radiation on *Quercus robur* and its insect and ectomycorrhizal associates. – *Global Change Biology* 5: 881-890.
- Nitsch, J.P. 1957. Photoperiodism in woody plants. *Proceedings of the American Society for Horticultural Science* 70:526-527.
- Nordli, Ø. Wielgolaski, F.E., Bakken A.K., Hjeltnes, S.H., Måge, F., Sivle, A. and Skre, O. 2008: Regional trends for bud burst and flowering of woody plants in Norway as related to climate change. – *International Journal of Biometeorology* 52: 625-639.
- Nybakken, L., Hörkkä, R., Julkunen-Tiitto, R. 2012: Combined enhancements of temperature and UVB influence growth and phenolics in clones of the sexually dimorphic *Salix myrsinifolia*. – *Physiologia Plantarum* 145: 551-564.
- Obeso, J.E. 2002: The costs of reproduction in plants. – *New Phytologist* 155: 321-348.

- Olsen J.E., Lee Y.K. 2011: Trees and boreal forests. In Temperature Adaptation in a Changing Climate: Nature at Risk(eds K.B. Storey & K.K.Tanino), pp. 160-178. CAB International, Wallingford, UK.
- Olsen, J. E. 2010: Light and temperature sensing and signaling in induction of bud dormancy in woody plants. –Plant Molecular Biology 73: 37-47.
- Olsen, J.E, Lee, Y., Junttila, O. 2014: Effect of alternating day and night temperature on short day-induced bud set and subsequent bud burst in long days in Norway spruce. –Frontiers in Plant Science 5,691.
- Oñate, M, Sergi Munné-Bosch. 2009: Influence of Plant Maturity, Shoot Reproduction and Sex on Vegetative Growth in the Dioecious Plant *Urtica Dioica*. – Annals of Botany 104.5: 945-956.
- Parmesan, C. and Yohe, G. 2003: A globally coherent fingerprint of climate change impacts across natural systems. – Nature 421: 37-42.
- Pauley, S.S. and T.O. Perry. 1954: Ecotypic variation of the photoperiodic response in *Populus*. – Journal of the Arnold Arboretum, 35:167-188.
- Pellis, A., Laureysens, I., Ceulemans, R. 2004: Genetic variation of the bud and leaf phenology of seventeen poplar clones in a short rotation coppice culture. – Plant Biology 6:38-46.
- Polgar, C., Gallinat, A., Primack, R. B. 2014: Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau’s Concord. –New Phytologist 202: 106-115.
- Quiring, D.T., 1994: Influence of inter-tree variation in time of budburst of white spruce on herbivory and the behavior and survivorship of *Zeiraphera canadensis*. –Ecological Entomology 19: 17-25.
- Randriamanana, T.R., Lavola, A., Julkunen-Tiitto., R. 2015b: Interactive effects of supplemental UV-B and temperature in European aspen seedlings: implications for growth, leaf traits, phenolic defense and associated organisms. –Plant Physiology and Biochemistry 93: 84-93.

- Randriamanana, T.R., Nissinen, K., Moilanen, J., Nybakken, L., Julkunen-Tiitto, R. 2015a: Long-term UV-B and temperature enhancement suggest that females of *Salix myrsinifolia* plants are more tolerant to UV-B than males. – Environmental and Experimental Botany.109: 295 -305.
- Ren, J., Yao, Y., Yang, Y., Korpelainen, H., Junttila, O., Li, C. 2006: Growth and physiological responses to supplemental UV-B radiation of two contrasting poplar species. Tree Physiology 26:665-672.
- Robson, M. T., Klem, K., Urban, O., Jansen, M. A. K. 2014: Re-interpreting plant morphological responses to UV-B radiation. –Plant, Cell and Environment 38(5): 856-66.
- Rohde, A., Bastien, C., Boerjan, W. 2011a: Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. –Tree Physiology 31: 472-482.
- Rohde, A., Storme, V., Jorge, V., Gaudet, M., Vitacolonna, N., Fabbrini, F., Bastien, C. 2011b: Bud set in poplar - genetic dissection of a complex trait in natural and hybrid populations. –New Phytologist 189:106-121.
- Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C. and Pounds, J. A. 2003: Fingerprints of global warming on wild animals and plants. – Nature 421: 57-60.
- Rosenthal, S.I. and Camm, E.L. 1996: Effects of air temperature, photoperiod and leaf age on foliar senescence of western larch (*Larixoccidentalis* Nutt.) in environmentally controlled chambers. – Plant, Cell and Environment, 19:1057-1065.
- Rozema, J., van de Staaij, J., Björn, L.O. & Caldwell, M. 1997: UV-B as an environmental factor in plant life: stress and regulation. – Trends in Ecology & Evolution 12: 22-28.
- Ruttink, T., Arend, M., Morreel, K., Storme, V., Rombauts, S., Fromm, J., Bhalerao, R.P., Boerjan, W., Rohde, A., 2007: A molecular timetable for apical bud formation and dormancy induction in poplar. –Plant Cell 19: 2370-2390.
- Sakai, A. and W. Larcher. 1987: Frost survival of plants: responses and adaptation to freezing stress. – Springer-Verlag, Berlin, 321 p.

- Savolainen, O., T. Pyhäjärvi and T. Knürr. 2007: Gene flow and local adaptation in trees. –Annual Review of Ecology, Evolution and Systematics 38: 595-619.
- Scheifinger, .H., Menzel, A., Koch, E., Peter, C. 2003: Trends of springtime frost events and phenological dates in Central Europe. –Theoretical and Applied Climatology74(1–2):41-51.
- Searles, P.S., Flint, S.D., Caldwell, M.M. 2001: A meta-analysis of plant field studies simulating stratospheric ozonedepletion. –Oecologia 127:1-10.
- Sicora, C., A. Szilárd, L. Sass, E. Turcsányi, A. Máté, I. Vass. 2006: UV-B and UV-A radiation effects on photosynthesis at the molecular level, p. 121-135. In: F. Ghetti, G. Checcucci, and J.F. Bornman (eds.). Environmental UV radiation: Impact on ecosystems and human health and predictive models. Springer, The Netherlands.
- Søgaard, G., Johnsen, Ø., Nilsen, J. & Junttila, O. 2008: Climatic control of bud burst in young seedlings of nine provenances of Norway spruce. –Tree Physiology 28: 311-320.
- Song, Y., Ma, K., Ci, D., Zhang, Z., Zhang, D. 2014: Biochemical, physiological and gene expression analysis reveals sex-specific differences in *Populus tomentosa* floral development. – PhysiologiaPlantarum 150:18-31.
- Strømme, C. B., Julkunen-Tiitto, R., Krishna, U., Lavola, A., Olsen, J. E. and Nybakken, L. 2015: UV-B and temperature enhancement affect spring and autumn phenology in *Populus tremula*. – Plant Cell &Environment 38: 867-877.
- Sugiura, T., Honjo, H. 1997: A dynamic model for predicting the flowering date developed using an endodormancy break model and a flower bud development model in Japanese pear. – Journal Agricultural Meteorologyv.54, n.5, p.897-900.
- Tanino, K.K., Kalcsits, L., Silim, S., Kendall, E. & Gray, G .R. 2010: Temperature driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. – Plant Molecular Biology 73: 49-65.

- Tikkanen, O. P. and Julkunen-Tiitto, R. 2003: Phenological variation as protection against defoliating insects: the case of *Quercus robur* and *Operopthera brumata*. – *Oecologia* 136: 244-251.
- Tognetti, R. 2012: Adaptation to climate change of dioecious plants: does gender balance matter? – *Tree Physiology* 32:1321-1324.
- Tossi, V., Lamattina, L., Cassia, R. 2009: An increase in the concentration of abscisic acid is critical for nitric oxide-mediated plant adaptive responses to UV-B irradiation. – *New Phytologist* 181:871-879.
- Ueno, S. Klopp, C., Noirod, C., Léger, V., Prince, E., Kremer, A., Plomion, C., Le Provost, G. 2011: Detection of genes involved in bud phenology in sessile oak (*Quercus petraea* Matt. Liebl) combining digital expression analysis and Q-PCR. *BMC Proceedings*, 5 (Suppl. 7), 1-2.
- Vince-Prue, D. 1975: Stem elongation and dormancy, p. 333-384. In: D. Vince-Prue (ed.). *Photoperiodism in plants*. McGraw Hill, New York.
- Vitasse, Y., Basler, D. 2013: What role for photoperiod in the bud burst phenology of European beech. – *European Journal of Forest Research* 132: 1-8.
- Vitasse, Y., Lenz, A., & Körner, C. 2014: The interaction between freezing tolerance and phenology in temperate deciduous trees. – *Frontiers in Plant Science*, 5: 541.
- Wang, J.W., Park, M.Y., Wang, L.J., Koo, Y., Chen, X.Y., Weigel, D., Poethig, R.S., 2011: miRNA control of vegetative phase in trees. – *PLoS Genetics* 7, e10002012.
- Wareing, P.F. 1953: Growth studies in woody species. V. Photoperiodism in dormant buds of *Fagus sylvatica*. – *Physiologia Plantarum* 6(4):692-706.
- Wareing, P.F. 1956: Photoperiodism in woody plants. – *Annual Review of Plant Physiology* 7: 191-214.
- Way, D.A. 2011: Tree phenology responses to warming: spring forward, fall back? – *Tree Physiology* 31: 469-471.
- Westergaard, L. Eriksen, E.N. 1997: Autumn temperature affects the induction of dormancy in first-year seedlings of *Acer platanoides* L. – *Scandinavian Journal of Forest Research*, 12: 11-16.

- Wilson, K.E., Thompson, J.E., Huner, N.P.A., Greenberg, B. M. 2001: Effects of ultraviolet-A exposure on ultraviolet-B-induced accumulation of specific flavonoids in *Brassica napus*. – *Photochemistry & Photobiology* 73: 678-684.
- Woodall, G.S., Stewart G.R.1998: Do anthocyanins play a role in UV protection of the red juvenile leaves of *Syzygium*? – *Journal of Experimental Botany* 49 (325): 1447-1450.
- Xu, X., Zhao, H., Zhang, X., Hänninen, H., Korpelainen, H. & Li C. 2010: Different growth sensitivity to enhanced UV-B radiation between male and female *Populus cathayana*. – *Tree Physiology* 30: 1489-149.
- Zawaski, C., Kadmiel, M., Pickens, J., Ma C., Strauss, S. & Busov, V. 2011: Repression of gibberellin biosynthesis or signaling produces striking alterations in poplar growth, morphology, and flowering. – *Planta* 234: 1285-1298.
- Zhang, X., Friedl, M.A., Schaaf, C.B., Strahler, A.H., 2004: Climate controls on vegetation phenological patterns in northern mid and high latitudes inferred from MODIS data. – *Global Change Biology* 10:1133-1145.
- Zhao, H.X., Li, Y., Duan B.L., Korpelainen, H., Li.,C.Y 2009: Sex-related adaptive responses of *Populus cathayana* to photoperiod transitions. – *Plant Cell & Environment* 32: 1401-1411.
- Zheng, C., Halaly, T., Acheampong, A. K., Takebayashi, Y., Jikumaru, Y., Kamiya, Y., et al. 2015: Abscisic acid (ABA) regulates grape bud dormancy, and dormancy release stimuli may act through modification of ABA metabolism. – *Journal of Experimental Botany* 66: 1527-1542.