Effects of maternal temperature on offspring performance of temperate forest trees

Sumitra Dewan

GHENT UNIVERSITY

FACULTY OF BIOSCIENCE ENGINEERING

To my father

Supervisors: Prof. dr. ir. Kris Verheyen
Department of Environment
Forest & Nature Lab

Prof. dr. ir. Pieter De Frenne
Department of Environment
Forest & Nature Lab

Dr. Kristine Vander Mijnsbrugge,
Research Institute for Nature and Forest (INBO),

Dean: Prof. dr. ir. Marc Van Meirvenne

Rector Prof. dr. ir. Rik Van de Walle

Sumitra Dewan

Effects of maternal temperature on offspring performance of temperate forest trees

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Effect van de maternale temperatuur op de nakomelingen van boomsoorten uit gematigde bossen

Illustration on the cover:

Front: Seedlings growing at the base of a mother tree in a temperate forest. This thesis explores their fate in a warming world.

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মুই এচ্ছে ইদ্দুর এই ন পাল্লুঙুন মর পরিবারঅ বল ছারা. এজাবত দ মু ফুদি তমারে ধন্যবাদ ন জানাঙ্. এচ্ছে সুজোগ পেলুঙ তমারে মর সিত দিঘলী ধন্যবাদ জানেবার্. বা এচ্ছে বাজি থেদ, তে বেচ খুঝি ওলুন্দি. বা-মা, তমারে ধন্যবাদ জানাঙর মরে সব সময় বল দিবাত্তেই, ম উঘুরে বিচ্ছেস রাঘেবাত্তেই. মা ইক্কু আর বেছ গুরি বার গরি পারিব-ম ঝিব Ph.D. খই খই. ম তিন বোন, সনাক্কু দি, শিবানী দি, বানু দি অলাক্কে মর তিন্ন লুদি. তমা বলে মুই এচ্ছে ইদ্দুর লুঙি পাচ্ছঙগে. তমারে মর ধন্যবাদ জানাঙর্. ম বোন ঝি উন: বৈজন্তী, সুমন্, চুক্তি, শিলু, ফাগুন তমারেও ধন্যবাদ মর ফর-মায়েশ পুরন গরি বাত্তে.

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Summary

The global mean surface temperature has increased by 0.87°C in the last century and is going to increase by another 1.1 °C to 6.4 °C by the end of the 21st century. Warming air and soil temperatures can have a significant impact in many terrestrial ecosystems. Key aspects affecting by climate change include plant's phenology (that is, the timing of biological events) and regeneration (from seeds or vegetatively). Warming in winter and spring due to climate change, for instance, advances the bud burst (leaf opening) time, changes reproductive success (germination) and seedling growth in many tree species in the temperate zone. In general, trees can respond to changing environmental conditions via phenotypic plasticity, a mechanism helping them to adapt in a new environment by altering phenotypes to a certain degree. However, phenotypic plasticity may not be enough for tree populations to cope with rapid climate change, and thus they need other mechanisms. One of the possibilities is via epigenetic variation. Epigenetic modifications are any mitotically or meiotically heritable contribution to the phenotype without changing the DNA sequence. Epigenetic variation is probably most relevant to the adaptation of long-lived trees since environmentally induced epigenetic variation during the development of long-lived trees can persist through mitotic cell divisions and can transfer the environmental memory to their offspring, which is also known as the maternal environmental effect. Despite the importance of maternal environmental effects, very few studies considered the maternal temperature conditions when testing responses of tree seedlings to warming.

In this thesis, we aim to understand the effects of maternal temperatures during reproduction on the responses (germination, phenological and growth responses) of seedlings and vegetative offspring (cuttings) of *Quercus robur*, *Fagus sylvatica*, *Populus nigra* and hybrid poplar (*Populus* spp.) to changing temperatures. Using a combination of the temperature differences across space (different sites in Belgium, and across Europe) and time (different years) and experimentally manipulated temperatures in maternal and in offspring generations, we studied germination, bud burst and growth cessation, and growth of the offspring in common gardens. In addition, we studied the global DNA methylation in vegetative offspring of hybrid poplar that was collected from maternal ramets with different environmental history to assess the environmentally induced epigenetic variation.

We used natural temporal temperature differences across different seed maturation years in forests in Belgium and applied warming to the seedlings of *Quercus robur* and *Fagus sylvatica* by elevating the soil surface temperature by 2.5°C between January-April for two years in a common garden. We observed an interaction between the maternal temperature during seed maturation and warming in bud burst time of both species suggesting that the bud burst of seedlings in response to warming was dependent on the maternal temperature.

Higher maternal temperature altered the growth of oak seedlings. Nevertheless, the responses of the seedlings to maternal temperature was species dependent.

Next, we studied the influence of maternal effects regarding local adaptation to the temperature and photoperiod on germination, budburst, and biomass of *Quercus robur* seedlings. In this experiment, we collected seeds of different provenances from a mature provenance trial. We monitored germination, bud burst, and shoot biomass of the seedlings in two common gardens, one in Denmark and the other in Belgium. These common gardens were situated at two different latitudes representing a mean annual temperature difference of nearly 2°C. There was an interaction between provenances and common gardens in seedlings' bud burst time, which suggests that the bud burst time of the seedlings of different provenances was dependent on the environmental condition of the seedlings (in the common gardens). The germination percentage and growth of the seedlings was lower in the Belgian common garden compared to the Danish common garden.

We performed a controlled crossing between three pairs of genotypes of *Populus nigra* in control (C) conditions and warming (+10°C) (W) and let seeds mature in the same environment. In addition, we applied another treatment where we pollinated in a control condition but seeds matured in warming (+10°C) (C>>W). Then, we assessed germination, bud burst and bud set of the seedlings in a common garden. We observed that warmer maternal temperature during seed maturation reduced seed germination and altered the bud burst and bud set time of the seedlings. At least in one genotype, we observed delayed bud burst and advanced bud set when the mother plants were exposed to higher temperatures during pollination and seed maturation. The effects of maternal temperature during seed maturation were different among genotypes.

Finally, to assess the transgenerational effects on bud burst and bud set time of vegetative offspring (stem cuttings), we performed a common garden experiment using vegetative cuttings of five different genotypes of hybrid poplars of the same provenance but with different environmental history (4.9 °C temperature and 3.5 hours photoperiod difference). We observed that increased maternal temperature of the coldest month advanced the bud burst and reduced the growing period of the vegetative cuttings across all five different genotypes. We did not detect any significant epigenetic variation in the cuttings of the mother trees within single genotypes growing under different climates. To understand the mechanism behind these changes further investigation using powerful molecular methods like whole-genome bisulphite sequencing techniques is necessary.

In sum, the results of this thesis indicate that the maternal temperature has the potential to influence the responses of offspring to climate change. The effects of maternal temperature vary among species and genotypes and depend on the temperature experienced by the offspring generation. Nevertheless, our results suggest that the timing of bud burst and

growth cessation will be contrastingly affected among studied species, and seed germination and growth of the seedlings will be reduced in response to climate warming.

Samenvatting

De wereldwijde gemiddelde oppervlaktetemperatuur is in de vorige eeuw met 0,87°C gestegen en zal tegen het einde van de 21e eeuw met nog eens 1,1°C tot 6,4°C toenemen. Opwarming van de lucht- en bodemtemperaturen kunnen een aanzienlijke impact hebben op verschillende terrestrische ecosystemen. De belangrijkste aspecten die beïnvloed worden door de klimaatverandering zijn de fenologie van planten (dat wil zeggen het tijdstip van biologische gebeurtenissen) en regeneratie (uit zaden of vegetatief). Temperatuurstijgingen in de winter en in de lente als gevolg van klimaatverandering bevordert onder andere de tijd van de knopuitbarsting (bladopening), veranderen het reproductieve succes (ontkieming) en de groei van zaailingen voor tal van boomsoorten in de gematigde zone. Over het algemeen kunnen bomen reageren op veranderende omgevingscondities via fenotypische plasticiteit, een mechanisme dat hen helpt zich aan te passen in een nieuwe omgeving door in een zekere maten hun fenotypen te veranderen. Echter, fenotypische plasticiteit is misschien niet voeldoende voor boompopulaties om zich aan de snelle klimaatverandering aan te passen. Bijgevolge hebben ze andere mechanismen nodig. Een mogelijk alternatief is epigenetische variatie. Epigenetische modificaties zijn elke mitotisch of meiotisch geproduceerde erfelijke bijdrage aan het fenotype zonder de DNA-sequentie te veranderen. Epigenetische variatie is waarschijnlijk het meest relevant voor de aanpassing van bomen met een lange levensduur, aangezien de epigenetische variatie veroorzaakt door milieuveranderingen tijdens hun ontwikkeling kan overleven via mitotische celdelingen. Bovendien kan het milieugeheugen worden overdragen aan hun nageslacht, ook bekend als het moederlijke milieueffect. Ondanks het belang van maternale milieueffecten, hebben zeer weinig studies de maternale temperatuursomstandigheden overwogen bij het testen van de reacties van boomzaailingen op kimaatopwarming.

In dit proefschrift proberen we te achterhalen hoe de maternale temperaturen tijdens de voortplanting zijn zich verhouden tot de reactie (kieming, fenologische en groeiresponsen) van zaailingen en vegetatieve nakomelingen (stekken) van *Quercus robur*, *Fagus sylvatica*, *Populus nigra* en hybride populier (*Populus* spp.) op de veranderende temperaturen. Met behulp van een combinatie van temperatuurverschillen in de ruimte (verschillende locaties in België en in heel Europa) en in de tijd (verschillende jaren) even als experimenteel gemanipuleerde temperaturen in moeder- en nageslacht, bestudeerden we kieming, knopuitbarsting en groeiactivering en groei van de nakomelingen in *common gardens* (een experiment om het effect van de omgeving te testen door twee soorten/ genotypen vanuit hun oorspronkelijke omgeving naar een gemeenschappelijke omgeving te verplaatsen). Daarnaast onderzochten we de wereldwijde DNA-methylatie in vegetatieve nakomelingen van hybride populier die werd verzameld van maternale *ramets* (een individu afkomstig van

een klonale populatie) met verschillende omgevingshistorie, met als doel de door het milieu veroorzaakte epigenetische variatie in kaart te brengen.

We maakten gebruik van natuurlijke temporele temperatuurverschillen over verschillende zaadrijpingsjaren in bossen in België en stelden de zaailingen van *Quercus robur* en *Fagus sylvatica* bloot aan een opwarming tussen januari en april gedurende twee jaar in een *common garden* met door de temperatuur van het bodemoppervlak met 2,5°C te verhogen. We observeerden een interactie tussen de temperatuur van de moeder tijdens de rijping van het zaad en de opwarming in de uitbarstingstijd van de knoppen van beide soorten. Dit suggereert dat de knopuitbarsting van zaailingen als gevolg van opwarming afhankelijk is van de maternale temperatuur. Hogere temperaturen van de moeder veranderden de groei van eiken zaailingen. Niettemin varieerde de respons van de zaailingen op de temperatuur van de moeder van soort tot soort.

Vervolgens hebben we de invloed van maternale effecten met betrekking tot lokale aanpassing aan de temperatuur en fotoperiode bestudeerd op de kieming, knopburst en biomassa van *Quercus robur* zaailingen. In dit experiment hebben we zaden van verschillende herkomsten verzameld uit een volwassen herkomstonderzoek. We onderzochten de ontkieming, de knopuitbarsting en de biomassa van de zaailingen in twee *common gardens*, waarvan een in Denemarken en een andere in België. Deze *common gardens* bevonden zich op twee verschillende breedtegraden, wat een gemiddelde jaarlijkse temperatuurverschil van bijna 2°C betekent. Er was een wisselwerking tussen de herkomsten en de *common gardens* in de uitbarstingstijd van de knoppen van de zaailingen. Dit suggereert dat de uitbarstingstijd van de knoppen van de zaailingen met verschillende herkomsten afhankelijk is van de milieucondities van de zaailingen (in de *common gardens*). Het kiempercentage en de groei van de zaailingen was lager in de Belgische *common garden* dan in de Deense *common garden*.

We voerden een gecontroleerde kruising uit tussen drie paren genotypen van *Populus nigra* in gecontroleerde omstandigheden (C) en opwarming (+ 10 ° C) (W) en lieten vervolgens de zaden rijpen in dezelfde omstandigheden. Daarnaast hebben we tevens een andere behandeling toegepast waarbij we de moederbomen bestoven in een controleconditie, maar de zaden lieten rijpen in opwarming (+ 10 ° C) (C >> W). Vervolgens onderzochten we opnieuw de kieming, knopuitbarsting en knoppenreeks van de zaailingen in een *common garden*. We stelden vast dat een warmere temperatuur van de moeder tijdens de rijping van het zaad de kieming van het zaad verminderde en de knopuitbarsting en het tijdstip van knopvorming van de zaailingen veranderde. Ten minste in één genotype, zagen we een vertraagde knopuitbarsting en geavanceerde knopvorming toen de moederplanten werden blootgesteld aan hogere temperaturen tijdens bestuiving en zaadrijping. De effecten

van temperatuur van de moeder tijdens de rijping van het zaad waren verschillend tussen genotypen.

Om de transgenerationele effecten op de knopuitbarsting en het tijdstip van knopvorming van vegetatieve nakomelingen (stengelstekken) te beoordelen, voerden we ten slotte een *common garden* experiment uit met behulp van vegetatieve stekken van vijf verschillende genotypes van hybride populieren van dezelfde herkomst, maar met verschillende omgevingshistorie (4,9 ° C-temperatuur en 3,5 uur verschil in fotoperiode). We stelden vast dat een verhoogde maternale temperatuur van de koudste maand ervoor zorgde dat de knop barstte en de groeiperiode van de vegetatieve stekken over alle vijf verschillende genotypen verminderde. We hebben geen significante epigenetische variatie ontdekt in de stekken van de moederbomen binnen enkele genotypes die in verschillende klimaten groeien. Om het mechanisme achter deze veranderingen te begrijpen, is verder onderzoek met behulp van krachtige moleculaire methoden zoals *whole-genome bisulfiet* sequentietechnieken noodzakelijk.

Tot slot geven de resultaten van dit proefschrift aan dat de maternale temperatuur de reacties van nakomelingen op de klimaatverandering potentieel kan beïnvloeden. De effecten van maternale temperatuur variëren tussen soorten en genotypen en zijn afhankelijk van de temperatuur die wordt ervaren door de generatie van het nageslacht. Desalniettemin gaven onze resultaten aan dat het tijdstip van knopuitbarsting en groeivertraging op contrasterende wijze zal worden beïnvloed door de bestudeerde soorten, en dat de zaadontkieming en de groei van de zaailingen zullen worden verminderd als reactie op klimaatopwarming.

List of Abbreviations

DNA	Deoxyribonucleic acid
IPCC	Intergovernmental Panel on Climate Change
RCP	Representative Concentration Pathway
CO_2	Carbon dioxide
Mm	Millimiter
m.a.s.l	Metres above sea level
NPK	Nitrogen, phosphorus and potassium
IR	Infrared
RD	Relative diameter
RH	Relative height
v/v	Volume to volume
TGP	Transgenerational plasticity
DOY	Days of the year
♀	Male symbol
♂	Female symbol
Cm	Centimetre
MSAP	Methylation Sensitive Amplified Fragment Length Polymorphism
L	Litre
MJanT	Mean monthly temperature for January
MJulyT	Mean monthly temperature for July
MAT	Mean annual temperature
DL	Day length
DL1May	Day length on 1 May
DL1Jan	Day length on 1 january
PCR	Polymerase Chain Reaction
AFLP	Amplified Fragment Length Polymorhism
NML	Nonmethylated loci
MSL	Methylation susceptible loci

1 Introduction

The introduction of this thesis is structured as follows: first climate change scenarios and possible impacts of global warming on the terrestrial ecosystem are discussed, followed by the definition of phenology (that is, the timing of biological events), its importance and measurement methods. Then, I introduce the factors controlling tree bud dormancy (or no physiological activity) related to the bud phenology of tree species. Next, I explain the available evidence of impacts of global warming on bud phenology (time to bud burst and growth cessation), growth performance and germination. Further, phenotypic plasticity (that is the ability of a single genotype to produce different phenotypes in different environments), the molecular mechanism of plasticity and the role of epigenetic variation (epigenetic modifications such as methylation of histones and DNA, are any mitotically or meiotically heritable contribution to the phenotype without changing the DNA sequence) regarding environmentally induced plasticity in the offspring generation are introduced. Afterward, I describe the available knowledge on maternal environmental effects on both sexually and asexually (vegetative) produced offspring and the relevance of such effects in the adaptation of trees in the light of the available knowledge from both annual and perennial species. Finally, I highlight the knowledge gaps concerning maternal temperature effects to understand the responses of tree populations to global warming. The main aim of this thesis, research questions and different chapters of the thesis are specified right at the end.

1.1 Climate change

The global mean surface temperature has increased by 0.87°C in the last century and is predicted to increase by another 1.1 °C to 6.4 °C by the end of 21st century (Figure 1.1) (IPCC, 2018). Along with increasing temperatures, precipitation patterns are changing as well (Figure 1.1). In addition, many extreme weathers and climate events such as heavy rainfalls, heat waves have increased since the 1950s (IPCC, 2013). The seasonal temperature anomalies are also increasing rapidly in most of the northern hemisphere (Cohen et al., 2012; Yu et al., 2018). Spring, summer, and autumn except for winter temperatures in the boreal zone have increased significantly during the last three decades (Cohen et al., 2012). Although, increased winter temperature may be pronounced in some regions (Yu et al., 2010). Warming is likely to influence the growth and recruitment of tree seedlings, leading to changes in the composition (Penuelas & Boada, 2003) and productivity of forests (Hanewinkel et al., 2013). Predicting the effects of climate change on species composition and quantifying how individual tree species respond to climate

variability is critical for understanding the future state of our forested ecosystems (Chen et al., 2011; Reich et al., 2015; Sittaro et al., 2017).

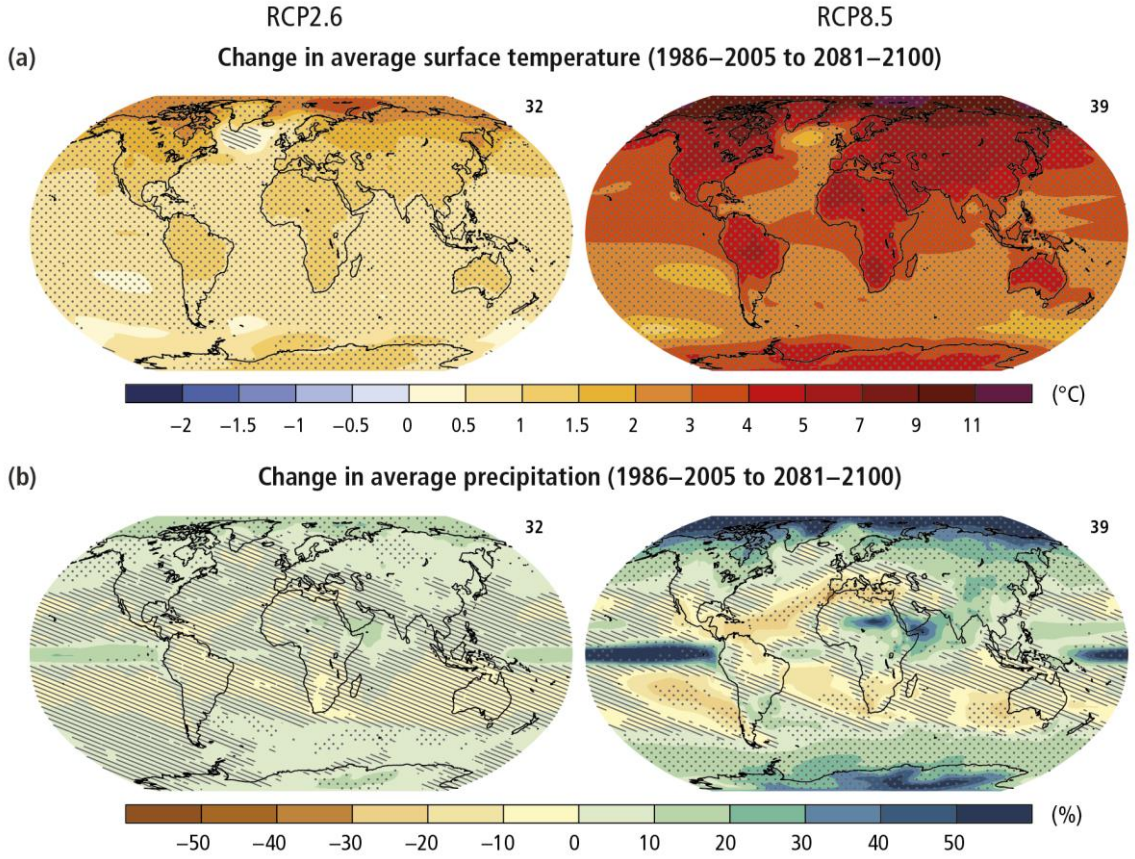

Figure 1.1 Maps of projected late 21st century annual mean surface temperature change and average percent change in annual mean precipitation. Adapted from IPCC (2013).RCP refers to 'Representative Concentration Pathway'. The four RCPs range from very high (RCP8.5) to relatively low (RCP2.6) future radiative forcing, where RCP2.6 pathway represents a strong mitigation scenario and is extended by assuming constant emissions after 2100 (including net negative CO2 emissions), leading to CO2 concentrations returning to 360 ppm by 2300.

1.2 Phenology, its importance, and measurements

Phenology is generally defined as the art of observing life cycle events in plants and animals in their temporal occurrence throughout the year (Lieth, 1974). In plants, that includes the study of biological events such as flowering, bud burst, seed set and dispersal, bud set and/or leaf fall in relation to climatic conditions or other drivers in the environment or plant (Vilhar et al., 2013). Plant phenology has been proposed as an indicator of climatic difference and global change by the European Environmental Agency and the Intergovernmental Panel on Climate Change (IPCC, 2018) and of great interest to the global change community (Menzel, 2002). Traditionally, in agriculture and forestry, phenological observations were carried out regarding the selection of suitable crops and cultivars (Chmielewski, 2003). Phenology plays a major role in environmental education and public information regarding climate change impacts (Menzel, 2002). For example, phenological

observations can provide an excellent understanding of the connection between climate and vegetation. In public health, phenology has facilitated the forecast of pollen shedding for many allergenic taxa (Chuine & Belmonte, 2004). Phenological data have frequently been using in important climate and vegetation models (Menzel, 2002; Fu et al., 2014b; Fitchett et al., 2015). The phenological cycle determines the density of foliage of plants and the leaf area index (LAI) (Rautiainen et al., 2012), and this in turn influences surface biophysical parameters, such as albedo, latent and sensible heat flux, momentum flux, CO2 flux, and net radiation (Pielke & Avissar, 1990; Davi et al., 2006). Different kinds of environmental models require these bio-geophysical parameters and often use phenological data either as a direct input or for phenological subroutine development (Menzel, 2002). Chuine and Beaubien (2001) predicted tree species distribution by a process-based model using the biological processes of survival and reproductive success as a function of phenology.

Phenology is probably the simplest way by which to track the changes in the behaviour of species. It can be assessed directly by regular observations using standard protocols. The assessments are carried out during the individual phases of phenological phenomena and are repeated until the phase is complete. Phenological observations can also be carried out through indirect techniques such as terrestrial digital image photography (Sonnentag et al., 2012), where digital camera systems were used to provide a permanent photographic record suitable for manual inspection by comparing with images or descriptions of standard phenological stages (Vilhar et al., 2013). Spectral vegetation indices (SVI) that combine visible and near-infrared light reflected by vegetation, such as the normalized difference vegetation index (NDVI), have been used to quantify the phenology of different ecosystems from the ground- (Soudani et al., 2012) and satellites (Delbart et al., 2006). Archive photos, videos and herbarium records can also be used as a means of indirect methods of phenology observation for understanding plant phenological responses to changes in temperature (MacGillivray et al., 2010; Davis et al., 2015; De Frenne et al., 2018). Besides, in-situ observation, phenology is frequently measured by applying climate manipulation experiments to predict the phenology of plants to warming (De Frenne et al., 2011; Fu et al., 2013; Man et al., 2014; Richardson et al., 2018). In this thesis, we focused on the bud phenology, which is bud burst as a measure of onset of spring growth, and leaf discolouration and bud set as measures of growth cessation.

1.3 Bud dormancy in trees

The length of the growing season, i.e., the time between spring bud break and fall bud set, of many tree species in the temperate and boreal zone is synchronized by dormancy. Dormancy is imposed by the variation in seasonal temperatures and photoperiod (day

length), and several molecular actors along with different genes and physiological pathways and circadian clock (Lang et al., 1987; McClung, 2006). The role of temperature in controlling dormancy is twofold. First, the experience of a certain amount of low temperatures makes plants receptive to warmer conditions as spring is approaching in temperate, Mediterranean and boreal ecosystems (known as “chilling” that is non-freezing temperatures in the range of 2-7°C). Second, warm temperatures are directly affecting the rates of development (known as “forcing” temperature) once, for instance, the chilling requirements are fulfilled. Dormancy is a vital strategy for many temperate tree species to avoid the unfavourable period for growth and ultimately limits the growth, wood production and quality (Lang et al., 1987). Lang (1987) described three types of dormancy: eco-dormancy, para-dormancy and endo-dormancy (Figure 1.2). Eco-dormancy is induced at the end of summer when the growth of buds and cambium meristems is ceased by the environmental conditions. In para-dormancy, growth suspension in a dormant structure is caused by another organ within the plant, but outside the dormant tissue. For example, the continuous inhibition of visible growth of lateral or axillary bud meristems is known by morphogenic factors, such as hormonal activity, which occurs through regulation and expression of multiple gene systems, produced in nearby organs such as apices (Lang et al. (1987), Figure 1.2). In some cases of para-dormacy, plants may readily resume growth when transferred to long-day conditions (Basler, 2015). After initiation of eco-dormancy, trees will form autumnal buds and gradually develop cold tolerance. Following eco-dormancy, trees enter into endo-dormancy. Endo-dormancy is caused by endogenous factors within the dormant tissue and no growth can be attained even in favourable environmental conditions. During this time, leaf senescence and formation of the bud occur in deciduous trees, and then, trees enter into dormancy and leaf fall. At this period, trees show their maximum adaptation to the cold. Endo-dormancy can be broken by the fulfilment of a chilling requirement. Photoperiod or day length is another important factor that controls the winter dormancy and thus the active growth of the vegetation (Saikkonen et al., 2012) (Figure 1.3). Because temperature is often an unpredictable cue of seasonality, while the course of late winter or spring temperature is strongly variable from year to year, which is also affected by climate change, most long-lived plant species native to areas outside the tropics have developed photoperiodism to safeguarding them against unpredictable temperature conditions (Saikkonen et al., 2012). The significance of photoperiodism increases with latitude, not only because the annual variation of the photoperiod becomes more pronounced, but also because of its biological function (Figure 1.3). One role of photoperiodism is to prevent bud burst from following the temperature as a risky environmental signal for development. It is insurance for plants against temperature-induced break of dormancy too early in the season, and induction of dormancy too late in the season. Chilling, forcing and photoperiod are part of complex interactions, e.g., a lack

of chilling may lead to an increased requirement of forcing temperatures for budburst, but may also be substituted by long days (Heide, 2008; Dantec et al., 2014). We are still far from fully understanding the full mechanism of bud dormancy processes (Cooke et al., 2012).

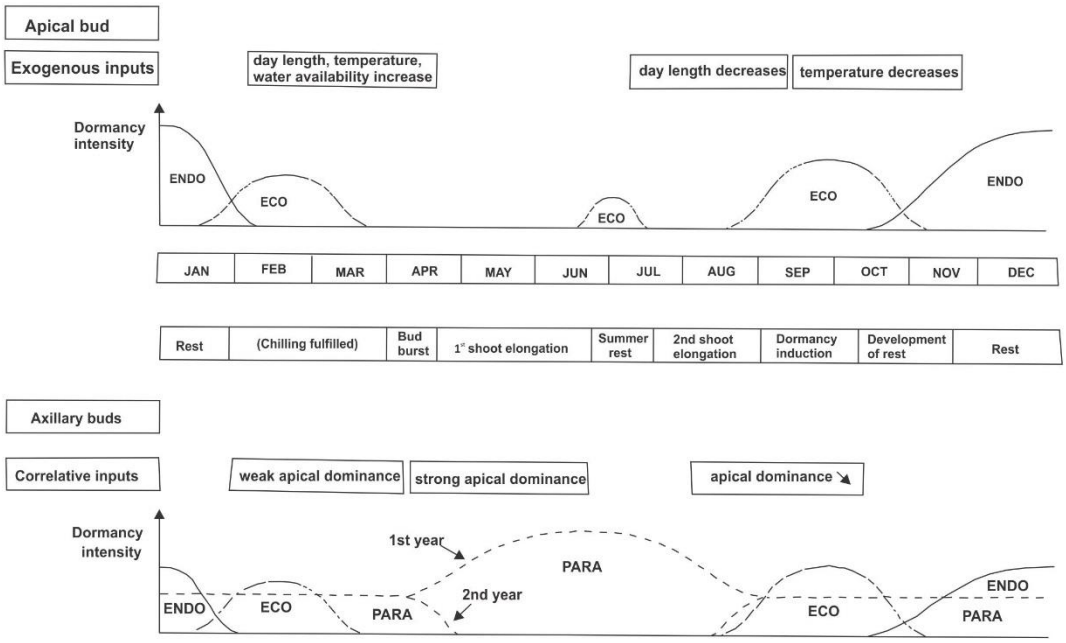

Figure 1.2 Diagram showing the seasonal occurrence of the different types of dormancy in the apical and the axillary buds in temperate climates. Adapted from (Rohde et al., 2000). Apical and axillary buds differ at entry into endodormancy in autumn: the apical bud changes from a growing via an ecodormant to an endodormant state, whereas the axillary buds change from a paradormant to an endodormant state.

The transition between the different phases of dormancy is gradual, and the environmental requirements for dormancy induction and release differs among species, provenances or ecotypes and even individuals (Körner, 2007; Uneo et al., 2013; Kramer et al., 2017). Some tree species may require photoperiodic signal such as reduced day length and some others need only low temperatures to enter into eco-dormancy (see Figure 1.4). Moreover, the ecological life strategy and successional status of a species may determine the response to warm temperatures in early spring (Korner & Basler, 2010). Late successional species such as *Abies alba*, *Picea abies*, *Fagus sylvatica*, *Tilia cordata* and one mid-successional species *Quercus petraea* were reported to delay their bud burst time under short photoperiod even after substantial chilling hours suggesting that the bud burst of the studied species was influenced by photoperiodism (Heide, 1993; Basler & Körner, 2012). Basler and Körner (2012) concluded that long-lived late successional species from milder, low elevation winters are more likely to adopt a more conservative strategy, relying more on

photoperiod than on temperature only to decrease the risk of frost damage when in future the climate becomes warmer. The experimental result of Laube et al. (2014) where the author showed that late successional species beech was sensitive to photoperiod when chilling requirements were not satisfied, which supports the conclusion of Basler and Körner (2012).

On the other hand, early successional species such as *Betula*, *Corylus*, *Larix*, *Prunus*, *Sorbus*, *Populus* are known to have no photoperiodism to bud burst (Basler & Körner, 2012; Soolanayakanahally et al., 2013). Therefore, it is suggested that these early successional species will become opportunistic to exploit the active growing season and will more likely respond to temperature only in spring, although the potential risk of frost damage may be larger (Korner & Basler, 2010; Basler & Körner, 2012). On the other hand, early successional species such as *Betula* can display photoperiod sensitivity when the chilling requirement was not fulfilled (Myking & Heide, 1995). Further, another early successional species *Populus* that are known to be photoperiod controlled for growth cessation (Wareing, 1956), temperature seems to alter the timing of growth cessation as well (Rohde et al., 2011a; Rohde et al., 2011b). The evidence in the literature shows that species according to their successional status as well as their adaptation to different ecotypes and provenances will have different threshold chilling temperatures to release dormancy and will exhibit heritable thermal responses (Vitasse et al., 2009; Dantec et al., 2014; Kramer et al., 2017).

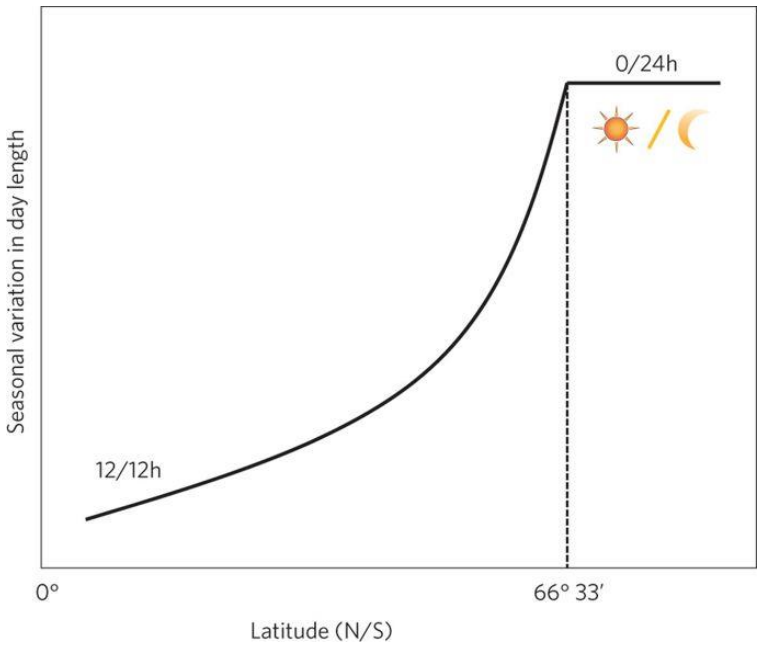

Figure 1.3 Latitudinal effects on the seasonality of day length. Day length varies insignificantly at lower latitudes while the seasonality of day length increases polewards. The X axis represents latitude and the Y axis represents seasonal variability in day length. Adapted from Saikkonen et al. (2012).

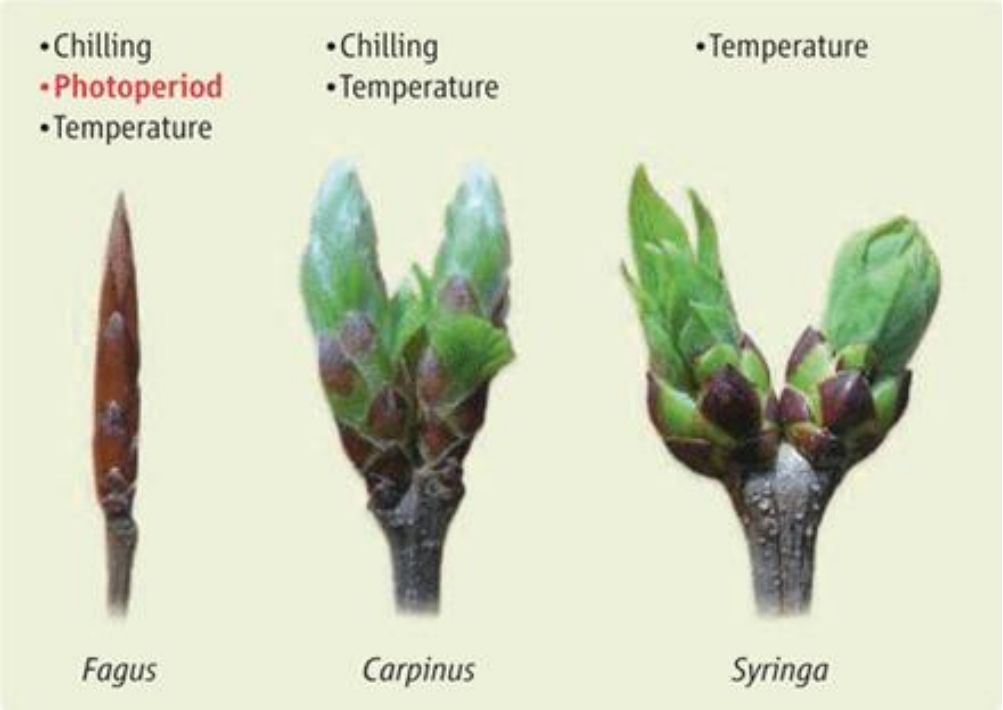

Figure 1.4 Not only temperature but especially the photoperiod controls bud burst in *Fagus*. In *Carpinus* bud burst is mainly controlled by chilling hours and forcing temperature, while the bud burst in *Syringa* primarily depends only on forcing temperature in spring. Adapted from Korner and Basler (2010).

1.4 Impact of global warming on bud phenology

One of the best-documented and observed effects of climate change is advancing bud phenology in the spring and often delayed phenology in the autumn. From more than 30 years of observation data since 1959, it was revealed that the spring growth advanced by six days while autumn growth cessation was delayed by 4.8 days in Europe (Menzel & Fabian, 1999). Later, this trend of advancing spring phenology was observed in a wide range of plant species across 19 European countries from 1971 through 2000, where advancement of spring/summer was 2.5 days decade^{-1} in Europe (Menzel et al., 2006). However, this spring advancing trend in response to warming across different vegetation types was found to be non-linear. For example, from a remote sensing analysis of growing season changes at high Northern latitudes, Delbart et al. (2006) found an advancing trend in the onset of spring greening between 1982 and 1991, followed by a delaying trend in spring onset between 1993 and 2004. Similarly, a non-linear response in spring greening up of the meadow and steppe vegetation was observed with a steady increase in winter and spring temperature in the Tibetan Plateau between 1982 and 2006 (Yu et al., 2010). In both vegetation types, the authors observed advanced spring growth from the early 1980's to mid-1990's and fairly delaying trend in spring growth since mid-1990's, which sustained until the end of the observation in 2006 (Yu et al., 2010). Such delaying onset of spring

growth can be linked to lack of chilling requirement due to increased temperatures in fall and winter months. As a manifestation of this lack of chilling requirement, plants extend the period to chilling delaying the onset of spring growth. A non-linear effect of warming on mean leaf unfolding date of seven dominant European tree species was also observed in 1,245 long-term in situ observation sites (Fu et al., 2015). Across all seven observed species, the mean leaf unfolding date decreased by circa 4 days during 1980-1994 and circa 2 days during 1999-2013 per °C of warming. It is likely that the seasonal phenological events will advance for many temperate tree species while for some species with high chilling requirements the phenological events may delay subsequently affecting the growing period with global warming.

Besides in situ observation, many studies are known in performing systematic model projections and climate manipulation experiments to better understand how the steady increase in global temperature is going to affect the phenological events along with the active vegetation growth. One of such model predictions suggests that climate change will affect leaf phenology in almost all 22 studied species in North America, with an average advancement during the 21st century by 5.0 days with warming of +3.2°C and 9.2 days with warming of +1°C (Morin et al., 2009). Advanced spring growth and delayed growth cessation were observed in a shrubland ecosystem by warming of 0.4–1.2°C (Prieto et al., 2009). Another whole ecosystem warming experiment on boreal and temperate species showed that elevated temperatures linearly correlate with advanced spring greening up and delayed autumn growth cessation (Richardson et al., 2018). However, Wolkovich et al. (2012) showed that climate manipulation experiments under-predict the phenological responses to temperature compared to in-situ observations by nearly 5 days per °C warming (Figure 1.5).

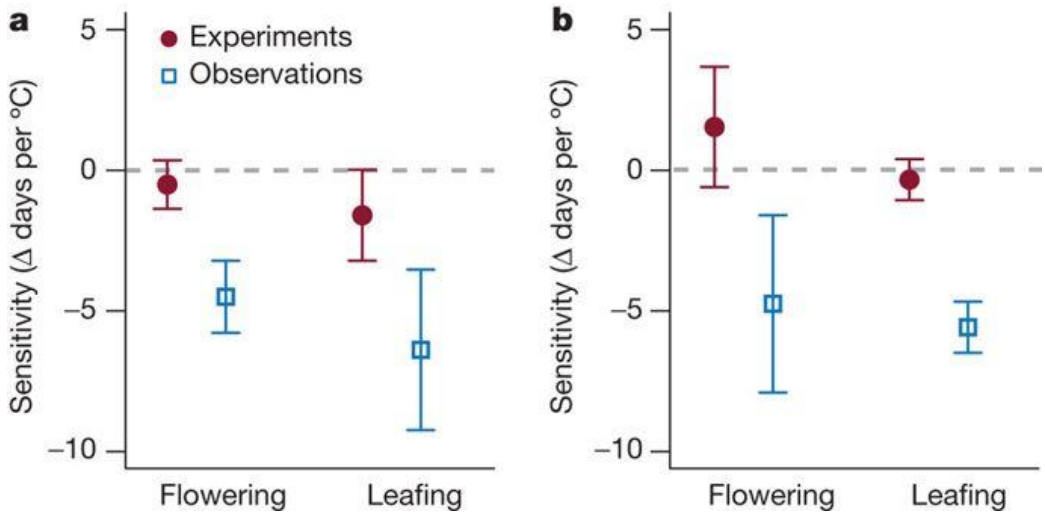

Figure 1.5 Estimates of the flowering and leafing sensitivities. Adapted from Wolkovich et al. (2012). Subpanel "a" represents plant responses to interannual temperature variation for all studied species and panel "b" represents plant responses for the species that are common to both experimental and observational data sets. Positive sensitivity (i.e., the region above the dashed gray line) means flowering and leafing are delayed with warming, whereas negative sensitivity (i.e., the region below the dashed line) indicates that the phenological events advance with warming.

The temperatures in late summer and early fall do not necessarily correlate with the timing of the first frost, and in such case, temperature does not always provide the right cue of initiation of eco- dormancy. Therefore, most of the trees need to complete sequentially as in *Populus* (see Figure 1.6) growth cessation (GC), bud set (BS) and leaf senescence (S), and dormancy (D) before winter and risk any physical damage. Way (2011) discussed how two warming scenarios are going to affect the sequence of these phenological events in *Populus*. In the first scenario (Warming 1) in Figure 1.6, across a latitudinal range of field sites, rising temperatures would delay growth cessation (GC) in poplar, but accelerate bud development (Rohde et al., 2011a). In a temperature manipulation experiment with the combination of elevated day and night temperatures on hybrid poplar, Kalcsits et al. (2009) showed that indeed warmer day temperature delayed the growth cessation, a combination of elevated day and night temperatures, however, advanced the growth cessation, followed by hastening of bud set, senescence and dormancy development (Warming 2 in Figure 1.6). In this scenario, the deeper winter dormancy is likely to delay the bud burst in spring while elevated spring temperature will advance the bud burst than expected (grey arrow in Figure 1.6) in the following spring, which suggests that elevated night temperature have a higher impact on growth cessation than elevated day temperature.

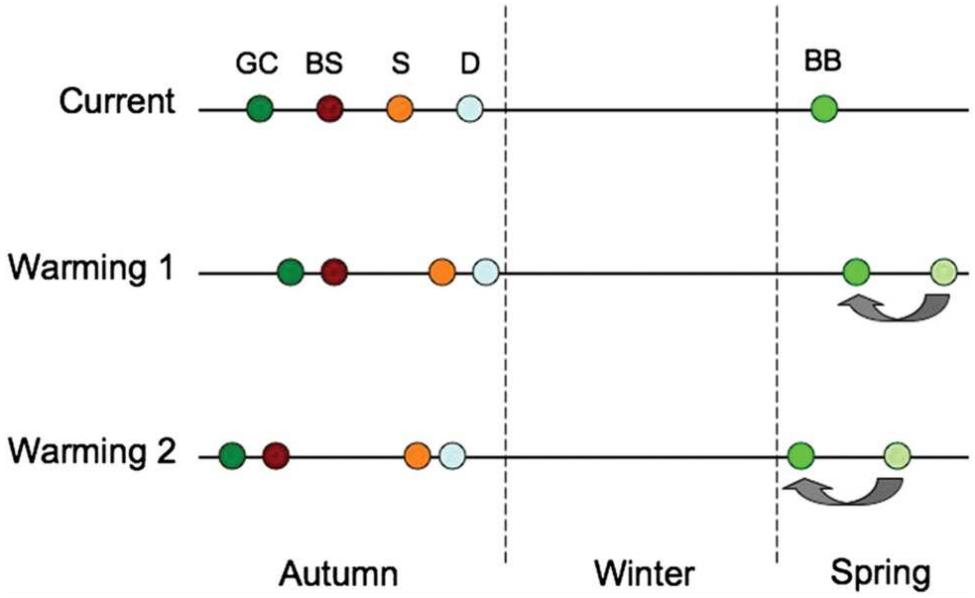

Figure 1.6 Prospective change in bud phenology in current and elevated temperatures. A sequential change in growth cessation (GC), bud set (BS), leaf senescence (S) and dormancy (D), which correlate with spring bud burst (BB). Warming 1 represents the effect of elevated temperatures in relation to a latitudinal (southward) transfer (Rohde et al., 2011a) while warming 2 represents the effect of a combination of elevated day and night temperatures (Kalcsits et al., 2009). Adapted from Way (2011) .

1.5 Impact of warming on germination and seedlings growth

Seedling recruitment via successful seed germination and emergence is critical as it allows genetic recombination and production of dispersal units to sustain population persistence and spread (Chen et al., 2014a; Frouz et al., 2015; Almazán-Núñez et al., 2016). Seed germination connects the successful life cycle of trees and is, like phenology, sensitive to temperature variation (Baskin & Baskin, 2001; Donohue, 2009). For many temperate tree species, flowering occurred simultaneously with leafing or followed by one another (Vanden Broeck, 2004; Packham et al., 2012), and timing of bud phenology have a clear link to the reproduction process. Global warming is likely not only to influence the phenology but will also influence seed germination, which ultimately will influence the seedling recruitment to population and thus can change population dynamics (Classen et al., 2010). Given decreasing crop production due to the frequent extreme events during spring when many species start flowering (Hedhly et al., 2009), it is expected that reproductive performance of the forest tree species is going to be affected by global warming. Increasing evidence showed the potential influence of global warming on seed germination and seedlings recruitment (Harsch et al., 2009; Milbau et al., 2009; Classen et al., 2010; Lett et al., 2018). Although, the effect of warming on germination often found to be dependent on other environmental factors such as moisture condition (Lett et al., 2018; Perez-Ruiz et al., 2018).

Elevated temperature often showed a positive effect on the growth of tree seedlings, although many mixed responses were also observed (Saxe et al., 2001; Piper et al., 2013; Lett et al., 2018). Species at their geographic range limit may show differential response to global warming. For example, using infra-red heating lamp and soil heating cable to increase the temperature by + 3.4° C for three growing seasons, Reich et al. (2015) showed that species to their warm range limit reduced growth with warming while species to their cold range limit displayed positive effect to warming. Warming along with dry condition is known to reduce the growth of tree seedlings along with a further increase of herbivory (Rodgers et al., 2018).

1.6 Phenotypic plasticity and parental environmental effects

In general, plants respond to a changing environment via phenotypic plasticity, adapt through natural selection or migrate to track the favourable conditions to which they are adapted in space. Adaptation through natural selection in long-lived trees is likely not an exclusive option given their long generation times; many species will be unable to keep up with the rapid climate change as expected at the end of 21st century (Corlett & Westcott, 2013; Sittaro et al., 2017). Phenotypic plasticity, the range of phenotypes a single genotype can express as a function of its environment, is genetically controlled, heritable and has potential importance to species' evolution (Nicotra et al., 2010). Three different types of environmentally induced plasticity are known: developmental plasticity, reversible plasticity (i.e., acclimation) and transgenerational plasticity (Donelson et al., 2018). The parental gametic and offspring embryonic environments induce developmental plasticity, whereas reversible plasticity occurs within juvenile or mature organisms. Both types of plasticity occur within a single generation and are mechanistically interlinked given developmental conditions not only change mean trait values but also modify the capacity for acclimation (Beaman et al., 2016). Transgenerational plasticity is a form of developmental plasticity where the environment experienced by earlier generations interacts with the environment of the current generation and influence the phenotype of offspring (Kuijper & Hoyle, 2015; Beaman et al., 2016). The magnitude of environmental conditions in both parent and offspring generations can affect the phenotypic responses differently within and across generations (Figure 1.7). Maternal effects are the most extensively studied transgenerational effects (Mousseau & Fox, 1998). Due to the long generation time, few studies assessed the parental environmental effects (i.e., transgenerational effect) in tree species (Skrøppa et al., 2010; Rix et al., 2012; Cendán et al., 2013). While it may be more relevant in tree adaptation via environmentally induced epigenetic inheritance regarding the rapid shift of climate (Brautigam et al., 2013). Because, in long-lived trees, an epigenetic change can occur in the relatively undifferentiated meristem cells, which give rise to both vegetative structures and the germ cell lineage (Jablonka, 2013). Such epigenetic variations

that were induced years earlier in long-lived trees can be transferred to their offspring (Jablonka, 2013).

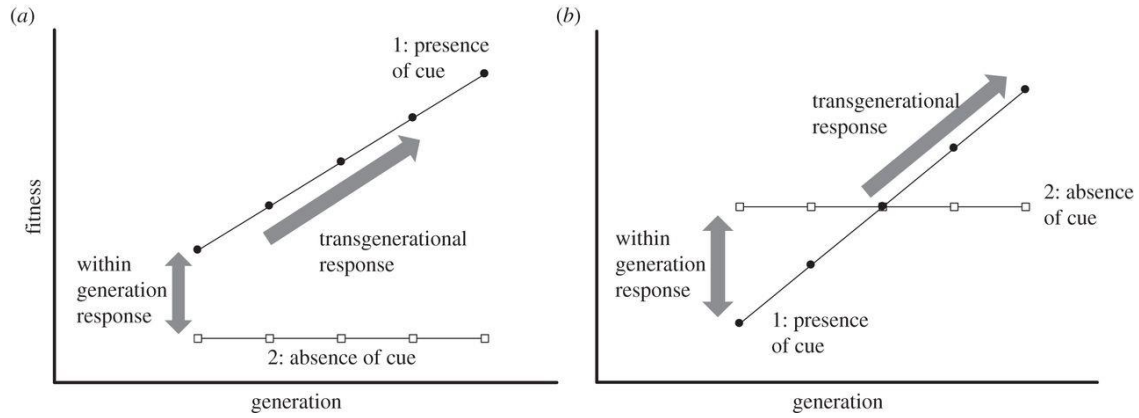

Figure 1.7 Graph showing predictions from theory for the evolution of within- and across-generation phenotypic plasticity. High environmental (temporal) variation is expected to favour increased within- and across-generational plasticity (a), while low temporal variation favours increased transgenerational plasticity (b). Adapted from Walsh et al. (2016). The X axis represents different generations and the Y axis represents the fitness of organisms within and across generations. The line with black circles denotes expectations for organisms reared in the presence of the environmental cue and the line with open squares denotes the expectations for organisms not exposed to the environmental cue.

1.7 Molecular mechanism of plasticity and the role of epigenetics

The ability of an organism to express plasticity in a given trait is mediated at the molecular level (Nicotra et al 2010), which is generally the result of environmentally sensitive gene expression and regulation of gene products (Beaman et al., 2016). The involved molecular process is referred to as “epigenetics”. Epigenetic modifications are any mitotically or meiotically heritable contribution to the phenotype without changing the DNA sequence (Beaman et al., 2016). Epigenetic modifications, such as methylation of histones and DNA by methyltransferases, are principal regulatory mechanisms that translate developmental cues into differential gene expression programs. Methylation of histones and DNA inhibit the binding of transcription factors to DNA and thereby influence gene expression (see Figure 1.8). During DNA methylation, a methyl group attached to one of the four bases in the DNA molecule (usually cytosine) followed by silencing of the gene activity (Figure 1.8). Methylation events are in turn regulated by noncoding RNAs, and modifications of RNA itself can induce plasticity. The effects of epigenetic modifications that occur in gamets, early during the development or rest of a life time of an organism can persist later in life and/or across generations and influence gene expression.

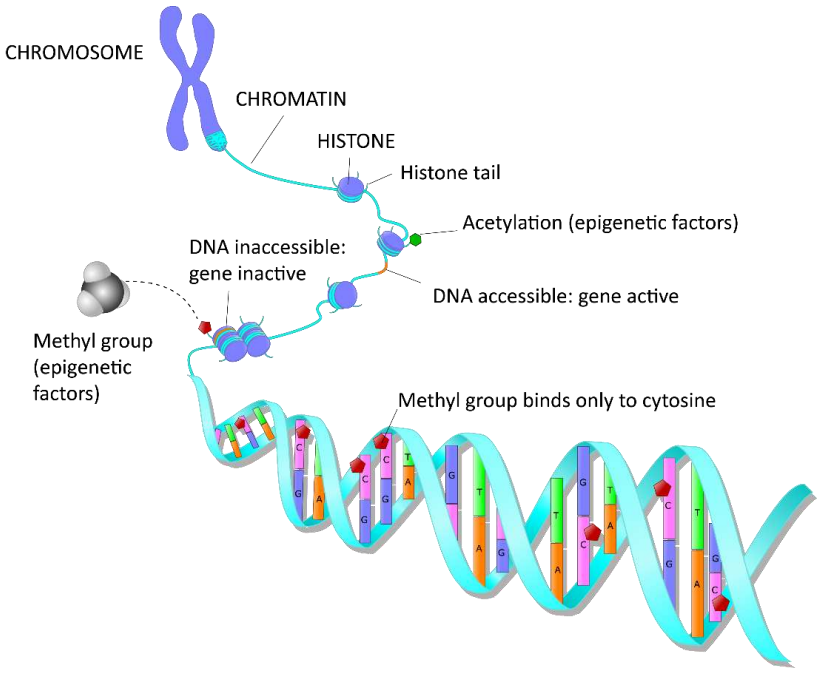

Figure 1.8 Representation of the chromatin structure, including histones and DNA, which become available to epigenetic marks.

Adapted from http://www.amsbio.com/epigenetics.aspx

1.8 Parental environmental effects on the responses of offspring

There is growing evidence showing the parental condition effects on the responses of offspring in annuals (Auge et al., 2017; Groot et al., 2017; Lampei et al., 2017), and perennials (González et al., 2017; Munzbergova & Hadincova, 2017). By manipulating both parental, grandparental and offspring temperature conditions in 14 genotypes of *Arabidopsis thaliana*, Groot et al. (2017) showed that the transgenerational effects increased flowering and influenced the flowering time in the third generation. In Norway spruce (*Picea abies*), the temperature during zygotic embryogenesis and seed maturation shifted the developmental program of the seeds, resulting in significant phenotypic changes, which lasted as long as 20 years (Skroppa & Johnsen, 2000; Skrøppa et al., 2010). An epigenetic memory mechanism was observed affecting the timing of bud burst phenology and the expression of bud burst related genes in genetically identical Norway spruce epitypes (an epigenetic alteration in a gene), allowing them to adapt rapidly to a changing environment (Carneros et al., 2017). The temperature sum experienced by the developing embryo and photoperiod conditions during embryogenesis epigenetically shifts the growth cycle of the embryos, giving rise to different epitypes from the same genotype (Yakovlev et al., 2014). Parental environmental effects were also reported in other conifer species such as white spruce (*Picea glauca* x *Picea engelmannii*) crosses, *Larix* spp., lodgepole pine (*Pinus contorta* var. latifolia), 'interior' spruce (*Picea glauca* (Moench) Voss x *Picea engelmannii* Parry ex. Engelm.) and western hemlock (*Tsuga heterophylla*) (Greenwood &

Hutchison, 1996; Webber et al., 2005; Liu & El-Kassaby, 2014). In addition, environmentally induced epigenetic variation was observed in natural populations of poplar (*Populus* spp.), valley oak (*Quercus lobate*) and mangrove tree species (*Laguncularia racemose*) (Lira-Medeiros et al., 2010; Raj et al., 2011; Platt et al., 2015). Epigenetic mechanisms can underpin plastic responses to environmental change, which may also be relevant for the persistence of clonal species (that are able to reproduce via vegetative means), those thriving in rapidly changing environments (Latzel et al., 2016). In vegetative reproduction, due to the absence of embryogenesis, the resetting of epigenetic modification can be avoided, and vegetative offspring can inherit epigenetic information of previous environmental interactions from the maternal ramet (Latzel et al., 2016). Using perennial clonal species (*Festuca rubra*) in a growth chamber, Munzbergova and Hadincova (2017) showed significant maternal condition effect on the species responses to climate change and the direction of the effect of the maternal climate was of different directions and intensities depending on plant origin and trait studied.

1.9 Knowledge gaps and hypotheses

Considering the steady increase in global surface temperature with an irregular pattern in seasonal temperatures, incorporating relevant magnitudes of transgenerational effects across different generations to environmental change are paramount to acquire the most relevant information for predicting the responses of species to future global change. Yet, our knowledge regarding the effect of the parental environment on the responses of seedlings to environmental change is limited to a few tree species. Despite the relevance of parental temperature effects in the responses of offspring to global warming, few studies included the parental temperature conditions to estimate the responses of tree seedlings to global warming. The successful establishment and recruitment of tree seedlings determine the succession of the forest (tree regeneration) (Chen et al., 2014a), which can be limited by germination and growth of the seedlings. Our knowledge on the role of the parental temperature on the germination success and growth of forest tree seedlings is still limited. The question also remains whether the tree seedlings would able to take advantage of elevated temperatures by extending the growing period. We still lack a good understanding of the extent to which epigenetic inheritance can occur for a wide range of tree species. In addition, we need to understand the role of the parental environment in controlling the mechanisms of how environmental signals being sensed and processed to alter the gene expression and thus related responses (e.g., phenological responses) of the offspring generation.

Given the influences of temperatures during seed maturation on the germination success (Donohue, 2009), we expect that the elevated maternal temperature would reduce the seed

germination in many temperate tree species. We still do not know whether the most observed trend of early spring bud burst and delayed growth cessation in response to global warming would persist across different temperate tree species if the mother trees exposed to warming. In general, the elevated temperature might advance bud burst time of some species, which would likely extend the growing season. In turn, it may increase the risk of late frost damage, which consequently is likely to reduce growth. Some other species such as late successional beech (*Fagus sylvatica*), which require longer chilling hours, may not be able to fulfil the required chilling hours due to the elevated winter temperature and would delay the bud burst time until the chilling hours fulfilled. Therefore, there would likely a trade-off between avoiding late frost damage and growth.

With global warming, however, photoperiod is not going to change. The question then arises as to how photoperiod sensitive tree species are going to respond to rapid global warming (Zohner et al., 2016). If we infer photoperiodic control on the onset of spring growth, then we can expect that many dominant forest tree species such as oak (*Q. robur*) and beech (*F. sylvatica*) would delay the onset of spring growth (i.e., bud burst) and reduce the growth. Whereas, the opportunistic species like *Populus* with no photoperiod sensitivity for spring growth might be able to take advantage of warming by starting the spring growth earlier.

1.10 Aim of the study

The main aim of this study was to understand the effects of maternal temperature on the germination, bud burst and growth cessation (here, leaf senescence in *Fagus* and *Quercus* and bud set in *Populus*) of tree seedlings and vegetative cuttings. This study was carried out on one late successional species, i.e., European beech (*Fagus sylvatica*), and one mid-successional species i.e., pedunculate oak (*Quercus robur*) and on several early successional species, i.e., black poplar (*Populus nigra*) and hybrid poplar (*Populus trichocarpa* × *P. deltoides*, *P. trichocarpa*). More specifically, we wanted to advance our understanding of the effects of elevated temperature in maternal and offspring generations on seed germination, growth and bud phenology of the seedlings.

The main research questions of this thesis were:

1) Will seed germination be affected if mother trees are exposed to elevated temperature during reproduction?
2) Do elevated maternal temperatures influence the timing of bud burst and growth cessation of the tree seedlings?
3) Does elevated maternal temperature influence the growth of the seedlings?
4) Does maternal environmental effects (cross-generational effects) on bud phenology persist in vegetative offspring (by stem cuttings)?

1.11 Outline of the thesis

This thesis is organized in four main chapters of studies, one chapter for the introduction of the studied species and at the end, a general discussion and conclusion. A box between chapter 3 and chapter 4 shows the responses of germination to warming of fructifying branches. Before focusing on the studies, a brief description of the taxonomy, ecology, geographical distribution, habitat, and reproduction of each species is given in a separate chapter (Chapter 2). A schematic diagram is presented in Figure 1.9 with the outline of the main four chapters along with brief experimental methods and duration of the studies. Two chapters (Chapter 4 and Chapter 6) represent observational studies; while the other two chapters (Chapter 3 and Chapter 5) represent experimental studies based on the application of heating treatments.

All four chapters (Chapter 3-6) focus on understanding the maternal effects on the response of tree seedlings in the face of global warming. Specifically, in Chapter 3, we studied the interactive effect of maternal temperature with the elevated temperature in offspring generation on the relative growth and time to bud burst and leaf discolouration in oak and beech seedlings. We used the temperature variation in space (at different forests in Flanders) and time (years) during seed maturation and applied a warming treatment using infrared heating lamps by elevating the soil surface temperature by 2.5°C between January-April for two years. In Chapter 4, we studied the maternal effects regarding local adaptation to the temperature and photoperiod on germination, bud phenology of the offspring using natural temperature differences across latitudes where seeds were collected from a mature provenance trial of four different provenances and seeds were grown in two common gardens at two different latitudes (temperature difference: 2°C).

Using controlled crossings between three pairs of genotypes and applying a combination of two maternal temperatures (warming during pollination and warming during seed maturation), we studied the effect of maternal temperature on seed germination, growth and the timing to bud burst and bud set of black poplar seedlings in Chapter 5. The maternal effect mediated by environmentally influenced epigenetic variation might be more relevant to vegetative offspring (Latzel & Klimešová, 2010a). Therefore, Chapter 6 was focused on exploring the maternal effect induced by different climates across latitudinal gradients (respective temperature and photoperiodic difference were 4.9 °C and 3.5 hours) on bud phenology of vegetative offspring of stem cuttings in a common garden. In addition, we assessed the global DNA methylation in the vegetative cuttings as a potential epigenetic variation induced by the environment. At last, the final chapter summarises the results of four studies (Table 7.1), provides general conclusions, and brings forward recommendations for potential future studies (Chapter 7).

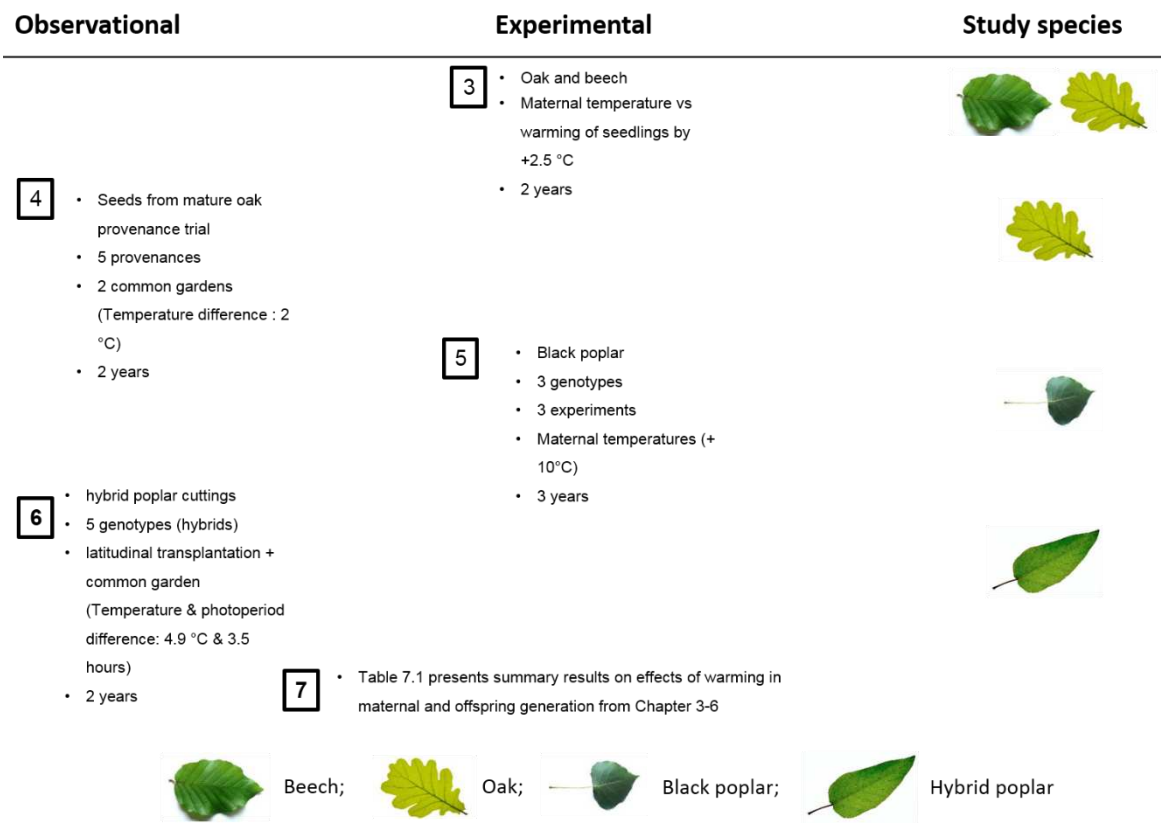

Figure 1.9 The outline of the main four chapters and general discussion of this Ph.D. thesis. The numbers with borders are the chapter numbers. The study species, the number of genotypes and experiments (where applicable), applied treatments and duration of each experiment are mentioned next to the number of chapters.

2 Study species

2.1 *Quercus robur*

2.1.1 Taxonomy

Quercus robur L., known as pedunculate or English oak, is in the genus *Quercus* and family Fagaceae (beech family). The Fagaceae originated in the montane tropics, migrated across the tropics via along the coast of the Atlantic and diverged into the principal living genera in the later Cretaceous. Rapid speciation of oaks commenced in the middle Eocene epoch (40-60 million years ago) as a response to the expansion of drier and colder climates (Axelrod, 1983; Johnson et al., 2002). Worldwide there are about 400 species of oaks (Johnson et al., 2002), and they are taxonomically divided into three groups: 1) the red oak group (*Quercus* section *Lobatae*; 2) the white oak group (*Quercus* section *Quercus* and 3) the intermediate group (*Quercus* section *Protobalanus*) (Johnson et al., 2002). *Quercus robur* is a member of the white oak section *Quercus*. All three groups include tree and shrub species. The red oaks and white oaks include evergreen and deciduous species, whereas the intermediate oaks are all evergreen.

2.1.2 Ecology

Based on different traits and principal component analysis, Leuschner and Meier (2018) grouped *Quercus robur* in mid-successional species. It is a light-demanding tree and can behave as a pioneer tree in open grassland (Praciak et al., 2013). The canopies of *Quercus* permit a good deal of light to pass through to the undergrowth, promoting the regeneration of many tree species and enriching forest diversity (Leuschner & Meier, 2018). Beech (*Fagus sylvatica*) is one substantial competitor of *Quercus*, in the presence of which the oaks are unable to predominate (Ligot et al., 2013). Compared to other mid and early successional species such as *Acer*, *Betula*, litter decomposition of *Quercus* is slower (Hobbie et al., 2006). *Quercus robur* supports a wide range of organisms that benefit from the food, support and shelter it supplies. Oak acorns are rich in starch and provide a good source of food to many wild creatures including jays, mice and squirrels (Ellenberg, 2009). Oak trees host hundreds of species of insects including leaf aphids and gypsy moths (Alford, 2012). In autumn, the soft leaves break down to form a rich leaf mould beneath the tree, which supports a wealth of invertebrates and fungi (Ellenberg, 2009).

Quercus has endogenous rhythmic growth such that it produces several shoots within each growing season (Collin et al., 1996; Leuschner & Ellenberg, 2017). It also displays endogenous rhythmic growth with alternating shoot and root growth flushes (Herrmann et al., 2015).

2.1.3 Geographical Distribution

Quercus robur is distributed throughout the lowland of Europe from the Iberian peninsula, France and Britain to the Urals in Russia (Jones, 1959). Its boundaries extend to northern Scotland, western Norway through the coastal belt of eastern Norway and southern Sweden, southern Finland, Livonia, and at Orsk in the Urals where it reaches to its eastern limit (Figure 2.1). Exact southern limits are uncertain on account of confusion with allied species (especially with *Q. pedunculiflora* K. Koch in the Balkans).

Figure 2.1 Geographical distribution of *Quercus* spp. in Europe (Meusel et al., 1965).Habitat

Quercus robur grows in areas with a mean temperature in the warmest month (July) of 15-22.9 °C and it can grow in areas with mean temperatures of the coldest months equalling -1 °C (Jones, 1959; Drobyshev et al., 2008; Miller et al., 2008). It grows in a wide range of precipitation (450 mm - 1960 mm per annum) and soil types (Dengler, 1930; Jones, 1959;

Friedrichs et al., 2009). Although according to the occurrence, it performs better in basic soils rich in mineral nutrients and prefers moist, heavy soils with the tolerance of a considerable degree of waterlogging and even flooding (Ellenberg, 2009). It forms forest on the floodplains of the great river valleys, where it may be associated with *Ulmus* spp., *Carpinus*, *Acer*, *Fraxinus*, etc., and occurs on a higher level than *Populus* spp. and *Salix* spp. (Ellenberg, 2009).

2.1.5 Reproduction

Q. robur is monoecious, i.e., it produces male and female flowers on the same tree (Johnson et al., 2002). The male flowers are grouped into loose pendulous catkins; the female flowers are grouped into short stiff spikes (Figure 2.2). The flowers open in May about 7-14 days after the buds have begun to open (Jones, 1959). In a given locality the flowering season lasts 2-3 weeks, which can vary based on location in the forest and elevation (Jones, 1959; Johnson et al., 2002). *Quercus* spp. are wind pollinated. The pollen is copiously produced and travels long distances (Jones, 1959), although, from the study of Moracho et al. (2016), we know that most pollination of *Quercus robur* occurs within stands, either between local mates (85.6%) or through selfing (6.8%). Individuals of *Quercus* species start to flower at the age of 15-35 years and in the warm climates of southern Europe, fruiting begins earlier than it does in the north (Jones, 1959; Johnson et al., 2002). Open-grown trees begin to fruit at a considerably earlier age (Kasprzyk et al., 2014). In the open, the individuals produce seed almost every year while in the stand it occurs at intervals of 3 or 4 years, and years in which there is an almost complete failure to produce seed are frequent (Wesołowski et al., 2014). Many factors such as site and stand conditions, crown size, tree position as well as climate history affect the inconsistent seed production (Martiník et al., 2013). A long warm growing season appears to be necessary for fruiting; hot late summer and autumn favours the laying down of flower buds and is often followed by abundant seed (masting) in the following year (Jones, 1959). High spring temperature in the masting year was found to be the essential weather cue for masting in oak (Nussbaumer et al., 2018). Pollen availability is known to control masting in *Quercus* species (Koenig et al., 2015). A significant increase in the seed production of temperate oaks with increasing spring temperature is observed over the last decade (Caignard et al., 2017). Acorns vary greatly in size between individual trees and years. The average number of acorns lying on the ground beneath oak canopy varies from 50—170 per sq. m. Hybridization often occurs between *Q. robur* and *Q. petraea* in natural population (Jones, 1959).

Acorns of *Q. robur* mature in one growing season. Six to nine weeks after pollination zygote develops into a globular-shaped embryo and morphologically the development of embryo completes 13 to 18 weeks after pollination (Prewein et al., 2005). Then, the acorns mature and reach the shedding phase 18-19 weeks after pollination. Usually the acorns of *Q. robur*

germinate soon after falling, after a somewhat longer delay; the radicle makes considerable growth during the late autumn and early winter (Johnson et al., 2002). There is no natural dormant period, and acorns will continue to grow throughout the winter in a greenhouse with adequate temperature (Johnson et al., 2002). Acorns are in some ways delicate fruit and are readily killed during the winter by unsuitable conditions. Acorn of *Quercus* is recalcitrant (Baskin & Baskin, 2001). That means the viability of acorns rapidly falls by losing more than 30 % of their fresh weight (Ozbingol, 2005). Further, a temperature of -6° C for 4 hours observed to kill 50% of the acorns (Ozbingol, 2005). *Q. robur* acorns will survive very well submerged in water which is not stagnant; no growth takes place, but even acorns which have begun to germinate before submergence keep reasonably well (Jones, 1959). It suffers severely from the fungus, partly because it is the more liable to be defoliated and to form Lammas shoots (that is a young leafy shoot produced usually in late summer).

Figure 2.2 Male and female flowers of pedunculated oak (left) and European beech (middle), and female flowers of black poplar (right).

2.2 *Fagus sylvatica*

2.2.1 Taxonomy

Fagus sylvatica L., the European beech or common beech, is part of the beech family Fagaceae. *Fagus* originated in the Early Tertiary in the northern Pacific Basin (Denk, 2003). *Fagus sylvatica* has been regarded as having two subspecies (ssp. sylvatica and ssp. orientalis (Lipsky) Greuter & Burdet, oriental beech) (Packham et al., 2012). Of the 13 species of *Fagus*, eleven are East Asian; *F. sylvatica* is European and *F. grandifolia* is North American (Shen, 1992).

2.2.2 Ecology

European beech (*Fagus sylvatica* L.), the main species in deciduous forests in Central Europe, is the most competitive tree species on sites with moderate soil moisture and acidity (Ellenberg, 2009). Beech, as many plant species of the cool-temperate central European

climate, experiences endogenously imposed periods of dormancy, in which cell division in the meristems is inhibited via genetic control (Leuschner & Meier, 2018). It is a late successional species (Leuschner & Meier, 2018) and is highly shade tolerant. It can be regenerated naturally in continuous cover silvicultural systems (Packham et al., 2012). Once beech has become the dominant species, this creates low light levels in the understory (Leuschner & Meier, 2018) where beech seedlings can survive better than other species. The large quantity of beech litter (ca. 900 g/m² per year), leads to the formation of a soil rich in humus (von Wühlisch, 2008). Beech litter decomposition is the slowest compared to other species such as *Acer*, *Betula* (Hobbie et al., 2006), which is due to the unpalatability of beech litter to earthworms, so beech forests, if undisturbed, show a thicker layer of humus than forests of any other species (Packham et al., 2012).

2.2.3 Geographical distribution

Beech is widely distributed in southern, central and western Europe (Figure 2.3). It reaches to its eastern distribution limit in eastern Poland, and to the north, it extends to southern Sweden, in the coastal strip southeast of Oslo, and isolated occurrence found in the region of Bergen in Norway (Packham et al., 2012). Within its main area of distribution, it is absent from the more continental climates of the Great Hungarian Plain and also from the lower Danube and Po valleys (Pidek et al., 2010).

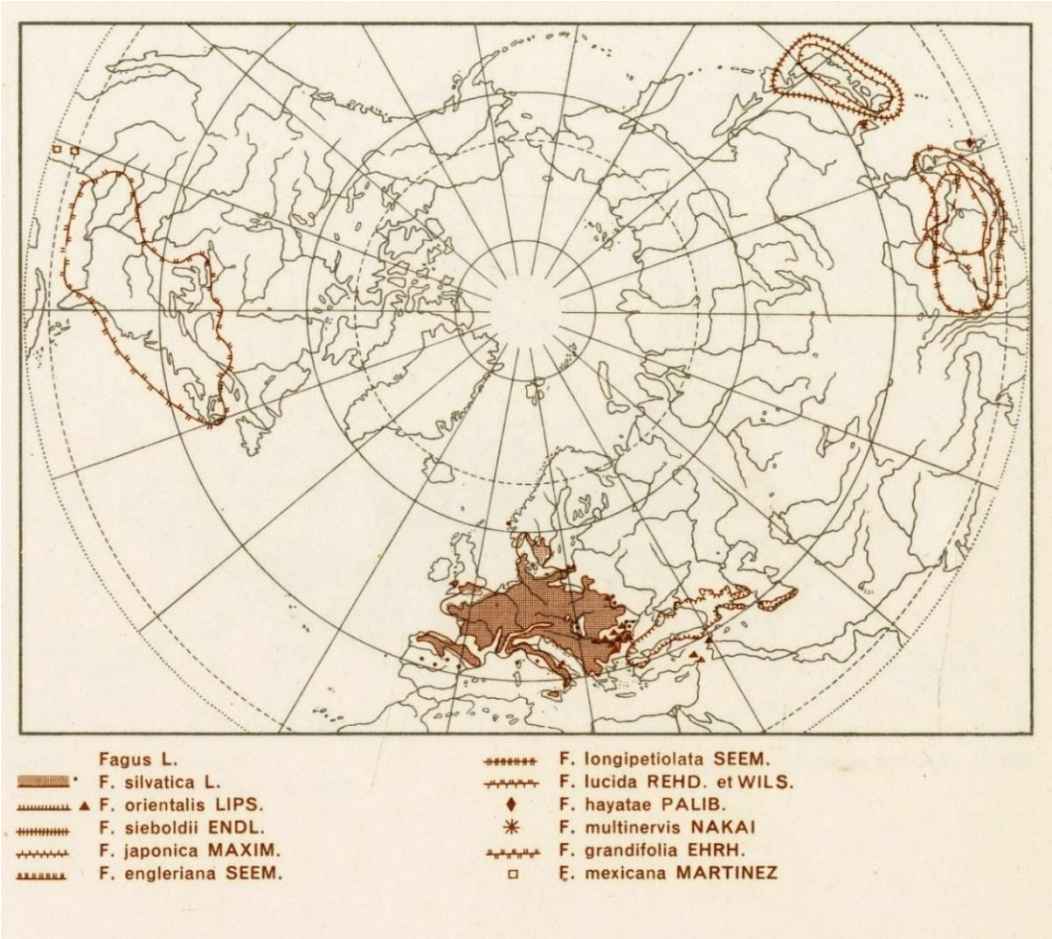

Figure 2.3 Geographical distribution of *Fagus* spp. (Meusel et al., 1965).

2.2.4 Habitat

Beech grows in regions with moist summers and mild winters, and it avoids the continental areas in Eastern Europe. For the growth of beech, an annual precipitation range of 520-1000 mm and a mean annual temperature of 4.5–6.0 °C is necessary with the mean temperature of 13–20 °C in the warmest months and minimum -2.3 °C in the coldest months (Leuschner et al., 2006; Packham et al., 2012). It grows in a wide range of soils and pH (3.5 to 8.5) over Europe, prefers well-drained soils and does not tolerate even relatively short-term flooding.

2.2.5 Reproduction

Beech is monoecious and protogynous (that is, the female reproductive organs come to maturity before the anthers) (Packham et al., 2012) . Male flowers are crowded into slender-stalked, pendent globose heads, female inflorescence usually with a cluster of two flowers, each with three styles (Figure 2.2). Beech is wind-pollinated (anemophilous) and self-incompatible (Nielsen & de Muckadeli, 1954). Pollen transport is generally limited to <500 m (Packham et al., 2012). The embryo develops completely 11 weeks after flowering and seeds mature and reach the phase of shedding 18 weeks after flowering (Pukacka &

Ratajczak, 2010). Beech begins flowering late in life, at approximately 40 years of age (Firbas, 1949).

The masting (the periodic synchronous production of very large seed crops) cycle is usually 4–8 years (Pidek et al., 2010). A cold and wet summer two years before a mast year and a dry and warm summer one year before a mast year are known to be important weather cues for masting of beech in Europe (Nussbaumer et al., 2018). Seed production in a mast year can range up to 1500–4000 seeds m^{-2} (Harmer, 1994). The viability of beech seeds is known to reduce at temperature below 0°C and growing humidity (Ratajczak & Pukacka, 2005). Seed germination in beech occurs in spring. Beech requires adequate moisture availability until germination had occurred. It has intermediate physiological dormancy, which can be broken by cold stratification (Suszka, 1966).

2.3 *Populus* spp.

2.3.1 Taxonomy

The genus *Populus* in the Salicaceae family appears in the fossil record after the Eocene (40 million years ago), and probably as early as the late Paleocene (Eckenwalder, 1996). *Populus* and *Salix* are the only genera in the family of Salicaceae. According to Eckenwalder (1996), there are 29 species of *Populus* divided into six sections namely *Abaso*, *Aigeiros*, *Leucoides*, *Populus*, *Tacamahaca*, and *Turanga* based on the morphological characters (Eckenwalder, 1996). Hybridisation is known to be common between species in different sections. There is broad disagreement about the number of species in the genus *Populus*, which arises probably because of the extensive phenotypic variation observed within broadly distributed *Populus* species, as well as the existence of many hybrids, which are sometimes misclassified as separate species (DiFazio et al., 2011). The number of *Populus* species currently recognized in the literature ranges from 22 to 85, with over 60 species in China alone (Fang et al., 1999; DiFazio et al., 2011).

2.3.2 Geographical distribution

Many species of *Populus* occur across broad geographic areas in the world (Stanton et al., 2010). In the section *Populus*, the transcontinental range of quaking aspen (*P. tremuloides*) extends in North America, from Alaska's sub-arctic region and Canada's Northwest Territory to central Mexico (Perala, 1990). Common aspen (*P. tremula*), the sibling species of *P. tremuloides*, has the most expansive range in the genus and is found throughout most of Europe and a substantial part of Asia. *P. nigra* in section *Aigeiros* is found over a large portion of Europe, the Mediterranean basin, Central Asia, Ukraine, Russia, and the northwest of China (Stanton et al., 2010) (Figure 2.4). Another species in this section, *P. deltoides* is distributed over 20°- 40° of latitude in North America between the Canadian

prairie and the Gulf of Mexico and between the Atlantic seaboard and the Great Plains (Cooper, 1990). The distribution of Black cottonwood (*P. trichocarpa*) in section *Tacamahaca* is also substantial spreading from Cook Inlet along the Alaskan Coast southward to Mexico's Baja Peninsula, and from the Rocky Mountains to the coast of southeast Alaska (DeBell, 1990).

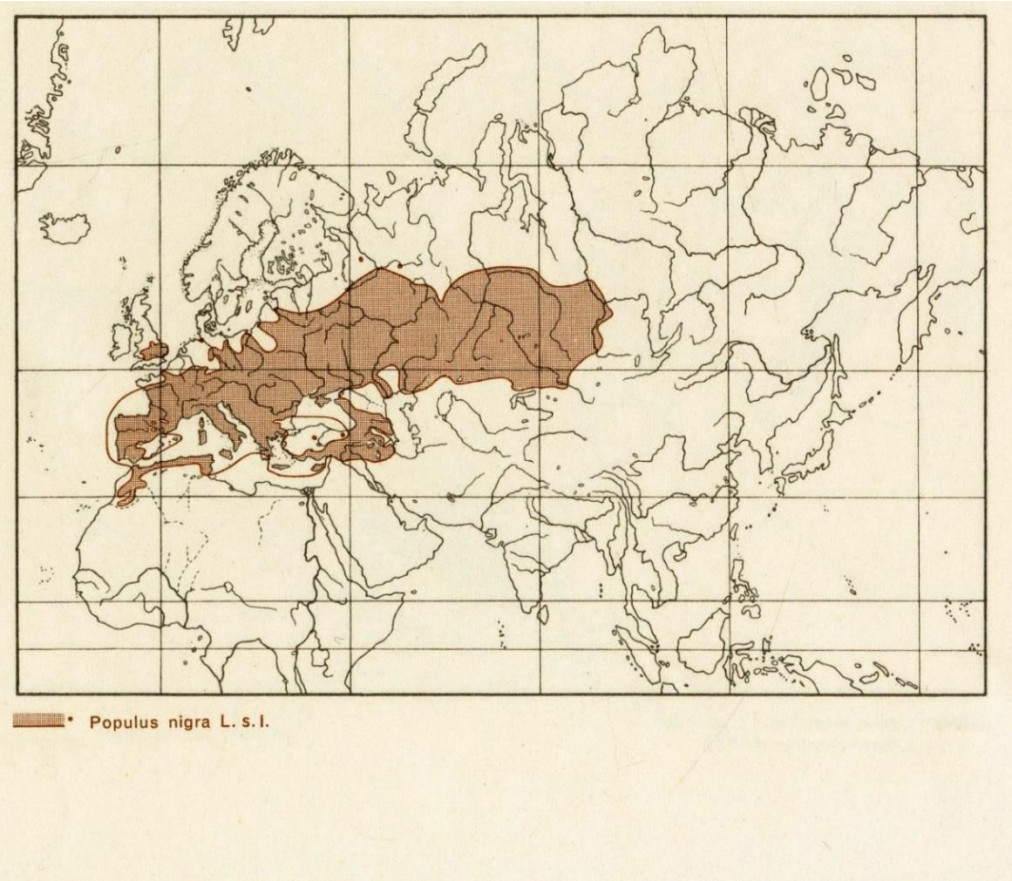

Figure 2.4 Geographical distribution of *Populus nigra* (Meusel et al., 1965)

2.3.3 Habitat

Populus species span a remarkable range of habitats: riparian, on establishment opportunities created by ice scouring and even fire, on upland sites (Bradshaw et al., 2000). Although, some species have more restricted habitat, such as *P. alba* is exclusively riparian in some parts of its range in Europe, some other species span entire continents across a wide range of environments, such as *P. tremuloides* in North America (Perala, 1990).

2.3.4 Ecology

Populus are usually dominant and pioneer species of riparian ecosystems due to their tolerance for complete even flooding (Karrenberg et al., 2002; Glenz, 2005; Isebrands & Richardson, 2014). The dynamics of the populations and the phases of colonization are directly related to the dynamics of the rivers (Karrenberg et al., 2002). On the active zones

of flood plains of large rivers, *Populus* plays important role in the patches of woody pioneer vegetation which are short lived (Bayard M. & F.H., 1991). Where stable conditions persist for more extended periods of time, pioneer softwood communities including *Populus* may be replaced by hardwood forests, often including species of *Acer*, *Ulmus* and *Fraxinus* (Ellenberg, 2009), or in boreal zone, by coniferous forests (Helm & Collins, 1997). *Populus* express episodic growth cessation in response to exogenous factors (Herrmann et al., 2015). Leaf litter production in *Populus* varies from 130- 640 g/m² per year depending on the environmental condition and site (Andersen et al., 2003; Cotrufo et al., 2005). Comparing to *Fraxinus* and *Alnus*, litter decomposition rate is known to be slower in *Populus* in Mediterranean riverine areas (Pérez-Corona et al., 2006).

2.3.5 Reproduction

Populus species are mostly dioecious (rarely monoecious), with separate male and female organs in different individuals and obligatory outcrossers (Bradshaw et al., 2000; Leplé et al., 2000). It starts flowering within 4–8 years in intensively managed plantations and within 10–15 years under favorable conditions in natural populations (Isebrands & Richardson, 2014). Flowering usually occurs before leaf emergence in early spring (Eckenwalder, 1996). Pollen is dispersed by wind, and the pollination distance is extensive (Vanden Broeck, 2004). Under normal field condition, full embryo development of *Populus* requires four weeks after pollination (Zenkteler et al., 2005). Seeds are produced in great numbers (> 25 million per tree per year) (Braatne et al., 1996), and their small size and cotton-like appendages facilitate dispersal over large distances by wind and water (Braatne et al., 1996; Karrenberg et al., 2002).

Seeds of *Populus* are recalcitrant, that is the viability of seeds falls when the moisture content of seeds drops below 30-65% (Baskin & Baskin, 2001). The viability of seeds retains for only 1–2 weeks in natural systems, and germination occurs within 24 hours under warm, moist conditions (Šiler et al., 2014). Vegetative propagation occurs extensively in *Populus* and probably this trait enable *Populus* to occupy intense habitats along river banks and to persist longterm in landscapes that are washed away or fragmented by powerful floods (Karrenberg et al., 2002; DiFazio et al., 2011).

3 Phenology and growth of *Fagus sylvatica* and *Quercus robur* seedlings in response to temperature variation in the parental vs. offspring generation

After: Sumitra Dewan, Pieter De Frenne, Olivier Leroux, Ivan Nijs, Kristine Vander Mijnsbrugge, Kris Verheyen (2019) Phenology and growth of *Fagus sylvatica* and *Quercus robur* seedlings in response to temperature variation in the parental vs. offspring generation. *Plant Biology*, in press.

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Abstract

Plants are known to respond to warming temperatures. Few studies, however, have included the temperature experienced by the parent plant in the experimental design, in spite of the importance of this factor for population dynamics. We investigated the phenological and growth responses of seedlings of two key temperate tree species (*Fagus sylvatica* and *Quercus robur*) to spatiotemporal temperature variation during the reproductive period (maternal temperature) and experimental warming of the offspring. To this end, we sampled oak and beech seedlings of different ages (one to five years) from isolated mother trees and planted the seedlings in a common garden. Warming of the seedlings advanced bud burst in both species. In oak seedlings, higher temperatures experienced by the mother trees during the reproductive period delayed bud burst in control conditions but advanced bud burst in heated seedlings. In beech seedlings, bud burst timing advanced both with increasing maternal temperature and with experimental warming of the seedlings. With higher maternal temperature, the diameter increment of the warmed seedlings decreased compared to the controlled oak seedlings. Overall, oak displayed more plastic responses to temperatures than beech. Our results underpin that the maternal temperature during the reproductive period can be a potential determinant of tree responses to climate change.

3.1 Introduction

Current climate change is affecting vegetation in terrestrial ecosystems across the globe (Peñuelas et al., 2007; Morin et al., 2009; Yu et al., 2010; Stocker et al., 2013). With increasing global surface temperatures many plant species advance the timing of their spring leaf flushing and first flowering (Morin et al., 2009; Prieto et al., 2009; Anderson et al., 2012; De Frenne et al., 2018). Global warming often enhances overall plant growth by affecting the growing-degree hours and the total number of freezing days, but at the same time, other factors of climate change for example decrease in water availability along with extreme temperatures might limit the plant growth (Mora et al., 2015). Plant responses are also influenced by the climatic conditions of the parental generation (Walter et al., 2016; Groot et al., 2017; Munzbergova & Hadincova, 2017).

The environmental conditions experienced by the parental generation interact with the environment of the offspring generation to influence the performance of the offspring, also known as non-genetic condition transfer effect (Donelson et al., 2018). Parental condition effects are known to influence germination, seed dormancy, flowering time, fecundity, growth, morphology and photosynthetic physiology in perennials and annuals (Galloway & Etterson, 2007; Springthorpe & Penfield, 2015; Walter et al., 2016; Imaizumi et al., 2017; Lampei et al., 2017; Ren et al., 2017; Singh et al., 2017). The mechanism behind such effects is known as epigenetic inheritance. An important aspect of the parental condition effect is that it involves condition-dependent parental investment in offspring and is the most widespread type of adaptive parental effects when selection favors increased parental investment (Bonduriansky et al., 2018). However, the role of such adaptive parental effects is still under debate due to their complex nature (Galloway, 2005; Dyer et al., 2010; González et al., 2017).

One possible first step towards estimating the magnitude of parental effects with varying parental and offspring environment is to compare offspring performance across multiple environments by manipulating both the parental and the offspring environment (Bonduriansky et al., 2018). This may reveal a change of the performance in offspring along environmental gradients. Such assessment can be performed by examining a broad range of ecologically relevant environments in both parental and offspring generations (Bonduriansky et al., 2018).

Temperature in early development (from fertilization to juvenile development) of the parents and during embryogenesis of the parental generation has been reported to influence the phenology of the next generation (Burton & Metcalfe, 2014; Carneros et al., 2017; Dewan et al., 2018; Donelson et al., 2018). Temperature is also one of the key cues for controlling the phenology in many temperate tree species (Korner & Basler, 2010; Morin et al., 2010;

De Frenne et al., 2011). Bud phenology (bud burst and leaf senescence and bud set in the autumn) controls the length of the growing season and has important impact on ecosystem productivity and functioning (Zhou et al., 2001; Polgar & Primack, 2011). Temperature requirement for bud burst is under strong genetic control, especially for the chilling requirement to break winter dormancy and sensitivity to temperature (Rousi & Pusenius, 2005; Sanz-Perez et al., 2009; Vitasse et al., 2009). It is also known that the temperature sum requirement and chilling hours to bud burst are negatively correlated (Dantec et al., 2014). The temperature experienced by the parental generation may provide parents information about the future environmental condition of the offspring and parents may, subsequently, alter the offspring response to temperature (Webber et al., 2005; Groot et al., 2017). Yet, few studies of global warming included both parental and offspring environmental condition in their experimental design to understand the response of plants to global warming and actually considered the fact that the environmental conditions of parents might reshape the performance of offspring.

Considering the influence of the parental condition (here temperature), we developed three hypotheses. First, we hypothesized that the maternal temperature condition has no influence on the offspring's bud burst timing, but bud burst would advance with the warming of the offspring. This would mean that there would be a genetic control on the bud burst timing and no maternal environmental effect. In the second hypothesis, bud burst would likewise advance with the warming of the offspring, but warmer maternal temperature conditions would provide additional advancement in an additive fashion. Our third hypothesis was that the two temperature effects interact: warmer maternal conditions would delay bud burst when the offspring grows in a cooler environment but would advance it when the offspring grows in a warmer environment. In this case, the temperature cue to bud burst as well as the direction of bud burst in the offspring generation would be altered by the maternal effect. We also assumed that maternal temperature would also influence the growth cessation of the offspring generation. Due to the change in bud burst timing, the growth of the offspring might also be affected by both maternal and offspring temperature conditions. Here, we assessed the effect of both offspring and maternal temperatures on the growth (stem diameter and height increment), bud burst and leaf senescence of oak and beech seedlings in a common garden experiment.

3.2 Materials and methods

3.2.1 Study sites and sampling protocol

To be able to relate offspring temperature to maternal temperature, we used seedlings of different ages from five different forests to take advantage of the temporal temperature variation in the seed maturation year. The description of the forests can be found in Table

3.1. For each of five forests, we selected three isolated mother trees except for Oostkamp Orchard with an undergrowth vegetation containing conspecific seedlings (Table 3.1). In total, we sampled 12 beech and 13 oak mother trees. We always selected isolated mother trees such that seedlings could easily be selected from one single mother tree. During the second week of October 2014, we sampled circa 20 seedlings per mother tree ranging from 1–5 years old based on visual observations of scars on the stem, the height, and diameter.

Table 3.1 Background information on the site where the seedlings were collected.

Collection site	Latitude (°)	Longitude (°)	Elevation (m a.s.l.)	No. of mother trees		Number of seedlings		Mean temperature (2007-2013)	Mean annual precipitation (2007-2013)
				Oak	Beech	Oak	Beech		
Aelmoeseneiebos	51.0	3.8	21	3	3	65	90	10.73	898
Brakelbos	50.8	3.7	140	3	3	89	86	10.57	871
Kloosterbos	50.8	3.8	66	3	3	68	44	10.63	848
Oostkamp Orchard	51.1	3.2	16	1	0	43		10.57	928
Raspaillebos	50.8	3.9	77	3	3	70	76	10.65	837

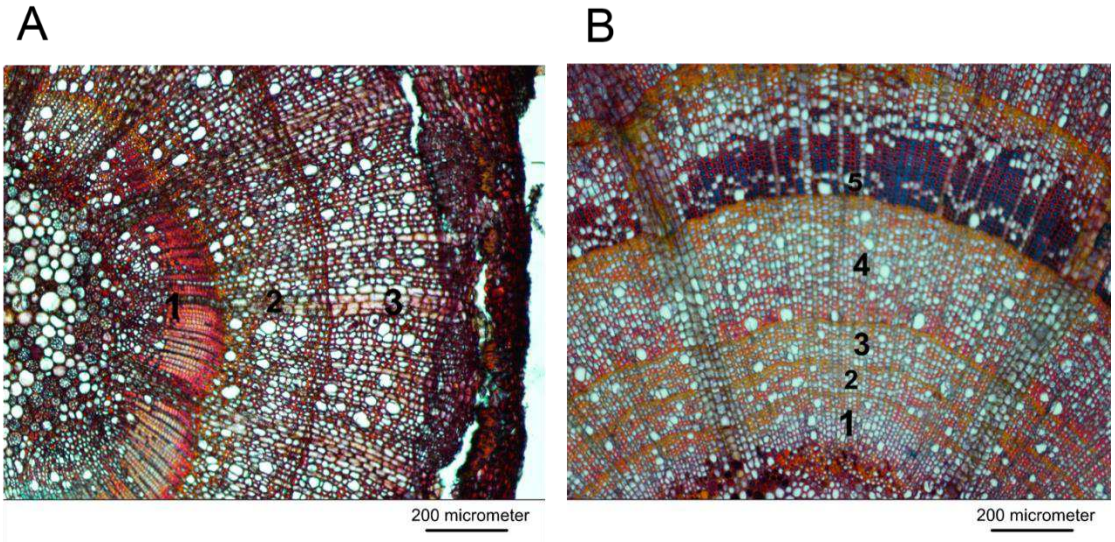


Figure 3.1 Photographs of two sections showing three growth rings of oak (A) and six growth rings of beech (B).

We selected 43 beech and 47 oak seedlings randomly based on their collar diameter for determination of age by hand-cut sectioning and growth rings counting following the method of Gruber (1998). Sections were observed and photographed using a Nikon Ni-U microscope equipped with a Nikon DS-Fi1c camera. Two photograhs of growth rings of oak and beech can be found in Figure 3.1. Out of the three allometric equations, the equation with the highest R^2 (Appendix Figure A.1) was selected to estimate the age of the remainder of 372 seedlings. We used collar diameter as a function of age in oak, whereas we used collar diameter and height as a function of age in beech (Appendix Table A.1).

3.2.2 Maternal temperatures

We used the best available gridded temperature data with a high spatial resolution for Belgium to couple the timing of phenology to the past climate (Delvaux et al., 2015). Data of weather stations based on daily maximum and minimum temperatures and daily precipitation amounts were used for the modeling. Data quality control procedures were first applied to ensure that only valid measurements were involved in the gridding process. Afterward, the set of unevenly distributed temperature data was interpolated using kriging on a 4 × 4 km² regular grid over Belgium (Wackernagel, 1995; Delvaux et al., 2015). From this model, we extracted site-specific data for maximum and minimum temperature (in °C) from 2007 to 2013, which covers the range of seed maturation years of the seedling in our study. The daily mean temperature (in °C) was approximated by calculating the average of the daily maximum and minimum temperature. The average daily minimum, mean and maximum temperatures during the reproduction period (April-September) (Appendix table A.2) and annual minimum, mean and maximum temperatures were used to assess the correlation between phenology of the seedlings, reproduction temperatures and annual temperatures (data not shown) of the mother trees. We used temperatures during April-September as reproduction period, because for both species flowering, pollination and seed maturation occur during this period. We only used the temperatures of the reproduction period for data analysis.

3.2.3 Experimental set-up

We planted 335 oak and 296 beech seedlings in 1.5 L plastic pots using standard potting soil (Peltracom, NPK 14:16:18). We removed as much soil as possible from the roots of each seedling by shaking. In January 2016, we supplied each pot 6 grams of Osmocote exact standard (NPK: 16-9-12+2MgO+TE), a slow releasing fertilizer. We watered all the seedlings during dry periods every second day at field capacity. Circa 200 seedlings (27%) died during two consecutive years of the study. Among them 14% died in the first year after transplantation, and the other 13% died mainly due to powdery mildew in the second year. There were 259 and 202 seedlings of oak and beech respectively at the end of the experiment.

Half of the plants were experimentally heated by circa + 2.5 °C using fifteen 150 W infrared (IR) heating lamps (Eider Landgeräte GmbH) during the period of end of January to April in both 2015 and 2016 (Appendix Figure A.2). Lamps were suspended using a wooden frame in four plots. The perpendicular distance between the IR lamps and the soil surface of the nearest pot was 100 cm. Effecter lamps did not emit photosynthetically active radiation (PAR). We randomly reshuffled the pots every second week within the treatments and plots, and twice between the plots during the treatment period to reduce biases. The soil surface

temperature was monitored randomly at fifteen points of each treatment every hour from 9:00 am to 16:00 pm once every week during the period of warming (January-April) using an IR thermometer (Linear laboratories model C-1600HP, accuracy = ± 1% of the reading plus one digit, emissivity = 1) (Rubio et al., 1997). To have an estimate of chilling days, we calculated the cumulative chilling days (C) between October 1 (t_1) and May 14 (t_2) during the experimental period in 2014 to 2016 following the method of Dantec et al. (2014) using the available climatic data from the weather station at Melle (www.kmi.be) , which is circa one km away from our experimental site. We calculated cumulative chilling days following the equation below and used two base temperatures (Tb): 5 and 10 °C (Dantec et al., 2014), and cumulative chilling days were presented in Appendix Figure A.3.

$$\mathrm{C} = \sum_{t2}^{t1} y(T)$$

$$\mathrm{y(T)} = \begin{cases} 0, \mathrm{T} > \mathrm{Tb} \\ 1, \mathrm{T} < \mathrm{Tb} \end{cases}$$

3.2.4 Phenology and plant growth

We monitored bud burst of each seedling during two successive years in 2015 and 2016. In addition, we monitored leaf discolouration in 2015. We scored the stages of bud burst for beech and oak following the adapted method of (Schüler et al., 2012) and (Wesołowski & Rowiński, 2006) (Appendix Table A.3). We monitored bud burst from 31 March onwards in both 2015 and 2016 twice per week until all the seedlings had opened their buds completely (that means when they reached the bud burst stage of five and six for oak and beech respectively (Appendix Table A.3). Leaf discolouration was quantified as the number of leaves that turned from green to yellow from 1 September once a week and continued until all the leaves discoloured. We considered a leaf completely discoloured when at least half of the leaves turned yellow and, at that point, counted how many leaves of each seedlings discoloured. We calculated the percentage of leaf discolouration of each seedling based on the total number of discoloured leaves. We measured the collar diameter (mm) and height (cm) in December 2014 before starting the heating experiment. At the end of the experiment in August 2016, we re-measured the collar diameter (mm) and height (cm) of the seedlings. We calculated diameter increment (i.e., the relative increment of collar diameter) and height increment (i.e., the relative height increment) of the seedlings based on the collar diameter and height at the beginning of the experiment. We used the following equations to assess the relative collar diameter and height increment of the oak and beech seedlings.

$$\mathrm{RD} = \frac{\mathrm{Db} - \mathrm{Da}}{\mathrm{Da}} \quad (1)$$

$$\mathrm{RH} = \frac{\mathrm{Hb} - \mathrm{Ha}}{\mathrm{Ha}} \quad (2)$$

RD and RH are relative collar diameter and height increment respectively. Db and Hb are respectively the diameter and height at the end of the experiment in August 2016; Da and Ha are diameter and height at the beginning of the experiment in 2014, respectively. We collected stem segments (at a height of 0.5 cm above the soil surface and of 5-10 cm length) from all the seedlings and the stem segments were stored in 70% (v/v) ethanol for age determination under the microscope.

3.2.5 Data analyses

All data analyses were performed in R version 3.3.3 (R Core Team, 2017). To analyse the interactive effect of maternal temperature with warming treatment on the traits, timing of bud burst, leaf discolouration, collar diameter and height increment of the seedlings, we used Linear mixed effects models (*lmer* function in the *lme4* package in R) (Bates et al., 2015) with Gaussian error distributions for each species separately. We used multiple random intercepts: *mother tree* nested in *site* and *seed maturation year* (as a representative for seedlings age, not nested) in the models. We used *lmerTest* package to extract the p values from the linear mixed effects models (Kuznetsova et al., 2017). We assessed the timing to bud burst (Days) and leaf discolouration (80%) from the starting day of observation (that means, total days needed to reach bud burst stage of 5 for oak and 6 for beech and 80% of the leaves of each individual turned yellow for both species from the start date of observation). The number of individuals per treatment was not the same in all response variables and years of observation due to mortality, bud damage due to mildew and frost damage (Appendix Table A.4). The age distribution of both species can be found in Appendix Figure A.4.

We estimated both marginal R^2 and conditional R^2 for all models to provide measures of goodness of fit of the models (i.e. how much of the variance they explain) where marginal R^2 indicates the amount of variation explained by only fixed effects and conditional R^2 indicates the amount of variation explained by the fixed and random effects (Nakagawa et al., 2013).

3.3 Results

3.3.1 Bud burst and leaf discolouration

We observed a significant interaction of maternal temperatures and warming on the seedlings bud burst time for both species in 2016 (Table 3.2, Figure 3.2 d-e, j). In oak, maternal temperatures affected the bud burst time of the control seedlings in both years (Figure 3.2 b,d-e). In 2016, warming advanced the bud burst time stronger when the mother trees were exposed to a higher minimum and mean temperature (Figure 3.2 d-e). In this year, control seedlings delayed their bud burst by 13 days with increasing minimum maternal temperatures while warmed seedlings advanced it by nearly two days (Table 3.2). However, the effect of maternal temperature was significant for minimum and mean daily temperatures.

In beech, the effect of maternal temperature on the seedlings bud burst time was not significant (Figure 3.2 g-l). The maternal temperature did not change the bud burst time of control seedlings. Overall, beech seedlings displayed different response to maternal temperature than oak (Figure 3.2 d-e, j and Table 3.2). We did not observe any effect for maximum maternal temperature on the bud burst time of both species. In addition, we did not observe any significant effects of maternal temperature on the time of leaf discolouration of oak and beech seedlings regardless of warming treatment (Figure 3.3 and Table 3.2).

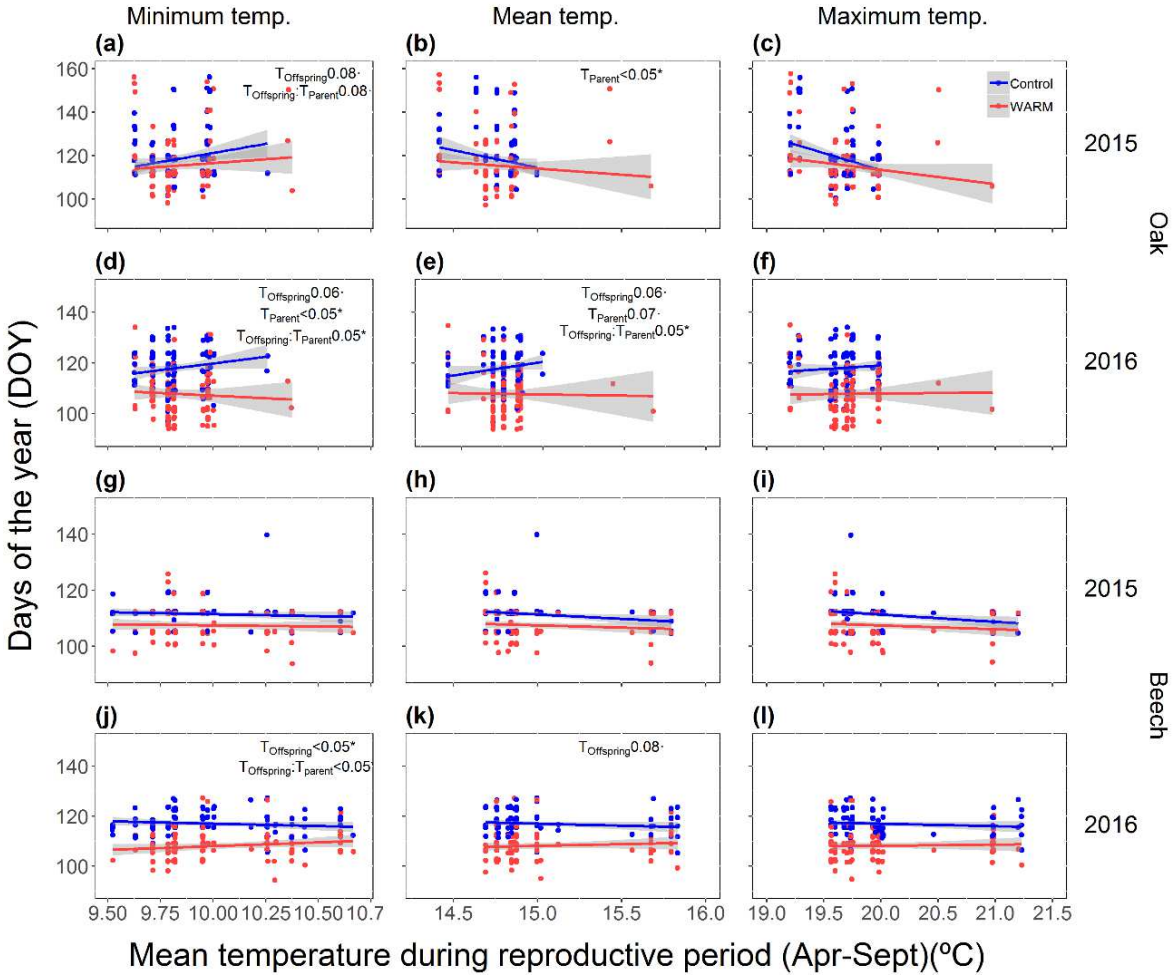


Figure 3.2 The effects of maternal temperatures (April-September, T_{Parent}) and warming of the offspring ($T_{Offspring}$) on the bud burst time in oak (a-f) and beech (g-l) seedlings.Red denotes heated seedlings and blue control. Grey polygons represent 95% confidence intervals. Values are p values from linear mixed effect models. Significance levels are denoted by 0.05 < p < 0.1, * p <0.05.

3.3.2 Plant growth

In oak, higher maternal temperatures increased the collar diameter increment of controlled seedlings but decreased it with warming (Figure 3.4 b and Table 3.2). This interaction was significant for mean daily reproductive temperature. However, we did not observe such interaction for minimum and maximum maternal temperatures (Figure 3.4 a, c and Table 3.2). In beech, maternal temperatures did not change the collar diameter increment of control and warm seedlings (Figure 3.4 d, e, f and Table 3.2). In both species, we did not observe any effect of maternal temperature on the height increment of neither controlled nor warmed seedlings (Appendix Figure A.5 and Appendix Table A.5). The variance of random effects of all models can be found in Appendix Table A.5, Appendix Table A.6.

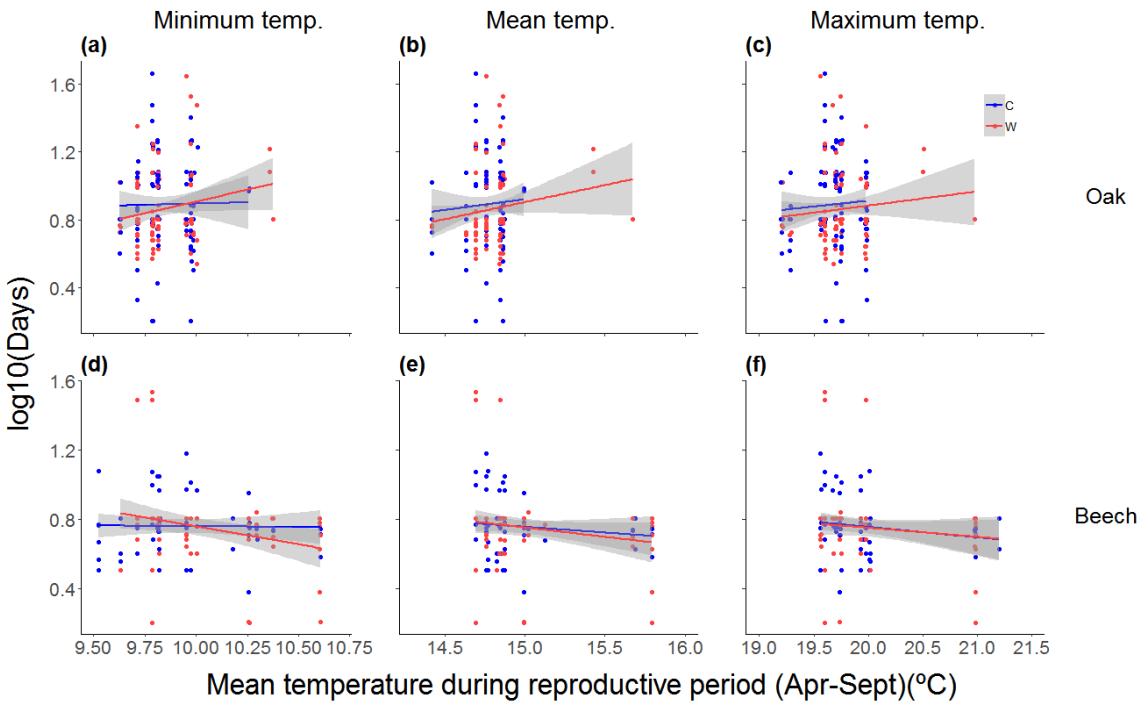


Figure 3.3 The relationship between the time of leaf discolouration in oak (a-c) and beech (d-f) seedlings and maternal temperatures (April-September). Colours denote seedling warming treatments with red heated and blue control. Log10(Days) means log10 transformation of total days needed for individual seedlings to reach 80% of leaf discolouration from start date of observation (1 September). Grey polygons represent 95% confidence intervals. Values are p values from linear mixed effect models. Significance levels are denoted by $0.05 < p < 0.1$, * $p < 0.05$.

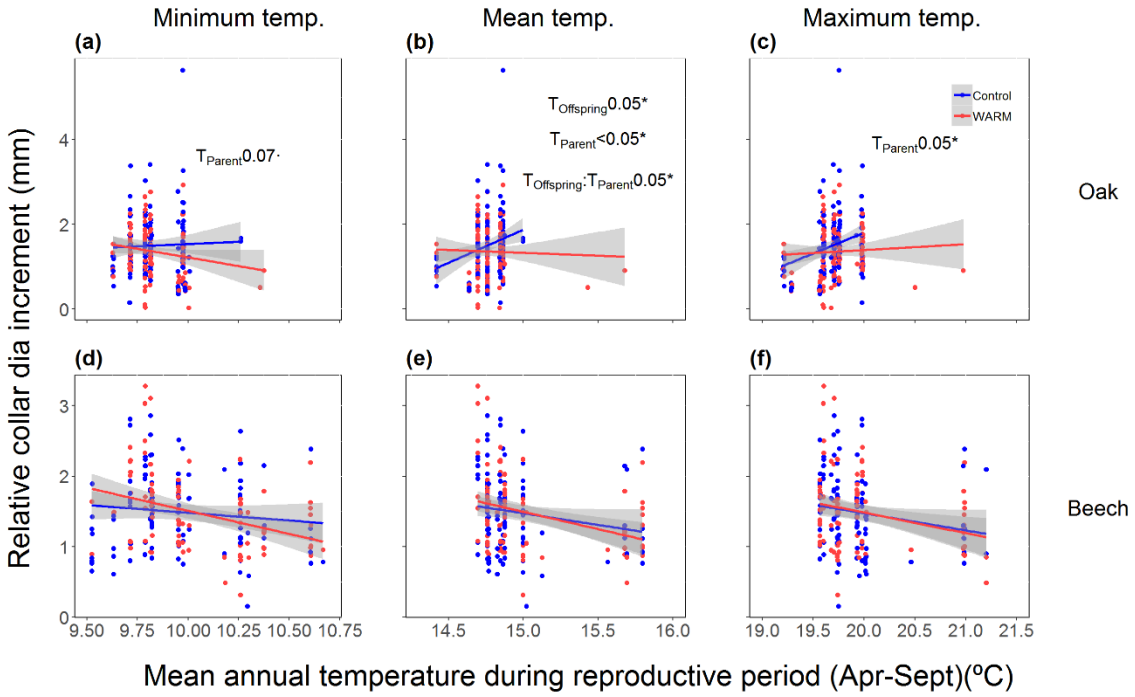


Figure 3.4 Relationship between relative collar diameter increment in oak and beech seedlings and maternal temperatures (April-September). Values are p values from linear mixed effect models. $T_{Offspring}$ means warming of the seedlings with red colours denoting heated seedlings and blue colours control seedlings, T_{Parent} means temperatures during reproductive period (April-September) of the mother trees. Grey polygons represent 95% confidence intervals. Significance are denoted by 0.05< p < 0.1, * p <0.05.

Table 3.2 The estimated parameters from linear mixed effects models on the phenology and growth of oak and beech seedlings as a function of maternal temperatures and warming condition in offspring generation. $T_{Offspring}$ means warming treatment of the seedlings and T_{Parent} means temperature maternal temperature and Leaf_discolour80 means the timing when 80% of the all the leaves of each seedling turned yellow. r²mar (only ramdom factors) and r²con (random and fixed factors together) indicate marginal and conditional r² respectively.

Fixed effects																			
Species	Response	**Effect**	Maternal temperature (°C)																
			Minimum						Mean						Maximum				
			Estimate ± Std. Error	df	t	p value	r²mar	r²con	Estimate± Std. Error	df	t	p value	r²mar	r²con	Estimate± Std. Error	df	t	p value	r²mar	r²con
Oak	Bud burst 2015	$T_{Offspring}$	154.6 ± 87.1	63.3	1.77	**0.08·**	0.05	0.39	179.7 ± 133.0	262.9	1.35	0.18	0.03	0.45	-0.8 ± 2.9	198.4	-0.25	0.80	0.02	0.38
		T_{Parent}	23.6 ± 8.2	1.1	2.86	0.20			18.6 ± 9.0	253.3	2.07	**0.04***			0.1 ± 0.1	44.9	0.69	0.49		
		$T_{Offspring}$: T_{Parent}	-16.02 ± 8.9	62.4	-1.81	**0.08·**			-12.4 ± 9.0	262.9	-1.37	0.17			0.04 ± 0.2	198.2	0.25	0.81		
	Bud burst 2016	$T_{Offspring}$	141.6 ± 75.9	235.3	1.86	**0.06·**	0.26	0.41	218.6 ±115.1	236.6	1.90	**0.06·**	0.27	0.40	73.8 ± 91.2	226.5	0.81	0.42	0.25	0.40
		T_{Parent}	13.5 ± 6.5	224.1	2.09	**0.04***			14.1 ± 7.6	139.1	1.86	**0.07·**			3.3 ± 5.2	64.6	0.64	0.52		
		$T_{Offspring}$: T_{Parent}	-15.4 ± 7.7	235.2	-2.00	**0.05***			-15.5 ± 7.8	236.6	-1.99	**0.05***			-4.3 ± 4.6	226.4	-0.92	0.36		
Beech	Bud burst 2015	$T_{Offspring}$	1.9 ±1.2	131.8	1.56	0.12	0.15	0.25	-19.6 ± 27.5	208.5	-0.71	0.48	0.19	0.24	-0.7 ± 1.4	128.4	-0.51	0.61	0.15	0.25
		T_{Parent}	0.04 ± 0.1	130.9	0.41	0.69			-2.8 ± 1.5	7.7	-1.84	0.10			-0.1 ± 0.1	129.2	-1.28	0.20		
		$T_{Offspring}$: T_{Parent}	-0.2 ± 0.1	131.7	-1.57	0.12			1.1 ± 1.8	208.4	0.57	0.57			0.03 ± 0.1	128.4	0.49	0.62		
	Bud burst 2016	$T_{Offspring}$	-59.3 ± 23.9	197.1	-2.48	**0.01***	0.43	0.48	-50.3 ± 28.6	192.7	-1.76	**0.08·**	0.43	0.47	-32.6 ± 27.3	191.1	-1.19	0.23	0.43	0.47
		T_{Parent}	-2.9 ± 2.1	10.0	-1.38	0.20			-1.7 ± 1.6	7.0	-1.01	0.34			-0.8 ± 1.2	6.4	-0.67	0.53		
		$T_{Offspring}$: T_{Parent}	5.0 ± 2.4	197.0	2.11	**0.04***			2.8 ± 1.9	192.3	1.45	0.15			1.2 ± 1.4	190.4	0.87	0.39		
Oak	Log 10 (Leaf_discolor80)	$T_{Offspring}$	-3.1 ± 2.4	223.7	-1.27	0.21	0.01	0.07	-2.2 ± 3.7	170.4	-0.58	0.56	0.01	0.07	-0.8 ± 2.9	198.4	-0.25	0.80	0.01	0.07
		T_{Parent}	-0.02 ± 0.2	139.0	-0.10	0.92			0.1 ± 0.2	66.9	0.40	0.69			0.1 ± 0.1	44.9	0.69	0.49		
		$T_{Offspring}$: T_{Parent}	0.3 ± 0.3	223.7	1.26	0.21			0.2 ± 0.3	170.3	0.57	0.57			0.04 ± 0.2	198.2	0.25	0.81		

Fixed effects																		
Species	Response	**Effect**	Maternal temperature (°C)															
			Minimum						Mean						Maximum					
			Estimate ± Std. Error	df	t	p value	r²mar	r²con	Estimate± Std. Error	df	t	p value	r²mar	r²con	Estimate± Std. Error	df	t	p value	r²mar	r²con
Beech	Log 10 (Leaf_discolor80)	$T_{Offspring}$	1.9 ± 1.2	131.8	1.56	0.12	0.02	0.24	0.3 ± 1.5	129.9	0.19	0.85	0.02	0.24	-0.7 ± 1.4	128.4	-0.51	0.61	0.02	0.27
		T_{Parent}	0.04 ± 0.1	130.9	0.41	0.69			-0.1 ± 0.1	130.9	-0.69	0.49			-0.1 ± 0.1	129.2	-1.28	0.20		
		$T_{Offspring}$: T_{Parent}	-0.2 ± 0.1	131.7	-1.57	0.12			-0.02 ± 0.1	129.8	-0.20	0.84			0.03 ± 0.1	128.4	0.49	0.62		
Oak	Relative collar dia. Increment	$T_{Offspring}$	10.2 ± 6.8	171.1	1.50	0.13	0.03	0.38	20.0 ± 10.0	100.8	2.00	**0.05***	0.04	0.38	10.3 ± 7.7	188.2	1.35	0.18	0.03	0.33
		T_{Parent}	1.1 ± 0.6	33.3	1.89	**0.07**·			1.6 ± 0.5	31.8	2.96	**0.01***			0.7 ± 0.3	30.3	2.01	**0.05***		
		$T_{Offspring}$: T_{Parent}	-1.1 ± 0.7	170.5	-1.53	0.13			-1.4 ± 0.7	100.6	-2.01	**0.05***			-0.5 ± 0.4	187.9	-1.37	0.17		
Beech		$T_{Offspring}$	2.4 ± 2.5	178.3	0.97	0.33	0.02	0.35	2.7 ± 3.1	177.6	0.87	0.38	0.05	0.38	2.1 ± 3.0	177.1	0.69	0.49	0.04	0.38
		T_{Parent}	-0.1 ± 0.3	18.8	-0.40	0.70			-0.3 ± 0.4	9.0	-0.80	0.45			-0.2 ± 0.3	7.6	-0.71	0.50		
		$T_{Offspring}$: T_{Parent}	-0.2 ± 0.2	178.2	-0.97	0.33			-0.2 ± 0.2	177.5	-0.88	0.38			-0.1 ± 0.2	177.1	-0.69	0.49		

Significance are denoted by . 0.05 < p < 0.1, * p <0.05;

3.4 Discussion

We showed that the maternal temperature altered the direct influence of warming on bud burst and growth of the offspring, in an interactive way. Furthermore, the seedlings of oak and beech seedlings displayed contrasting response to maternal temperature.

3.4.1 Effect of temperature on the phenology of the seedlings

Our results suggest that warming indeed advanced seedlings' bud burst time in both species. However, maternal temperature influenced the direction of this change thereby rejecting our first and second hypothesis. The interaction between maternal temperatures and warming showed that the effect of maternal temperatures and warming interdependent in seedlings' bud burst time. This result supports the third hypothesis of bud burst time as we described in the introduction. In oak, higher maternal temperature additionally advanced bud burst time of warmed seedlings but delayed this time in control seedlings. This interaction suggests that the warmer maternal temperature might be able to reshape the temperature cue to start bud burst in offspring generation (Yakovlev et al., 2014). For example, in Norway spruce, Carneros et al. (2017) found that an epigenetic memory mechanism affects the timing of bud burst phenology and the expression of bud burst related genes in genetically identical Norway spruce epitypes (individuals only differing in an epigenetic alteration in a gene), allowing them to adapt rapidly to a changing environment. The temperature sum experienced by the developing embryo and photoperiod conditions during embryogenesis epigenetically shift the growth cycle of the embryos, giving rise to different epitypes from the same genotype (Yakovlev et al., 2014).

In beech, however, higher maternal temperature did not change the bud burst time of control seedlings but delayed those of warm seedlings. This result is significant for minimum temperature in 2016 while the warming effect was stronger in the second year. Different response to the maternal temperature between oak and beech could link to varying temperature sensitivity and chilling requirement with species and genotypes (Vitasse et al., 2009; Dantec et al., 2014; Kramer et al., 2017). Given lower chilling requirement to bud burst in oak, it is likely that oak seedlings had sufficient chilling hours before starting the warming treatment (i.e., in end of January) in our study (Dantec et al., 2014) (see Appendix Figure A.3) and then warming of the seedlings provided the forcing temperature to initiate bud burst earlier in the first year of observation (Figure 3.2b). While in the second year, the required chilling hours to initiate bud burst probably was likely compensated by the forcing temperature via warming. As a result, the time to bud burst was even earlier in the second year than the first year (Figure 3.2 d, e). The effect of maternal temperature differed between two years in both species, which could be due to the stronger effect of warming in the

second year. Alternatively, it could also be that the effect of maternal environment varies over the life cycle of the progenies (Latzel & Klimešová, 2010b). Since we did not follow the response of full-sib families, the results of different bud burst time in this study was likely partly influenced by the genetic variability in different populations (Jump et al., 2006). However, the results of our study can help in estimating the probable magnitude of the maternal effects in a broader sense. More studies are necessary to assess the magnitude of maternal effects and to understand the response of tree seedlings to global warming by including extended ecologically relevant environments and more species while controlling for genotypes.

The results of our study in both species were probably the result of temperature memory mediated by the related genes and gene expression. However, we do not know the mechanism behind the interactive effect of maternal temperatures with warming on the seedlings bud burst time in this study. Although environmentally induced epigenetic variation could be a possible candidate for the phenological plasticity in our study (Verhoeven et al., 2010; Guarino et al., 2015; Gonzalez et al., 2016; Gugger et al., 2016; Carneros et al., 2017), which suggest the necessity of further studies to explore the molecular mechanism of such plasticity. An increase in minimum and mean temperatures during the reproductive period seems to have more influence than increasing maximum temperature during the reproductive period in oak seedlings. However, Gugger et al. (2016) found that average maximum temperature is correlated most for environmental association with epigenetic variation and local adaptation.

The maternal temperature did not change leaf discolouration time in the seedlings of both species regardless of warming treatment. Our results suggest that maternal temperature during the reproductive period probably was not a strong predictor for leaf senescence of the offspring. The absence of a warming treatment effect on the leaf discolouration in our study contrast with the result of Fu et al. (2018), where the authors reported a significant delay in leaf senescence in beech saplings with warming. Further, other factors such as precipitation, photoperiod and variation in bud burst time, were shown to control leaf senescence in oak and beech (Vitasse et al., 2010; Archetti et al., 2013; Fu et al., 2014a).

3.4.2 Maternal conditions affect the growth of the seedlings

In oak, the diameter increment of the warmed seedlings reduced compared to control seedlings with higher maternal temperatures suggesting that the maternal effect contrasts with the effects of the offspring's environment. This result also indicates that warming might decrease the tree performance. In beech, however, higher maternal temperatures and warming did not change the growth of the seedlings. Also, Webber et al. (2005) observed no effect of elevated maternal temperature on the plant height of *Picea glauca* × *Picea*

engelmannii hybrid in an ambient environment, which corroborates the findings of our result in beech. Contrasting responses between the two species studied here is probably linked to their growth behaviour (Čufar et al., 2008; Puchałka et al., 2017). In general, the diameter growth in oak seedlings is favoured by early cambium growth irrespective to leaf flush receiving an additional advantage in terms of growth while in beech both cambial growth and leaf flushing occurs at the same time (Čufar et al., 2008; Puchałka et al., 2017). In general, we expect increased plant growth with warming, which may, though, be limited by water availability during the growing season (Martinez-Sancho et al., 2017). However, the growth of the seedlings in warmer conditions can be affected by many different factors. Increasing temperature above optimum can alter the metabolic rate and energy expenditure, which probably reduce the growth of the plant (Atkinson & Sibly, 1997). Our result of growth reduction in oak seedlings in response to warming of the seedlings is also in line with the study of Martinez-Sancho et al. (2017), in which the authors reported that warming the air by 1-2°C decreased the diameter increment of *Quercus robur* and *Q. petraea* seedlings by 40% in the second year of warming. Reduction of growth could also be due to nitrogen deficiency resulting from reduced net photosynthetic rates in warmer conditions (Leon-Sanchez et al., 2016). Our result of oak suggested that the reproductive condition of the mother trees also have the potential to influence the growth of seedlings and should be considered when assessing plant growth responses to global warming.

Our results show that the interaction between the temperature in the maternal and offspring environment has the potential to change seedlings' bud burst time and growth. That means the response of tree species might be non-linear to the steady increase in global surface temperature (Stocker et al., 2013). The genotypes that adapted already to the warmer environment might respond differently to further global warming; even within species across the distribution range, this can affect interactions with pathogen and insects, tree competition and species dynamics.

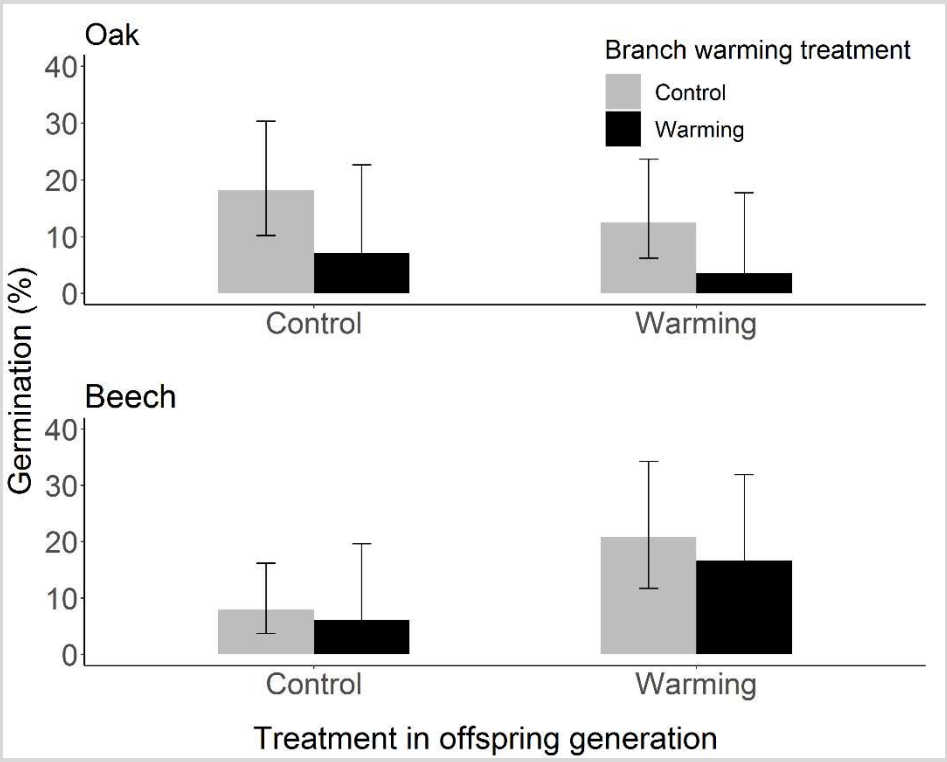
In sum, we showed that changes of temperature during reproduction may substantially affect the seedlings' phenology in two key temperate tree species and that the reproductive environment of mother trees is a potential determinant of the phenology and growth of trees in a warmer world. However, the contradictory responses exhibited by beech and oak emphasize that the maternal effect can lead the offspring's behaviour into different directions, which needs to take into consideration when estimating the future response of tree species to climate change.

Box 1 - Experimental warming of fruits on fructifying twigs of 'in situ' trees (oak and beech)

The influence of temperature during seed production on reproductive success (Penfield & MacGregor, 2017) is the focus of this Box 1. We here heated the fruits on fructifying twigs to simulate a warmer reproductive environment to observe the effect of maternal temperature on seed germination. We selected one oak (*Quercus robur*) and one beech (*Fagus sylvatica*) at the Aelmoeseneiforest, Gontrode, Belgium (51.0 °N, 3.8 °E). We chose both trees in such a way that flower-bearing branches could easily be reached. Three fructifying branches of the oak tree were experimentally heated by circa +2 °C using three 150 W infrared (IR) heating lamps (Eider Landgeräte GmbH) from June to September in 2015 (Box 1 Figure 1). During the same period in 2015 and 2016, we warmed four branches of beech tree by using the same infrared heating lamps as used in oak (Box 1 Figure 1). In October 2015, we collected seeds from both heated and non-heated branches of the oak tree using mesh bags, and seeds were stored cold (5°C) until sowing. In beech, there was no viable seed production in 2015. Therefore, we repeated the same procedure in 2016 to harvest beech seeds in October 2016. We sowed seeds within a week after collection in 2015 for oak and 2016 for beech in seed trays containing 28 cells in each tray using standard potting soil (Peltracom, NPK 14:16:18). In total, there were 56 heated and 111 non-heated seeds from oak, and 69 heated and 124 non-heated seed from the beech tree. We sowed one seed in each cell of the seed tray. The dimension of each tray was 51 by 28 cm and 15 cm depth. We watered the seed trays thoroughly after sowing the seed. We applied soil heating to increase soil temperature by circa + 4°C using heating mats (Carón et al., 2015) to half of each seed type (either heated or non-heated). We monitored seed germination (emergence of the shoot) twice a week between 7 April 2015 and 1 July 2015 for oak. In beech, we monitored seed germination (emergence of the shoot) twice a week from 17 March 2016 and continued until 29 May 2016.

In oak, the combination of elevated maternal temperature during the reproduction period and soil warming did not affect the germination success (Box 1 Figure 2). In beech, the soil warming treatment significantly increased the germination success of seeds (Z value = 2.024, $p = 0.043$) (Box 1 Figure 2). We did not observe a difference in germination success in response to branch warming.

Box 1 Figure 1 Branch warming of fructifying twigs of oak (A) and beech (B) trees



Box 1 Figure 2 Germination percentage in response to higher maternal temperature and soil warming in oak and beech. Germination percentage was based on total number of seedlings emerged from sowed acorns. Error bars denote the confidence intervals.

4 Weak but persistent provenance effects modulate the response of *Quercus robur* seedlings to elevated temperatures

After: Sumitra Dewan, Pieter De Frenne, Sebastian Kepfer Rojas, Safaa Wasof, Kristine Vander Mijnsbrugge, Kris Verheyen (2018) Persistent provenance effects modulate the response of *Quercus robur* seedlings to elevated temperatures. (submitted to *Plant Ecology & Evolution*)

Abstract

A clinal variation in bud phenology and growth has repeatedly been reported in many common garden experiments for dominant and codominant tree species. The response of the seedlings generated from such translocated trees has not been studied yet, despite its relevance regarding the role of transgenerational plasticity in the adaptation of long-living trees in the face of climate change. Here, to understand the response and performance of tree seedlings of different origins (provenances) grown in the same maternal environment, we assessed seed germination, bud burst time and biomass of seedlings in two common gardens. We collected seeds from a mature provenance trial of five different provenances of oak (*Quercus robur*, Fagaceae) and seeds were grown in two common gardens at two different latitudes representing a mean annual temperature difference of nearly 2°C. We observed an interaction between provenances and common gardens in seedlings' bud burst time indicating the prevalence of an environmental effect at the origin (provenance), which depends on the seedlings growing environment. We observed a marginal effect of the provenance on seedlings' bud burst time. The germination success and biomass were reduced across all provenances in the southern common garden. Biomass increased and seedlings' bud burst time advanced in seedlings resulting from heavier acorns. Our results indicate that the environment of origin influences the performance and bud phenology of seedlings and these effects were dependent on the seedlings' growing environment and were probably genotype specific. In addition, our results suggest that the effect of global warming might differ with provenances and that the environmental history of the predecessor generations is likely to influence the response of tree seedlings as well.

4.1 Introduction

Global surface temperatures are increasing at a rate of circa 0.2°C per decade since the 1970s (Hansen et al., 2006; IPCC, 2013). The extent to which populations persist in a warming climate depends on the ability of plant populations to respond via migration, adaptive evolution, and phenotypic plasticity (Hoffmann & Sgro, 2011). The present velocity of species' migrations and adaptations in response to global warming is, for many species, too slow to cope with rapid climate change (Kullman, 2002; Delzon et al., 2013). This slow migration is especially true for long-lived organisms such as trees that also exhibit slow genetic adaptation. Especially such species may benefit from high phenotypic plasticity in the face of rapid climate change.

Phenotypic plasticity is defined as the range of phenotypes that a single genotype can express as a function of its environment (Bradshaw, 1965; Nicotra et al., 2010). One form of phenotypic plasticity is known as transgenerational plasticity (TGP), in which the environment of earlier generations influences the offspring growth and its responses to environmental conditions independent from genetic changes (Beaman et al., 2016). TGP is known to have a positive influence on plant adaptation particularly during rapid shifts of climatic conditions (Kuijper & Hoyle, 2015). More and more evidence is accumulating that the parent plant's environment influences the performance of the offspring (Groot et al., 2016; Groot et al., 2017; Lampei et al., 2017; Munzbergova & Hadincova, 2017). Most TGP studies are on annuals and species with vegetative reproduction abilities. There are few studies on TGP in trees; mostly due to their long generation times. Nonetheless, TGP may be vital for tree adaptation, given that long-lived trees may be able to pass to their offspring, through their germ cells, epigenetic variations that were induced years earlier and had already been somatically tested (Herman & Sultan, 2011; Jablonka, 2013).

Provenance trials or common garden experiments are traditionally used to study local adaptation assuming a higher fitness of dominant to co-dominant tree species at the home site in comparison to the translocated sites (Whitlock, 2015). In such common garden experiments, populations often display clinal variation in bud burst and growth of different provenances along gradients of the temperature at their origin (Olson et al., 2013; Saenz-Romero et al., 2017; Wilkinson et al., 2017). It is likely that when the trees of such transplant experiments reproduce, some of the adaptive traits such as bud burst time, growth and survival are influenced not only by the environmental conditions of the seedling but also by the past environment of the mother plant. Studies focusing on the response of the offspring of translocated tree population in transplant experiments can reveal the possible role of transgenerational plasticity in evolutionary tree adaptation.

Many common garden experiments showed in situ patterns in bud burst, where trees originating from different geographic origins start flushing according to the temperature at their origin (Kremer, 2016; Wilkinson et al., 2017). It is not known whether the progeny of such translocated individuals displays the provenance effect when the mother trees have been growing in a common environment for nearly half a century. In addition, we do not know how the seedlings of these translocated individuals of different provenances would respond in a warmer world.

Here we took advantage of an existing mature provenance trial of oak (*Quercus robur*) in Nyskov, Denmark, to study the responses of the offspring of five different oak provenances that have been growing for more than 50 years in a common environment (Jensen, 2010). We sampled acorns in this provenance trial and replanted them at two different common gardens: one close to the maternal common garden (situated in Copenhagen, Denmark at 50 km from the maternal common garden) and the other one in Gontrode, Belgium. The latter represents a climate warming scenario (+1.8°C mean annual temperature) that is predicted to occur in Denmark by c. 2080 (Christensen et al., 2013) . In both gardens, we monitored germination, bud burst, and biomass of the seedlings for two years. We also followed the bud burst time of individual mother trees to compare the variability in bud burst time between mother trees and seedlings. We expected existing variability in bud burst time among different provenances in mother trees as well as in seedlings. The seedlings bud burst time would likely advance with warming. Since bud burst trait controls the start of growing season, changing bud burst time would likely affect the growth of the seedlings with warming. Further, we assumed that warming would affect the germination success of the acorns of different provenances.

4.2 Materials and methods

4.2.1 Study site and seed collection

In November 2015, we collected nearly 1200 acorns from the oak (*Quercus robur*) provenance trial located at Nyskov, Denmark containing two Danish (Bregentved and Wedellsborg), one Swedish (Visingsö) and one Dutch provenance. There were two replicate plots for the Bregentved provenance. We kept the plots separately and recognized them as Bregentved 1 and Bregentved 2, and counted them as separate provenance in this paper. The provenance trial was established between 1940 and 1947 with the primary purpose to test the production and wood quality for tree breeding. We selected the mother trees such that the canopy was separated from the other trees and the acorns could easily be separately collected from a single mother tree. We collected circa 60 healthy acorns per mother tree from the forest floor around the base of the stem of the mother trees. Four

mother trees per provenance (except, only three mother trees in Bregentved 1) were selected, resulting in nineteen mother trees. The seeds were stored cold (5°C) until sowing.

4.2.2 Common garden and experimental design

Within a few days following collection, we measured the fresh acorn mass of each acorn and sowed seeds at circa one cm depth in trays using standard potting soil (Peltracom, NPK 14:16:18). Each tray contained 28 cells and dimension of each tray was 51 by 28 cm and 15 cm depth, and the volume of each cell was circa 13.2 cm^3. We sowed one acorn per cell while we randomly distributed the provenances in each tray. In total, there were 42 trays, and we kept the trays at the edge of a forest at Forest & Nature Lab, Ghent University, Gontrode, Belgium (Figure 4.1) (Table 4.1). In December 2015, we transported half of the trays (21 in total) to the northern common garden at the University of Copenhagen, Denmark located circa 50 km away from the provenance trial with the purpose to expose the seedlings to a similar climate as the mother trees (Figure 4.1) (Table 4.1). The remainder of the seed trays were kept at the southernmost garden in Belgium. The description of the provenance trial and common gardens can be found in Table 4.1. Plants were protected from birds and rodents in both countries using a fence around, and a cage/net above the pots. We watered the plants twice per week in both common gardens except during rainy days.

Daily maximum and minimum temperatures and total daily precipitation for 2016 were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information (http://www.ncei.noaa.gov/) for Danish common garden and weather station at Melle (www.kmi.be) for Belgian common garden. In 2016, the mean annual temperature difference between two common gardens was 1.8°C (Appendix Figure B.1): the mean annual temperatures in the Belgian and Danish common garden were 11.1 °C and 9.3°C respectively. In the same year, total precipitation was 1001 mm and 623 mm in the Belgian and Danish common garden respectively.

Table 4.1 The background information of the provenance trial and the common gardens. Climatic data were extracted from the WorldClim version 2 dataset (Fick & Hijmans, 2017).

Site	Location/country	Latitude (°)	Longitude (°)	Elevation (m a. s. l.)	Mean annual temperature (°C)	Mean annual precipitation (mm)
Provenance trial	Nyskov/ Denmark	55.33	12.07	19	8.4	597
Common garden 1	Copenhagen/Denmark	55.68	12.54	9	8.6	612
Common garden 2	Gontrode/Belgium	50.98	3.81	21	10.2	795

Figure 4.1 The experimental design in two common gardens. A is the common garden in Denmark, B is the common garden in Belgium, C represents a sample tray with one-year-old seedlings in the Danish common garden and D represents the trays with one-year-old seedlings in the Belgian common garden.

4.2.3 Monitoring germination, bud burst and biomass

We monitored seed germination (emergence of the shoot) twice a week between 5 April 2016 and 22 July 2016 in both common gardens, and germination percentage was quantified based on the number of seedlings emerged from sowed seeds. We measured the bud burst of the seedlings at both common gardens from 27 March 2017 until all the seedlings and mother trees had completely opened their buds (stage 3) (Table 4.2). We scored the stages of bud burst once (Denmark) to twice (Belgium) per week following the adapted method of Wesołowski and Rowiński (2006) (Table 4.2). For seedlings, bud burst was monitored on the apical bud of each seedling. For mother trees, each observation was ideally performed on ten apical buds on the southern, lowermost part of the crown. At the end of the experiment in August 2017, we harvested all seedlings and quantified the shoot biomass of each seedling in both common gardens after drying them in an oven at 70°C for 24 hours.

Table 4.2 Description of the scoring systems of bud burst in the seedlings of oak based on visual observation adapted after Wesołowski and Rowiński (2006).

Score	Description	Stage
1	Undeveloped, all stages from sleeping bud, to a bud with broken scales, tips of leaves visible but still forming a single bud tip	
2	Broken-from small leaves with bases still hidden in bud scales but tips detached from the bud axis, till small leaves with folded (incompletely unfolded) leaf blades	
3	Developed, small completely unfolded leaf blade	

4.2.4 Data analysis

All data analyses were performed in R version 3.3.3 (R Core Team, 2017). We used Generalised Linear mixed effects models (*glmer* function in the *lme4* package in R) with Binomial distributions for germination percentage and with Poisson error distributions for seedlings' bud burst time as a function of *provenance*, *common garden* and their interaction, and *acorn mass* (Bates et al., 2015). In both models for germination and bud burst, we used *mother tree* (individual ID) and *Tray* as non-nested random effects. We calculated the number of days to bud burst of the seedlings from the starting of the observations (27 March). In addition, we used Linear mixed effects models (*lmer* function in the *lme4* package in R) with Gaussian error distributions and *mother tree* (individual ID) as a random effect to assess the effect of *provenance* on acorn mass (Bates et al., 2015). Next, we assessed the effects of *provenance*, *common garden* and their interaction, and *acorn mass* on biomass, by using Linear mixed effects models (*lmer* function in the *lme4* package in R) with Gaussian error distributions, and *mother tree* and *Tray* as non-nested random effects. The number of seedlings in different provenances and mother trees was not evenly distributed in the analysis of bud burst and biomass (Appendix Table B.1).

To test the overall effect of common garden, provenance and their interaction, and acorn mass on the germination, bud burst time and biomass we performed a likelihood ratio test. We used the full model including *Common garden*, *Provenance*, *Acorn mass* and interactions between *Common garden* and *Provenance* with always *mother tree* and *Tray* as non-nested random effects and compared it with the reduced model by dropping each variable and interaction term at a time to get the effect size of the variable in question.

4.3 Results

4.3.1 Bud burst

Seedlings of the Bregentved 2 and Wedellsborg provenances had delayed bud burst time compared to Bregentved 1 (Table 4.3). There was an interaction between provenances and common gardens in seedlings' bud burst time suggests that the effect of provenances depend on common gardens (Table 4.4, Figure 4.2). However, we found a week marginal effect of provenance on the seedlings' bud burst time. The seedlings displayed nearly four weeks earlier bud burst in the Belgian common garden than the Danish common garden (Figure 4.4). Seedlings in the Danish common garden displayed similar bud burst time as the mother trees (Figure 4.4). Seedlings germinated from higher acorn mass displayed earlier bud burst in both common gardens (Table 4.4, Appendix Figure B.3).

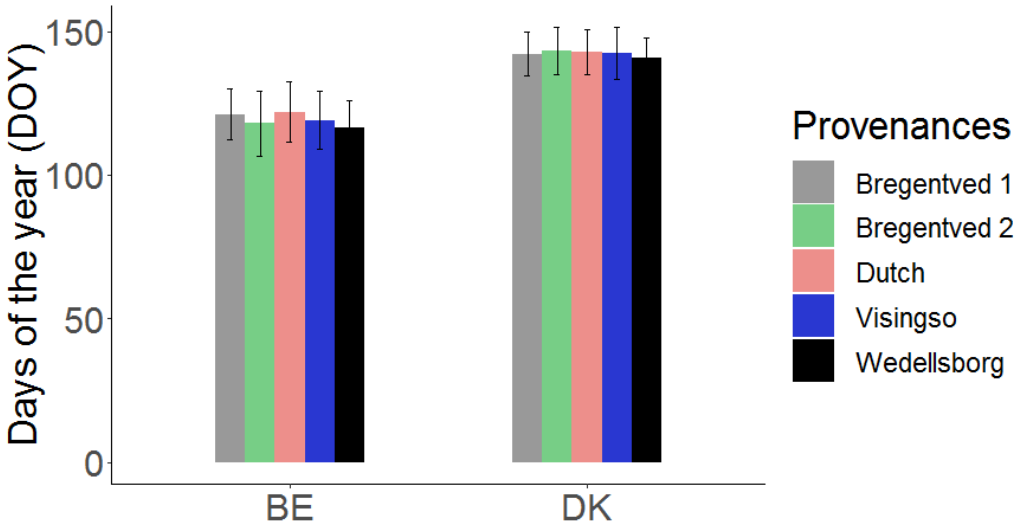


Figure 4.2 Bud burst dates (DOY) of the 5 provenances in the Belgian (BE) and Danish (DK) common gardens. The seedlings in the Belgian common garden displayed earlier bud burst than Danish common garden. The error bars denote the standard deviation.

Table 4.3 The estimated parameters from (Generalised) Linear mixed effects model for the bud burst and germination, and biomass of the seedlings as a function of common garden, provenance and acorn mass.

Fixed effects					
Response	Effect	Estimate	Std. Error	z value	p value
Bud burst	(Intercept)	3.63	0.04	85.97	< 0.001
	Common garden DK	0.49	0.04	13.60	< 0.001
	Bregentved 2	-0.10	0.05	-2.13	<0.05
	Dutch	0.02	0.05	0.43	0.67
	Visingso	-0.08	0.06	-1.39	0.17
	Wedellsborg	-0.11	0.06	-2.03	< 0.05
	Acorn mass	-0.02	0.01	-3.48	< 0.001
	Common garden DK : Bregentved 2	0.10	0.04	2.32	< 0.05
	Common garden DK : Dutch	-0.02	0.05	-0.42	0.68
	Common garden DK: Visingso	0.06	0.06	1.0	0.32
	Common garden DK: Wedellsborg	0.10	0.05	1.84	0.07
		Estimate	Std. Error	z value	p value
Germination	(Intercept)	-0.28	0.47	-0.59	0.56
	Common garden DK	1.10	0.48	2.31	< 0.05
	Bregentved 2	0.21	0.43	0.49	0.63
	Dutch	-0.63	0.44	-1.43	0.15
	Visingso	-0.23	0.49	-0.46	0.64
	Wedellsborg	-0.44	0.45	-0.98	0.33
	Acorn mass	-0.02	0.07	-0.30	0.76
	Common garden DK : Bregentved 2	0.54	0.47	1.17	0.24
	Common garden DK : Dutch	0.35	0.45	0.78	0.44
	Common garden DK: Visingso	0.58	0.54	1.07	0.29
	Common garden DK: Wedellsborg	-0.09	0.46	-0.2	0.84
		Estimate	Std. Error	t value	p value
Biomass	(Intercept)	-2.55	0.71	-3.57	< 0.001
	Common garden DK	3.11	0.65	4.82	< 0.001
	Bregentved 2	0.21	0.75	0.29	0.78
	Dutch	0.16	0.81	0.20	0.84
	Visingso	0.15	0.91	0.16	0.87
	Wedellsborg	-1.41	0.87	-1.63	0.11
	Acorn mass	0.97	0.12	8.1	< 0.001
	Common garden DK : Bregentved 2	0.74	0.74	1.00	0.32
	Common garden DK : Dutch	1.18	0.82	1.43	0.15
	Common garden DK: Visingso	-0.78	0.92	-0.84	0.40
	Common garden DK: Wedellsborg	1.61	0.87	1.85	0.07

Table 4.4 The effects of common garden, provenance, acorn mass and the interaction between common gardens and provenances on germination, bud burst time and shoot biomass of oak seedlings resulting from the likelihood ratio test.

Response	Effect	χ^2-value	df	p value
Germination	Common garden	14.9	5	< 0.05 *
	Provenance	10.9	8	0.21
	Common garden x Provenance	3.3	4	0.51
	Acorn mass	0.1	1	0.76
Bud burst	Common garden	132.3	5	< 0.001***
	Provenance	13.8	8	0.09 ·
	Common garden x Provenance	11.4	4	< 0.05 *
	Acorn mass	11.9	1	< 0.01**
Biomass	Common garden	61.0	5	< 0.001***
	Provenance	18.2	8	< 0.05*
	Common garden x Provenance	8.5	4	0.07 ·
	Acorn mass	63.1	1	< 0.001***

4.3.2 Germination percentage and acorn mass

The probability of seed germination differed significantly between the two common gardens being lower in the Belgian common garden (Table 4.3 and Figure 4.3). There was no significant difference in the probability of seed germination between the provenances (Table 4.3 and Table 4.4). We did not observe any difference in acorn mass among the provenances (F= 1.80, p=0.19 and Appendix Figure B.2).

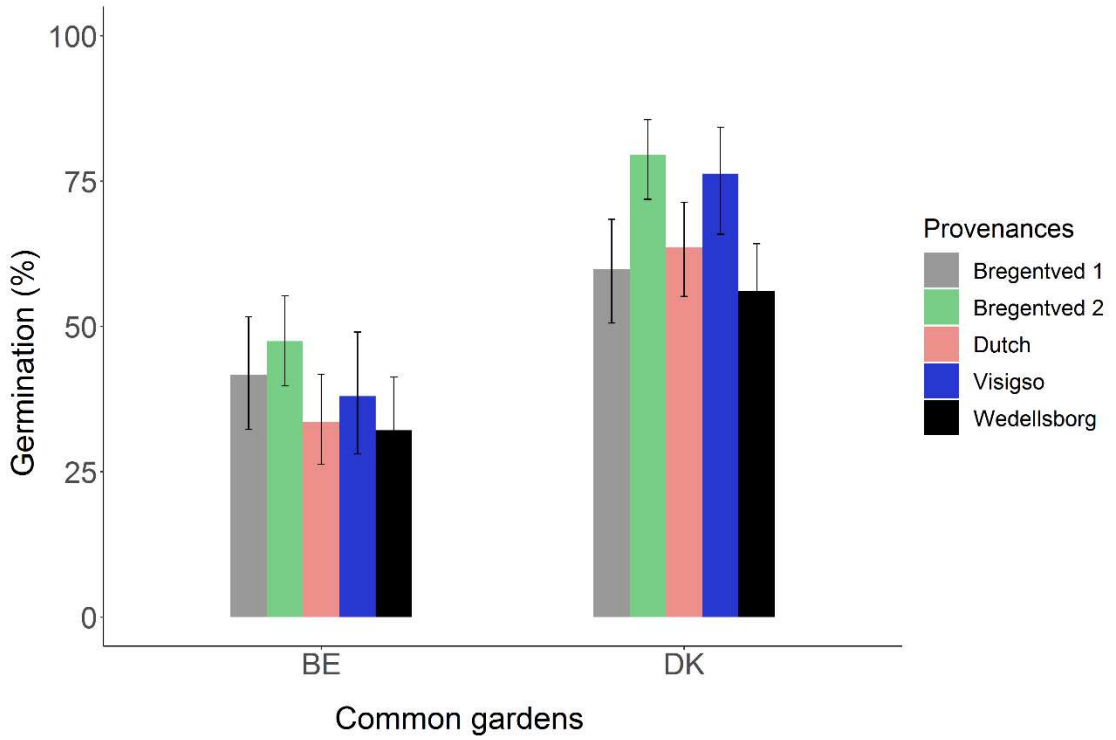


Figure 4.3 Seed germination percentage of five different provenances in the Belgian (BE) and the Danish (DK) common gardens. Germination percentage was quantified based on the total number of seedlings emerged from sowed acorns. Error bars denote the confidence intervals.

4.3.3 Biomass

The seedlings in the Belgian common garden had significantly lower biomass compared to the seedlings in the Danish common garden (Table 4.3 and Figure 4.5). The biomass of the seedlings was significantly different among provenances (Table 4.3). We observed a marginal interaction between Wedellsborg provenance and common gardens (Table 4.3). Heavier acorns produced larger seedlings (Table 4.3).

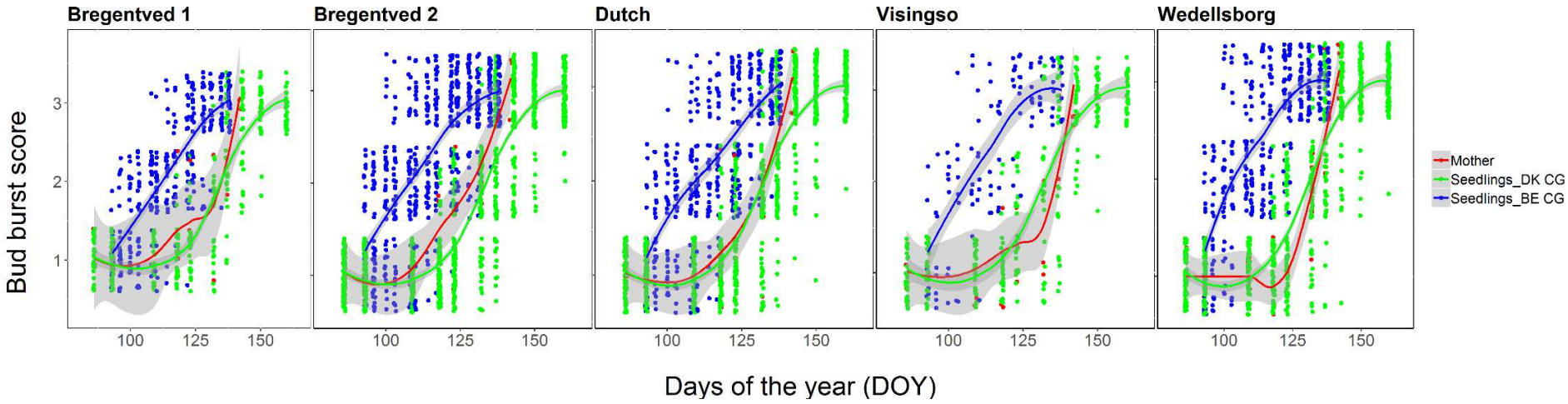


Figure 4.4 The bud burst score in the mother trees and the seedlings in two common gardens in five different provenances. “Seedlings_DK CG” and “Seedlings_BE CG” refer to seedlings in Danish common garden and seedlings in Belgian common garden respectively. The curves and respective confidence interval (95%) were fitted using the method ‘loess’ in R. We jittered the data points for clarity.

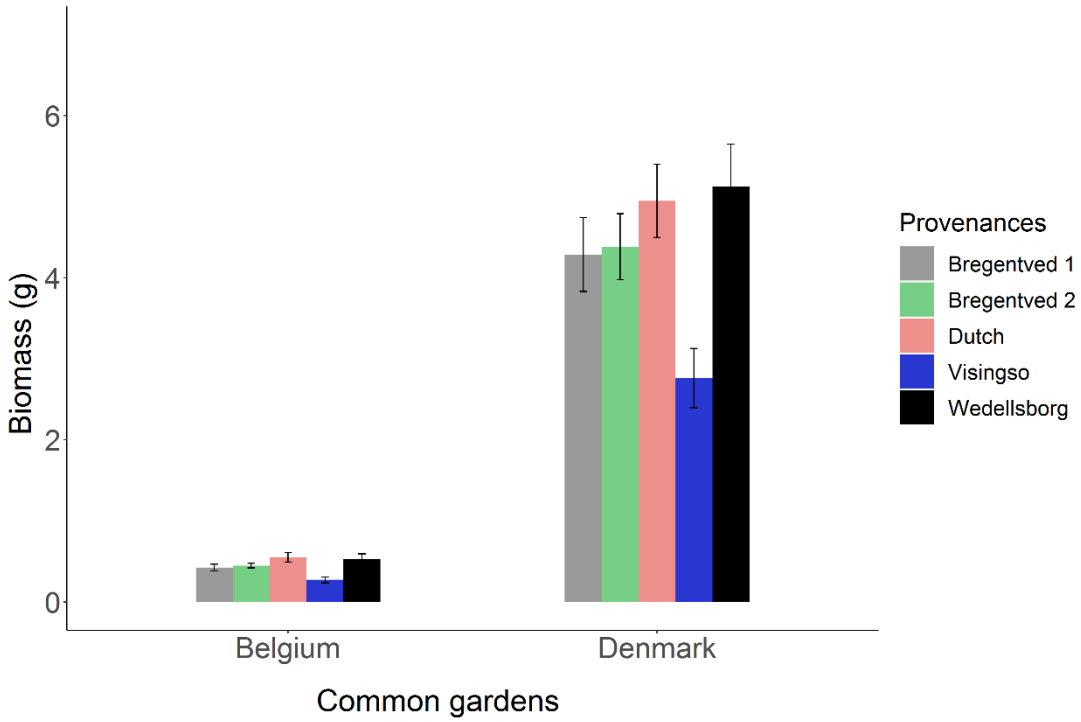


Figure 4.5 Biomass for the 5 provenances in the Danish and Belgian common garden. The biomass of the seedlings in all provenances in the Belgian common garden was reduced compared to the seedlings in the Danish common garden. Error bars denote the standard error.

4.5 Discussion

In the Belgian common garden, the bud burst time of all provenances was nearly four weeks earlier than the Danish common garden. There was an interaction between provenances and common gardens in seedlings' bud burst time. In addition, we observed higher germination rates and biomass of the seedlings in the Danish common garden compared to the Belgian common garden.

4.5.1 Bud burst

We observed earlier bud burst in the Bregentved 2 provenance compared to the Bregentved 1 provenance, which further differs, based on the common gardens. In the Belgian common garden, seedlings of the Bregentved 2 provenance displayed earlier bud burst compared to the Bregentved 1 provenance while in the Danish common garden, seedlings of this provenance delayed bud burst compared to Bregentved 1 provenance. This interaction suggests that the effect of provenance depends on common gardens. Using *Arabidopsis thaliana* and elevated temperature exposure in three generations, Groot et al. (2017) showed that the parental effect changed the flowering time of the offspring and the effect was genotype specific. In addition, the expression of maternal effects can depend on the offspring environment (Groot et al., 2016). In our study, it is likely that the provenances have a different genetic background (Bischoff et al., 2008). That means the provenance effect on seedlings' bud burst time can be a result of genetic variabilities in different provenances. In addition, we observed that the seedlings at the Danish common garden displayed similar bud burst as the mother trees, which differed from the seedlings' response at Belgian one (see Figure 4.4). Given the advantageous parental effects on offspring performance when the parent and offspring environments are correlated (Lampei et al., 2017), our results showed that the seedlings at the Danish common garden would display higher fitness compared to Belgian one. In our study, we do not know whether maternal effect in seedlings bud burst time will be beneficial or not. Advancing bud burst time in a warmer environment would provide an advantage to the offspring generation by starting the growing season earlier. Although, it may increase the chance of early frost damage, which ultimately will reduce seedlings growth and thus reduce overall fitness (Richardson et al., 2018). It might be more interesting to know how the seedlings would respond to warming if the mother trees would be exposed to an elevated temperature. This needs further investigation. Earlier bud burst in the Belgian common garden was expected, as the temperature sum requirement would have fulfilled earlier (Wuehlisch et al., 1995). Besides, photoperiod likely influenced the bud burst time in the Belgian common garden as well since the seedlings in the Belgian common garden received 36 to 14.4 minutes day length longer between 1 February and 1 March compared to those in the Danish common garden (Schreiber et al.,

2013; Schueler & Liesebach, 2014). Seedlings in Belgian common garden displayed nearly four weeks earlier bud burst than seedlings in Danish common garden, which suggests temperature along with photoperiod may have influenced the bud burst in oak.

The marginal difference in seedlings' bud burst time among the provenances indicate that the influence of the provenance prevailed even after growing one generation in a common environment, which supports our expectation. This result also indicates that there was still genetic control of different provenances in bud burst time. Further, increased genetic diversity can increase the variability in bud burst time among the seedlings within the provenances, which can be expected in an oak stand due to the extensive gene flow and cross-pollination (Elshibli et al., 2015). The mating in European beech, another wind pollinating species, was reported to be restricted to nearby trees (Ouayjan & Hampe, 2018). In oak, Gerber et al. (2014) reported a high level of gene flow with variation in individual and in stands. That means, in our study, there was a high possibility of increased genetic diversity among the seedlings.

Acorn mass displayed a significant positive influence on the bud burst time by advancing the bud burst time. Although the effect of seed trait may only be relevant during early development of seedlings and will disappear after four years (Zhang et al., 2017), it may have substantial influence in later life stage (Burton & Metcalfe, 2014). In our study, the effect of acorn mass on the bud burst time was likely resulted from the increased growth of the seedlings.

4.5.2 Germination and acorn mass

The observed lower germination in the Belgian common garden compared to Danish one suggests that the environment in common gardens mostly influenced germination. This result supports our expectation regarding the change in germination success with warming. Reduced germination with increasing temperature is known in *Quercus* species (Rao, 1988). The elevated temperature may be advantageous for some species through increasing reproductive success (Milbau et al., 2009), and the responses to an elevated temperature may be different across the species distribution range (De Frenne et al., 2011). The threshold temperature for germination can differ among species and provenances (Baskin & Baskin, 2001; Durr et al., 2015) although we did not find any provenance effect on seed germination. Another critical factor that controls germination of oak acorns is their moisture content. Acorn germination is reduced by decreasing the moisture content below 30 to 50 percent (Olson & Boyce, 1971; Ozbingol, 2005). Higher precipitation in the Belgian common garden than the Danish common garden suggests that the moisture content was not the limiting factor for germination in Belgian common garden. Nevertheless, our results corroborate that warming may decrease germination in oak (Perez-Ruiz et al., 2018).

There was no difference in acorn mass among the provenances suggesting that acorn mass was probably influenced by the climatic condition of the provenance trial (Borgman et al., 2014). In general, acorn mass in oak is mostly influenced by the local climate (Caignard et al., 2017) and can thus vary across years due to temperature variation (Caignard et al., 2017) and between individuals due to genetic differences (Nikolic & Orlovic, 2002).

4.5.3 Biomass

Biomass of the seedlings differed across the provenances, which could be the result of genetic variability in different provenances. Reduced biomass in Belgian common garden compared to the seedlings in Danish common garden, indeed, indicate the negative effect of warming on the growth, which is probably linked with confounding effects such as increase pest and diseases with warming (Quarles, 2007). This result contrasts the general expectation of increased growth with warming (Richardson et al., 2018). Our results indicate that the warming effect on tree growth might be correlated to the temperature gradient along the species distribution range (Reich & Oleksyn, 2008), and oak populations from colder than optimum temperature range may receive the positive effect of warming (Saenz-Romero et al., 2017). The marginal interaction between common gardens and Wedellsborg provenance suggests the biomass of the seedlings of this provenance differed in two common gardens. Given the growth responses to temperature can vary with genetic differences (Housset et al., 2018), we infer that the growth response of the seedlings of Wedellsborg provenance in two common gardens was related to the genetic effect. Although, very low variability among the provenances at Belgian common garden in biomass production indicates that growing environmental condition (e.g., temperature, light) influenced more in biomass production than genetic differences. Environmental factors, such as light availability, can influence the growth of oak seedlings as well (Leuschner & Meier, 2018). The different light condition in two common gardens was likely to influence the growth of the seedlings. The evidence from literature showed that exposure to the sun increases the photosynthesis and carbohydrate concentration of oak seedlings (Baber et al., 2014), which can influence the growth and thus overall fitness of the seedlings. Seedlings in the Belgian common garden received more shade as the common garden was located at the edge of a forest and seedlings in the Danish common garden received almost no shade, as the common garden was located in the open area, which of course was not the ideal condition for the recruitment of oak seedlings. Therefore, there was a possible overestimation in our results. Additionally, we observed high fungal infestation (powdery mildew) in the Belgian common garden (no data available) likely due to the existence of oak stand at the vicinity, which could reduce the growth of the seedlings (Bert et al., 2016). Nevertheless, global warming, in general, might be beneficial to some species but not for all species as warming and drought may increase other growth limiting factors such as pest

and diseases (Quarles, 2007; Reich & Oleksyn, 2008; Dale & Frank, 2017; Perez-Ruiz et al., 2018; Richardson et al., 2018).

In addition, we observed a strong positive correlation between acorn mass and seedling biomass, which can be an indication of maternal influence on seedlings trait (Roach & Wulff, 1987). Due to higher maternal seed provisioning for seedling establishment in heavier acorns, these are usually selected for afforestation purposes via seedling (Bruno et al., 2006; González-Rodríguez et al., 2011). Maternal seed provisioning refers the allocation of carbohydrates, lipids, proteins and mineral nutrients by the mother plant into the developing seed, where these reserves of nutrients are mobilized to the embryo germinating seedlings to produce the shoot and root systems (Herman & Sultan, 2011). The strong correlation between inter-annual seed mass and seedling traits in two pine species provides further evidence of parental influence in seedlings performance (Borgman et al., 2014).

4.5.4 Conclusions

In sum, our results indicate that the effects of global warming on oak depend on the provenances. The direction of change in seedlings bud burst time depends on the temperature condition of the offspring. The results of this study have implications in understanding the evolutionary plant adaptation to climate change and call for further studies to understand the role of transgenerational effects on tree adaptation to climate change.

5 Maternal temperature during seed maturation affects seed germination and timing of bud set in seedlings of European black poplar

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Abstract

The maternal temperature during seed development can significantly affect seed dormancy, germination and seedling performance. While the response of germination and seedling phenology to maternal temperatures has been well studied for annuals and conifers, very few studies focus on deciduous trees. To understand the responses of seedlings to variation in maternal temperature during seed maturation, we assessed the germination, bud phenology (bud burst, bud set) and height of full sib families in a common garden. We performed three controlled crosses between three different pairs of genotypes of European black poplar (*Populus nigra*) to achieve full sib families in three experiments in warm (+10°C) and cold (control) maternal environments during the crossing and seed maturation. Warmer (+10°C) maternal temperatures decreased seed germination success. The seedlings from the warmer maternal environment also displayed later bud burst and earlier bud set, but only in one out of the three crossings (Proven ♀ x Horrues ♂). Our results indicate that the maternal environment can considerably affect seed germination and the phenological responses of even two-year old seedlings suggesting the existence of memory of maternal temperature during seed maturation. The seedlings resulting from the colder maternal environment grew taller than those from the warmer environment did during the first, but not second, growing season. Our results further our understanding of the responses of deciduous forest trees to rapid climate change, but more research is needed to better understand the mechanisms behind the observed effects of maternal warming.

5.1 Introduction

The phenology of trees affects key forest ecosystem processes such as biomass production, carbon sequestration, plant-animal interactions and linkages with the understorey biodiversity. Leafing out (bud burst) and growth cessation and senescence (bud set) in particular are key tree phenophases at the start and end of the growing period. Many plant species advanced their spring bud burst in the last decades due to climate warming (Menzel et al., 2006; Zhang et al., 2007; Prieto et al., 2009; Yu et al., 2010). However, the response to increasing temperature varies among species and genotypes within species (Hedhly et al., 2009; Vitasse et al., 2009; Yu et al., 2010; Fu et al., 2013). Recent findings indicate that the phenology of plants is not only controlled by environmental cues and genetic factors, but also the maternal environment during seed development may determine the phenology of progenies (Yakovlev et al., 2012; Cendán et al., 2013; Latzel et al., 2014; Penfield & MacGregor, 2017).

The temperature of the maternal environment during seed production plays a significant role and can alter the germination success, phenology, establishment and fitness of the progenies (Roach & Wulff, 1987; Fenner, 1991; Hedhly et al., 2007; Rix et al., 2012; Cendán et al., 2013; Chen et al., 2014b; Lemke et al., 2015; Gruwez et al., 2016; Penfield & MacGregor, 2017). Yet, the effect of the maternal temperature during seed production on seed germination, seedling performance and phenology is complex. Whereas we know quite well how germination, growth, flowering and yield in annual crops and fruit trees are affected, very few studies focus on forest trees (El-Keblawy et al., 1996; Greenwood & Hutchison, 1996; Owens et al., 2001; Johnsen et al., 2005; Hedhly et al., 2009; Rix et al., 2012). Annual plants have relatively short life cycles. This provides them with the opportunity to adapt faster to a changing environment through natural selection while trees are limited in this respect as generation times are much longer. Trees generally only flower and fruit after 5-20 yrs, which can be longer in forest stands (up to 50 years for some species) (den Ouden et al., 2010). Thus, it is likely that contrasting strategies exist between annuals and trees to adapt to a rapidly changing environment.

Variation in maternal temperature during seed development first affects seed dormancy and germination and later the phenology and growth of the seedlings (Roach & Wulff, 1987; Greenwood & Hutchison, 1996; Chen et al., 2014b; Penfield & MacGregor, 2017). Maternal effects on the seedlings can also occur via direct transmission of cytoplasmic DNA (Roach & Wulff, 1987). Other maternal effects can also originate via the endosperm (Roach & Wulff, 1987). The endosperm contains enzymes that are important for germination and that provide nutrients to the developing embryo (Roach & Wulff, 1987). A zygote may inherit epigenetic states from the mother plant which are affected by temperature in the tissues of

the seed (Penfield & MacGregor, 2017). In *Arabidopsis thaliana*, it has been shown that seed dormancy and germination is controlled via a long-term temperature memory, which is established before seed fertilization and is integrated into the tissue of the fruit (silique) (Chen et al., 2014b). There are also interactions between the maternal and zygotic environment that influence embryo development and seed germination (Evans & Kermicle, 2001; Penfield & MacGregor, 2017). Germination of seeds then links the pathway from mother plant to offspring.

In conifers, it has been shown that the phenology is affected by the so-called adaptive epigenetic memory, the memory from the time of embryo development and seed maturation. These effects are known to persist for up to 20 years after germination (Yakovlev et al., 2012). Johnsen et al. (2005) reported that warmer maternal environments during embryogenesis delay the formation of terminal buds of the next generation in Norway spruce (*Picea abies*) through epigenetic variation, a change in gene expression without any change in DNA structure. Greenwood and Hutchison (1996) also observed similar epigenetic effects on the growth (height) induced by maternal temperature in *Larix* spp.. However, many differences exist between gymnosperms and angiosperms in their life cycle, megasporangium structure and seed development (Yakovlev et al., 2012). Considering their contrasting life histories and ecology, we can question whether the performance (germination and height) and phenological responses of broadleaves to the maternal temperature would display similar responses as conifers or not.

To understand the responses of tree seedlings to the maternal temperature during seed development and maturation, we here assessed the phenological responses (bud burst and bud set) and performance (germination and growth) of seedlings of black poplar (*Populus nigra*). Poplar is a dioecious species providing the opportunity to perform controlled crosses in specific environmental conditions, enabling us to study of the response of full-sib families to environmental variation, and eliminating the potential variation caused by genetic diversity. Since the prevailing maternal environment during embryogenesis and seed maturation may influence the performance of the progenies, application of different temperatures during the crossing and seed maturation may result in deviating phenological responses and performance in the progenies (seedlings). To assess the response of full-sib families to temperature, we performed controlled crosses in warm (+10˚C, W) and cold (control, C) maternal environments. Following these different temperature treatments during seed maturation, we sowed all the seeds in a common garden and assessed the performance of the resulting seedlings.

We hypothesised that (i) seed germination success of poplar seeds depends on the maternal environment during seed development, (ii) the seedlings of black poplar generated from the seeds of warmer maternal environments will display earlier bud burst and later bud

set, (iii) the height of the seedlings will vary due to the variation in timing of bud burst and bud set.

5.2 Materials and methods

5.2.1 Study species

European black poplar (*Populus nigra*) is a keystone species of riparian ecosystems in Europe. It has a wide distribution in Europe and is found in northern Africa and central and western Asia as well (Vanden Broeck, 2003). Black poplar is an important species for the breeding program of hybrid poplar clones in western Europe, that are planted for wood and biomass production, windbreaks, and soil protection (Vanden Broeck, 2004; Vanden-Broeck et al., 2012). In Europe, the hybrid poplar plantations cover circa 800,000 ha and among them, nearly 50% are for industrial production of round wood; 12% are for environmental protection such as windbreaks, to control soil erosion etc. (FAO, 2012).

5.2.2 Crosses

In two succeeding years, we performed crosses between three pairs of Black poplar genotypes (Proven ♀ x Horrues ♂ - Cross 1, Meers ♀ x Elst ♂ - Cross 2, Oosterzele ♀ x Remicourt ♂ - Cross 3, see Table 5.2) in three different experiments using grafts taken from trees that were growing in the field that were grafted on potted rootstocks in 2013 and 2014 (Table 5.1). We wanted to test whether the response of the offspring to maternal temperature would be similar across genotypes. The rootstocks were potted into 5L-pots using standard potting soil (Sanilor pro, NPK 12-14-24). The preparation of plant materials (grafts), performance of controlled crosses and subsequent monitoring were done at the Research Institute for Nature and Forest (INBO), Geraardsbergen, Belgium (50.763 °N, 3.879 °E ; 19.8 m above sea level). The controlled crosses and production of clonal grafts were performed following Vanden-Broeck et al. (2012).

Table 5.1 Detail description of the year of crossing, genotypes and treatments in three different treatments.

Year	Experiment	Cross	Treatment
2013	1	Proven ♀ x Horrues ♂	**C** : Pollination and seed maturation at cold (C) **W** : Pollination and seed maturation at warm (W) (+10°C)
2014	2	Proven ♀ x Horrues ♂	**C** : Pollination and seed maturation at cold (C) **C>>W** : Pollination in cold (C) but seed maturation at Warm (W) **W** : Pollination and seed maturation at warm (W) (+10°C)
2014	3	(Meers ♀ x Elst ♂) and (Oosterzele ♀ x Remicourt ♂)	**C** : Pollination and seed maturation at cold (C) **C>>W** : Pollination in cold (C) but seed maturation at Warm (W) **W** : Pollination and seed maturation at warm (W) (+10°C)

Table 5.2 Background of the clones used for controlled crosses in both 2013 and 2014.

Sex	Clone name	Province/Country	Collection site	Latitude (°N)	Longitude (°E)
♀ x	Proven	West-Flanders, Belgium	Proven	50.90	2.64
♂	Horrues	Hainaut, Belgium	Thoricourt	50.61	3.99
♀ x	Meers	Limburg, Nederlands	Meers	50.96	5.72
♂	Elst	East-Flanders, Belgium	Elst	50.82	3.74
♀ x	Oosterzele	East-Flanders, Belgium	Oosterzele	50.95	3.84
♂	Remicourt	Hainaut, Belgium	Twee Akren	50.73	3.87

Figure 5.1 'A' represents manual pollination in the greenhouse, 'B' represents mother plants with maturing seeds in the greenhouse and 'C' represents seedlings generated from control and warm maternal environment in a greenhouse.

5.2.3 Experiment 1: pollination and seed maturation in cold and warm condition

At the beginning of February 2013, we produced the clonal grafts. After a couple of weeks, we performed the first controlled crosses in the greenhouse starting from 21 February between the clones Proven and Horrues (Cross 1) in warm (W) and cold (C) conditions. The mean temperature difference between warm and cold conditions was 10°C during day and night. We used two separate glasshouse compartments for the application of the warming treatments where cold and warm conditions were maintained as close as possible to 10 ºC and 20 ºC, respectively. In warm sunny days, the temperature difference between cold and warm compartments in the greenhouse, however, was smaller than 10° C. The crosses were performed between 21 - 28 February. We collected branches bearing staminate inflorescences from the selected paternal genotypes, adult trees growing in the field (Table 5.2) and placed the branches in tap water baths in the same warm or cold conditions as the female plant (graft). Fresh pollen was collected in a glass jar daily from each temperature treatment and hand pollination of the female flowers was performed using a soft paintbrush (Raphaël no. 4) (Figure 5.1). Black poplar pollens lose their viability quickly but can be stored at cool temperatures of 3°C. We stored pollen 3-6 days at 3°C depending on the maturity of the female flowers. Seeds developed and matured in the same conditions as pollination was performed. As soon as the seeds were mature, seeds were collected (between 17 April and 28 May 2013). We stored seeds in the fridge at 3°C until they were sown on 16 June. We sowed in total circa 3500 seeds in germination boxes (rectangular wooden boxes filled with potting soil) in a greenhouse (Appendix Table C.1). We assessed the total germination percentage of each germination box after completion of germination. The seedlings were transplanted in July to boxes each containing 24 seedlings. The boxes were kept in the greenhouse until December 2013 when they were transplanted in raised beds filled with sandy soil outside in a common garden at the Research Institute for Nature

and Forest (INBO), Geraardsbergen, Belgium (Figure 5.1). The mean annual temperature and annual precipitation at the common garden were 10.13˚C, 11.84˚C and 11.18˚C; and 843.28 mm, 836.14 mm and 736.81 mm respectively in 2013, 2014 and 2015 (Delvaux et al., 2015). We monitored the bud set in 2014 and 2015 and bud burst of the progenies in 2015 (Appendix Table C.1). We measured the plant height (cm) at the end of each growing season in 2014 and 2015. We assessed the bud burst and bud set once in a week (starting in the beginning of April for bud burst and in the beginning of August for bud set) until all the progenies completed bud burst (beginning of May) and bud set (beginning of October). The bud set and bud burst were monitored by scoring each plant according to the method described in Table 5.3. We always observed the buds at one third of the total height of the stem below the apical bud and gave a score of 60-80 % of the buds within the same stage. The common garden was regularly irrigated and fungicide (Caddy- Cyproconazole) was applied as necessary during the growing seasons.

5.2.4 Experiment 2: extra temperature treatment

In 2014, we performed the second experiment by repeating the crossing in February (graft preparation on 11 February and pollination on 23 February) between Proven and Horrues (Cross 1) (the grafts were taken from exactly the same tree as in the first experiment). In this experiment, there was one extra treatment (C » W) where we performed the crosses in a cold (control) environment and let the seeds to develop and mature in a warm (+10°C) maternal environment to disentangle the effect of maternal temperature during pollination vs. during seed maturation. Performance of controlled crosses, germination and growth of seedlings were the same as in Experiment 1. Sowing, germination and transplantation of the seedlings to different boxes in the greenhouse followed the same procedure as in the first experiment. We sowed in total circa 2100 seeds from all three treatments (Appendix Table C.1). We also assessed the total germination percentage in each treatment after the completion of germination in July. In 2014, we started to assess the bud set of the progenies and the assessment of the bud set was followed once per week from August onwards until all of the seedlings completed the bud set (score 0). After the completion of bud set, we transplanted the seedlings during December 2014 in a common garden (same location as Experiment 1) where we monitored the bud burst in 2015, 2016 and bud set in 2015 (Appendix Table C.1). We assessed the bud burst and bud set of the progenies each week by following the same visual observation as in Table 5.3. We also measured the height (cm) of the seedlings after each growing season in December 2014 and 2015. We managed the site of the common garden as similar to Experiment 1.

Table 5.3 Description of the scoring systems of bud burst and bud set in poplar cuttings based on visual observations adapted after Pellis et al. (2004), Rohde et al. (2011b).

Bud burst score	Description of visual evaluation
0	Dormant bud; no sign of any physiological activity
1	Buds were slightly swollen and the bud scales reddishly coloured
2	Buds were fully swollen and turned towards a rounded shape, no sign of breakage of buds
3	Buds started breaking, wet and sticky, tip of reddish shoots appeared
4	Bud burst and reddish shoots turned towards a green colour, very young leaves could be observed
5	Green leaves started growing and venation of leaf could be observed
Bud set score	
3	More than two rolled-up leaves
2	Last leaf (partially) rolled-up, other leaves fully stretched
1	Bud well visible, bud scales predominantly green colour, all leaves are stretched
0	Apical bud reddish-brown colour

5.2.5 Experiment 3: extra genotypes

We performed the third experiment in 2014 using crosses between Meers and Elst (Cross 2) and between Oosterzele and Remicourt (Cross 3) (Table 5.2) following the same method and temperature treatments as described above in the second experiment. We performed hand pollination on 23 February for Cross 3 and at the beginning of March for the Cross 2 due to the late maturation of pistillate inflorescence and continued for a week. The pollen was stored in the fridge at 3˚C before pollination. The temperatures during the crossing, seed development and maturation, time of sowing, transplantation of the seedlings and assessment of germination percentage, bud burst and bud set were all similar to the second experiment. We sowed circa 3800 and 2050 seeds from Cross 2 and Cross 3 respectively (Appendix Table C.1). We monitored bud burst of the progenies in 2015 and 2016, bud set in 2014 and 2015 and we measured the height (cm) of the progenies after each growing season in December 2014 and 2015. We managed the site of the common garden similar as in Experiment 1. Bud burst data of 2016 from Cross 3 and treatment C » W were removed because more than 50 % of the buds remained dormant for the entire observation period.

5.2.6 Statistical analysis

All statistical analyses were performed in R version 3.3.3 (R Core Team, 2017). We used Generalised Linear Mixed-Effects models (*glmer* function in the *lme4* package in R) with Poisson error distributions to analyse the effects of temperatures of the maternal environment during crossing, seed development and maturation on the stages of bud burst

and bud set. We used *plant ID* as a random factor in this model to account for the repeated measurements of the same individual over time. We calculated the number of days (days) to bud burst and bud set from the starting day of observation. The start dates of observations for bud burst and bud set were 7 April and 13 August respectively. We analysed the effects of maternal temperatures on the number of days to bud burst and bud set using Generalised Linear models (*glm* function in the *lme4* package in R) with Poisson error distributions. The effects of the maternal temperatures on the height (cm) of the progenies and germination percentage were analysed by linear regression models. The germination percentage was assessed for each germination box based on the number of seeds sown and number of seeds germinated; these data were then used to estimate the effect of maternal temperature on germination percentages using a linear model with Gaussian error distribution. To assess the effects of changed timing of bud burst and bud set on the height of the progeny, we analysed the relationship between the height of the progenies and the score of bud burst, bud set, and the number of days to bud burst and set by using Generalised Linear models with Poisson error distributions. All analyses were similar for the three crosses in all three experiments. The number of individuals per treatment was not same in all experiments and years of observation due to mortality, bud damage due to insect attack and dormant buds (as described in section 5.2.5) (more detail is available in Appendix Table C.1). To quantify the overall maternal environmental effect as well as the influence of genotypic variation and year to year variation, we performed likelihood ratio tests by including maternal temperature, genotype (Cross) and year as fixed effect in all three experiments in Generalised Linear models and compared the original with the reduced model by dropping each variable (genotype, year and maternal temperature) one by one. Model fit was assessed using chi-square tests on the log-likelihood values to compare different models.

5.3 Results

5.3.1 Experiment 1

Maternal warming (treatments W) resulted in lower germination success of the seeds than in the control (C) maternal environment (Table 5.4, Figure 5.2). We observed delayed bud burst and advanced bud set in seedlings of black poplar when we warmed the mother plant during the crossing, seed development and maturation (Figure 5.3). However, a significant difference was observed for bud burst scores only (Table 5.5).

Table 5.4 The effects of maternal environment (C, C » W, W) on germination percentages of the seeds in Cross 1 (Proven ♀ x Horrues ♂), Cross 2 (Meers ♀ x Elst ♂) and Cross 3 (Oosterzele ♀ x Remicourt ♂) in Experiment 1, Experiment 2 and Experiment 3.

Experiment	Cross	(Intercept)			C » W			W		
		Estimate (mean±SE)		P	Estimate (mean±SE)		p	Estimate (mean±SE)		p
1	1	60.248	± 4.653	<0.001***	Treatment not included			-39.509	± 6.008	<0.001***
2	1	52.142	± 5.225	0.002 **	-48.460	± 6.399	0.005 **	-43.286	± 6.033	0.006 **
3	2	22.058	± 2.778	<0.001***	-11.369	± 3.675	0.009**	-18.664	± 3.258	<0.001***
3	3	39.896	± 6.344	0.008**	-19.273	± 8.190	0.1000	-37.790	± 10.988	0.041*

Note : Significance are denoted by · 0.05 < p < 0.1, * p<0.05, ** p < 0.01, *** p<0.001.

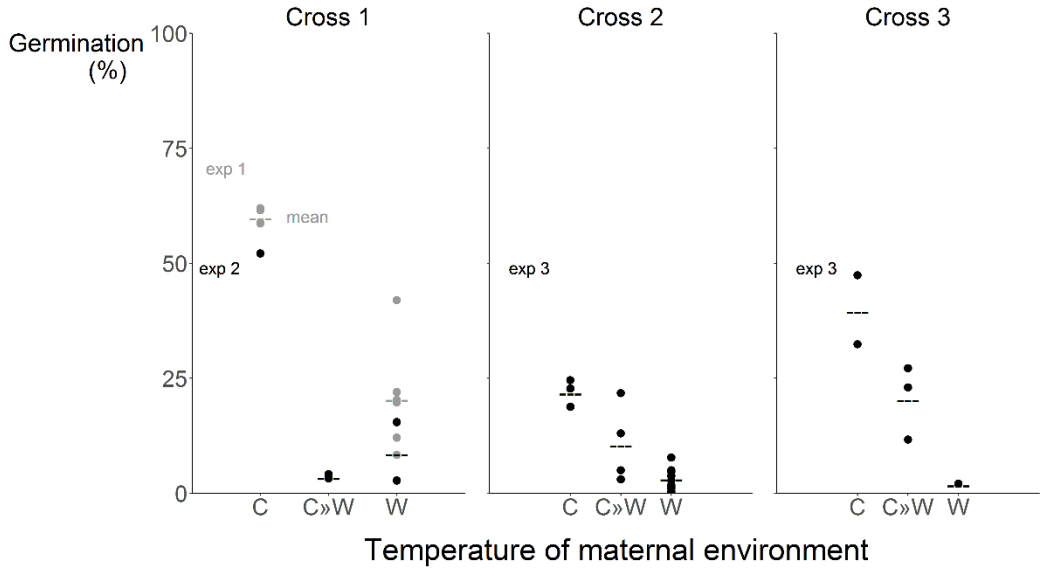

Figure 5.2 Germination percentages were higher in control (C) maternal environment than in the warmer maternal environment (C » W and W) in all three experiments and crosses (Cross 1= Proven ♀ x Horrues ♂, Cross 2 = Meers ♀ x Elst ♂ and Cross 3 = Oosterzele ♀ x Remicourt ♂). Each point represents the germination percentage in each germination box. There was only one germination box for the control treatment (C) in Experiment 2 and Cross 1. The dashed lines represent the mean germination across germination boxes.

The warmer maternal temperature had a negative effect on the height of the seedlings in both 2014 (estimate = -5.797, SE = 2.173 and p= 0.008) and 2015 (estimates -20.28, SE = 3.93 and p <0.001) (data can be found in Appendix Table C.2). Due to the earlier bud burst and later bud set the seedlings also grew taller (Table 5.6).

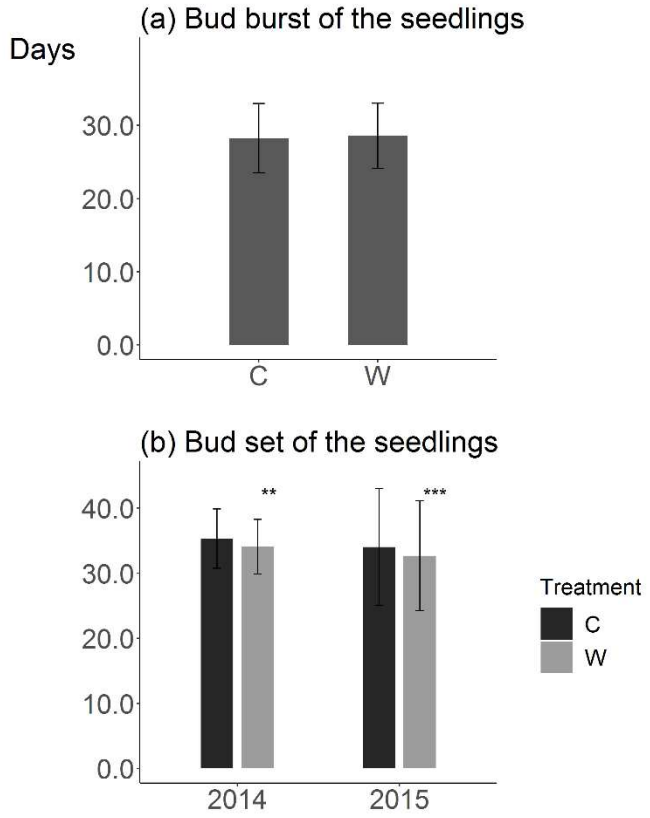

Figure 5.3 Effects of temperatures during the crossing, seed development and maturation on bud burst (a); and bud set (b) in Cross 1 (Proven ♀ x Horrues ♂) of *Populus nigra* in Experiment 1. Days represents the total days needed for the bud to completely burst (reach score 5) and set (reach score 0) since the start of the observations. The start dates of observations for bud burst and set were 7 April and 13 August respectively. Error bars denote standard deviation. Significance are denoted by ** p < 0.01, *** p<0.001.

Table 5.5 The effects of maternal environment on the bud burst score, bud set score and number of days to bud burst and bud set in 2014, 2015 and 2016 of the progenies of the Cross 1 (Proven ♀ x Horrues ♂), Cross 2 (Meers ♀ x Elst ♂) and Cross 3 (Oosterzele ♀ x Remicourt ♂) in Experiment 1, Experiment 2 and Experiment 3.

Experiment	Cross	Year of observation	Parameter	(Intercept)		C » W		W	
				z	p	z	p	z	p
Experiment 1	Cross 1		Bud burst						
		2015	Score	100.95	< 0.001 ***			-2.18	0.030 *
			Days	413.24	< 0.001***	Treatment not included		0.87	0.384
			Bud set						
		2014	Score	20.61	< 0.001***			-4.27	<0.001 ***
			Days	504.57	< 0.001***	Treatment not included		-3.11	0.002**
		2015	Score	-12.50	< 0.001***			-2.78	0.005**
			Days	391.17	< 0.001***			-3.47	<0.001 ***

Experiment	Cross	Year of observation	Parameter	(Intercept)		C » W		W	
				z	p	z	p	z	p
Experiment 2	Cross 1		Bud burst						
		2015	Score	29.49	<0.001***	0.18	0.854	-0.25	0.800
			Days	98.24	<0.001***	-0.59	0.553	1.48	0.139
		2016	Score	29.78	<0.001***	0.69	0.488	-0.62	0.534
			Days	120.92	<0.001***	-1.08	0.283	1.68	0.092 .
			Bud set						
		2014	Score	16.44	<0.001***	-0.41	0.681	-1.19	0.236
			Days	284.85	<0.001***	0.16	0.872	-2.16	0.031*
		2015	Score	6.97	<0.001***	-2.29	0.022*	-3.96	<0.001***
			Days	155.16	<0.001***	-3.26	0.001**	-5.05	<0.001***
Experiment 3	Cross 2		Bud burst						
		2015	Score	33.87	<0.001***	0.31	0.760	-0.27	0.787
			Days	103.57	<0.001***	-0.11	0.914	0.22	0.822
		2016	Score	26.76	<0.001***	1.24	0.216	-0.05	0.961
			Days	128.98	<0.001***	-1.78	0.076 .	0.35	0.728
			Bud set						
		2014	Score	13.91	<0.001***	0.06	0.950	0.98	0.329
			Days	279.12	<0.001***	-0.27	0.788	1.58	0.114
		2015	Score	-3.37	<0.001***	-0.65	0.513	1.34	0.182
			Days	121.64	<0.001***	0.52	0.607	1.14	0.253
	Cross 3		Bud burst						
		2015	Score	23.19	<0.001***	0.55	0.585	-0.31	0.758
			Days	76.97	<0.001***	-1.12	0.264	0.14	0.891
		2016	Score	50.60	<0.001***	Data discarded #	Data discarded #	0.96	0.335
			Days	140.61	<0.001***	Data discarded #	Data discarded #	-1.75	0.081 .
			Bud set						
		2014	Score	8.27	<0.001***	0.58	0.565	0.10	0.917
			Days	245.21	<0.001***	1.45	0.147	0.11	0.909
		2015	Score	-6.49	<0.001***	0.74	0.457	0.31	0.753
			Days	108.54	<0.001***	2.47	0.013*	-0.32	0.750

Note : Significance are denoted by . 0.05 < p < 0.1, * p<0.05, ** p < 0.01, *** p<0.001. # Data were removed because more than 50 % of the buds remained dormant for the entire observation period

Table 5.6 The relationship between the height of the progenies and the scores of bud burst, bud set and number of days to bud burst, bud set from the start of observation in 2014, 2015 and 2016 of the progenies from Cross1 (Proven ♀ x Horrues ♂), Cross 2 (Meers ♀ x Elst ♂) and Cross 3 (Oosterzele ♀ x Remicourt ♂) in all three experiments.

Experiment	Year of observation	Parameter	(Intercept) (Mean ± S.E.)		Height (Mean ± S.E.)	
Experiment 1						
Cross 1		Bud burst				
	2015	Score	0.873	± 0.028***	0.002	± 0.0003***
		Days	3.468	± 0.021***	-0.001	± 0.0002 ***
		Bud set				
	2014	Score	-0.171	± 0.03 ***	0.004	± 0.0003 ***
		Days	3.368	± 0.018***	0.002	± 0.0002 ***
	2015	Score	-1.251	± 0.045 ***	0.007	± 0.0003 ***
		Days	2.794	± 0.019 ***	0.003	± 0.0001 ***
Experiment 2						
Cross 1		Bud burst				
	2015	Score	1.105	± 0.073***	0.0007	± 0.0026
		Days	3.309	± 0.061***	-0.002	± 0.0022
	2016	Score	0.912	± 0.086 ***	0.0014	± 0.0008 ·
		Days	3.422	± 0.065 ***	-0.0006	± 0.0006
		Bud set				
	2014	Score	0.117	± 0.071 ·	0.006	± 0.0018 ***
		Days	3.458	±0.040 ***	0.003	± 0.001 **
	2015	Score	-0.541	± 0.093 ***	0.007	± 0.0008 ***
		Days	2.942	± 0.064 ***	0.006	± 0.0006 ***
Experiment 3						
Cross 2						
		Bud burst				
	2015	Score	1.108	± 0.059***	0.002	±0.003
		Days	3.280	± 0.051 ***	-0.005	± 0.002 *
	2016	Score	0.931	± 0.075***	0.0008	± 0.0007
		Days	3.431	± 0.054 ***	-0.00008	± 0.000
		Bud set				
	2014	Score	0.173	± 0.066 **	0.0045	± 0.0019 *
		Days	3.452	± 0.036 ***	0.002	± 0.001 *
	2015	Score	-0.68	± 0.089 ***	0.0052	± 0.0009 ***
		Days	3.036	± 0.052 ***	0.003	± 0.000 ***
Cross 3						
		Bud burst				
	2015	Score	0.939	± 0.097 ***	0.010	± 0.004 *
		Days	3.454	± 0.078 ***	-0.01	± 0.004 ***
	2016	Score	1.077	± 0.086 ***	0.001	± 0.0009 ·
		Days	3.377	± 0.076 ***	-0.002	± 0.0008 **
		Bud set				
	2014	Score	0.103	± 0.070	0.003	± 0.002 ·

Experiment	Year of observation	Parameter	(Intercept) (Mean ± S.E.)		Height (Mean ± S.E.)	
		Days	3.409	± 0.039 ***	0.001	± 0.001
	2015	Score	-0.67	± 0.111 ***	0.003	± 0.001 **
		Days	3.083	± 0.063 ***	0.002	± 0.0007 **

Note : Significance are denoted by . 0.05 < p < 0.1, * p<0.05, ** p < 0.01, *** p<0.001

5.3.2 Experiment 2

Germination percentages of the seeds matured in a warmer maternal environment were lower than the seeds matured in a colder maternal environment (Table 5.4, Figure 5.2). The pattern of later bud burst and earlier bud set with maternal warming (W treatment: crossing, seed development and maturation in the warmer environment) was similar to the same Cross 1 (Proven and Horrues) in Experiment 1 (Figure 5.4 and Table 5.5). However, there were no significant effects of maternal temperature (C » W, W) on bud burst. When seeds matured in a warmer environment (W) with crossing in a cold (control) environment (treatment C » W), the resulting seedlings showed earlier bud burst. In 2015, we also found earlier bud set in the seedlings generated in the treatment of C » W compared to those that were crossed, developed and matured in the control treatment.

We observed negative effects of warmer maternal temperatures on the height of the seedlings (estimate = -5.377, SE = 1.382 and p = <0.001) in the first year after germination. However, in the following year, there was no significant effect of the maternal temperature on the height of the seedlings (estimate = -3.999, SE=5.688 and p = 0.484) (data can be found in Appendix Table C.2). Still taller seedlings set buds later in 2014 and 2015 (Table 5.6).

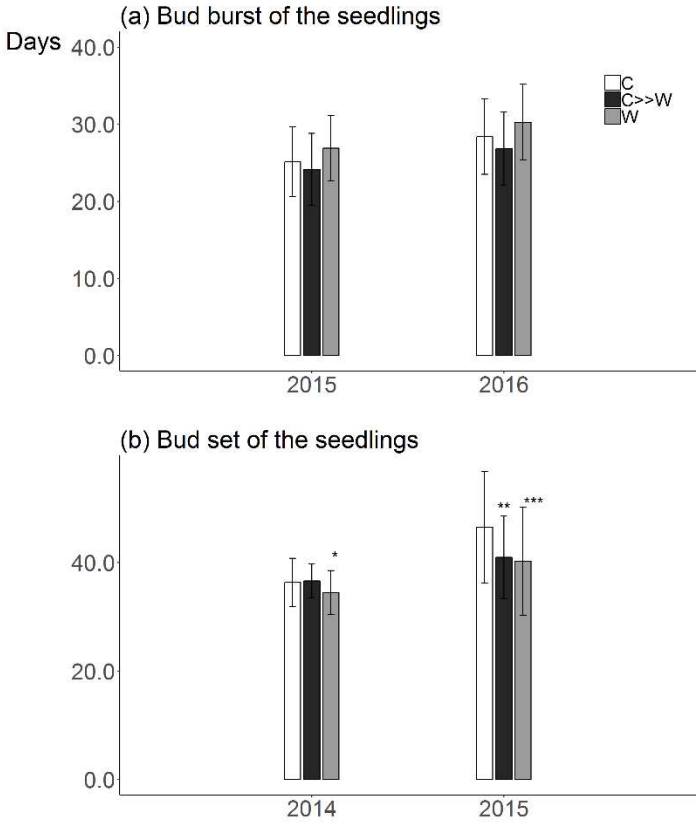

Figure 5.4 Effects of temperatures during the crossing, seed development and maturation on bud burst (a); and bud set (b) in Cross 1 (Proven ♀ x Horrues ♂) of *Populus nigra* in Experiment 2. Days represents the total days needed for the bud to completely burst (reach score 5) and set (reach score 0) since the start of the observations. The start dates of observations for bud burst and set were 7 April and 13 August respectively. Error bars denote standard error (S.E.). Significance denoted by * p<0.05, ** p < 0.01, *** p<0.001

5.3.3 Experiment 3

We observed lower germination of the seeds matured in a warmer maternal environment (treatment W; C » W) for both Cross 2 and Cross 3 than in control (C) maternal environment (Table 5.4, Figure 5.2). There were no significant effects of maternal temperatures on bud burst and bud set in the progenies of both Cross 2 and Cross 3 (Figure 5.5, Table 5.5). However, the seedlings of these crosses in the treatment of C » W showed comparatively earlier bud burst than other treatments (C, W), which seems to be a similar response as in Cross 1 (Figure 5.4 and Figure 5.5). Unlike in Experiment 1 and 2 for Cross 1 (Proven and Horrues), here the seedlings of Cross 2 resulting from seeds produced in warmed mother trees (W treatment) displayed later bud set in both 2014 and 2015, whereas later bud set was observed in the C » W treatment for Cross 3 in 2015 (Figure 5.5 and Table 5.5).

There was no significant effect of warmer maternal temperatures (W treatment) on the height of the seedlings for both Cross 2 and Cross 3. In the first year (2014), we observed negative effects of the C » W treatment on the height of the progenies for both Cross 2 (estimate = -6.703, SE = 1.07 and p = <0.001) and Cross 3 (estimate = -2.492, SE = 0.895

and p = 0.006) (data can be found in Appendix Table C.2). This effect was not significant in the next year, however. The height of the seedlings increased due to the early bud burst and late bud set and extended growing season (Table 5.6).

5.3.4 Effect of the maternal temperature in all experiments

The genotype, year and maternal temperature had a significant influence on the time to bud burst of the offspring as indicated by the likelihood ratio tests: the effect of genotype: deviance = -89.056, p < 0.001; the effect of year: deviance = -59.154, p < 0.001; the effect of maternal temperature: deviance = -14.018, p < 0.001, by removing genotype, year and temperature at a time from the full model.

We also observed that the timing of bud set varied due to the genotype (deviance = -210.98, p < 0.001), year (deviance = -167.72, p < 0.001) and maternal temperature (deviance = -19.774, p < 0.001), as displayed by the likelihood ratio tests.

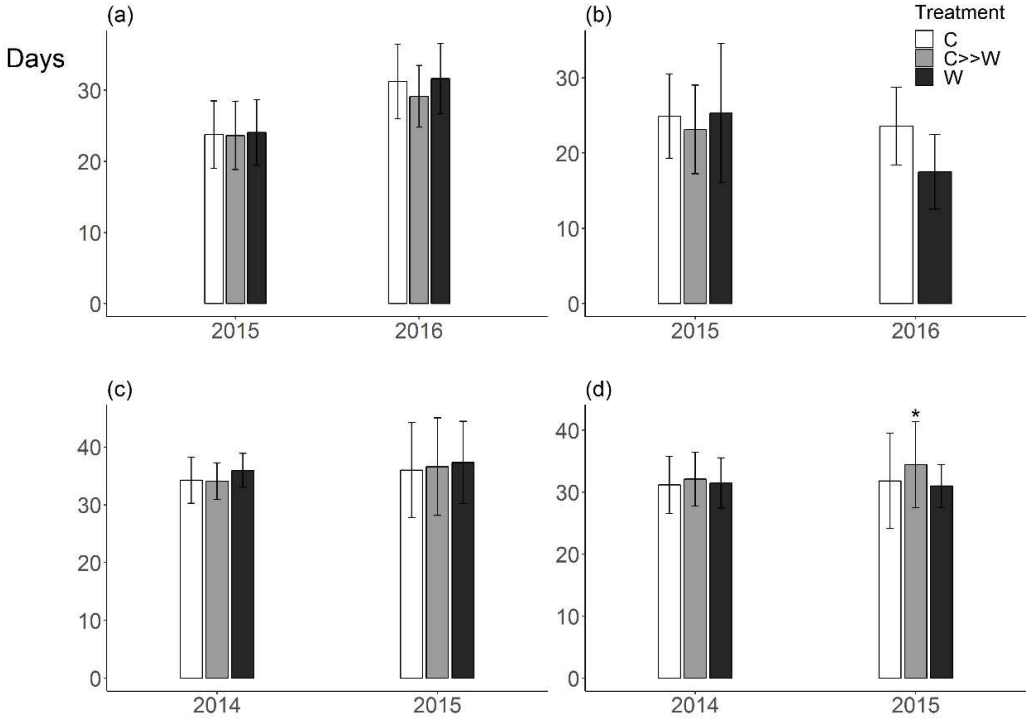

Figure 5.5 Effects of temperatures during crossing, seed development and maturation on bud burst (a, b) and bud set (c, d) in the progenies of Cross 2 (Meers ♀ x Elst ♂) and Cross 3 (Oosterzele ♀ x Remicourt ♂) of *Populus nigra* in Experiment 3, whereas a and c represent Cross 2; b and d represent Cross 3. Days means the total days needed for the bud to completely burst (reach score 5) and set (reach score 0) since the start of the observations. The start dates of observations for bud burst and set were 7 April and 13 August respectively. Error bars denote standard deviation. Significance denoted by * p<0.05.

5.4 Discussion

There was a lower seed germination success when the mother plant experienced warmer maternal temperatures during maturation of the seeds than in control maternal environment. Lower germination percentages may have resulted from a changed moisture content in the seeds, improper embryo development and hormone activity. Poplar seeds are recalcitrant, meaning that if the moisture content in the seeds drops below 30-65 %, the viability of the seeds is lost (Baskin & Baskin, 2001). The warmer maternal environment may have desiccated seeds more compared to the cold environment. However, the warmer maternal environment can also change the germination success substantially due to improper development of the embryo and hormonal activity via the variation in genetic or epigenetic features, which can also be controlled by protein activity. Recent studies in *Arabidopsis thaliana* confirmed that the environment of the mother plant plays a central role in controlling seed dormancy. The environment of the mother plants is integrated into a long term temperature memory via the Flowering Locus in the fruit tissue even before the development of the flowers, and thus before fertilisation and seed development (Chen et al., 2014b). On the other hand, Lacey and Herr (2000) and Zhang et al. (2012) reported increased germination by up to 35 % in *Plantago lanceolata* and *Carduus nutans* as a result of post-fertilisation high temperatures experienced by the zygote. The positive effects of elevated temperatures on embryo development in Norway spruce were reported by Kvaalen and Johnsen (2008). They found higher survival of the matured embryo after transplantation when they kept the culture at 28˚C compared to 23˚C and therefore they suggested that the optimal temperature requirement for maturation and production of a high quality embryo might be higher than 23˚C in Norway spruce. Elevated temperatures can also negatively affect seed viability of the conifer shrub *Juniperus communis* due to the interrupted growth of the pollen tube, female gametophyte and fertilization and thus failure of the embryo development (Gruwez et al., 2016).

The life cycle of black poplar is probably affected by global warming in different ways. Since we observed in this study that different genotypes responded differently to a warmer environment, there is a possibility that warming will delay the timing of pollen maturation and dispersal in genotypes with low sensitivity to temperature. The germination success of black poplar is highly dependent on moist and warm conditions, which is mostly maintained along rivers after flooding events (Corenblit et al., 2014). Therefore, the predicted changes in the climatic system such as winter warming and drought in spring and summer (Stocker et al., 2013) might substantially affect the seed germination, seedling recruitment and early establishment of the species, which is a vital stage for early successional species like black poplar.

Our results from full-sib families indicate that the maternal environment during seed maturation at least partly influenced the timing of bud set of the progenies. Full-sib families set buds earlier (in Cross 1) when the mother plant was kept in a warm (W) environment during seed maturation, underpinning that the changed phenology was due to the maternal environment, which was genotype specific. However, the perception of environmental variation could be via the mother plant, or via the developing zygote itself. Without genetic analyses, it is difficult to differentiate the effects mediated by the mother plant from the direct effects of environmental variation on the developing zygote. However, many studies on flowering time, drought tolerance, germination and disease resistance show that the maternal environment at the origin influences the responses of the offspring (Cendán et al., 2013; Vivas et al., 2013; González et al., 2017; Groot et al., 2017). In our study, the environment of the mother tree (from where grafts were taken) might also influence the phenological response of the offspring by interacting with the environment during seed maturation. The maternal temperature during pollination (prefertilisation environment) also has additional effects on the phenological response of the seedlings, which can be compared between the treatments of W (crossing, seeds developed and matured in warmer environment) and C » W (crossing in cold (control) environment, seeds developed and matured in warmer environment). This result of our study is in line with the study of Imaizumi et al. (2017), who showed that the photoperiod during the prereproductive and reproductive stages influences the germination behaviour of *Arabidopsis thaliana*. The weather experienced during earlier stages of the life cycle has been reported to influence the germination of *Arabidopsis thaliana* and two perennial species (*Genista tinctoria* and *Calluna vulgaris*) (Walter et al., 2016; Auge et al., 2017).

The earlier bud set in the seedlings of Cross 1 in response to maternal warming contrasts with findings in Norway spruce by Kvaalen and Johnsen (2008). We assume that the earlier bud set in poplar seedlings (in Cross 1) that experienced a warmer maternal temperature during seed development may be due to the memory effects that established during seed development and persisted at least until the second year after germination. However, without a molecular study (e.g., methylation-sensitive amplified polymorphism technique) exploring the mechanisms that maintain such memory, we cannot confirm that the change in the timing for bud set in poplar's seedlings here was due to the memory effect. Slower growth of the seedlings from a warmed mother plant might have led to earlier bud set. Bud phenology in Norway spruce (*Picea abies*) is regulated by epigenetic mechanisms, the memory established by the temperature sum during zygotic or somatic embryogenesis (Yakovlev et al., 2012; Yakovlev et al., 2016). Epigenetic memory effects mediated by maternal temperatures have also been reported in other conifers such as *Picea glauca* × *P. engelmannii* (Webber et al., 2005) and *Larix* spp. (Greenwood & Hutchison, 1996) and

annuals such as *Arabidopsis thaliana* (Whittle et al., 2009). However, the temperature sensitivity during reproduction might differ between conifers and broadleaves because of the different reproductive system and genetic structures. Angiosperms such as poplar contain less epigenetic regulator genes than gymnosperms as Norway spruce (Yakovlev et al., 2016); perhaps this mechanism provides lower plasticity as well as opposite response to varying temperature condition as previously reported in some conifers. The larger genome size with expanded regulator gene families resulting from the longer evolutionary history in conifers might have caused this difference (Nystedt et al., 2013).

Surprisingly, there were nearly no significant effects of maternal warming (treatments W and C » W) on the bud burst of the seedlings, but in general the timing to bud burst was delayed in warmer maternal environments. Delayed bud burst and earlier bud set due to warmer maternal environments could lead to a reduction in growing season length and lower biomass production. Delaying bud burst can be a trade-off against frost damage by reducing growth. In a study on the sapling of *Fagus sylvatica*, the authors showed a negative correlation between frost damage and bud burst time (Gömöry & Paule, 2011). However, the absence of a significant effect of maternal warming on bud burst suggests that the effects of maternal warming were less prominent on poplar's bud burst, probably due to the lower sensitivity to environmental cues to initiate spring growth. Nevertheless, a recent study on Norway spruce demonstrated that buds in plants originating from a cold (18˚C) embryogenic environment burst almost 2 weeks earlier than those originating from a warm (28˚C) embryogenic environment (Carneros et al., 2017), most likely resulting from the epigenetic memory of temperature during embryogenesis.

Variation in phenological responses among different crosses with different genotypes in our study suggests that the sensitivity to warmer maternal temperature might differ among genotypes. In addition, we also observed through the likelihood ratio test that along with the maternal environment, genotype also significantly contribute to influence the phenological responses of the offspring. In case of Cross 2, we performed the pollination nearly two weeks later than in Cross 1 and Cross 3 due to the late maturation of the pistillate flower, which resulted in a longer day length during the crossing. Sensitivity to photoperiod has been reported to be higher in species native to milder climates (that is, less than six months with an average temperature below 5˚C) and bud burst of only 35% out of 173 species were reported to be sensitive to the photoperiod (Zohner et al., 2016). Black poplar might have lower sensitivity to the photoperiod in terms of its bud burst, but on the other hand, the photoperiod in the maternal environment (longer days due to the late maturation of the pistillate flower in Cross 2) may have interacted with warmer temperatures to change the sensitivity to temperature. Nevertheless, there were no significant effects of the W and C » W treatments on the timing to bud burst and set in both Cross 2 and Cross 3.

5.5 Conclusion

In sum, our study will help to better understand and predict the responses of trees to rapid climate change. Our results can, for instance, be used in models predicting forest phenology in a warmer world by simulating the bud burst or bud set date based on the response of bud growth or growth cessation to temperature. The sensitivity to maternal temperature and the direction of the effect of maternal warming might differ among species. Black poplar seems to be sensitive to maternal temperatures and such sensitivity varies among different genotypes, which suggests that during the selection of provenances, preparation of planting materials for regeneration and breeding programmes, we need to consider the temperature sensitivity of the species and genotypes (Sixto et al., 2016). We here furthered our insights in the performance and phenological responses of the seedlings of a model forest tree species like *Populus nigra* to maternal warming. In terms of future research, our study will provide the opportunity to further explore what genetic factors regulate the phenological response of the seedlings and related factors that control the sensitivity to the warmer maternal environment.

6 Transgenerational effects in asexually reproduced offspring of *Populus*

After: Sumitra Dewan, Pieter De Frenne, An Vanden Broeck, Marijke Steenackers, Kristine Vander Mijnsbrugge, Kris Verheyen (2018) Transgenerational effects in asexually reproduced offspring of *Populus*. *Plos One*, 13(12): e0208591.

Abstract

The response of trees to a changing climate can be affected by transgenerational phenotypic plasticity, i.e. phenotypic variation that is conserved and transferred to the offspring. Transgenerational plasticity that is influenced by epigenetics (heritable changes in gene function that do not result from changes in DNA sequence) during both sexual and asexual reproduction are of major relevance for adaptation of plants to climate change. To understand the transgenerational effects on the responses of vegetatively propagated poplar (*Populus deltoides* and *P*. *trichocarpa*) ramets (cuttings) to a changing environment, we tested whether the temperature and photoperiod experienced by the mother trees (genets) persistently affects the phenology of the cuttings grown in a common environment. We weekly monitored the bud phenology of the cuttings collected from the parent trees that have been growing across Europe along a >2100 km latitudinal gradient for at least 18 years. In addition, we asked whether there was variation in DNA methylation as measured by Methylation Sensitive Amplified Fragment Length Polymorphism (MSAPs) in the clones due to the different environmental conditions experienced by the parent trees. Our results indicate a transgenerational effect on bud phenology in the asexually reproduced offspring (vegetative cuttings). The temperatures experienced by the parent tree clones (from different geographic regions) altered the bud flush of the cuttings in the common garden. However, no significant epigenetic variation was detected in the cuttings of the parent trees within single genotypes growing under different climates. In sum, our results show that trees have the potential to respond to rapid climate change but the mechanism behind these changes need to be further investigated by more powerful molecular methods like whole-genome bisulphite sequencing techniques.

6.1 Introduction

Phenotypic plasticity is an important mechanism for trees to cope with climate change and heterogeneous environments (Matesanz et al., 2010). Bud phenology (here defined as the timing of bud flush and bud set) in particular is a very important phenotypic trait to assess plant responses to climate change since it controls the growing period available to plants. Along with other phenotypic traits, it received increased attention in the light of global warming (Yu et al., 2010; Wolkovich et al., 2012; Basler & Korner, 2014; Sivadasan et al., 2017). Bud phenology can be influenced by global warming and interactions between the climate and photoperiod (Yu et al., 2010; Rohde et al., 2011a; Way, 2011). However, the phenology of tree species does not show simple linear responses to warming temperatures (Chuine et al., 2010). In the case of a latitudinal transfer from south to north, i.e., if plants experience colder than optimal temperatures, longer time would be required for the duration of bud formation and the cessation of growth can be delayed (Rohde et al., 2011a). One component of phenotypic plasticity is transgenerational effects when the parent's environment influences offspring responses to environmental conditions independent of genetic changes (Groot et al., 2017; Donelson et al., 2018), which is also known as transgenerational phenotypic plasticity (TGP). It is particularly important for tree responses to climate change (Donelson et al., 2018). Most former studies (on plants) have focused on transgenerational (particularly maternal) effects of sexually reproduced offspring, examining the effects of the maternal environment on seed germination and flowering time (Galloway, 2005; Galloway & Etterson, 2007; Herman & Sultan, 2011). Various studies, by comparing the performance of the progeny in maternal and non-maternal environments, have shown that maternal effects are likely adaptive (Galloway & Etterson, 2007; Latzel & Klimešová, 2010a). However, in heterogeneous environments where the progeny environment is hard to predict, the phenotypes with low variance of fitness get a selective advantage which is also known as bet hedging phenomena, which involves a trade-off between the mean and the variance of fitness and in such conditions the advantage of parental effects might not be observed (Philippi & Seger, 1989). For example, when the fitness of a genotype varies over generations, the appropriate measure of its relative growth rate is its geometric mean fitness, rather than its arithmetic mean fitness (Philippi & Seger, 1989). The geometric mean of n numbers is the nth root of their product. If the numbers vary, then the geometric mean is always less than the arithmetic mean; in general, the geometric mean becomes smaller as the numbers being averaged become more variable. Thus, the geometric mean fitness of a genotype can be increased by reducing the variance of its fitness over generations. Although thus far this effect has been studied mainly in non-clonal plants, transgenerational plasticity is also applicable to asexually generated progeny (Latzel & Klimešová, 2010a).

Asexual reproduction lacks the variation-generating mechanisms of meiotic recombination and segregation, which reduces the potential for genetically based adaptation (Latzel et al., 2016). Nevertheless, many clonal species persist and successfully expand in a range of different environments. Some of the most successful invasive plant species are clonal (Richards et al., 2012a; Wang et al., 2017). Clonal individuals have the advantage of resource sharing (such as water, carbohydrates and mineral nutrients) between the connected ramets, an effect known as clonal integration, which is probably one reason of their invasion success (Wang et al., 2017). Furthermore, epigenetic variation might contribute to adaptation of asexual plants in a wide range of, particularly stressful, environments (Verhoeven & Preite, 2014). It is suggested that, given the absence of meiotic resetting of epigenetic modification in clonal reproduction, vegetative offspring can inherit epigenetic information of previous environmental interactions from the maternal ramet (Latzel et al., 2016). Transgenerational effects were observed in clonal offspring of *Festuca rubra* and *Trifolium repens* and some transgenerational effects in clonal plants of *Trifolium repens* were reported as adaptive (González et al., 2017; Munzbergova & Hadincova, 2017). DNA methylation, the addition of a methyl group to one of the four bases in the DNA molecule (usually cytosine), is recognized as one of the prime epigenetic mechanisms to correlate with gene expression and might play an important role in transgenerational effects. However, the processes behind transgenerational effects are not well understood (Jablonka & Lamb, 1995; Latzel & Klimešová, 2010a; Verhoeven & Preite, 2014). Yet, transgenerational effects in clonal plants are important for forest management, reforestation programmes and nurseries, because many tree species (e.g. poplars, willows and many fruit trees) are reproduced vegetatively by means of cuttings or grafting.

Poplars (*Populus* sp.), member of the Salicaceae, have a wide distribution in the world and can easily be propagated vegetatively. The species therefore serves as an ideal model to study responses of asexually reproducing plants to climate change. *Populus* is a fast growing genus important for biomass production, and hybrid poplar can produce 70-105 tons of aboveground dry biomass per hectare after 10-15 years following hybrid poplar stands in 41 locations in Sweden (Johansson & Karačić, 2011). The hybrid poplars are also planted widely in Europe for wood production, windbreaks and soil protection (Vanden Broeck, 2004; Vanden-Broeck et al., 2012).

Here we set up a common garden experiment to assess maternal effects (further referred to as parental effect) on bud phenology of cuttings collected from genets growing in different climatic environments (further referred to as parent trees because all genotypes were not female) and representing five different clones of *Populus trichocarpa* × *P*. *deltoides* and *Populus trichocarpa* (further referred to as: genotypes). Parent trees representing a single genotype were grown across a latitudinal gradient of >2100 kilometres (corresponding to a

4.9 °C temperature difference and different photoperiods of up to 3.5 hours), and cuttings of these parent trees were then grown in the common garden. We hypothesized a transgenerational effect of temperature experienced by the parent trees on bud phenology of the vegetatively (by stem cuttings) produced offspring within genotypes. Furthermore, we investigated DNA methylation variation as a potential epigenetic mechanism for transgenerational effects (in this case, epigenetic variation in the generations of cuttings) using Methylation Sensitive Amplified Fragment Length Polymorphism (MSAP) analysis (Guarino et al., 2015). The aim of the molecular part of this study was to look for significant natural variation in genome-wide DNA methylation patterns within individuals plants of a single clone (genotype) and vegetatively propagated from parent trees with different histories (that is, grown in contrasting macroclimates across Europe), that lasted after growing for four years in a common environment.

6.2 Materials and Methods

6.2.1 Description of the clones

Between the 1980s to 1990s cuttings of five hybrid poplar genotypes namely Beaupré, Raspalje and Unal (all *Populus trichocarpa* × *P*. *deltoides*), Fritzy Pauley (*P*. *trichocarpa*), and Trichobel (*P*. *trichocarpa* × *P*. *trichocarpa*) were produced from adult trees under the renowned poplar breeding programme at the Research Institute for Nature and Forest (INBO), Geraardsbergen in Belgium. The adult trees that resulted from the original seedlings selected in the breeding programme (further referred to as 'provenance trees') remained in Grimminge, Belgium and were not transferred. Asexually reproduced offspring of the provenance trees (onwards referred to as 'parent trees' or 'genets') were taken as cuttings and planted in Spain (near Madrid), Italy (Casale Monferrato), France (Saint-Usage, Beuxes, Gueméne Penfao) and Sweden (Uppsala) to establish stool beds for the purpose of tree breeding (Table 6.1). Later, new stool beds were established in the vicinity of the old stool beds from cuttings of the former beds.

6.2.2 Sample (cuttings) collection

Between February and April 2014 before the start of bud burst, we collected 817 one year old cuttings of 22 cm length of the above mentioned five *Populus* genotypes growing at seven sites; including the local site of the provenance trees in Grimminge, Belgium (Table 6.1). Not all five genotypes were present in all the seven sites and the number of cuttings of each genotype was not evenly distributed either due to mortality or bud damage. Each cutting was measured (collar diameter) and planted in individual 5 L pots containing standard potting soil (Saniflor pro, NPK 12-14-24) and monitored in a common garden outside the Research Institute for Nature and Forest (INBO), Geraardsbergen, Belgium

(Table 6.1). The cuttings were regularly irrigated and fungicide ('Caddy'- Cyproconazole) was applied two times on 5/05/2015 and 26/08/2015 during the growing season in the common garden. It was known that the application of the above fungicide (Cyproconazole) influenced the gene expression of the fungal pathogens, but the effect on the DNA methylation or on the gene expression of the plant community is yet to be discovered (Zhan et al., 2006).

Table 6.1 Background information of the study sites across Europe where poplar trees were transplanted and sampled.

Country	Site	Latitude (°)	Longitude (°)	Elevation (m)	MJanT[a] (°C)	MJulyT[b] (°C)	MAT[c] (°C)	DL[d] (h) 1 Jan.	DL[d] (h) 1 May	Cuttings planted (year)
France	Beuxes	47.09	0.18	43	5.42	19.17	11.99	8.36	14.25	1994
Spain	Madrid	40.68	-4.10	988	3.46	21.98	11.92	9.14	13.79	1996
France	Gueméne Penfao	47.63	1.89	139	3.54	19.04	11.04	8.29	14.30	1985
Belgium	Grimminge	50.78	3.93	28	3.96	18.18	10.86	7.81	14.58	Provenance trees
Italy	Casale Monferrato	45.08	8.30	161	1.49	18.94	9.46	8.63	14.10	1982
France	Saint-Usage	47.08	5.24	178	0.69	18.22	9.10	8.36	14.25	1994
Sweden	Uppsala	59.86	17.64	18	-0.87	16.97	7.01	5.71	15.70	1990
Belgium	Geraardsbergen	50.78	3.88	29	3.96	18.18	10.86	7.81	14.58	Common garden

[a]MJanT- mean monthly temperature for January,
[b]MJulyT- mean monthly temperature for July,
[c]MAT- mean annual temperature
[d]DL-Day length

6.2.3 Observation of growth and bud phenology

We quantified autumn phenology (bud set) in 2014 and both spring and autumn phenology (bud burst and bud set) in 2015. The bud burst and bud set was assessed once a week (starting on 17 March 2015 i.e. Day of the year (DOY) 76 for bud burst and on 13 August 2014 (DOY 225) and 21 August 2015 (DOY 233) for bud set) until all the cuttings completed bud burst and bud set. The bud set and bud burst was monitored by scoring each plant according to the method of Pellis et al. (2004); Rohde et al. (2011b) with a few modifications as detailed in Appendix Table D.1. The scoring was done on one-third of the total height of stem below the apical bud and determined the score when 60-80% of the buds reach in the same stage. We measured the height (cm) of the cuttings in December 2014 after end of the growing season.

6.2.4 Temperature and day length data

The mean 1982-2014 annual temperature (˚C) (MAT), mean monthly temperature for July (MJulyT), mean monthly temperature for January (MJanT) at the seven sites where the

clones had been growing were calculated using the RFc package in R version 3.3.3 (Dmitry, 2016). We chose mean July and January temperatures to represent the warmest and coldest month of the year, because it has been shown before that minimum and maximum temperatures, rather than mean annual temperatures, better predict leafing, flowering and growth of several plant species (Dreesen et al., 2012; Siegmund et al., 2016; Körner & Hiltbrunner, 2018). Day length on 1 May (DL1May) and day length on 1 January (DL 1Jan) at all seven sites were calculated according to Schreiber et al. (2013).

The growing period of each plant for 2015 was calculated by counting the number of calendar days between complete bud burst (bud burst score equal to 5) and complete bud set (bud set score equal to 0).

6.2.5 Determination of DNA methylation

6.2.5.1 Plant materials and sample collection

DNA methylation variation was studied within the poplar clones growing in the common garden experiment. On 17 April 2017 (that is, after four growing seasons in the common garden), 54 leaf samples (young, freshly developed leaves) were collected from one year old stem of the cuttings in the common garden (Appendix Table D.2). Just after collection, fresh leaves were stored in silica gel until DNA extraction.

6.2.5.2 DNA samples

DNA samples were obtained from the same plant tissue and collected at identical developmental stage (newly expanded, fully-grown leaves). Total genomic DNA was extracted from these leaves with the QuickPickTM Plant DNA kit (Isogen Life Science, De Meern, Nederland). The integrity of the DNA was assessed on 1.5% agarose gels, and DNA quantification was performed with Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies) using a Synergy HT plate reader (BioTek).

6.2.5.3 Verifying clone (genotype) identity with microsatellite markers

Twelve nuclear microsatellite loci (SSRs) were used to verify the identity of the genotypes. We selected SSRs that were found useful for the identification of *Populus* genotypes in former studies (Smulders et al., 2001; Liesebach et al., 2010). PCR products were run on an ABI 3500 analyzer with the GeneScan-600 LIZ size standard and analyzed using GeneMapper 4.1 (Thermo Fisher Scientific). Details on microsatellites and PCR-conditions are given in Appendix Table D.3.

6.2.5.4 Methylation-sensitive Amplified Length Polymorphism

The Methylation Sensitive Amplified Length Polymorphism (MSAP) method, a modified version of the Amplified Fragment Length Polymorphism (AFLP) DNA fingerprinting

technique (Vos et al., 1995), was adapted from Guarino et al. (2015). Briefly, we initially tested 32 primer combinations on a subset of 16 samples using two sets of restriction and ligation reactions. Seven combinations of EcoRI (labelled primers) / HpaII - MspI primers (Appendix Table D.4) were selected for the MSAP analysis of the total 54 samples, based on clarity and reproducibility of amplified bands and the presence of polymorphism. Eleven samples were replicated, starting from the same leaf sample and two different DNA-extractions to assess reproducibility. PCR amplicons were fluorescently labeled with one of two dyes: NED and VIC, and were run in simplex on an ABI 3500 analyzer with the GeneScan-600 LIZ size standard (Thermo Fisher Scientific). The EcoRI - MspI and EcoRI - HpaII DNA fingerprinting profiles were processed per primer combination. Only fragments ≥150 bp in size were considered to reduce the potential impact of size homoplasie (Vekemans et al., 2002). The genotyping error rate was estimated for each primer combination according to Bonin et al. (2004) and based on the 11 replicates.

6.2.6 Statistical analysis

6.2.6.1 Bud phenology

All statistical analyses were performed in R version 3.3.3 (R Core Team, 2017). Linear mixed effects models (*lmer* function in the *lme4* package in R) were used to analyse the relationship between phenology (bud burst and bud set) and temperature (MJanT, MJulyT, MAT), stem diameter (mm) and day length (DL1May and DL1Jan) of the seven sites (parental environment) where the parent trees of the different genotypes were growing (Bates et al., 2015). The number of cuttings (ramets) per genotype collected at each of the seven parental environments, however, was not evenly distributed (Appendix Table D.2). So, instead of one combined model with genotype as random factor, we applied the above model for each genotype separately to reduce the bias of small vs. large sample sizes in the model. Moreover, the phenological response can also differ depending on the genotype. Since the relationship between the phenology and temperature variables may change over the gradual progress of bud set or bud burst (Carneros et al., 2017), we applied the same linear mixed effects model for each observation day (days of the year, DOY) separately for 2014 and 2015. We used *site* as a random effect in the models. The weighted mean of the slopes of all genotypes for each temperature variable was then calculated by using the slopes from the mixed models by bootstrapping 500 times (*boot* function in the *boot* package in R) (Canty & Ripley, 2017) (see box A for detail). Similarly, we used linear mixed effect models to analyse the relationship between the length of the growing season and temperature variables (MJanT, MJulyT, MAT) and day length (DL1May and DL1Jan) using *site* as random effect for each genotype. Then, we calculated the weighted mean of the slopes from the models again by using bootstrapping as above. We also analysed the

relationship between height (cm) of the cuttings after one growing season that was in 2014 and total days (day) needed to open the bud (time to bud burst score 5) starting from 17 March (= observation day 1) in 2015 using Generalised Linear models with Poisson error distributions.

Box A Bootstrapping (based on Efron and Tibshirani (1993) and Canty and Ripley (2017)):

Bootstrapping can be a very useful tool in statistics and it is very easily implemented in R. Bootstrapping comes in handy when there is doubt that the usual distributional assumptions and asymptotic results are valid and accurate. Bootstrapping is a nonparametric method, which lets us compute estimated standard errors, confidence intervals and hypothesis testing.

Generally, bootstrapping follows the same basic steps of 1) resample a given data set a specified number of times, 2) calculate a specific statistic from each sample, 3) find the standard deviation of the distribution of that statistic.

Here, we performed bootstrapping for weighted mean of the slopes of the relationship between average maternal temperatures and bud phenology of all genotypes.

Given a vector of values and a vector of weights, we can compute the weighted mean by the built-in function ***weighted.mean(x, w)*****,** where x is the vector of values and w is the vector of weights. In our analysis, we set the weights as per the number of samples, set function for weighted mean calculation as ***function(x, w) sum(x*w)*** and ran the bootstrapping for 999 times to get the weighted mean of the slopes.

6.2.6.2 DNA methylation

We used GeneMapper v3.7 (Thermo Fisher Scientific) for the sizing of the DNA fragments (raw data) and the RawGeno v 2.0 R CRAN package (Arrigo et al., 2009) for automatic scoring of the variation in the sized fragment patterns and to transform the fragment profiles into a binary character matrix, using 0 or 1 to define the absence or the presence of a specific DNA band, respectively. The msap was used to assess the cytosine CpG methylation profile of CCGG motifs for each sample and to analyze the data (Perez-Figueroa, 2013). The presence of both EcoI / MspI and EcoRI / HpaII products (pattern 1/1) denotes an unmethylated state, the presence of only one of the EcoRI / HpaII (1/0) or EcoRI / MspI (0/1) products represent methylated states (hemimethylated or internal C methylation) and absence from both EcoRI / MspI and EcoRI / HpaII products (0/0) is considered as an uninformative state, as it could be caused by either fragment absence or hypermethylation (Perez-Figueroa, 2013). The epigenetic state scoring error rate was estimated for each primer combination from discordant scores in MspI and HpaII profiles of 11 individuals that were processed twice from different DNA extracts. Following the

procedure in Herrera and Bazaga (2010), every loci was then classified as either Methylation-susceptible loci (MSL) or Nonmethylated loci (NML), depending on whether the observed proportion of methylated states across all samples exceeded the estimated error rate. Only samples without missing data for the seven primer combinations and for both enzyme combinations are included in the analysis. This resulted in 52 samples analyzed (replicated samples excluded) for in total 233 MSAP-fragments.

The analysis was performed by grouping the clones per country. The amount of genetic and epigenetic variation was estimated using the Shannon diversity index (S). The epigenetic differentiation between groups (φ_{ST}) was tested using analyses of molecular variance (AMOVA) based on 1000 permutations. A Mantel test was performed to obtain the correlation between MSL and NML and to shed light on how much epigenetic variation was influenced by the genetic background. The genetic (NML) and epigenetic (MSL) structure was assessed by a principal coordinates analysis (PCoA).

6.3 Results

6.3.1 Bud burst, bud set and growing season

We observed earlier bud burst (that means a higher bud burst score) in cuttings in 2015 where the parent tree experienced warmer mean January, July and mean annual temperature across almost all poplar genotypes, which was presented by positive slope of the relationship between temperature and mean bud burst score (Figure 6.1). The plots showing the relationship between mean annual temperatures (MAT) experienced by parent trees and mean bud burst score of the cuttings of four genotypes on day of the year 83 are available in Appendix Figure D.1.

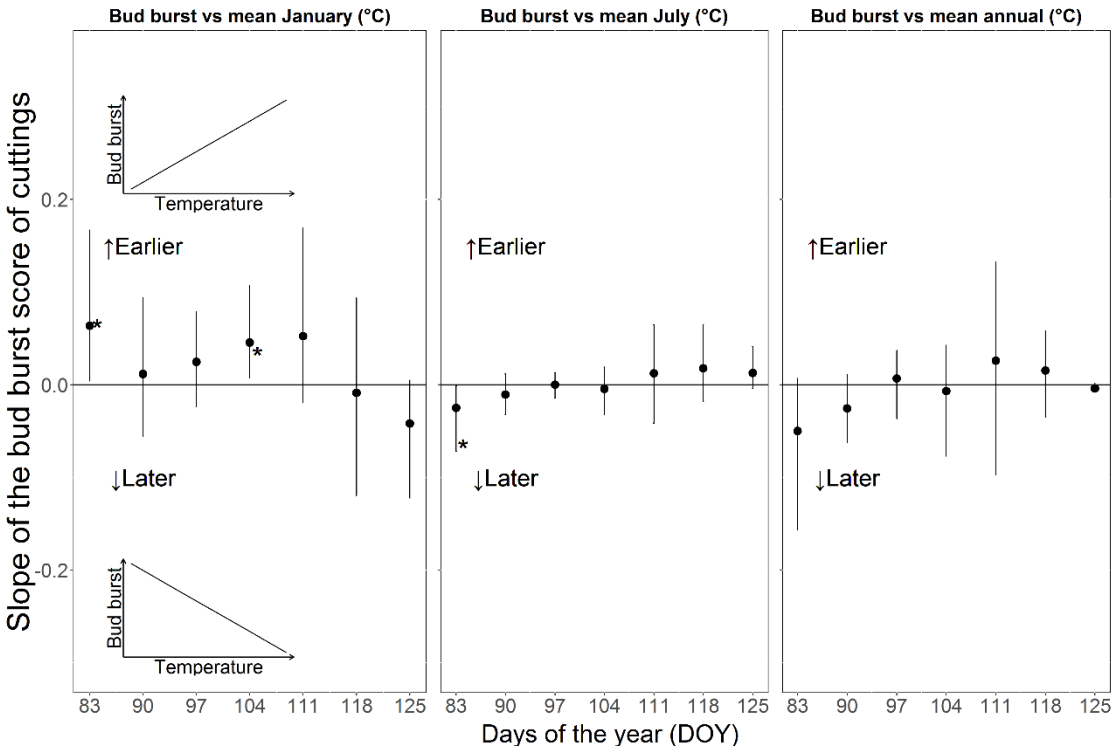

Figure 6.1 Mean weighted (bootstrapped) slopes of the relationship between the mean bud burst score in 2015 and mean January, July and mean annual temperatures experienced by the parent trees. Error bars denote 95% confidence intervals (upper and lower) across the 500 bootstrapped values. Significances at the 95% level are denoted by *. 'Earlier' means that buds burst earlier (higher bud burst score) with increasing temperatures and 'Later' means that buds burst later (lower bud burst score) with increasing temperatures.

Taller plants exhibited significantly earlier bud burst in the second growing season (Appendix Figure D.2). The cuttings also set buds earlier (that means lower bud set score) with warmer January and mean annual temperatures in parental environment, which was indicated by a positive slope of the relationship between temperature and mean bud set score (Figure 6.2). The significant difference in bud set was observed only on one day during the second growing season. We did not observe any consistent relationship between bud burst and day length (1 May and 1 January) of the sites where the parent trees have been growing (Appendix Figure D.3). However, the day lengths on 1 May and 1 January of the growing sites of the parent trees had a significant influence on bud set in 2014 (only on day of the year 247), but in the following year no significant effect was observed (Appendix Figure D.4).

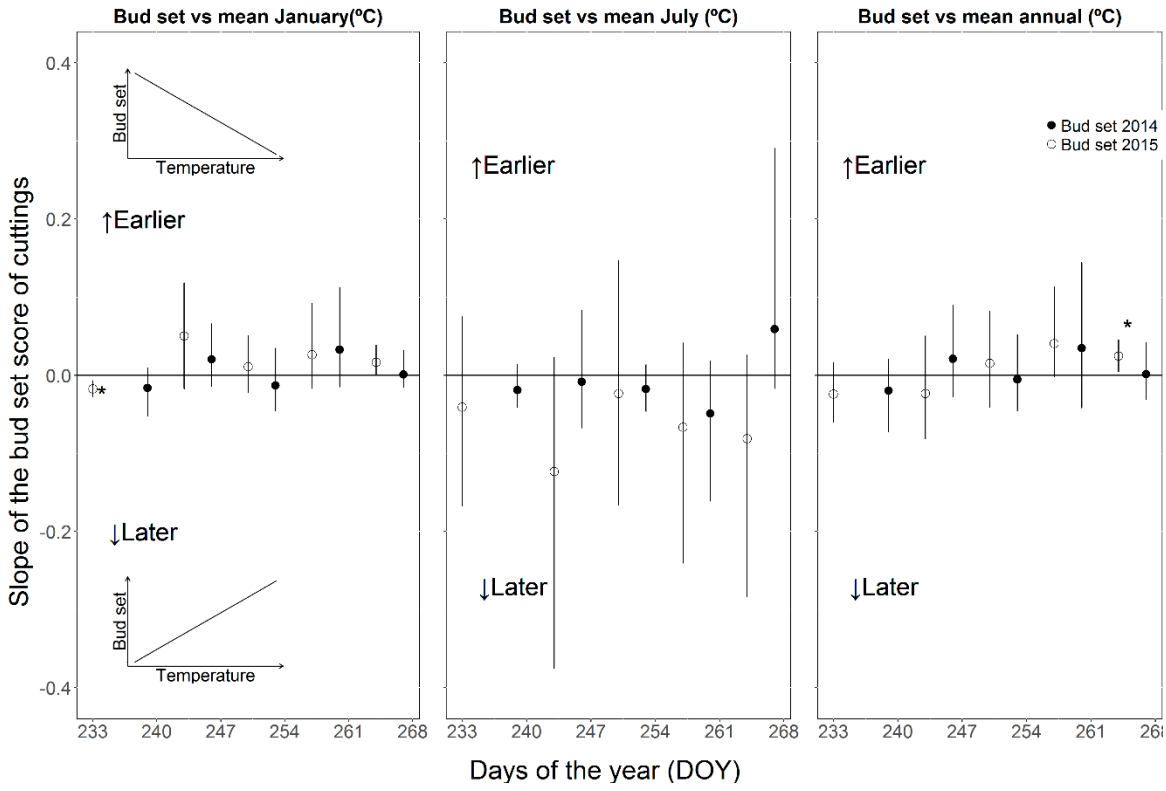

Figure 6.2 Mean weighted (bootstrapped) slopes of the relationship between the mean bud set score in 2014 and 2015 and mean January, mean July and mean annual temperatures experienced by the parent trees. Error bars denote 95% confidence interval (upper and lower) across the 500 bootstrapped values. Significances at the 95% level are denoted by *. 'Earlier' means that buds set earlier (lower bud set score) with increasing temperatures and 'Later' means that buds set later (higher bud set score) with increasing temperatures.

The results of linear mixed effect models for each genotype on each observation day (DOY) for bud burst in 2015 and bud set in 2014 and 2015 can be found in Appendix Table D.5 and Appendix Table D.6.

Parental temperature also significantly affected the length of the growing season of poplar cuttings probably due to earlier bud set. The length of the growing season was significantly shorter with warmer mean annual temperature of the translocated sites across all the genotypes (the weighted mean = -0.509, upper and lower confidence intervals were -0.07046 and -1.37171 respectively across 500 bootstrapped values) (Figure 6.3). Remarkably, there was no effect of day length on the length of the growing season (Appendix Figure D.5).

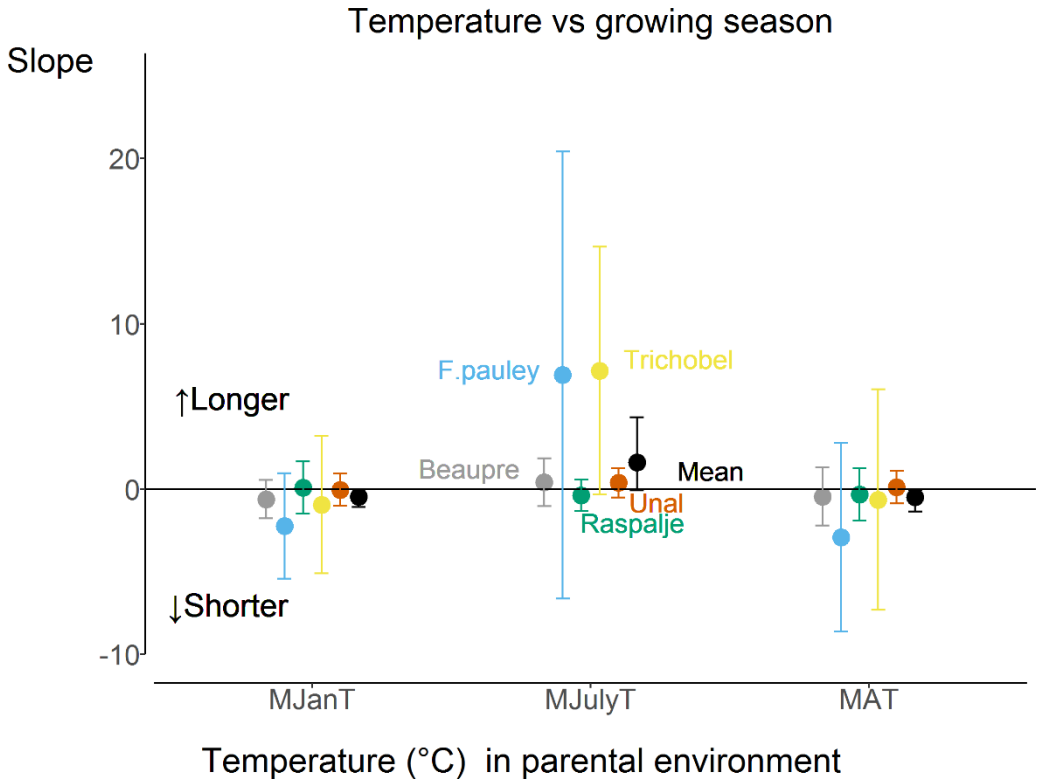

Figure 6.3 Slopes of the relationship between the length of growing season of five genotypes (Beaupre, Fritzy Pauley, Raspalje, Trichobel and Unal) in 2015 and mean January, July and annual temperatures experienced by the parent trees. Mean weighted (bootstrapped) slopes are indicated by black points and error bars denote 95% confidence intervals (upper and lower) across 500 bootstrapped values. Error bars denote 95% confidence intervals. 'Shorter' means that growing season is shorter with increasing temperature and 'Longer' means that growing season is longer with increasing temperature.

6.3.2 Clone verification and DNA methylation

The clone identification was confirmed by the results of the microsatellite analysis (Appendix Table D.3). The mean estimated genotyping error rate was 0.022 (Appendix Table D.4). Of these 233 MSAP-fragments, 142 were Methylation Susceptible Loci (MSL) (of which 114 (80%) were polymorphic) and 91 were Nonmethylated Loci (NML) (70 (77%) were polymorphic). There was no higher degree of epigenetic variation compared to genetic variation (Appendix Figure D.5). In contrast, the mean Shannon's diversity index for MSL (Mean ± SE = 0.403 ± 0.165) was significantly lower than the corresponding figure for NML (0.494 ±0.162) (Wilcoxon rank sum test with continuity correction: W = 2631.5, P < 0.001). The AMOVA-based estimate of epigenetic differentiation between groups was low and not statistically significant (φ_{ST} = 0.0189, p = 0.198). The Mantel test indicated a high correlation between MSL and NML (r = 0.845, p < 0.001, nperm = 1000). Similarly, the PCoA based on the methylated loci was very similar to the PCoA for the non-methylated loci, suggesting a high dependence between genetic and epigenetic variation.

6.4 Discussion

The results of this study indicate the presence of a transgenerational effect mediated by parental environment on the bud phenology of asexually produced offspring (vegetative cuttings) of different *Populus* genotypes. Previous studies in *Populus* sp. have shown that trees from more southern locations display earlier bud burst and shoot growth cessation later in the summer compared to trees from more northern origins (Farmer, 1996 ; Rohde et al., 2011b; Evans et al., 2016). In this study, the temperatures experienced by the parent trees in different regions across Europe likely altered the bud flush of the cuttings in the common garden. Although growing in a common environment, we found a correlation of earlier bud burst with warmer January, July and mean annual temperatures of the parent environment. This indicates that the parental environment may have played an important role in altering the timing of bud burst. Processes responsible for such transgenerational effects are not yet perfectly understood, but the most important processes are likely the nutrient conditions of the parent plant and epigenetic inheritance (Latzel, 2015). In Norway spruce, Carneros et al. (2017) found that an epigenetic memory mechanism affects the timing of bud burst phenology and the expression of bud burst related genes in genetically identical Norway spruce epitypes (an epigenetic alteration in a gene), allowing them to adapt rapidly to a changing environment. The temperature sum experienced by the developing embryo and photoperiod conditions during embryogenesis epigenetically shift the growth cycle of the embryos, giving rise to different epitypes from the same genotype (Yakovlev et al., 2014). Although the latter studies provide evidence for the stable inheritance of epigenetic marks under sexual reproduction, several studies also demonstrate the stable transmission of DNA methylation from parent to clonal offspring in asexually reproducing plant species (Richards et al., 2012b; Verhoeven & Preite, 2014). Significant variability in DNA-methylation patterns as well as significant variation in bud phenology was found in Lombardy poplar, a clone of *Populus nigra* that is worldwide distributed since the 18th century (Vanden Broeck et al., 2018). However, in this study using MSAP we did not find evidence for variation in genome-wide DNA methylation patterns within plants of the same genotype and propagated from parent trees with different environmental histories. It is possible that epigenetic variation was (at least partly) erased (e.g. by epigenetic resetting resulting in reduced polymorphisms in DNA methylation) at the time the leaves were collected for molecular analysis (after almost four years in the common environment and two years after the assessment of bud phenology). In addition, the environmental clone history of the parent trees might have been too short to shape strong differences in genome-wide DNA methylation patterns in response to the environmental history. More powerful molecular techniques, such as bisulphite high-throughput

sequencing techniques (Schield et al., 2016), are needed to further investigate the mechanisms behind the transgenerational effects observed in this study.

The programming to start spring growth is actually set during fall when the plant enters dormancy. In poplar, it is known that photosynthate amounts present in the stem and root in the late autumn can contribute substantially to growth and overwintering carbohydrate storage (Nelson & Isebrands, 1983). Increased growth likely influenced the bud burst time, which was suggested with our observation that taller cuttings (after one year of growth) burst buds earlier in the second growing season. Therefore, there was a possibility that the observed phenological changes were a result of developmental plasticity, which could also be considered as within-generational plasticity.

We did not observe any effect of day length on bud burst. Absent to low photoperiod-sensitivity to bud burst of two poplar species was also reported in Zohner et al. (2016). However, the photoperiod signal was known to have an influence in reducing the temperature sum requirement to bud burst in European beech (Schueler & Liesebach, 2014). If we infer that the genotypes that were exposed to a warmer parental environment might require more accumulated heat for bud burst, then this means that bud burst of these genotypes will be delayed until reaching the sufficient heat sum (Olson et al., 2013). Winter warming can influence the bud burst time via affecting the dormancy and the chilling requirement (Fu et al., 2012). Advancing bud burst with winter warming, therefore suggests that chilling requirement was fulfilled earlier and temperature sum may have played important role in controlling the bud burst time (Richardson et al., 2018).

We unexpectedly observed earlier bud set with warmer mean annual temperatures experienced by the parent trees, but the difference was observed only on one day during the second growing season. In general, the growth cessation in many temperate species was delayed with global warming and an extension of the growing season was observed (Menzel & Fabian, 1999; Richardson et al., 2018). Following the report of Munzbergova and Hadincova (2017), the change in bud phenology detected here might be influenced by origin (effect of tree provenance), parental environment (different geographic regions) and the offspring‘s environment.

Earlier bud set in our study was linked to earlier bud burst by allowing the plants an earlier start of dormancy for the chilling accumulation, which can be a trade-off between avoiding late frost damage and extending growing season. Earlier bud break translated into earlier bud set, which was also reported in *Quercus robur* L. and *Fagus sylvatica* (Fu et al., 2014a). Although, in poplar, a delayed bud set with warming was found by Rohde et al. (2011a). Equatorward transfer of a genotype, in general, shortens the period of active growth due to the reduction of the growing-season photoperiod, thereby advancing autumn growth cessation (Rohde et al., 2011a; Olson et al., 2013). Temperature can also interact with the

photoperiod to alter the photoperiodic signal to growth cessation in poplar (Rohde et al., 2011a; Rohde et al., 2011b). Though, from the study of Richardson et al. (2018), we know that the growth cessation in many temperate species is not constrained by photoperiod. In our study, more likely the interactive effect of photoperiod and temperature had an additional influence on the earlier growth cessation (Rohde et al., 2011a). The phenological changes in our study are probably not adaptive and might change over time. Adaptive plasticity is also dependent on the accuracy of the environmental cues, the degree of environmental heterogeneity and stable epigenetic marks at least within an individual's lifetime (Herman et al., 2014).

The shortening of the growing season in cuttings of which the parent trees experienced warmer mean annual temperatures was mostly due to the earlier bud set. In some species including poplar, photosynthesis and biomass growth can be sustained until leaf senescence (Nelson & Isebrands, 1983), which plays an important role in winter dormancy and spring growth by providing sufficient resources and root growth. It is likely that the earlier bud set resulted in lower growth thereby providing limited resources for winter storage and subsequent spring growth. Higher January temperatures would then fail to promote growth - even though the trees burst buds earlier - with limited biomass storage. Unlike our findings, earlier studies reported an extended growing season despite earlier spring growth and leaf senescence (Fu et al., 2014a). However, climate warming may have a positive effect on the length of the growing season as observed by many observational and climate manipulation experiments (Menzel & Fabian, 1999; Menzel et al., 2006; Morin et al., 2010; Yu et al., 2010; Gunderson et al., 2012; Fu et al., 2014a; Richardson et al., 2018).

6.5 Conclusion

In sum, our results indicate that a latitudinal transfer of poplar clones resulted in different phenological responses to temperature. Together with other factors such as genetic variability, variable temperature sensitivity among species, the environmental condition of parent trees needs to be taken into account to better predict the response of trees to climate change. Nevertheless, the mechanism behind the shift of the timing of bud phenology remains complex and unclear, which provides the opportunity to further investigate the mechanism behind the phenological shift due to heterogenous parental environments and whether such phenological variation is adaptive.

7 General discussion and conclusion

Despite the importance of maternal environmental effects on tree adaptation (Mousseau & Fox, 1998; Hoyle & Ezard, 2012; Brautigam et al., 2013; Penfield & MacGregor, 2017), few studies have assessed the performance and responses of tree seedlings to changing the maternal environment. Environmental conditions during the embryogenesis and reproduction period are known to influence the reproductive success (Lacey et al., 1997; González-Rodríguez et al., 2011), offspring performance (Galloway & Etterson, 2007) and phenology of the offspring (Groot et al., 2017) suggesting potential influence of maternal environment in modifying offspring performance and responses in the face of climate change. Beside sexually reproduced seedlings the responses of vegetative offspring are known to influence through parental condition (Latzel & Klimešová, 2010a; Latzel et al., 2016; Munzbergova & Hadincova, 2017), which might have more relevance in adaptive forestry practice in the face of global warming as many tree species such as poplar, willow etc. reproduce by stem cuttings. Despite the maternal effect on the performance of its offspring, yet, few studies have considered the effect of maternal temperature in estimating the response of trees to global warming.

7.1 Main results

Here, I assessed the response of seedlings of three temperate tree species to the elevated maternal temperature in control and warmer environment using natural temperature difference in space and time and using temperature manipulation experiments. I was able to show that elevated temperature experienced by the mother trees altered the performance and phenological responses of the offspring produced via sexual and vegetative reproduction (Chapter 3 to Chapter 6). In addition, I showed that the effects of the maternal environment depend on the environmental conditions of the offspring generation (Chapter 3, 4). However, using an observational method for oak and beech (by sampling seedlings from the base of isolated mother trees), I was not able to distinguish whether the altered response in the seedlings was due to genotypic variation or maternal environmental effect or in the combination of both. In this last chapter, I summarise the germination and growth performance, and phenological responses of the three temperate forest trees investigated here. In addition, I provide important implications of the results of this thesis in conservation and forest management in the face of climate change; discuss the limitation of the studies and further research opportunities.

Table 7.1 The effect of higher maternal temperature and seedlings environment on the response of seedlings of three temperate tree species. ↓ means the effect is significantly negative, ↑ means the effect is significantly positive, 0 means that we observed no significant effect, n/a means the effect was not tested, *Earlier* means the time of bud phenology (i.e., bud burst, bud set or leaf discolouration) is significantly earlier, and *Later* means the time of bud phenology is later. The column "Chapter" denotes the Chapter and Box number where the effect was tested.

Species	Variable	Interaction (Maternal environment X Seedlings environment)	Effect of elevated maternal temperature	Effect of warming seedlings	Chapter
Oak (*Q. robur*)	Germination	0	0	↓	Box 1(p 45), Chapter 4
	Growth	yes	↓	↓	Chapter 3, Chapter 4
	Bud burst	yes	later	earlier	Chapter 3, Chapter 4
	Leaf discolouration	0	0	0	Chapter 3
Beech (*F. sylvatica*)	Germination	0	0	↑	Box 1 (p 45)
	Growth	0	0	0	Chapter 3
	Bud burst	yes	0	earlier	Chapter 3
	Leaf discolouration	0	0	0	Chapter 3
Black poplar (*P. nigra*)	Germination	n/a	↓	n/a	Chapter 5
	Growth	n/a	↓	n/a	Chapter 5
	Bud burst	n/a	later	n/a	Chapter 5
	Bud set	n/a	earlier	n/a	Chapter 5
Hybrid poplar (*Populus* spp.)	Growth	n/a	↓	n/a	Chapter 6
	Bud burst	n/a	earlier/later	n/a	Chapter 6
	Bud set	n/a	earlier/later	n/a	Chapter 6

7.2 Effects of elevated maternal temperature

7.2.1 Will seed germination be affected if mother trees are exposed to elevated temperature during reproduction?

Increasing evidence shows that maternal environment influences the germination success (Donohue, 2009; Penfield & MacGregor, 2017), which ultimately can influence the seedling recruitment and population dynamics (Huang et al., 2016). Since this life stage is sensitive to temperature, global warming is likely to influence the seed germination. Therefore, understanding the role of maternal temperature in this life stage of trees is essential in the face of climate change. Yet, our understanding of maternal temperature effect on seed germination is limited to a few species. Few climate manipulation experiments considered the maternal environmental condition in estimating the germination performance of tree species.

Here, we showed that elevated maternal temperature, particularly during reproduction has a differential effect on germination success in the three studied species (Table 7.1): negative effect for European black poplar (*Populus nigra*) (Chapter 5) and no effect for oak (*Q. robur*) and beech (*Fagus sylvatica*) (Box 1) suggesting the effect of elevated maternal temperature in germination success differs with species. In our study, we applied extreme maternal temperature (+10°C) treatment for *Populus nigra* that does not represent the realistic warming condition and therefore, it may provide a bias estimation of the response of the offspring to warming. Alike our findings an effect of seed source environment on germination success was observed across two pairs of alpine and low land species (Meineri et al., 2013), where the authors observed the maternal environmental effect on one out of four species. A negative effect of warmer reproductive temperatures on the reproduction success was observed in *Juniperus communis* (Gruwez et al., 2016), where the authors suggested the observed negative effect was likely due to the disrupted growth of the pollen tube which leads to the failure of fertilization and embryo development. In some species such as *Carduus nutans* where germination is restricted by seed dormancy may receive the benefit of maternal warming and display higher germination rate as seeds from warmed maternal plants are less dormant than seeds from ambient environment (Zhang et al., 2012). Similar results are known from the study of Donohue et al. (2007) and reviewed by Donohue (2009), where the authors showed that in two genotypes of *Arabidopsis thaliana*, low seed maturation temperature induces greater seed dormancy resulting lower germination (see Figure 7.1). In genotype Col, the dormancy was broken in half of the seeds by a short cold treatment, and in another genotype (Ler), this cold-induced dormancy was broken only after a cycle of warm followed by cold treatment (Figure 7.1). In *Arabidopsis thaliana*, the temperature dependent seed germination was known to control by the

Flowering locus T (FT), the same pathway that controls flowering time (Chen et al., 2014b). Here, the authors found that mother plant plays a central role in controlling progeny seed germination via activating FT and integrating long-term temperature memories in fruit tissues (Chen et al., 2014b). Maternal genotypes and the maternal environment was known to affect the germination percentage and the timing of germination in a conifer (*Pinus pinaster*) (Cendán et al., 2013) and herb species (Galloway et al., 2009). Another example of maternal provisioning in controlling reproductive success was reported by Redmond et al. (2012). By comparing field observation data in 1974 with the data collected in 2008, the authors showed that seed cone production among pinyon pine (*Pinus edulis*), a masting species, declined by 40% from 1974 to 2008 throughout New Mexico and northwestern Oklahoma, which was highly correlated to increase late summer temperature during the time of cone initiation.

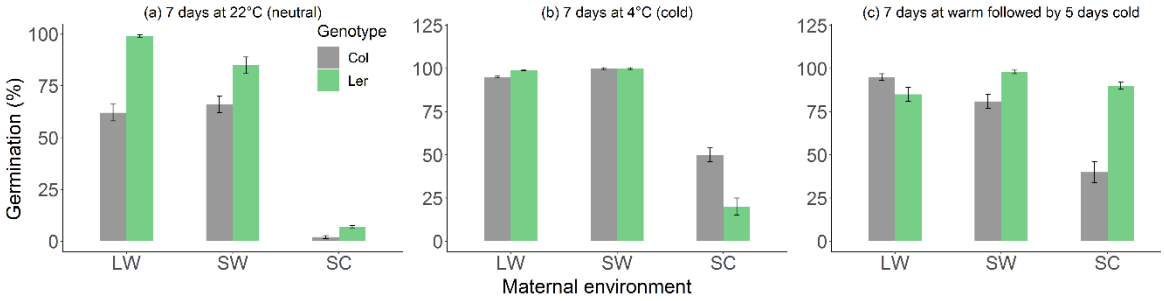

Figure 7.1 Graph showing a combined effect of temperature and day lengths in parental environment during seed maturation on germination in two genotypes of *A. thaliana* (Ler- Landsberg *erecta* and Col- Columbia ecotype). LW means long day length in warming (at 22°C), SW means short day length in warming (at 22°C) and SC means short day length in cool temperature (at 10 °C). Adapted from Donohue (2009).

In addition, local environmental variation especially the temperature variation affects seed germination (Baskin & Baskin, 2001). We observed a different effect of warmer growing environment on seed germination across species (Chapter 4, Box 1 and Table 7.1). In oak, warmer growing environment displayed negative effect on seed germination (Chapter 4 and Box 1) while in beech, it displayed a positive effect on seed germination (Box 1). These different results suggest that the effect of global warming will be dependent on species (Classen et al., 2010) and across the species distribution range (De Frenne et al., 2011). However, overall, global warming is likely to reduce the seed germination of many tree species (Chapter 3,4, Carón et al. (2015), Perez-Ruiz et al. (2018)) affecting the seedlings recruitment and succession of forest, and will likely change the composition of forest (Peñuelas et al., 2007).

7.2.2 Do elevated maternal temperatures influence the timing of bud burst and growth cessation of the tree seedlings?

Using controlled crossings between three pairs of genotypes at ambient and +10°C temperatures (Chapter 5), we were able to study the effects of elevated maternal temperature on the bud burst and bud set time of the *Populus nigra* seedlings which were generated from the seeds that were exposed to two different temperatures during the crossing and seed maturation. As a result, the elevated temperature during the crossing and seed maturation delayed the timing to bud burst and advanced bud set (see Table 7.1) in one genotype, but no effect was observed in other two genotypes (Chapter 5). These results suggest that the maternal effect is genotype specific which also corroborates the findings of Galloway (2001); Latzel et al. (2014). On the other hand, Galloway (2001) showed that the response of different families (group experienced the same environment) of *Campanula americana* differed to maternal nutrient, and paternal light environments and Latzel et al. (2014) showed that maternal genotypes differed in their flowering phenology in a perennial plant (*Plantago lanceolata*). In addition, in oak and beech, we observed that the maternal effect depends on the environmental condition of the offspring similar to the findings of Groot et al. (2016). On the other hand, Munzbergova and Hadincova (2017) showed that the maternal climate interacted more intensively with the climate of origin (of source plant) than with the offspring climate. We observed an interaction between maternal temperature and warming of the seedlings of oak and beech (Chapter 3), showing the effect of elevated maternal temperature on seedlings bud burst time, in fact, dependent on the offspring environment (Chapter 3). Using spatiotemporal maternal temperatures and warming treatment on the seedlings collected from the base of isolated mother trees, in this chapter, we showed that the bud burst time of oak seedlings delayed in control condition but this time advanced in warmer condition when the mother trees experienced elevated reproductive temperature. This result mainly provides us with important information that the response of certain genotypes, which are already adapted to a warmer environment will respond differently to global warming than those adapted in a colder environment. However, the different response in beech seedlings regarding the bud burst time to elevated reproductive temperature suggests that the maternal effect on the response of the offspring differs with species. Indeed, warming treatment applied on seedlings advanced the bud burst time in both oak and beech seedlings (Chapter 3 and Chapter 4), which supports the general trend of advancing onset of spring growth with warming (Sanz-Perez et al., 2009; Richardson et al., 2018).

Bud dormancy is a vital adaptation strategy of many temperate species to seasonal change (Lang et al., 1987) and thus control the annual growth and plant production. The regulation of bud burst and bud set integrates with eco- and endodormancy signals such as hormone

levels, day length, and temperature (Lang et al., 1987; Tanino, 2004). Temperature sensitivity in plants known to control by H2A.Z hormone (Kumar & Wigge, 2010). In sessile oak (*Quercus petraea*), the differentially expressed genes and the gene expression are known to control the bud dormancy (Uneo et al., 2013). Through the evolution of molecular studies, we have learned that the regulation of bud burst and bud set correlates with the change in gene activity and the expression of genes and epigenetic modification (Santamaria et al., 2009; Herman & Sultan, 2011). The mother plant has an important role in the regulation of offspring responses by controlling the activity of related genes and gene expression (Chen et al., 2014b; Auge et al., 2017). There is evidence showing that elevated temperature during embryogenesis resulted in different epitypes (an epigenetic alteration in a gene) from the same genotype in Norway spruce (Yakovlev et al., 2014). The epitype of Norway spruce generated at warmer environment delayed bud burst time by two weeks (Figure 7.2) and that the effect in altering bud burst time was mediated by epigenetic memory and bud burst related gene expression (Carneros et al., 2017). Further evidence of maternal effects was found in *Arabidopsis thaliana* where the authors showed that the exposure of parental and grandparental generations to elevated temperature altered the flowering time of the offspring (Groot et al., 2017). The environment mediated phenotypic variation through epigenetic modification was observed in natural population of white mangrove (*Laguncularia racemose*) (Lira-Medeiros et al., 2010), poplar (*Populus nigra*) (Vanden Broeck et al., 2018), and oak (*Quercus lobate*) (Platt et al., 2015) suggesting the contribution of epigenetic variation to the adaptive potential of tree species.

Figure 7.2 Figures showing the embryogenesis(a), plantation (b) with the epitypes and spring bud burst (c) in two epitypes generated from embryogenesis in warm (WE) and in cold environments(CE). Adapted from Carneros et al. (2017).

In both oak and beech seedlings, we did not observe maternal temperature effect on leaf discolouration of seedlings (Table 7.1, Chapter 3). Other biotic and abiotic factors such as pest infestation (e.g., powdery mildew in oak) (Marçais & Desprez-Loustau, 2012), and water availability (Robertson, 1992; Archetti et al., 2013) are likely related to leaf discolouration or growth cessation in autumn.

In our studies (Chapter 3- Chapter 5), the observed correlation between phenological change and maternal environment supports the potential influence of maternal environment determining the offspring bud phenology to environmental change suggesting the necessity to include maternal environment while estimating the response of tree species to climate change.

7.2.3 Does elevated maternal temperature influence the growth of the seedlings?

Following the change in the time of seedling's bud phenology with the elevated maternal temperature, one would expect that the growth of the seedlings would be affected as well. We observed that elevated reproductive temperatures reduced the diameter growth of the oak seedlings (*Quercus robur*) (Chapter 3) and reduced the height of black poplar seedlings (*Populus nigra*) (Chapter 5), but no effect on the growth of beech seedlings was observed (Chapter 3). We also observed an interaction between maternal environment and warming of the seedlings in the growth of oak seedlings (Chapter 3), which indicates the seedlings growth depends on both maternal and seedlings growing environment. These results lead us to believe that, indeed, maternal temperatures have the potential to influence the growth of the seedlings to some extent and that this effect varies among species. Johnsen et al. (1995) compared Norway spruce progeny created from exactly the same genotypes under the warmer conditions of a greenhouse to those from a nearby outdoor seed orchard and found no difference in height between plants from the greenhouse and the outdoor seed orchard. Nonetheless, in three *Larix* spp. a significant growth difference was observed between inside and outside a greenhouse environment where the temperature inside averaged 4°C above the outside temperature (Greenwood & Hutchison, 1996). Whereas in the hybrid of *Picea glauca* × *Picea engelmannii*, it has been reported that there is no effect of elevated reproductive temperatures on the plant height (Webber et al., 2005). The expression of transgenerational effects may not be confined to the seedling stage and differ in the adult stage following year to year variation of maternal effects in a perennial weed (*Plantago lanceolata*) (Latzel & Klimešová, 2010b). Although, Galloway et al. (2009) showed that maternal environment influenced the seed trait while maternal genetic effect influenced the offspring size trait within a life cycle of *Campanulastrum americanum*. A genetic pathway is involved in the regulation of growth cessation and thus controls the annual growth of tree species (Ke et al., 2017; Ding et al., 2018). For example, in *Populus*, phytochromes (phys) are early-acting components in the photoperiodic pathway controlling short day (SD)-induced growth cessation and GIGANTEA-like genes are known to control this pathway and control the growth of many tree species including *Populus* (Ding et al., 2018). The observed effect of maternal temperature on the growth of the seedlings could be influenced by the maternal genotypes, which we were not able to differentiate in our

study in open pollinated offspring of oak and beech. Besides, tree growth can be influenced by many other biotic and abiotic factors. Increasing temperature above optimum can alter the metabolic rate and energy expenditure, which probably reduce the growth of the plant (Atkinson & Sibly, 1997). In addition, soil water availability along with warming is known to limit the growth of tree species (Martinez-Sancho et al., 2017). Fungal diseases (Bert et al., 2016) and insect damages (Skuhravy et al., 1998) can have the subsequent possible damaging effect on tree growth. Beside maternal temperature, we also observed different growth responses of oak and beech seedlings (Table 7.1 and Chapter 3, 4) to warming, which indicates global warming will reduce the growth of some but not all species.

7.2.4 Does maternal environmental effect (cross-generational effect) on bud phenology prevail in vegetative offspring (by stem cuttings)?

In addition to sexually reproduced offspring, we studied the maternal environmental effects on bud phenology and growth of vegetative offspring (produced by stem cuttings) of *Populus* spp. (Chapter 6 and Table 7.1) and assessed the global DNA methylation in the vegetative cuttings as a potential epigenetic variation induced by the environment. It is suggested that through avoiding meiosis and its associated epigenetic resetting, vegetative reproduced offspring can inherit epigenetic information of previous environmental interactions from the maternal ramet (Latzel et al., 2016). We observed that the temperature condition along with the photoperiod experienced by the mother plants at different geographical sites influenced the bud phenology and growth of vegetative offspring (cuttings), which indicate a transgenerational effect on the bud phenology of vegetative offspring. Similar to our findings, environmental history is known to influence the response of vegetative offspring in other species including *Populus*. For example, drought legacies can affect the responses of vegetative cuttings of two economically important hybrid genotypes of *Populus* spp. (Raj et al., 2011). The environmentally induced epigenetic modification was observed in the natural population of another species (*Laguncularia racemosa*) (Lira-Medeiros et al., 2010). In Lombardy poplar, a clone of *Populus nigra* that is worldwide distributed since the 18th century, Vanden Broeck et al. (2018) observed variation in DNA methylation pattern and bud set time in vegetative cuttings, where the authors showed significantly delayed bud set in vegetative offspring with increased mean temperature of January of parental environment (Figure 7.3). Although in our study, we did not observe a difference in global DNA methylation in the plants of same genotypes originated from mother plants with different environmental history, the mechanism behind transgenerational effects is known to being epigenetic variation mediated by DNA methylation as observed in the above studies. The most pronounced differences in transcript abundance patterns in response to drought condition was observed to be correlated to the longest time of establishment of genotype (Raj et al., 2011). It was possible

that in our study, the environmental clone history of the mother trees might have been too short to shape strong differences in genome-wide DNA methylation patterns in response to environmental history. In addition, another possibility of epigenetic resetting was likely growing in a common garden for nearly four growing seasons before we performed the MSAP analysis. Powerful methods such as bisulphite high-throughput sequencing techniques are necessary to detect the mechanism behind the transgenerational effect on the bud phenology of vegetative cuttings.

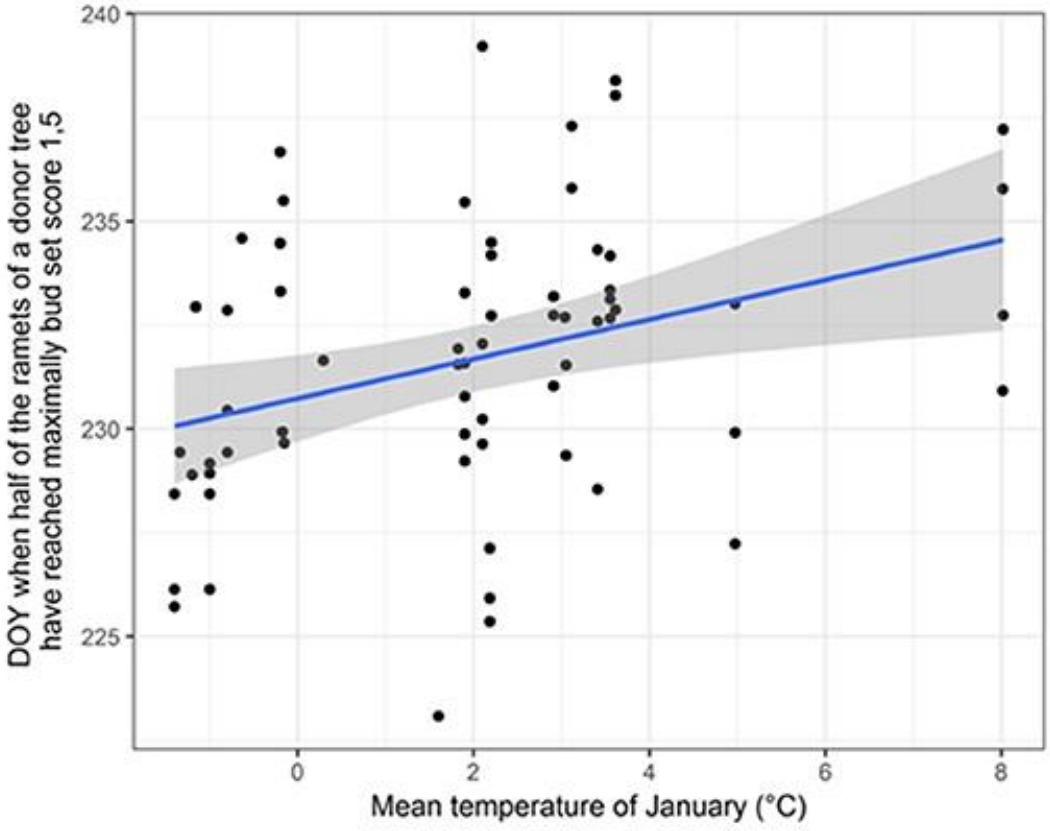

Figure 7.3 Graph showing the correlation between mean January temperature in the parental environment and days of the year (DOY) when half of the ramets reach bud set score of 1.5. Reprinted from Vanden Broeck et al. (2018).

Based on theories of phenotypic plasticity within and across generations (Figure 1.7), the cross-generational plasticity can increase when there is less environmental variability across generations. The offspring generation may integrate different environmental cues experienced by the offspring, cues passed by the maternal and grandmaternal effects as well as genetic cues (effects of alleles at one or more polymorphic loci) by giving different weights to different cues in determining the phenotype (Leimar & McNamara, 2015). Therefore, maternal effects may depend on the stability of environmental condition across generations (Walsh et al., 2016), where the authors reported that temporal variation in predator-induced mortality of *Daphnia ambigua* selects for within-generation plasticity while consistently strong (or weak) mortality selects for increased transgenerational plasticity. In Chapter 3, the observed interdependency of maternal and offspring temperature condition

on the offspring's bud burst time was probably the integration of maternal and offspring cues. Similarly, we observed the interdependent effect of common garden and provenance on bud burst time suggest the possible integration of different environmental cues. In animal studies, the most extensively studied example of the integration of maternal and offspring cues is the induction of defence against predation (Mikulski & Pijanowska, 2010; Walsh et al., 2016). In plants, a stronger phenotypic response to a combination of maternal and juvenile offspring cues was found in the shade avoidance phenotype of *Campanulastrum americanum* where maternal light influenced the expression of most adult traits but had the strongest effect when plants were germinated in natural environments. (Galloway & Etterson, 2009). In our study, we did not compare the phenology between maternal and offspring generation which could provide understanding the extent of contribution of maternal and offspring cues in determining the phenology of the offspring in our study species.

In this thesis, we studied the effect of elevated maternal temperature on germination, bud phenology and growth of one late successional species (Beech, *Fagus sylvatica*) (Chapter 3, Box 1), one mid-successional species (European oak, *Quercus robur*) (Chapter 3, 4, Box 1) and several early successional species such as European black poplar (*Populus nigra*) (Chapter 5) and hybrid poplar (*Populus trichocarpa* × *P*. *deltoides*, *P*. *trichocarpa* × *P*. *trichocarpa*) (Chapter 6). We observed different bud phenology in response to elevated maternal temperature across studied species (Table 7.1) where elevated reproductive temperature reduced growth and changed bud phenology across different successional species. In general, the timing of bud phenology varies due to the species-specific requirement for the breaking of bud dormancy based on the environmental signal (Lang et al., 1987). Elevated temperature was known to have greater influence in altering the bud phenology of many temperate tree species than photoperiod (Richardson et al., 2018), although photoperiod is known to control the growth cessation in *Populus* (Pauley & Perry, 1954) and bud burst of late successional species (Korner & Basler, 2010). Since early successional species as *Populus* spp. was not constrained by photoperiod to bud burst, early successional species are likely to receive the benefit of warming by an earlier onset of growth. Temperature can interact with photoperiod in changing the bud phenology in both late and early successional species (Kalcsits et al., 2009; Sanz-Perez et al., 2009; Rohde et al., 2011b; Basler & Korner, 2014). In our study, oak, a mid-successional species, was found to be more sensitive to temperature than beech (Chapter 3). Along with temperature, longer day lengths seems to accelerate bud burst time of oak seedlings (Chapter 4). The more plastic behaviour of oak regarding the timing of bud burst and less sensitive to drought may facilitate oak to adapt than that of less plastic and more drought sensitive beech to projected climate change (IPCC, 2018; Vanhellemont et al., 2019). Changing the time of

bud phenology is correlated with the successful establishment and succession of tree species (Cardoso et al., 2018). Given the light preference of oak seedlings (Leuschner & Meier, 2018), early bud burst in response to warming may provide oak seedlings at the forest floor a better chance for the successful establishment by eliminating possible competition for light by capturing a significant amount of solar radiation before the canopy trees produce leaves. Conversely, even though beech has a high tolerance of shade, it can receive the further advantage of earlier bud burst by capturing more light. However, earlier bud burst in response to warming (Richardson et al. (2018), Table 7.1), the most observed trend in bud phenology of many temperate species, will increase the chance of late frost damage (Gömöry & Paule, 2011).

7.3 Implications for forest management

Here, I provide some possible implications of the results of this thesis for forest management.

Reproduction success through successful germination and establishment of seedlings confirms the continuous succession of forest trees (regeneration), while germination can be affected by as little as 1°C temperature rise (Springthorpe & Penfield, 2015). The observed reduced germination percentage in black poplar (*P. nigra*) and oak (*Q. robur*) in response to higher maternal temperature and warming (Chapter 4-6) suggests that warming might reduce seed germination percentage of these tree species. These results could be implemented by extending the available knowledge regarding higher maternal temperature effect on reduced germination in breeding and afforestation programme. For example, during the selection of seed source and planting materials for reforestation programme and nurseries in the face of climate change the maternal temperature condition needs to be considered for more adaptive forest in the face of climate change.

Further, maternal effects depend on the stability of environmental condition across generations (Figure 1.7 and Leimar and McNamara (2015); Chapter 3, Chapter 5) and phenological response in offspring generation can be a result of the integration of different environmental cues (acquired by the offspring itself and passed cue by maternal or grandmaternal effect). Therefore, depending on the environmental condition within and across generations, it may be possible that we will not observe a direct maternal effect. In forest management, we can apply the knowledge of maternal effects in selecting the traits (such as growth) according to the match or mismatch of environmental condition in maternal and its subsequent generation as we can expect better fitness in offspring when maternal environment correlates to offspring environment (Chapter 4 and Galloway and Etterson (2009)).

Forest trees offer excellent opportunities to relate epigenetic variation and phenotypic variation in natural populations, and the role of epigenetic variation in evolutionary processes, particularly in the context of a rapid shift of climate (Brautigam et al., 2013). The results of this study concerning the parental environmental effects have implications in understanding the evolutionary plant adaptation to climate change and induce further studies to understand the role of transgenerational effects on tree adaptation to climate change. Given the phenological changes in the seedlings and vegetative cuttings in response to elevated parental temperature (Chapter 3-6), we are confident that maternal temperature can be a potential predictor in estimating the phenology of the tree seedlings to warming. The interaction between parental environment and the seedlings' environment

suggests that the genotypes that are already adapted to a warmer environment can display a different response to warming than those distributed in the relatively colder environment. Therefore, we need to consider different responses among species and genotypes to warming across their distribution ranges while estimating responses of tree seedlings to warming. The contradictory responses exhibited by beech and oak emphasize that the maternal effects can lead to different offspring's behaviour, which also needs to take into consideration when estimating the future response of tree species to climate change and during the selection of adaptive traits in breeding programmes in the face of climate change.

Since, the phenology of insects (leaf herbivores) synchronize with the bud burst time of many dominant to co-dominant tree species (Ivashov et al., 2002), forest managers can utilize the knowledge of bud burst time to apply precautionary measures to control the insect outbreak in a forest ecosystem. For example, forest managers can introduce genotypes with considerable phenological variation within a *Q. robur* stand to limit the colonisation of neighbouring trees by dispersing larvae of spring-active insects (Tikkanen & Julkunen-Tiitto, 2003).

Black poplar (*Populus nigra*), as well as European oak (*Quercus robur*), seem to be sensitive to parental temperatures in terms of their germination and bud phenology. The sensitivity to parental temperature was different across species and genotypes, which suggests that during the selection of provenances, preparation of planting materials for regeneration and breeding programmes, we need to consider the temperature sensitivity of the species and genotypes (Sixto et al., 2016).

In general, bud phenology controls the annual growth of many temperate tree species. Given the reduced growth of the tree seedlings in response to elevated maternal temperature (Table 7.1), we believe that the growth of tree species will reduce with global warming and drought condition (Maes et al., 2018). The influence of parental environment in tree growth potentially improves our understanding of the importance of parental environmental effect on ecosystem carbon and nutrient cycles (Niinemets & Tamm, 2005). Since the growth and successful recruitment of the seedlings can influence the population dynamics (Huang et al., 2016) and composition of forest (Leak & Graber, 1976), the estimated growth response of seedlings in response to warming can further our understanding regarding the possible impact of tree growth on the population dynamics and forest composition in a warmer world.

7.4 Perspectives for future research

Here, I provide some further research opportunities based on the methods and results of this thesis to extend our understanding regarding the role of maternal environment on the responses of forest tree species in the face of climate change.

The greatest challenge to study the maternal environmental effect on the seedlings of forest tree species is the long generation times. The age at first reproduction of temperate trees varies from 8 to 40 years (Braatne et al., 1996; Kaliniewicz & Tylek, 2018) while most of the research projects run for a short period (varies between 4-6 years). In addition, to be able to disentangle the environmental effect from other potential factors such as genetic variation (Verhoeven et al., 2016), which can alter the responses (phenological and growth performance) of seedlings, someone needs to study a single genotype across different environmental gradients. To study the role of epigenetic modification concerning phenological change through environmental stimuli (maternal environment), we require both molecular techniques and experimental methods to differentiate between phenological variation due to genetic control and due to epigenetic modification. In this thesis, for open pollinated oak and beech seedlings, we were not able to distinguish the genetic variation from the maternal temperature variation in modifying phenological responses and growth performance. However, this method by using spatiotemporal temperature variation in the maternal environment would be a potential method combining with molecular analysis to extend our understanding the effect of maternal temperature on the response of offspring of long-living trees. Using controlled crosses between dioecious individuals of *Populus nigra*, we were able to differentiate genetic effect from the maternal environmental effect. Although, to assess maternal environmental effect on the net performance of offspring one should manipulate both maternal and offspring environment across a broad range of ecologically relevant environments (Bonduriansky et al., 2018). Therefore, further studies are necessary to study the magnitude of maternal environmental effects across different temperature gradients by manipulating both maternal and offspring environments, which can be done using the different experimental approach such as controlled crosses, and common garden approach along with advanced molecular techniques.

In this thesis, using seeds from a mature common garden, we were able to study the effect of the environment at origin on the seedlings in response to different environments, but we were not able to determine the effect of parental environment. The results of common gardens concerning local adaptation may have the relevance to environmentally induced transgenerational effect (Latzel, 2015). Existing mature common gardens of a single genotype across different environmental gradients provide the opportunity to study the effect of parental environment on the seedlings responses (e.g., phenological and growth

performance). Additionally, the role of epigenetic modification in relation to such responses can be explored by examining whole genomes and whole epigenomes to thoroughly explore the genetic basis for DNA methylation and its environmental plasticity (Verhoeven et al., 2016).

In addition, we need to extend studies of maternal effects by including more tree species given the environmental requirement for optimum growth and performance varies with species (Baskin & Baskin, 2001; Leuschner & Meier, 2018). The relevant epigenetic modification in relation to different responses across a range of different species thus will extend our understanding concerning the role of epigenetic modification and will help us to estimate the responses of long-lived tree species to environmental changes.

To minimise the genetic control over the epigenetic modification the clonal species or completely inbred species were studied (Herrera et al., 2016; Munzbergova & Hadincova, 2017), which can provide the indication that the resulted epigenetic variation induced by the environment but not from the genetic differences (Verhoeven et al., 2016). In our study, using vegetative cuttings of single genotypes of the different parental environment at different geographical sites, the observed results indicated an environmentally induced transgenerational effect. However, here, we lack the sufficient number of samples including the well-balanced experimental design, whereas the time of the establishment of parental cuttings in different geographic sites were different and thus exposed to different environmental condition for different periods, which can influence the related epigenetic variation based on establishment period (Raj et al., 2011). Thus, further studies are recommended by applying a combination of extensive experimental designs and advanced molecular techniques to explore further the inherent mechanism related to phenological variation.

In this thesis, I focused on increasing parental temperature in combination with offspring temperature. However, climate change does not only consist of temperature changes but is a combination of changes in precipitation changes and drought, enhanced atmospheric CO_2 concentrations, atmospheric N deposition, together with temperature (IPCC, 2018). Such interactive effects remain for future exploration. Besides, IR heating lamp, natural temperature gradients across space and time as we used in this thesis, other available techniques such as open-top chambers, passive night-time warming method using automatic scaffolding cover during the night can be used to simulate the climate change. Therefore, further studies including more than one climatic driver can help to increase our understanding of the responses of the forest trees to climate change.

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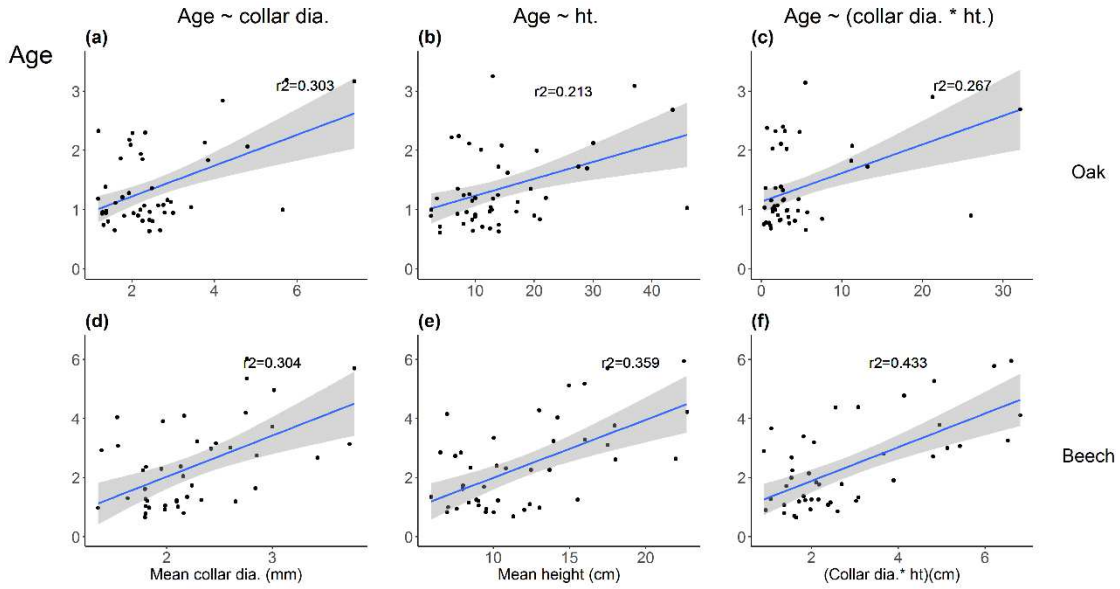
Appendix A Phenology and growth of *Fagus sylvatica* and *Quercus robur* seedlings in response to temperature variation in the parental vs. offspring generation

Appendix Table A.1 The R2-values of four allometric equation models applied to the oak and beech seedlings. The bold values represent the highest R2 of the models including both collar diameter and height of the seedlings.

No.	Oak (n = 48)		Beech (n = 43)	
	Equation	R^2	Equation	R^2
1	Age = 0.7027+0.2602 . collar diameter	0.303	Age = -0.7566 +1.3934. collar diameter	0.304
2	Age =0.948474+ 0.028587 . height	0.213	Age =0.03765+ 0.19579. height	0.359
3	Age = 1.13457+ 0.04831. (collar diameter*height)[1]	0.267	Age = 0.74863+ 0.05701. (collar diameter*height)[1]	0.433

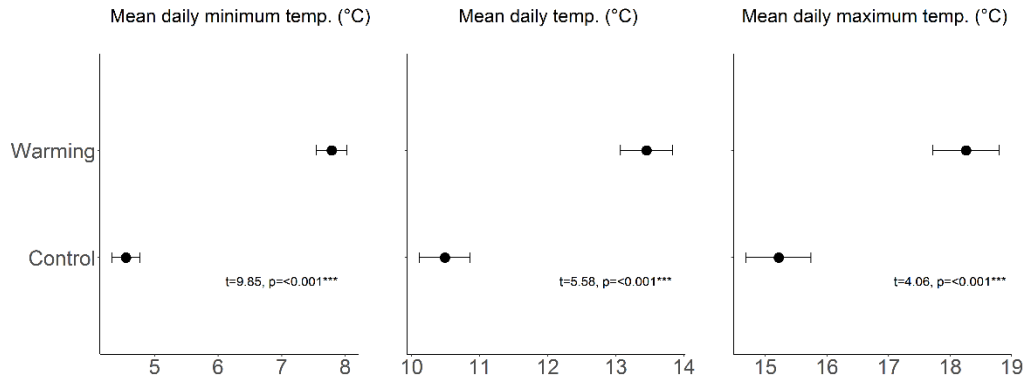
Note: [1] Collar diameter and height of each seedlings were multiplied before performing the regression analysis.



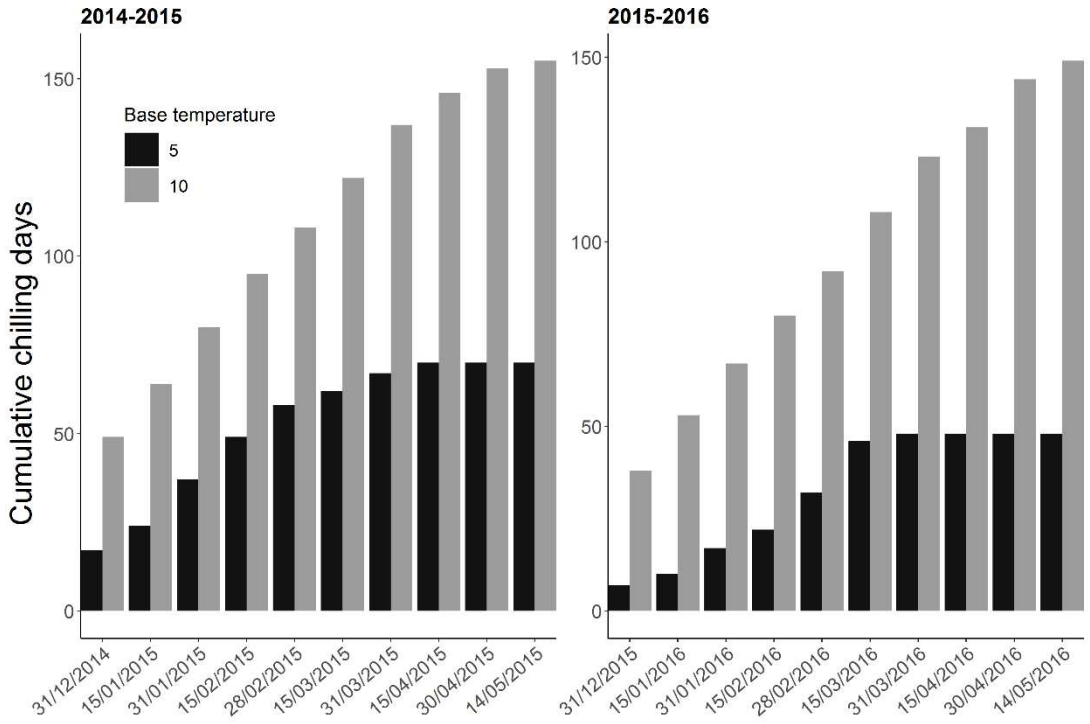
Appendix Figure A.1 The relationship between age and growth parameters of the oak and beech seedlings. In (c) and (f), we multiplied collar diameter and height of each seedling before using in the model.

Appendix table A.2 Average daily minimum, mean and maximum temperature during reproductive period (April-September) at five different sites and seed maturation years.

Site	Species	Seed maturation year	Average daily minimum temperature	Average daily mean temperature	Average daily maximum temperature
Aelmoeseneiebos	Beech	2007	10.67	15.56	20.46
Aelmoeseneiebos	Beech	2008	10.30	15.12	19.95
Aelmoeseneiebos	Beech	2010	9.63	14.83	20.02
Aelmoeseneiebos	Beech	2011	10.61	15.79	20.98
Aelmoeseneiebos	Beech	2012	10.26	15.00	19.74
Aelmoeseneiebos	Beech	2013	9.97	14.86	19.75
Aelmoeseneiebos	Oak	2012	10.26	15.00	19.74
Aelmoeseneiebos	Oak	2013	9.97	14.86	19.75
Brakelbos	Beech	2008	10.29	15.02	19.75
Brakelbos	Beech	2009	10.44	15.83	21.23
Brakelbos	Beech	2010	9.52	14.77	20.01
Brakelbos	Beech	2011	10.37	15.68	20.98
Brakelbos	Beech	2012	9.95	14.76	19.56
Brakelbos	Beech	2013	9.79	14.69	19.60
Brakelbos	Oak	2011	10.37	15.68	20.98
Brakelbos	Oak	2012	9.95	14.76	19.56
Brakelbos	Oak	2013	9.79	14.69	19.60
Klosterbos	Beech	2012	10.00	14.84	19.68
Klosterbos	Beech	2013	9.81	14.76	19.70
Klosterbos	Oak	2012	10.00	14.84	19.68
Klosterbos	Oak	2013	9.81	14.76	19.70
Oostkamp orchard	Oak	2011	10.36	15.43	20.50
Oostkamp orchard	Oak	2012	9.99	14.64	19.29
Oostkamp orchard	Oak	2013	9.63	14.42	19.21
Raspailiebos	Beech	2011	10.18	15.69	21.20
Raspailiebos	Beech	2012	9.82	14.87	19.93
Raspailiebos	Beech	2013	9.71	14.85	19.98
Raspailiebos	Oak	2012	9.82	14.87	19.93
Raspailiebos	Oak	2013	9.71	14.85	19.98

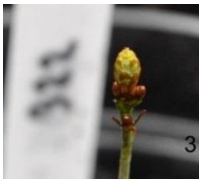


Appendix Figure A.2 Soil surface temperatures in our experiment. Daily minimum, mean and maximal temperatures, in the warming and control pots. Values are t values and p values from linear mixed effect models where random effect was measurement points. Error bars indicate standard errors. Significance are denoted by *** p<0.001.



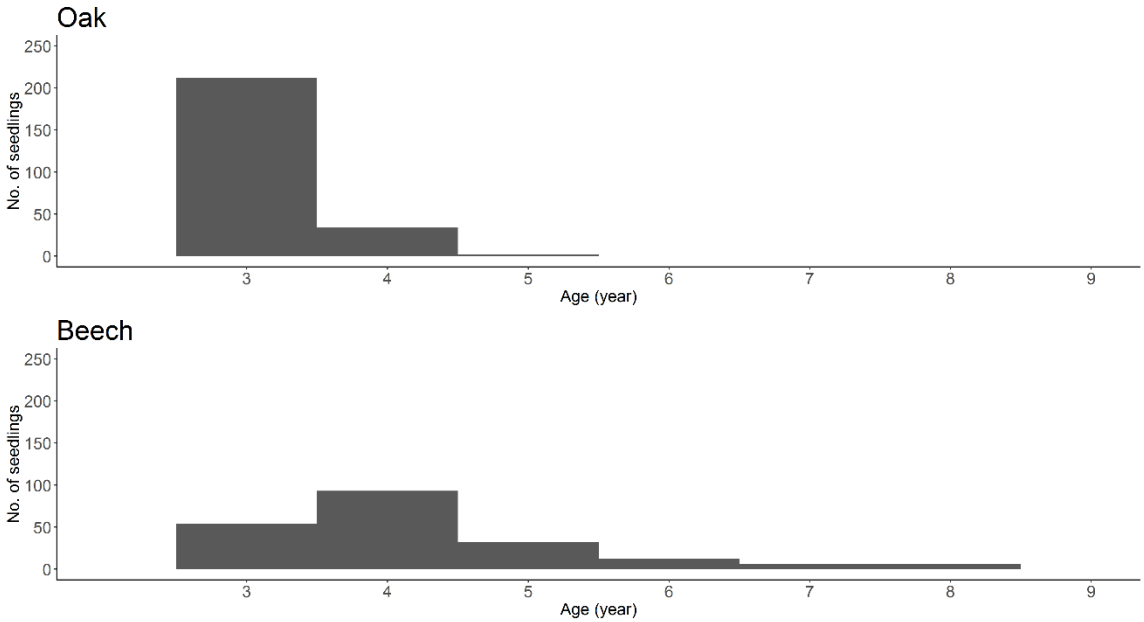
Appendix Figure A.3 Cumulative chilling days calculated between October 1 to 14 May using two base temperatures: 5 and 10°C.

Appendix Table A.3 Description of the scoring systems of bud burst in the seedlings of oak and beech based on visual observation adapted after (Wesołowski & Rowiński, 2006; Schüler et al., 2012).

Score	Oak (1-5 stages)	Oak (stages)	Beech (1-6 stages)	Beech (stages)
1	Dormant buds		Dormant buds	
2	Bud-swollen		Buds swollen and elongated	
3	Buds expanding and green		Buds scales broken	
4	Bud-burst		Leaves emerging	
5	At least one leaf unfolded		Leaves spread out	
6			Leaves unfolded, smooth and bright	

Appendix Table A.4 The number of individuals for each species and responses used in the analysis

Species	Response	Number of individuals
Oak	Bud burst 2015	274
	Bud burst 2016	248
	Leaf discolouration (80%) 2015	231
	Relative collar diameter increment	238
	Relative height increment	238
Beech	Bud burst 2015	220
	Bud burst 2016	205
	Leaf discolouration (80%) 2015	140
	Relative collar diameter increment	195
	Relative height increment	195



Appendix Figure A.4 The distribution of age class in oak and beech seedlings.

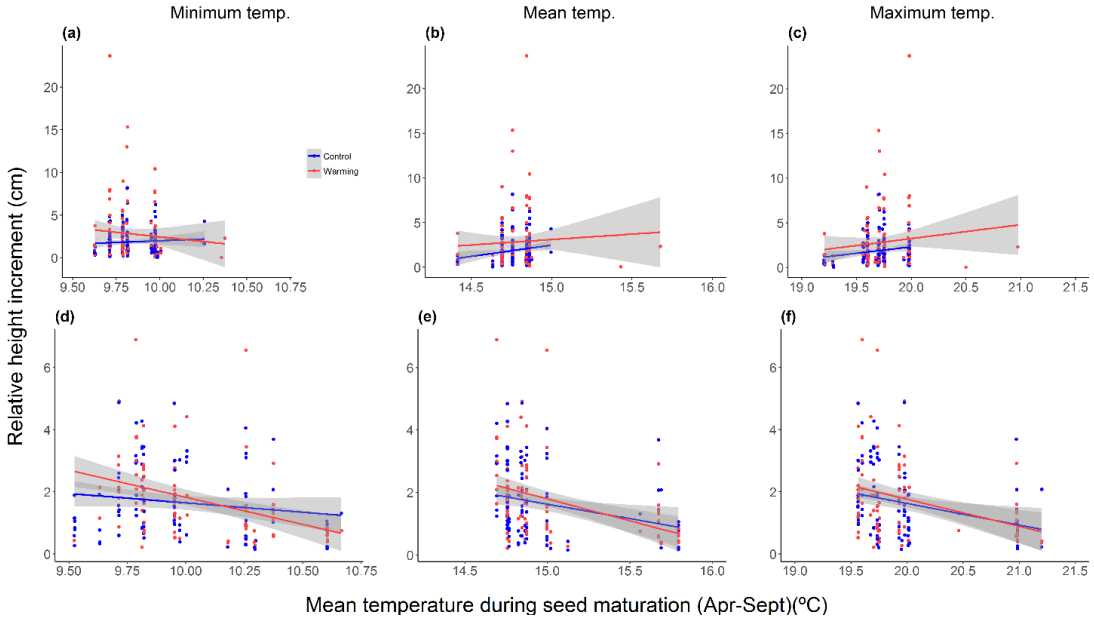
Appendix Table A.5 The effect of warming treatment and temperature during reproduction period on the relative height increment of oak and beech seedlings where TOffspring means warming treatment in offspring generation and TParent means temperature during reproduction period in parental generation and Leaf_discolour80 means the timing when 80% of the all the leaves of each seedlings turned yellow.

Fixed effects																		
Species	Response	Effect	Parental reproductive temperature (°C)															
			Minimum						Mean						Maximum					
			Estimate ± Std. Error	df	t	p value	r^2_{mar}	r^2_{con}	Estimate ± Std. Error	df	t	p value	r^2_{mar}	r^2_{con}	Estimate± Std. Error	df	t	p value	r^2_{mar}	r^2_{con}
Oak	Relative height Increment	$T_{Offspring}$	26.3± 5.4	188.7	1.0	0.30	0.03	0.14	4.0± 38.0	198.2	0.1	0.92	0.03	0.14	-14.2± 29.5	226.8	-0.5	0.63	0.04	0.14
		T_{Parent}	0.8± 2.2	16.8	0.3	0.74			1.0± 2.3	51.6	0.4	0.66				42.0	0.2	0.81		
		$T_{Offspring}$: T_{Parent}	-2.6± 2.6	188.2	-1.0	0.32			-0.2± 2.6	198.0	-0.1	0.94			0.8± 1.5	226.8	0.5	0.61		
Beech		$T_{Offspring}$	7.1± 5.2	179.0	1.4	0.18	0.01	0.33	7.5± 6.7	178.5	1.1	0.26	0.04	0.34	5.1± 6.4	178.5	0.8	0.43	0.05	0.34
		T_{Parent}	0.03± 0.6	12.7	0.1	0.96			-0.5± 0.7	7.9	-0.7	0.52			-0.4± 0.5	6.4	-0.9	0.40		
		$T_{Offspring}$: T_{Parent}	-0.7± 0.5	178.8	-1.3	0.19			-0.5± 0.5	178.4	-1.1	0.27			-0.3± 0.3	178.5	-0.8	0.43		
Random effects	Species	Effect	Variance															
			Minimum (°C)						Mean (°C)						Maximum (°C)					
Relative height increment	Oak	Mother tree : Site	0.55						0.47						0.47					
		Site	0.00						0.00						0.00					
		Seed maturation year	0.18						0.30						0.23					
	Beech	Mother tree: Site	0.19						0.17						0.19					
		Site	0.01						0.00						0.01					
		Seed maturation year	0.32						0.30						0.26					

Appendix Table A.6 The variance terms from linear mixed effects models on the phenology and growth of oak and beech seedlings as a function of reproductive temperatures in parental generation and warming condition in offspring generation.

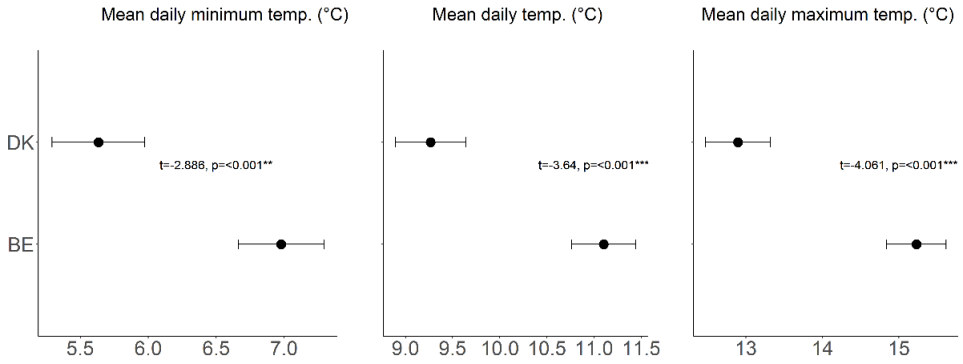
Random effects					
Species	Response	Effect	Variance		
			Minimum (°C)	Mean (°C)	Maximum (°C)
Oak	Bud burst15	Mother tree : Site	3.09	3.21	3.14
		Site	44.16	61.68	47.91
		Seed maturation year	0.59	0.00	1.51
	Bud burst16	Mother tree: Site	0.39	0.39	0.24
		Site	14.32	12.33	15.17
		Seed maturation year	0.00	0.00	0.00
Beech	Bud burst15	Mother tree : Site	0.65	0.70	0.72
		Site	1.28	0.79	0.43
		Seed maturation year	0.92	0.29	0.35
	Bud burst16	Mother tree: Site	0.12	0.09	0.13
		Site	0.97	0.84	0.81
		Seed maturation year	1.18	0.97	0.94
Oak	Log 10 (Leaf_discolour80)	Mother tree : Site	0.00	0.00	0.00
		Site	0.00	0.00	0.00
		Seed maturation year	0.00	0.00	0.00
Beech		Mother tree: Site	0.01	0.01	0.01
		Site	0.00	0.00	0.00
		Seed maturation year	0.00	0.00	0.00

Oak	Relative collar dia. increment	Mother tree : Site	0.02	0.01	0.02
		Site	0.00	0.00	0.00
		Seed maturation year	0.19	0.20	0.15
Beech		Mother tree: Site	0.03	0.03	0.03
		Site	0.00	0.00	0.01
		Seed maturation year	0.09	0.09	0.09



Appendix Figure A.5 The relationship between relative height increment in oak and beech seedlings and parental reproductive temperature (April-September). (a-c) represent the response in oak seedlings and (d-f) represent the response to beech seedlings to mean minimum, mean and mean maximum temperature (°C) during seed maturation period (Arpil-September).

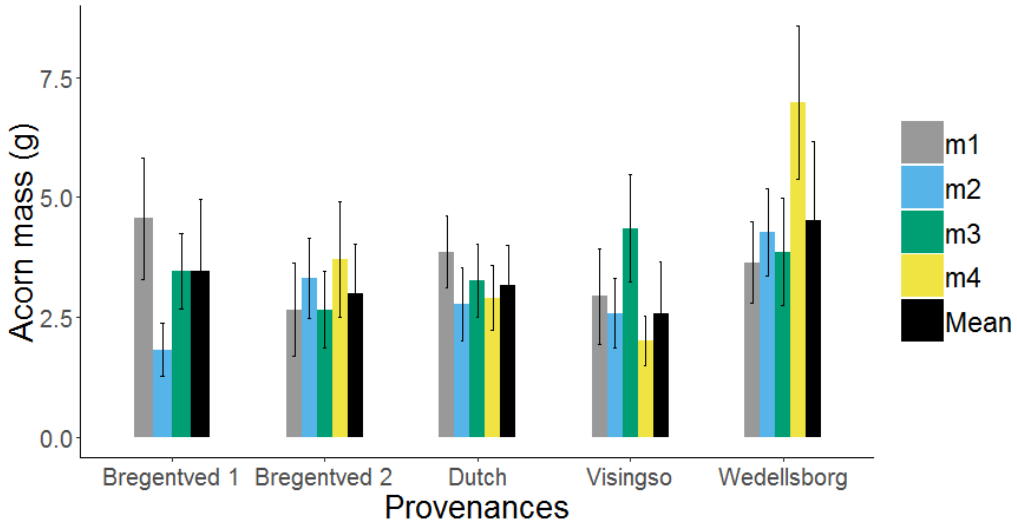
Appendix B Weak but persistent provenance effects modulate the response of *Quercus robur* seedlings to elevated temperatures



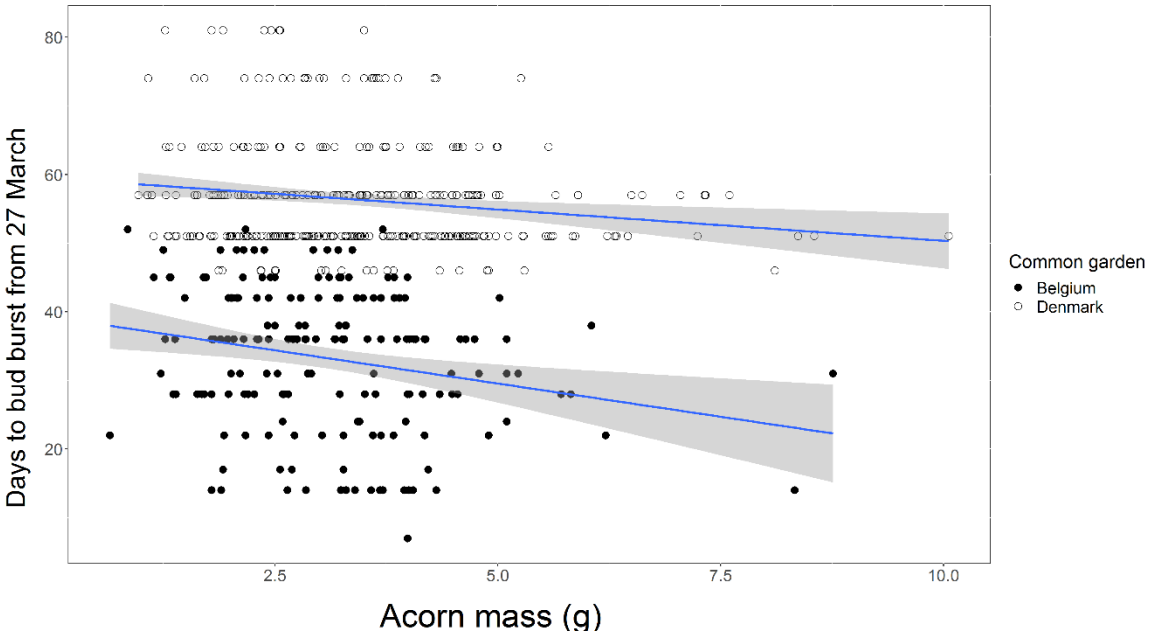
Appendix Figure B.1 Daily minimum, mean and maximal temperatures in Danish common garden (DK) and Belgian common garden (BE) in 2016. Values are t values and p values from linear models Error bars indicate standard errors. Significance are denoted by ** $p < 0.01$, *** $p<0.001$

Appendix Table B.1 Number of seedlings for bud burst and biomass analysis in common gardens, mother tree and sites. No acorn germinated from mother tree 3 of Visingso provenance in Belgian common garden.

	Site	id_mother	Common garden	Number of seedlings
1	Bregentved 1	b1m1	Belgium	13
2	Bregentved 1	b1m1	Denmark	27
3	Bregentved 1	b1m2	Belgium	12
4	Bregentved 1	b1m2	Denmark	15
5	Bregentved 1	b1m3	Belgium	12
6	Bregentved 1	b1m3	Denmark	15
7	Bregentved 2	b2m1	Belgium	11
8	Bregentved 2	b2m1	Denmark	12
9	Bregentved 2	b2m2	Belgium	8
10	Bregentved 2	b2m2	Denmark	15
11	Bregentved 2	b2m3	Belgium	23
12	Bregentved 2	b2m3	Denmark	52
13	Bregentved 2	b2m4	Belgium	17
14	Bregentved 2	b2m4	Denmark	20
15	Dutch	nm1	Belgium	7
16	Dutch	nm1	Denmark	18
17	Dutch	nm2	Belgium	6
18	Dutch	nm2	Denmark	23
19	Dutch	nm3	Belgium	9
20	Dutch	nm3	Denmark	14
21	Dutch	nm4	Belgium	9
22	Dutch	nm4	Denmark	21
23	Visingso	sm1	Belgium	6
24	Visingso	sm1	Denmark	9
25	Visingso	sm2	Belgium	2
26	Visingso	sm2	Denmark	7
27	Visingso	sm3	Denmark	10
28	Visingso	sm4	Belgium	14
29	Visingso	sm4	Denmark	27
30	Wedellsborg	wm1	Belgium	4
31	Wedellsborg	wm1	Denmark	12
32	Wedellsborg	wm2	Belgium	9
33	Wedellsborg	wm2	Denmark	12
34	Wedellsborg	wm3	Belgium	9
35	Wedellsborg	wm3	Denmark	22
36	Wedellsborg	wm4	Belgium	4
37	Wedellsborg	wm4	Denmark	12



Appendix Figure B.2 Variability of acorn mass (g) among the provenances. m1, m2, m3 and m4 represent the different mother trees in each provenance, and black circles are the mean of all the mother trees. Error bars denote the standard error.



Appendix Figure B.3 The relationship between acorn mass and bud burst time (total days from 27 March) of the seedlings.

Appendix C Maternal temperature during seed maturation affects seed germination and timing of bud set in seedlings of European black poplar

Appendix Table C.1 The number of individuals per treatment and experiment monitored for bud burst and bud set in each year of observation. * Data were removed because more than 50% of the buds remained dormant for the entire observation period (see main text). See Table 5.1 for the meaning of C, C>>W and W.

	Measured trait and year	C	C » W	W
Experiment 1				
Cross 1	No. of seeds sown	1440		2015
	Bud burst			
	2015	542	Treatment not included	295
	Bud set			
	2014	567		328
	2015	521		284
Experiment 2				
Cross 1	No. of seeds sown	537	600	1010
	Bud burst			
	2015	37	35	13
	2016	46	19	49
	Bud set			
	2014	173	13	69
	2015	45	43	99
Experiment 3				
Cross 2	No. of seeds sown	1070	985	1818
	Bud burst			
	2015	45	39	20
	2016	45	43	44
	Bud set			
	2014	182	90	37
	2015	45	44	46
Cross 3				
	No. of seeds sown	960	895	190
	Bud burst			
	2015	23	16	3
	2016	84	Data discarded*	2
	Bud set			
	2014	163	143	4
	2015	46	48	4

Appendix Table C.2 Height of the seedlings in 2015 under different treatments and experiments

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	1	13.001	25°C	109
Experiment 1	**Proven**	**Horrues**	2	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	3	13.001	25°C	72
Experiment 1	**Proven**	**Horrues**	4	13.001	25°C	108
Experiment 1	**Proven**	**Horrues**	5	13.001	25°C	75
Experiment 1	**Proven**	**Horrues**	6	13.001	25°C	48
Experiment 1	**Proven**	**Horrues**	7	13.001	25°C	122
Experiment 1	**Proven**	**Horrues**	8	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	9	13.001	25°C	121
Experiment 1	**Proven**	**Horrues**	10	13.001	25°C	156
Experiment 1	**Proven**	**Horrues**	11	13.001	25°C	100
Experiment 1	**Proven**	**Horrues**	12	13.001	25°C	79
Experiment 1	**Proven**	**Horrues**	13	13.001	25°C	122
Experiment 1	**Proven**	**Horrues**	14	13.001	25°C	129
Experiment 1	**Proven**	**Horrues**	15	13.001	25°C	38
Experiment 1	**Proven**	**Horrues**	16	13.001	25°C	63
Experiment 1	**Proven**	**Horrues**	17	13.001	25°C	70
Experiment 1	**Proven**	**Horrues**	18	13.001	25°C	79
Experiment 1	**Proven**	**Horrues**	28	13.001	25°C	56
Experiment 1	**Proven**	**Horrues**	29	13.001	25°C	59
Experiment 1	**Proven**	**Horrues**	30	13.001	25°C	55
Experiment 1	**Proven**	**Horrues**	53	13.001	25°C	72
Experiment 1	**Proven**	**Horrues**	54	13.001	25°C	51
Experiment 1	**Proven**	**Horrues**	55	13.001	25°C	127
Experiment 1	**Proven**	**Horrues**	56	13.001	25°C	155
Experiment 1	**Proven**	**Horrues**	57	13.001	25°C	43
Experiment 1	**Proven**	**Horrues**	58	13.001	25°C	134
Experiment 1	**Proven**	**Horrues**	59	13.001	25°C	125
Experiment 1	**Proven**	**Horrues**	60	13.001	25°C	55
Experiment 1	**Proven**	**Horrues**	66	13.001	25°C	55
Experiment 1	**Proven**	**Horrues**	67	13.001	25°C	44
Experiment 1	**Proven**	**Horrues**	68	13.001	25°C	71
Experiment 1	**Proven**	**Horrues**	112	13.001	25°C	137
Experiment 1	**Proven**	**Horrues**	113	13.001	25°C	158
Experiment 1	**Proven**	**Horrues**	114	13.001	25°C	128
Experiment 1	**Proven**	**Horrues**	115	13.001	25°C	159
Experiment 1	**Proven**	**Horrues**	116	13.001	25°C	95
Experiment 1	**Proven**	**Horrues**	117	13.001	25°C	80
Experiment 1	**Proven**	**Horrues**	118	13.001	25°C	104
Experiment 1	**Proven**	**Horrues**	119	13.001	25°C	152
Experiment 1	**Proven**	**Horrues**	120	13.001	25°C	172
Experiment 1	**Proven**	**Horrues**	121	13.001	25°C	64
Experiment 1	**Proven**	**Horrues**	122	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	123	13.001	25°C	108
Experiment 1	**Proven**	**Horrues**	124	13.001	25°C	137

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	125	13.001	25°C	76
Experiment 1	**Proven**	**Horrues**	126	13.001	25°C	48
Experiment 1	**Proven**	**Horrues**	127	13.001	25°C	131
Experiment 1	**Proven**	**Horrues**	128	13.001	25°C	132
Experiment 1	**Proven**	**Horrues**	129	13.001	25°C	121
Experiment 1	**Proven**	**Horrues**	130	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	131	13.001	25°C	105
Experiment 1	**Proven**	**Horrues**	132	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	133	13.001	25°C	89
Experiment 1	**Proven**	**Horrues**	134	13.001	25°C	116
Experiment 1	**Proven**	**Horrues**	135	13.001	25°C	98
Experiment 1	**Proven**	**Horrues**	136	13.001	25°C	91
Experiment 1	**Proven**	**Horrues**	137	13.001	25°C	112
Experiment 1	**Proven**	**Horrues**	138	13.001	25°C	121
Experiment 1	**Proven**	**Horrues**	139	13.001	25°C	92
Experiment 1	**Proven**	**Horrues**	140	13.001	25°C	92
Experiment 1	**Proven**	**Horrues**	141	13.001	25°C	102
Experiment 1	**Proven**	**Horrues**	142	13.001	25°C	134
Experiment 1	**Proven**	**Horrues**	143	13.001	25°C	110
Experiment 1	**Proven**	**Horrues**	144	13.001	25°C	115
Experiment 1	**Proven**	**Horrues**	145	13.001	25°C	192
Experiment 1	**Proven**	**Horrues**	146	13.001	25°C	130
Experiment 1	**Proven**	**Horrues**	147	13.001	25°C	97
Experiment 1	**Proven**	**Horrues**	148	13.001	25°C	206
Experiment 1	**Proven**	**Horrues**	149	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	150	13.001	25°C	198
Experiment 1	**Proven**	**Horrues**	151	13.001	25°C	134
Experiment 1	**Proven**	**Horrues**	152	13.001	25°C	139
Experiment 1	**Proven**	**Horrues**	153	13.001	25°C	157
Experiment 1	**Proven**	**Horrues**	154	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	155	13.001	25°C	107
Experiment 1	**Proven**	**Horrues**	156	13.001	25°C	150
Experiment 1	**Proven**	**Horrues**	157	13.001	25°C	148
Experiment 1	**Proven**	**Horrues**	158	13.001	25°C	100
Experiment 1	**Proven**	**Horrues**	159	13.001	25°C	99
Experiment 1	**Proven**	**Horrues**	160	13.001	25°C	116
Experiment 1	**Proven**	**Horrues**	161	13.001	25°C	167
Experiment 1	**Proven**	**Horrues**	162	13.001	25°C	63
Experiment 1	**Proven**	**Horrues**	163	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	164	13.001	25°C	120
Experiment 1	**Proven**	**Horrues**	165	13.001	25°C	143
Experiment 1	**Proven**	**Horrues**	208	13.001	25°C	118
Experiment 1	**Proven**	**Horrues**	209	13.001	25°C	59
Experiment 1	**Proven**	**Horrues**	210	13.001	25°C	64
Experiment 1	**Proven**	**Horrues**	211	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	212	13.001	25°C	100
Experiment 1	**Proven**	**Horrues**	213	13.001	25°C	101

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	214	13.001	25°C	78
Experiment 1	**Proven**	**Horrues**	215	13.001	25°C	88
Experiment 1	**Proven**	**Horrues**	216	13.001	25°C	86
Experiment 1	**Proven**	**Horrues**	217	13.001	25°C	25
Experiment 1	**Proven**	**Horrues**	218	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	219	13.001	25°C	60
Experiment 1	**Proven**	**Horrues**	220	13.001	25°C	95
Experiment 1	**Proven**	**Horrues**	221	13.001	25°C	95
Experiment 1	**Proven**	**Horrues**	222	13.001	25°C	80
Experiment 1	**Proven**	**Horrues**	223	13.001	25°C	63
Experiment 1	**Proven**	**Horrues**	224	13.001	25°C	104
Experiment 1	**Proven**	**Horrues**	225	13.001	25°C	104
Experiment 1	**Proven**	**Horrues**	226	13.001	25°C	124
Experiment 1	**Proven**	**Horrues**	227	13.001	25°C	94
Experiment 1	**Proven**	**Horrues**	228	13.001	25°C	132
Experiment 1	**Proven**	**Horrues**	229	13.001	25°C	160
Experiment 1	**Proven**	**Horrues**	375	13.001	25°C	129
Experiment 1	**Proven**	**Horrues**	392	13.001	25°C	183
Experiment 1	**Proven**	**Horrues**	393	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	394	13.001	25°C	95
Experiment 1	**Proven**	**Horrues**	395	13.001	25°C	138
Experiment 1	**Proven**	**Horrues**	396	13.001	25°C	79
Experiment 1	**Proven**	**Horrues**	397	13.001	25°C	82
Experiment 1	**Proven**	**Horrues**	398	13.001	25°C	196
Experiment 1	**Proven**	**Horrues**	399	13.001	25°C	156
Experiment 1	**Proven**	**Horrues**	400	13.001	25°C	52
Experiment 1	**Proven**	**Horrues**	401	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	402	13.001	25°C	140
Experiment 1	**Proven**	**Horrues**	403	13.001	25°C	120
Experiment 1	**Proven**	**Horrues**	404	13.001	25°C	150
Experiment 1	**Proven**	**Horrues**	405	13.001	25°C	89
Experiment 1	**Proven**	**Horrues**	406	13.001	25°C	140
Experiment 1	**Proven**	**Horrues**	407	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	408	13.001	25°C	216
Experiment 1	**Proven**	**Horrues**	409	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	410	13.001	25°C	120
Experiment 1	**Proven**	**Horrues**	411	13.001	25°C	95
Experiment 1	**Proven**	**Horrues**	412	13.001	25°C	147
Experiment 1	**Proven**	**Horrues**	413	13.001	25°C	180
Experiment 1	**Proven**	**Horrues**	414	13.001	25°C	133
Experiment 1	**Proven**	**Horrues**	415	13.001	25°C	195
Experiment 1	**Proven**	**Horrues**	416	13.001	25°C	104
Experiment 1	**Proven**	**Horrues**	417	13.001	25°C	92
Experiment 1	**Proven**	**Horrues**	418	13.001	25°C	62
Experiment 1	**Proven**	**Horrues**	419	13.001	25°C	105
Experiment 1	**Proven**	**Horrues**	420	13.001	25°C	170

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	421	13.001	25°C	88
Experiment 1	**Proven**	**Horrues**	422	13.001	25°C	116
Experiment 1	**Proven**	**Horrues**	423	13.001	25°C	192
Experiment 1	**Proven**	**Horrues**	424	13.001	25°C	116
Experiment 1	**Proven**	**Horrues**	425	13.001	25°C	80
Experiment 1	**Proven**	**Horrues**	426	13.001	25°C	167
Experiment 1	**Proven**	**Horrues**	427	13.001	25°C	103
Experiment 1	**Proven**	**Horrues**	428	13.001	25°C	162
Experiment 1	**Proven**	**Horrues**	429	13.001	25°C	80
Experiment 1	**Proven**	**Horrues**	430	13.001	25°C	124
Experiment 1	**Proven**	**Horrues**	431	13.001	25°C	191
Experiment 1	**Proven**	**Horrues**	432	13.001	25°C	160
Experiment 1	**Proven**	**Horrues**	433	13.001	25°C	88
Experiment 1	**Proven**	**Horrues**	434	13.001	25°C	151
Experiment 1	**Proven**	**Horrues**	435	13.001	25°C	68
Experiment 1	**Proven**	**Horrues**	436	13.001	25°C	110
Experiment 1	**Proven**	**Horrues**	437	13.001	25°C	163
Experiment 1	**Proven**	**Horrues**	438	13.001	25°C	182
Experiment 1	**Proven**	**Horrues**	439	13.001	25°C	115
Experiment 1	**Proven**	**Horrues**	440	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	481	13.001	25°C	89
Experiment 1	**Proven**	**Horrues**	482	13.001	25°C	102
Experiment 1	**Proven**	**Horrues**	483	13.001	25°C	115
Experiment 1	**Proven**	**Horrues**	484	13.001	25°C	119
Experiment 1	**Proven**	**Horrues**	485	13.001	25°C	110
Experiment 1	**Proven**	**Horrues**	486	13.001	25°C	65
Experiment 1	**Proven**	**Horrues**	487	13.001	25°C	121
Experiment 1	**Proven**	**Horrues**	488	13.001	25°C	88
Experiment 1	**Proven**	**Horrues**	489	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	490	13.001	25°C	112
Experiment 1	**Proven**	**Horrues**	491	13.001	25°C	85
Experiment 1	**Proven**	**Horrues**	492	13.001	25°C	62
Experiment 1	**Proven**	**Horrues**	493	13.001	25°C	105
Experiment 1	**Proven**	**Horrues**	494	13.001	25°C	79
Experiment 1	**Proven**	**Horrues**	495	13.001	25°C	71
Experiment 1	**Proven**	**Horrues**	496	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	497	13.001	25°C	145
Experiment 1	**Proven**	**Horrues**	498	13.001	25°C	58
Experiment 1	**Proven**	**Horrues**	499	13.001	25°C	52
Experiment 1	**Proven**	**Horrues**	500	13.001	25°C	92
Experiment 1	**Proven**	**Horrues**	501	13.001	25°C	61
Experiment 1	**Proven**	**Horrues**	502	13.001	25°C	135
Experiment 1	**Proven**	**Horrues**	503	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	504	13.001	25°C	91
Experiment 1	**Proven**	**Horrues**	505	13.001	25°C	107
Experiment 1	**Proven**	**Horrues**	506	13.001	25°C	77
Experiment 1	**Proven**	**Horrues**	507	13.001	25°C	148

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	508	13.001	25°C	128
Experiment 1	**Proven**	**Horrues**	509	13.001	25°C	136
Experiment 1	**Proven**	**Horrues**	510	13.001	25°C	176
Experiment 1	**Proven**	**Horrues**	511	13.001	25°C	111
Experiment 1	**Proven**	**Horrues**	512	13.001	25°C	138
Experiment 1	**Proven**	**Horrues**	513	13.001	25°C	78
Experiment 1	**Proven**	**Horrues**	605	13.001	25°C	137
Experiment 1	**Proven**	**Horrues**	606	13.001	25°C	124
Experiment 1	**Proven**	**Horrues**	607	13.001	25°C	107
Experiment 1	**Proven**	**Horrues**	608	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	609	13.001	25°C	200
Experiment 1	**Proven**	**Horrues**	610	13.001	25°C	120
Experiment 1	**Proven**	**Horrues**	611	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	612	13.001	25°C	173
Experiment 1	**Proven**	**Horrues**	613	13.001	25°C	75
Experiment 1	**Proven**	**Horrues**	614	13.001	25°C	41
Experiment 1	**Proven**	**Horrues**	640	13.001	25°C	62
Experiment 1	**Proven**	**Horrues**	641	13.001	25°C	169
Experiment 1	**Proven**	**Horrues**	642	13.001	25°C	108
Experiment 1	**Proven**	**Horrues**	643	13.001	25°C	85
Experiment 1	**Proven**	**Horrues**	648	13.001	25°C	90
Experiment 1	**Proven**	**Horrues**	649	13.001	25°C	103
Experiment 1	**Proven**	**Horrues**	650	13.001	25°C	139
Experiment 1	**Proven**	**Horrues**	651	13.001	25°C	88
Experiment 1	**Proven**	**Horrues**	652	13.001	25°C	95
Experiment 1	**Proven**	**Horrues**	653	13.001	25°C	48
Experiment 1	**Proven**	**Horrues**	654	13.001	25°C	156
Experiment 1	**Proven**	**Horrues**	677	13.001	25°C	232
Experiment 1	**Proven**	**Horrues**	686	13.001	25°C	128
Experiment 1	**Proven**	**Horrues**	689	13.001	25°C	86
Experiment 1	**Proven**	**Horrues**	700	13.001	25°C	146
Experiment 1	**Proven**	**Horrues**	701	13.001	25°C	118
Experiment 1	**Proven**	**Horrues**	702	13.001	25°C	92
Experiment 1	**Proven**	**Horrues**	703	13.001	25°C	170
Experiment 1	**Proven**	**Horrues**	704	13.001	25°C	180
Experiment 1	**Proven**	**Horrues**	705	13.001	25°C	148
Experiment 1	**Proven**	**Horrues**	706	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	707	13.001	25°C	178
Experiment 1	**Proven**	**Horrues**	708	13.001	25°C	140
Experiment 1	**Proven**	**Horrues**	709	13.001	25°C	194
Experiment 1	**Proven**	**Horrues**	710	13.001	25°C	189
Experiment 1	**Proven**	**Horrues**	711	13.001	25°C	154
Experiment 1	**Proven**	**Horrues**	717	13.001	25°C	100
Experiment 1	**Proven**	**Horrues**	718	13.001	25°C	88
Experiment 1	**Proven**	**Horrues**	731	13.001	25°C	151
Experiment 1	**Proven**	**Horrues**	732	13.001	25°C	228

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	735	13.001	25°C	265
Experiment 1	**Proven**	**Horrues**	744	13.001	25°C	168
Experiment 1	**Proven**	**Horrues**	747	13.001	25°C	102
Experiment 1	**Proven**	**Horrues**	748	13.001	25°C	112
Experiment 1	**Proven**	**Horrues**	763	13.001	25°C	153
Experiment 1	**Proven**	**Horrues**	764	13.001	25°C	185
Experiment 1	**Proven**	**Horrues**	765	13.001	25°C	158
Experiment 1	**Proven**	**Horrues**	766	13.001	25°C	168
Experiment 1	**Proven**	**Horrues**	767	13.001	25°C	146
Experiment 1	**Proven**	**Horrues**	768	13.001	25°C	164
Experiment 1	**Proven**	**Horrues**	775	13.001	25°C	69
Experiment 1	**Proven**	**Horrues**	776	13.001	25°C	80
Experiment 1	**Proven**	**Horrues**	777	13.001	25°C	102
Experiment 1	**Proven**	**Horrues**	778	13.001	25°C	75
Experiment 1	**Proven**	**Horrues**	779	13.001	25°C	106
Experiment 1	**Proven**	**Horrues**	780	13.001	25°C	105
Experiment 1	**Proven**	**Horrues**	781	13.001	25°C	41
Experiment 1	**Proven**	**Horrues**	800	13.001	25°C	142
Experiment 1	**Proven**	**Horrues**	801	13.001	25°C	150
Experiment 1	**Proven**	**Horrues**	802	13.001	25°C	215
Experiment 1	**Proven**	**Horrues**	806	13.001	25°C	183
Experiment 1	**Proven**	**Horrues**	814	13.001	25°C	192
Experiment 1	**Proven**	**Horrues**	815	13.001	25°C	162
Experiment 1	**Proven**	**Horrues**	816	13.001	25°C	208
Experiment 1	**Proven**	**Horrues**	817	13.001	25°C	196
Experiment 1	**Proven**	**Horrues**	818	13.001	25°C	188
Experiment 1	**Proven**	**Horrues**	819	13.001	25°C	162
Experiment 1	**Proven**	**Horrues**	820	13.001	25°C	115
Experiment 1	**Proven**	**Horrues**	821	13.001	25°C	141
Experiment 1	**Proven**	**Horrues**	822	13.001	25°C	181
Experiment 1	**Proven**	**Horrues**	837	13.001	25°C	122
Experiment 1	**Proven**	**Horrues**	838	13.001	25°C	146
Experiment 1	**Proven**	**Horrues**	839	13.001	25°C	139
Experiment 1	**Proven**	**Horrues**	840	13.001	25°C	211
Experiment 1	**Proven**	**Horrues**	841	13.001	25°C	204
Experiment 1	**Proven**	**Horrues**	842	13.001	25°C	160
Experiment 1	**Proven**	**Horrues**	870	13.001	25°C	240
Experiment 1	**Proven**	**Horrues**	876	13.001	25°C	244
Experiment 1	**Proven**	**Horrues**	887	13.001	25°C	172
Experiment 1	**Proven**	**Horrues**	888	13.001	25°C	206
Experiment 1	**Proven**	**Horrues**	889	13.001	25°C	173
Experiment 1	**Proven**	**Horrues**	890	13.001	25°C	155
Experiment 1	**Proven**	**Horrues**	906	13.001	25°C	164
Experiment 1	**Proven**	**Horrues**	908	13.001	25°C	280
Experiment 1	**Proven**	**Horrues**	909	13.001	25°C	226
Experiment 1	**Proven**	**Horrues**	910	13.001	25°C	228
Experiment 1	**Proven**	**Horrues**	920	13.001	25°C	220

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	921	13.001	25°C	265
Experiment 1	**Proven**	**Horrues**	922	13.001	25°C	184
Experiment 1	**Proven**	**Horrues**	923	13.001	25°C	154
Experiment 1	**Proven**	**Horrues**	924	13.001	25°C	200
Experiment 1	**Proven**	**Horrues**	931	13.001	25°C	61
Experiment 1	**Proven**	**Horrues**	948	13.001	25°C	271
Experiment 1	**Proven**	**Horrues**	19	13.001	15°C	132
Experiment 1	**Proven**	**Horrues**	20	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	21	13.001	15°C	63
Experiment 1	**Proven**	**Horrues**	22	13.001	15°C	93
Experiment 1	**Proven**	**Horrues**	23	13.001	15°C	51
Experiment 1	**Proven**	**Horrues**	24	13.001	15°C	118
Experiment 1	**Proven**	**Horrues**	25	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	26	13.001	15°C	56
Experiment 1	**Proven**	**Horrues**	27	13.001	15°C	75
Experiment 1	**Proven**	**Horrues**	31	13.001	15°C	88
Experiment 1	**Proven**	**Horrues**	32	13.001	15°C	48
Experiment 1	**Proven**	**Horrues**	33	13.001	15°C	71
Experiment 1	**Proven**	**Horrues**	34	13.001	15°C	84
Experiment 1	**Proven**	**Horrues**	35	13.001	15°C	172
Experiment 1	**Proven**	**Horrues**	36	13.001	15°C	73
Experiment 1	**Proven**	**Horrues**	37	13.001	15°C	109
Experiment 1	**Proven**	**Horrues**	38	13.001	15°C	68
Experiment 1	**Proven**	**Horrues**	39	13.001	15°C	95
Experiment 1	**Proven**	**Horrues**	40	13.001	15°C	147
Experiment 1	**Proven**	**Horrues**	41	13.001	15°C	127
Experiment 1	**Proven**	**Horrues**	52	13.001	15°C	68
Experiment 1	**Proven**	**Horrues**	61	13.001	15°C	54
Experiment 1	**Proven**	**Horrues**	62	13.001	15°C	52
Experiment 1	**Proven**	**Horrues**	63	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	64	13.001	15°C	196
Experiment 1	**Proven**	**Horrues**	65	13.001	15°C	160
Experiment 1	**Proven**	**Horrues**	69	13.001	15°C	50
Experiment 1	**Proven**	**Horrues**	70	13.001	15°C	123
Experiment 1	**Proven**	**Horrues**	71	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	72	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	73	13.001	15°C	106
Experiment 1	**Proven**	**Horrues**	74	13.001	15°C	46
Experiment 1	**Proven**	**Horrues**	75	13.001	15°C	95
Experiment 1	**Proven**	**Horrues**	76	13.001	15°C	58
Experiment 1	**Proven**	**Horrues**	77	13.001	15°C	50
Experiment 1	**Proven**	**Horrues**	78	13.001	15°C	103
Experiment 1	**Proven**	**Horrues**	79	13.001	15°C	73
Experiment 1	**Proven**	**Horrues**	80	13.001	15°C	142
Experiment 1	**Proven**	**Horrues**	81	13.001	15°C	116
Experiment 1	**Proven**	**Horrues**	82	13.001	15°C	102

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	83	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	84	13.001	15°C	93
Experiment 1	**Proven**	**Horrues**	85	13.001	15°C	106
Experiment 1	**Proven**	**Horrues**	86	13.001	15°C	118
Experiment 1	**Proven**	**Horrues**	87	13.001	15°C	116
Experiment 1	**Proven**	**Horrues**	88	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	89	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	90	13.001	15°C	85
Experiment 1	**Proven**	**Horrues**	91	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	92	13.001	15°C	95
Experiment 1	**Proven**	**Horrues**	108	13.001	15°C	134
Experiment 1	**Proven**	**Horrues**	109	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	110	13.001	15°C	185
Experiment 1	**Proven**	**Horrues**	166	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	167	13.001	15°C	125
Experiment 1	**Proven**	**Horrues**	168	13.001	15°C	183
Experiment 1	**Proven**	**Horrues**	169	13.001	15°C	110
Experiment 1	**Proven**	**Horrues**	170	13.001	15°C	191
Experiment 1	**Proven**	**Horrues**	171	13.001	15°C	135
Experiment 1	**Proven**	**Horrues**	172	13.001	15°C	144
Experiment 1	**Proven**	**Horrues**	173	13.001	15°C	103
Experiment 1	**Proven**	**Horrues**	174	13.001	15°C	121
Experiment 1	**Proven**	**Horrues**	175	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	176	13.001	15°C	126
Experiment 1	**Proven**	**Horrues**	177	13.001	15°C	157
Experiment 1	**Proven**	**Horrues**	178	13.001	15°C	126
Experiment 1	**Proven**	**Horrues**	179	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	180	13.001	15°C	198
Experiment 1	**Proven**	**Horrues**	181	13.001	15°C	153
Experiment 1	**Proven**	**Horrues**	182	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	183	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	184	13.001	15°C	75
Experiment 1	**Proven**	**Horrues**	185	13.001	15°C	157
Experiment 1	**Proven**	**Horrues**	186	13.001	15°C	141
Experiment 1	**Proven**	**Horrues**	187	13.001	15°C	170
Experiment 1	**Proven**	**Horrues**	188	13.001	15°C	117
Experiment 1	**Proven**	**Horrues**	189	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	190	13.001	15°C	110
Experiment 1	**Proven**	**Horrues**	191	13.001	15°C	137
Experiment 1	**Proven**	**Horrues**	192	13.001	15°C	161
Experiment 1	**Proven**	**Horrues**	193	13.001	15°C	55
Experiment 1	**Proven**	**Horrues**	194	13.001	15°C	95
Experiment 1	**Proven**	**Horrues**	195	13.001	15°C	100
Experiment 1	**Proven**	**Horrues**	196	13.001	15°C	113
Experiment 1	**Proven**	**Horrues**	197	13.001	15°C	105
Experiment 1	**Proven**	**Horrues**	198	13.001	15°C	125
Experiment 1	**Proven**	**Horrues**	199	13.001	15°C	125

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	200	13.001	15°C	201
Experiment 1	**Proven**	**Horrues**	201	13.001	15°C	96
Experiment 1	**Proven**	**Horrues**	202	13.001	15°C	94
Experiment 1	**Proven**	**Horrues**	203	13.001	15°C	136
Experiment 1	**Proven**	**Horrues**	204	13.001	15°C	176
Experiment 1	**Proven**	**Horrues**	205	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	206	13.001	15°C	113
Experiment 1	**Proven**	**Horrues**	207	13.001	15°C	101
Experiment 1	**Proven**	**Horrues**	230	13.001	15°C	91
Experiment 1	**Proven**	**Horrues**	231	13.001	15°C	100
Experiment 1	**Proven**	**Horrues**	232	13.001	15°C	58
Experiment 1	**Proven**	**Horrues**	233	13.001	15°C	153
Experiment 1	**Proven**	**Horrues**	234	13.001	15°C	130
Experiment 1	**Proven**	**Horrues**	235	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	236	13.001	15°C	95
Experiment 1	**Proven**	**Horrues**	237	13.001	15°C	89
Experiment 1	**Proven**	**Horrues**	238	13.001	15°C	53
Experiment 1	**Proven**	**Horrues**	239	13.001	15°C	172
Experiment 1	**Proven**	**Horrues**	240	13.001	15°C	46
Experiment 1	**Proven**	**Horrues**	241	13.001	15°C	91
Experiment 1	**Proven**	**Horrues**	242	13.001	15°C	90
Experiment 1	**Proven**	**Horrues**	243	13.001	15°C	106
Experiment 1	**Proven**	**Horrues**	244	13.001	15°C	70
Experiment 1	**Proven**	**Horrues**	245	13.001	15°C	70
Experiment 1	**Proven**	**Horrues**	246	13.001	15°C	63
Experiment 1	**Proven**	**Horrues**	247	13.001	15°C	63
Experiment 1	**Proven**	**Horrues**	248	13.001	15°C	80
Experiment 1	**Proven**	**Horrues**	249	13.001	15°C	82
Experiment 1	**Proven**	**Horrues**	250	13.001	15°C	108
Experiment 1	**Proven**	**Horrues**	251	13.001	15°C	91
Experiment 1	**Proven**	**Horrues**	252	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	253	13.001	15°C	80
Experiment 1	**Proven**	**Horrues**	254	13.001	15°C	85
Experiment 1	**Proven**	**Horrues**	255	13.001	15°C	110
Experiment 1	**Proven**	**Horrues**	256	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	257	13.001	15°C	142
Experiment 1	**Proven**	**Horrues**	258	13.001	15°C	50
Experiment 1	**Proven**	**Horrues**	259	13.001	15°C	102
Experiment 1	**Proven**	**Horrues**	260	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	261	13.001	15°C	82
Experiment 1	**Proven**	**Horrues**	262	13.001	15°C	127
Experiment 1	**Proven**	**Horrues**	263	13.001	15°C	48
Experiment 1	**Proven**	**Horrues**	264	13.001	15°C	105
Experiment 1	**Proven**	**Horrues**	265	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	266	13.001	15°C	139
Experiment 1	**Proven**	**Horrues**	267	13.001	15°C	86

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	268	13.001	15°C	156
Experiment 1	**Proven**	**Horrues**	269	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	270	13.001	15°C	103
Experiment 1	**Proven**	**Horrues**	271	13.001	15°C	121
Experiment 1	**Proven**	**Horrues**	272	13.001	15°C	144
Experiment 1	**Proven**	**Horrues**	273	13.001	15°C	95
Experiment 1	**Proven**	**Horrues**	274	13.001	15°C	58
Experiment 1	**Proven**	**Horrues**	275	13.001	15°C	54
Experiment 1	**Proven**	**Horrues**	276	13.001	15°C	109
Experiment 1	**Proven**	**Horrues**	277	13.001	15°C	179
Experiment 1	**Proven**	**Horrues**	278	13.001	15°C	114
Experiment 1	**Proven**	**Horrues**	279	13.001	15°C	62
Experiment 1	**Proven**	**Horrues**	280	13.001	15°C	109
Experiment 1	**Proven**	**Horrues**	281	13.001	15°C	113
Experiment 1	**Proven**	**Horrues**	282	13.001	15°C	79
Experiment 1	**Proven**	**Horrues**	283	13.001	15°C	94
Experiment 1	**Proven**	**Horrues**	284	13.001	15°C	147
Experiment 1	**Proven**	**Horrues**	285	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	286	13.001	15°C	82
Experiment 1	**Proven**	**Horrues**	287	13.001	15°C	190
Experiment 1	**Proven**	**Horrues**	288	13.001	15°C	114
Experiment 1	**Proven**	**Horrues**	289	13.001	15°C	89
Experiment 1	**Proven**	**Horrues**	290	13.001	15°C	148
Experiment 1	**Proven**	**Horrues**	291	13.001	15°C	114
Experiment 1	**Proven**	**Horrues**	292	13.001	15°C	90
Experiment 1	**Proven**	**Horrues**	293	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	294	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	295	13.001	15°C	102
Experiment 1	**Proven**	**Horrues**	296	13.001	15°C	106
Experiment 1	**Proven**	**Horrues**	297	13.001	15°C	158
Experiment 1	**Proven**	**Horrues**	298	13.001	15°C	150
Experiment 1	**Proven**	**Horrues**	299	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	300	13.001	15°C	102
Experiment 1	**Proven**	**Horrues**	301	13.001	15°C	135
Experiment 1	**Proven**	**Horrues**	302	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	303	13.001	15°C	141
Experiment 1	**Proven**	**Horrues**	304	13.001	15°C	163
Experiment 1	**Proven**	**Horrues**	305	13.001	15°C	100
Experiment 1	**Proven**	**Horrues**	306	13.001	15°C	128
Experiment 1	**Proven**	**Horrues**	307	13.001	15°C	147
Experiment 1	**Proven**	**Horrues**	308	13.001	15°C	114
Experiment 1	**Proven**	**Horrues**	309	13.001	15°C	129
Experiment 1	**Proven**	**Horrues**	310	13.001	15°C	128
Experiment 1	**Proven**	**Horrues**	359	13.001	15°C	103
Experiment 1	**Proven**	**Horrues**	360	13.001	15°C	141
Experiment 1	**Proven**	**Horrues**	361	13.001	15°C	157
Experiment 1	**Proven**	**Horrues**	362	13.001	15°C	165

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	363	13.001	15°C	156
Experiment 1	**Proven**	**Horrues**	364	13.001	15°C	160
Experiment 1	**Proven**	**Horrues**	365	13.001	15°C	150
Experiment 1	**Proven**	**Horrues**	366	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	367	13.001	15°C	169
Experiment 1	**Proven**	**Horrues**	368	13.001	15°C	197
Experiment 1	**Proven**	**Horrues**	369	13.001	15°C	157
Experiment 1	**Proven**	**Horrues**	370	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	371	13.001	15°C	175
Experiment 1	**Proven**	**Horrues**	372	13.001	15°C	121
Experiment 1	**Proven**	**Horrues**	373	13.001	15°C	173
Experiment 1	**Proven**	**Horrues**	374	13.001	15°C	145
Experiment 1	**Proven**	**Horrues**	376	13.001	15°C	77
Experiment 1	**Proven**	**Horrues**	377	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	378	13.001	15°C	88
Experiment 1	**Proven**	**Horrues**	379	13.001	15°C	99
Experiment 1	**Proven**	**Horrues**	380	13.001	15°C	160
Experiment 1	**Proven**	**Horrues**	387	13.001	15°C	216
Experiment 1	**Proven**	**Horrues**	388	13.001	15°C	196
Experiment 1	**Proven**	**Horrues**	389	13.001	15°C	204
Experiment 1	**Proven**	**Horrues**	390	13.001	15°C	125
Experiment 1	**Proven**	**Horrues**	391	13.001	15°C	105
Experiment 1	**Proven**	**Horrues**	441	13.001	15°C	128
Experiment 1	**Proven**	**Horrues**	442	13.001	15°C	88
Experiment 1	**Proven**	**Horrues**	443	13.001	15°C	126
Experiment 1	**Proven**	**Horrues**	444	13.001	15°C	143
Experiment 1	**Proven**	**Horrues**	445	13.001	15°C	113
Experiment 1	**Proven**	**Horrues**	446	13.001	15°C	108
Experiment 1	**Proven**	**Horrues**	447	13.001	15°C	108
Experiment 1	**Proven**	**Horrues**	448	13.001	15°C	188
Experiment 1	**Proven**	**Horrues**	449	13.001	15°C	64
Experiment 1	**Proven**	**Horrues**	450	13.001	15°C	100
Experiment 1	**Proven**	**Horrues**	451	13.001	15°C	156
Experiment 1	**Proven**	**Horrues**	452	13.001	15°C	67
Experiment 1	**Proven**	**Horrues**	453	13.001	15°C	107
Experiment 1	**Proven**	**Horrues**	454	13.001	15°C	85
Experiment 1	**Proven**	**Horrues**	455	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	456	13.001	15°C	111
Experiment 1	**Proven**	**Horrues**	457	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	458	13.001	15°C	161
Experiment 1	**Proven**	**Horrues**	459	13.001	15°C	118
Experiment 1	**Proven**	**Horrues**	460	13.001	15°C	84
Experiment 1	**Proven**	**Horrues**	461	13.001	15°C	86
Experiment 1	**Proven**	**Horrues**	462	13.001	15°C	87
Experiment 1	**Proven**	**Horrues**	463	13.001	15°C	180
Experiment 1	**Proven**	**Horrues**	464	13.001	15°C	127

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	465	13.001	15°C	94
Experiment 1	**Proven**	**Horrues**	466	13.001	15°C	86
Experiment 1	**Proven**	**Horrues**	467	13.001	15°C	116
Experiment 1	**Proven**	**Horrues**	468	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	469	13.001	15°C	129
Experiment 1	**Proven**	**Horrues**	470	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	471	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	472	13.001	15°C	136
Experiment 1	**Proven**	**Horrues**	473	13.001	15°C	107
Experiment 1	**Proven**	**Horrues**	474	13.001	15°C	180
Experiment 1	**Proven**	**Horrues**	475	13.001	15°C	55
Experiment 1	**Proven**	**Horrues**	476	13.001	15°C	176
Experiment 1	**Proven**	**Horrues**	477	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	478	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	479	13.001	15°C	161
Experiment 1	**Proven**	**Horrues**	480	13.001	15°C	112
Experiment 1	**Proven**	**Horrues**	514	13.001	15°C	113
Experiment 1	**Proven**	**Horrues**	515	13.001	15°C	177
Experiment 1	**Proven**	**Horrues**	516	13.001	15°C	192
Experiment 1	**Proven**	**Horrues**	517	13.001	15°C	180
Experiment 1	**Proven**	**Horrues**	518	13.001	15°C	96
Experiment 1	**Proven**	**Horrues**	519	13.001	15°C	140
Experiment 1	**Proven**	**Horrues**	520	13.001	15°C	36
Experiment 1	**Proven**	**Horrues**	521	13.001	15°C	154
Experiment 1	**Proven**	**Horrues**	522	13.001	15°C	84
Experiment 1	**Proven**	**Horrues**	523	13.001	15°C	35
Experiment 1	**Proven**	**Horrues**	524	13.001	15°C	66
Experiment 1	**Proven**	**Horrues**	525	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	526	13.001	15°C	80
Experiment 1	**Proven**	**Horrues**	527	13.001	15°C	55
Experiment 1	**Proven**	**Horrues**	528	13.001	15°C	98
Experiment 1	**Proven**	**Horrues**	529	13.001	15°C	119
Experiment 1	**Proven**	**Horrues**	530	13.001	15°C	129
Experiment 1	**Proven**	**Horrues**	531	13.001	15°C	123
Experiment 1	**Proven**	**Horrues**	532	13.001	15°C	172
Experiment 1	**Proven**	**Horrues**	533	13.001	15°C	119
Experiment 1	**Proven**	**Horrues**	534	13.001	15°C	105
Experiment 1	**Proven**	**Horrues**	535	13.001	15°C	145
Experiment 1	**Proven**	**Horrues**	536	13.001	15°C	55
Experiment 1	**Proven**	**Horrues**	537	13.001	15°C	107
Experiment 1	**Proven**	**Horrues**	538	13.001	15°C	110
Experiment 1	**Proven**	**Horrues**	539	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	540	13.001	15°C	83
Experiment 1	**Proven**	**Horrues**	541	13.001	15°C	60
Experiment 1	**Proven**	**Horrues**	542	13.001	15°C	42
Experiment 1	**Proven**	**Horrues**	543	13.001	15°C	85
Experiment 1	**Proven**	**Horrues**	544	13.001	15°C	55

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	545	13.001	15°C	108
Experiment 1	**Proven**	**Horrues**	546	13.001	15°C	123
Experiment 1	**Proven**	**Horrues**	547	13.001	15°C	195
Experiment 1	**Proven**	**Horrues**	548	13.001	15°C	147
Experiment 1	**Proven**	**Horrues**	549	13.001	15°C	
Experiment 1	**Proven**	**Horrues**	550	13.001	15°C	106
Experiment 1	**Proven**	**Horrues**	551	13.001	15°C	64
Experiment 1	**Proven**	**Horrues**	552	13.001	15°C	72
Experiment 1	**Proven**	**Horrues**	553	13.001	15°C	137
Experiment 1	**Proven**	**Horrues**	554	13.001	15°C	56
Experiment 1	**Proven**	**Horrues**	555	13.001	15°C	171
Experiment 1	**Proven**	**Horrues**	556	13.001	15°C	186
Experiment 1	**Proven**	**Horrues**	557	13.001	15°C	186
Experiment 1	**Proven**	**Horrues**	558	13.001	15°C	153
Experiment 1	**Proven**	**Horrues**	559	13.001	15°C	154
Experiment 1	**Proven**	**Horrues**	560	13.001	15°C	110
Experiment 1	**Proven**	**Horrues**	561	13.001	15°C	140
Experiment 1	**Proven**	**Horrues**	562	13.001	15°C	177
Experiment 1	**Proven**	**Horrues**	563	13.001	15°C	179
Experiment 1	**Proven**	**Horrues**	564	13.001	15°C	166
Experiment 1	**Proven**	**Horrues**	565	13.001	15°C	115
Experiment 1	**Proven**	**Horrues**	566	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	567	13.001	15°C	124
Experiment 1	**Proven**	**Horrues**	568	13.001	15°C	130
Experiment 1	**Proven**	**Horrues**	596	13.001	15°C	86
Experiment 1	**Proven**	**Horrues**	597	13.001	15°C	142
Experiment 1	**Proven**	**Horrues**	598	13.001	15°C	164
Experiment 1	**Proven**	**Horrues**	599	13.001	15°C	100
Experiment 1	**Proven**	**Horrues**	600	13.001	15°C	226
Experiment 1	**Proven**	**Horrues**	601	13.001	15°C	181
Experiment 1	**Proven**	**Horrues**	602	13.001	15°C	234
Experiment 1	**Proven**	**Horrues**	603	13.001	15°C	176
Experiment 1	**Proven**	**Horrues**	604	13.001	15°C	163
Experiment 1	**Proven**	**Horrues**	615	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	616	13.001	15°C	172
Experiment 1	**Proven**	**Horrues**	617	13.001	15°C	190
Experiment 1	**Proven**	**Horrues**	618	13.001	15°C	70
Experiment 1	**Proven**	**Horrues**	619	13.001	15°C	200
Experiment 1	**Proven**	**Horrues**	620	13.001	15°C	147
Experiment 1	**Proven**	**Horrues**	621	13.001	15°C	157
Experiment 1	**Proven**	**Horrues**	622	13.001	15°C	167
Experiment 1	**Proven**	**Horrues**	623	13.001	15°C	134
Experiment 1	**Proven**	**Horrues**	624	13.001	15°C	220
Experiment 1	**Proven**	**Horrues**	625	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	626	13.001	15°C	179
Experiment 1	**Proven**	**Horrues**	627	13.001	15°C	179

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	628	13.001	15°C	204
Experiment 1	**Proven**	**Horrues**	629	13.001	15°C	218
Experiment 1	**Proven**	**Horrues**	630	13.001	15°C	134
Experiment 1	**Proven**	**Horrues**	631	13.001	15°C	81
Experiment 1	**Proven**	**Horrues**	636	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	637	13.001	15°C	231
Experiment 1	**Proven**	**Horrues**	638	13.001	15°C	218
Experiment 1	**Proven**	**Horrues**	639	13.001	15°C	171
Experiment 1	**Proven**	**Horrues**	644	13.001	15°C	122
Experiment 1	**Proven**	**Horrues**	645	13.001	15°C	115
Experiment 1	**Proven**	**Horrues**	646	13.001	15°C	114
Experiment 1	**Proven**	**Horrues**	647	13.001	15°C	80
Experiment 1	**Proven**	**Horrues**	655	13.001	15°C	167
Experiment 1	**Proven**	**Horrues**	656	13.001	15°C	118
Experiment 1	**Proven**	**Horrues**	657	13.001	15°C	107
Experiment 1	**Proven**	**Horrues**	658	13.001	15°C	50
Experiment 1	**Proven**	**Horrues**	659	13.001	15°C	52
Experiment 1	**Proven**	**Horrues**	660	13.001	15°C	161
Experiment 1	**Proven**	**Horrues**	661	13.001	15°C	105
Experiment 1	**Proven**	**Horrues**	662	13.001	15°C	98
Experiment 1	**Proven**	**Horrues**	663	13.001	15°C	127
Experiment 1	**Proven**	**Horrues**	664	13.001	15°C	140
Experiment 1	**Proven**	**Horrues**	665	13.001	15°C	170
Experiment 1	**Proven**	**Horrues**	666	13.001	15°C	166
Experiment 1	**Proven**	**Horrues**	667	13.001	15°C	128
Experiment 1	**Proven**	**Horrues**	668	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	674	13.001	15°C	275
Experiment 1	**Proven**	**Horrues**	675	13.001	15°C	228
Experiment 1	**Proven**	**Horrues**	676	13.001	15°C	230
Experiment 1	**Proven**	**Horrues**	678	13.001	15°C	193
Experiment 1	**Proven**	**Horrues**	679	13.001	15°C	125
Experiment 1	**Proven**	**Horrues**	680	13.001	15°C	194
Experiment 1	**Proven**	**Horrues**	681	13.001	15°C	198
Experiment 1	**Proven**	**Horrues**	682	13.001	15°C	144
Experiment 1	**Proven**	**Horrues**	683	13.001	15°C	144
Experiment 1	**Proven**	**Horrues**	687	13.001	15°C	98
Experiment 1	**Proven**	**Horrues**	688	13.001	15°C	92
Experiment 1	**Proven**	**Horrues**	690	13.001	15°C	14
Experiment 1	**Proven**	**Horrues**	691	13.001	15°C	93
Experiment 1	**Proven**	**Horrues**	692	13.001	15°C	170
Experiment 1	**Proven**	**Horrues**	693	13.001	15°C	102
Experiment 1	**Proven**	**Horrues**	694	13.001	15°C	127
Experiment 1	**Proven**	**Horrues**	695	13.001	15°C	104
Experiment 1	**Proven**	**Horrues**	697	13.001	15°C	149
Experiment 1	**Proven**	**Horrues**	698	13.001	15°C	143
Experiment 1	**Proven**	**Horrues**	699	13.001	15°C	162
Experiment 1	**Proven**	**Horrues**	712	13.001	15°C	78

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	713	13.001	15°C	133
Experiment 1	**Proven**	**Horrues**	714	13.001	15°C	186
Experiment 1	**Proven**	**Horrues**	715	13.001	15°C	168
Experiment 1	**Proven**	**Horrues**	716	13.001	15°C	102
Experiment 1	**Proven**	**Horrues**	719	13.001	15°C	100
Experiment 1	**Proven**	**Horrues**	720	13.001	15°C	59
Experiment 1	**Proven**	**Horrues**	721	13.001	15°C	61
Experiment 1	**Proven**	**Horrues**	722	13.001	15°C	203
Experiment 1	**Proven**	**Horrues**	723	13.001	15°C	203
Experiment 1	**Proven**	**Horrues**	729	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	730	13.001	15°C	107
Experiment 1	**Proven**	**Horrues**	733	13.001	15°C	200
Experiment 1	**Proven**	**Horrues**	734	13.001	15°C	246
Experiment 1	**Proven**	**Horrues**	736	13.001	15°C	193
Experiment 1	**Proven**	**Horrues**	737	13.001	15°C	236
Experiment 1	**Proven**	**Horrues**	738	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	742	13.001	15°C	210
Experiment 1	**Proven**	**Horrues**	743	13.001	15°C	183
Experiment 1	**Proven**	**Horrues**	745	13.001	15°C	193
Experiment 1	**Proven**	**Horrues**	746	13.001	15°C	237
Experiment 1	**Proven**	**Horrues**	749	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	750	13.001	15°C	80
Experiment 1	**Proven**	**Horrues**	751	13.001	15°C	120
Experiment 1	**Proven**	**Horrues**	752	13.001	15°C	191
Experiment 1	**Proven**	**Horrues**	753	13.001	15°C	161
Experiment 1	**Proven**	**Horrues**	754	13.001	15°C	215
Experiment 1	**Proven**	**Horrues**	757	13.001	15°C	170
Experiment 1	**Proven**	**Horrues**	758	13.001	15°C	259
Experiment 1	**Proven**	**Horrues**	759	13.001	15°C	243
Experiment 1	**Proven**	**Horrues**	760	13.001	15°C	212
Experiment 1	**Proven**	**Horrues**	761	13.001	15°C	232
Experiment 1	**Proven**	**Horrues**	769	13.001	15°C	135
Experiment 1	**Proven**	**Horrues**	770	13.001	15°C	159
Experiment 1	**Proven**	**Horrues**	771	13.001	15°C	146
Experiment 1	**Proven**	**Horrues**	772	13.001	15°C	168
Experiment 1	**Proven**	**Horrues**	773	13.001	15°C	163
Experiment 1	**Proven**	**Horrues**	774	13.001	15°C	131
Experiment 1	**Proven**	**Horrues**	782	13.001	15°C	132
Experiment 1	**Proven**	**Horrues**	783	13.001	15°C	125
Experiment 1	**Proven**	**Horrues**	784	13.001	15°C	103
Experiment 1	**Proven**	**Horrues**	785	13.001	15°C	140
Experiment 1	**Proven**	**Horrues**	786	13.001	15°C	184
Experiment 1	**Proven**	**Horrues**	787	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	788	13.001	15°C	119
Experiment 1	**Proven**	**Horrues**	789	13.001	15°C	107
Experiment 1	**Proven**	**Horrues**	790	13.001	15°C	121

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	791	13.001	15°C	116
Experiment 1	**Proven**	**Horrues**	797	13.001	15°C	228
Experiment 1	**Proven**	**Horrues**	798	13.001	15°C	220
Experiment 1	**Proven**	**Horrues**	799	13.001	15°C	208
Experiment 1	**Proven**	**Horrues**	803	13.001	15°C	170
Experiment 1	**Proven**	**Horrues**	804	13.001	15°C	216
Experiment 1	**Proven**	**Horrues**	805	13.001	15°C	198
Experiment 1	**Proven**	**Horrues**	807	13.001	15°C	228
Experiment 1	**Proven**	**Horrues**	808	13.001	15°C	265
Experiment 1	**Proven**	**Horrues**	811	13.001	15°C	240
Experiment 1	**Proven**	**Horrues**	812	13.001	15°C	225
Experiment 1	**Proven**	**Horrues**	813	13.001	15°C	245
Experiment 1	**Proven**	**Horrues**	823	13.001	15°C	135
Experiment 1	**Proven**	**Horrues**	824	13.001	15°C	164
Experiment 1	**Proven**	**Horrues**	825	13.001	15°C	247
Experiment 1	**Proven**	**Horrues**	826	13.001	15°C	176
Experiment 1	**Proven**	**Horrues**	827	13.001	15°C	250
Experiment 1	**Proven**	**Horrues**	828	13.001	15°C	212
Experiment 1	**Proven**	**Horrues**	829	13.001	15°C	145
Experiment 1	**Proven**	**Horrues**	830	13.001	15°C	210
Experiment 1	**Proven**	**Horrues**	831	13.001	15°C	171
Experiment 1	**Proven**	**Horrues**	832	13.001	15°C	169
Experiment 1	**Proven**	**Horrues**	833	13.001	15°C	200
Experiment 1	**Proven**	**Horrues**	834	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	835	13.001	15°C	153
Experiment 1	**Proven**	**Horrues**	836	13.001	15°C	80
Experiment 1	**Proven**	**Horrues**	843	13.001	15°C	138
Experiment 1	**Proven**	**Horrues**	844	13.001	15°C	234
Experiment 1	**Proven**	**Horrues**	845	13.001	15°C	193
Experiment 1	**Proven**	**Horrues**	846	13.001	15°C	128
Experiment 1	**Proven**	**Horrues**	847	13.001	15°C	174
Experiment 1	**Proven**	**Horrues**	848	13.001	15°C	154
Experiment 1	**Proven**	**Horrues**	849	13.001	15°C	211
Experiment 1	**Proven**	**Horrues**	850	13.001	15°C	184
Experiment 1	**Proven**	**Horrues**	851	13.001	15°C	208
Experiment 1	**Proven**	**Horrues**	852	13.001	15°C	215
Experiment 1	**Proven**	**Horrues**	853	13.001	15°C	196
Experiment 1	**Proven**	**Horrues**	854	13.001	15°C	204
Experiment 1	**Proven**	**Horrues**	855	13.001	15°C	143
Experiment 1	**Proven**	**Horrues**	856	13.001	15°C	225
Experiment 1	**Proven**	**Horrues**	857	13.001	15°C	206
Experiment 1	**Proven**	**Horrues**	858	13.001	15°C	173
Experiment 1	**Proven**	**Horrues**	859	13.001	15°C	233
Experiment 1	**Proven**	**Horrues**	860	13.001	15°C	232
Experiment 1	**Proven**	**Horrues**	861	13.001	15°C	209
Experiment 1	**Proven**	**Horrues**	862	13.001	15°C	274
Experiment 1	**Proven**	**Horrues**	865	13.001	15°C	278

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	866	13.001	15°C	226
Experiment 1	**Proven**	**Horrues**	867	13.001	15°C	156
Experiment 1	**Proven**	**Horrues**	868	13.001	15°C	190
Experiment 1	**Proven**	**Horrues**	869	13.001	15°C	141
Experiment 1	**Proven**	**Horrues**	871	13.001	15°C	150
Experiment 1	**Proven**	**Horrues**	872	13.001	15°C	224
Experiment 1	**Proven**	**Horrues**	873	13.001	15°C	228
Experiment 1	**Proven**	**Horrues**	874	13.001	15°C	144
Experiment 1	**Proven**	**Horrues**	875	13.001	15°C	161
Experiment 1	**Proven**	**Horrues**	877	13.001	15°C	221
Experiment 1	**Proven**	**Horrues**	878	13.001	15°C	205
Experiment 1	**Proven**	**Horrues**	879	13.001	15°C	175
Experiment 1	**Proven**	**Horrues**	880	13.001	15°C	138
Experiment 1	**Proven**	**Horrues**	882	13.001	15°C	223
Experiment 1	**Proven**	**Horrues**	883	13.001	15°C	223
Experiment 1	**Proven**	**Horrues**	885	13.001	15°C	271
Experiment 1	**Proven**	**Horrues**	886	13.001	15°C	256
Experiment 1	**Proven**	**Horrues**	891	13.001	15°C	258
Experiment 1	**Proven**	**Horrues**	892	13.001	15°C	196
Experiment 1	**Proven**	**Horrues**	893	13.001	15°C	196
Experiment 1	**Proven**	**Horrues**	894	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	895	13.001	15°C	151
Experiment 1	**Proven**	**Horrues**	896	13.001	15°C	261
Experiment 1	**Proven**	**Horrues**	897	13.001	15°C	217
Experiment 1	**Proven**	**Horrues**	898	13.001	15°C	211
Experiment 1	**Proven**	**Horrues**	899	13.001	15°C	255
Experiment 1	**Proven**	**Horrues**	900	13.001	15°C	285
Experiment 1	**Proven**	**Horrues**	903	13.001	15°C	280
Experiment 1	**Proven**	**Horrues**	904	13.001	15°C	254
Experiment 1	**Proven**	**Horrues**	905	13.001	15°C	174
Experiment 1	**Proven**	**Horrues**	907	13.001	15°C	238
Experiment 1	**Proven**	**Horrues**	911	13.001	15°C	195
Experiment 1	**Proven**	**Horrues**	912	13.001	15°C	265
Experiment 1	**Proven**	**Horrues**	913	13.001	15°C	190
Experiment 1	**Proven**	**Horrues**	914	13.001	15°C	178
Experiment 1	**Proven**	**Horrues**	918	13.001	15°C	230
Experiment 1	**Proven**	**Horrues**	919	13.001	15°C	232
Experiment 1	**Proven**	**Horrues**	925	13.001	15°C	232
Experiment 1	**Proven**	**Horrues**	926	13.001	15°C	252
Experiment 1	**Proven**	**Horrues**	927	13.001	15°C	154
Experiment 1	**Proven**	**Horrues**	928	13.001	15°C	235
Experiment 1	**Proven**	**Horrues**	929	13.001	15°C	211
Experiment 1	**Proven**	**Horrues**	930	13.001	15°C	122
Experiment 1	**Proven**	**Horrues**	932	13.001	15°C	262
Experiment 1	**Proven**	**Horrues**	933	13.001	15°C	245
Experiment 1	**Proven**	**Horrues**	934	13.001	15°C	165

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 1	**Proven**	**Horrues**	935	13.001	15°C	185
Experiment 1	**Proven**	**Horrues**	936	13.001	15°C	225
Experiment 1	**Proven**	**Horrues**	937	13.001	15°C	230
Experiment 1	**Proven**	**Horrues**	939	13.001	15°C	295
Experiment 1	**Proven**	**Horrues**	940	13.001	15°C	216
Experiment 1	**Proven**	**Horrues**	941	13.001	15°C	206
Experiment 1	**Proven**	**Horrues**	942	13.001	15°C	201
Experiment 1	**Proven**	**Horrues**	943	13.001	15°C	187
Experiment 1	**Proven**	**Horrues**	944	13.001	15°C	192
Experiment 1	**Proven**	**Horrues**	945	13.001	15°C	250
Experiment 1	**Proven**	**Horrues**	946	13.001	15°C	275
Experiment 1	**Proven**	**Horrues**	947	13.001	15°C	241
Experiment 1	**Proven**	**Horrues**	949	13.001	15°C	290
Experiment 1	**Proven**	**Horrues**	950	13.001	15°C	250
Experiment 1	**Proven**	**Horrues**	951	13.001	15°C	244
Experiment 1	**Proven**	**Horrues**	952	13.001	15°C	275
Experiment 2	Proven	Horrues	50	14.001	15°C	129
Experiment 2	Proven	Horrues	51	14.001	15°C	69
Experiment 2	Proven	Horrues	52	14.001	15°C	133
Experiment 2	Proven	Horrues	53	14.001	15°C	88
Experiment 2	Proven	Horrues	54	14.001	15°C	88
Experiment 2	Proven	Horrues	55	14.001	15°C	90
Experiment 2	Proven	Horrues	56	14.001	15°C	51
Experiment 2	Proven	Horrues	57	14.001	15°C	113
Experiment 2	Proven	Horrues	58	14.001	15°C	56
Experiment 2	Proven	Horrues	59	14.001	15°C	62
Experiment 2	Proven	Horrues	60	14.001	15°C	60
Experiment 2	Proven	Horrues	61	14.001	15°C	106
Experiment 2	Proven	Horrues	62	14.001	15°C	72
Experiment 2	Proven	Horrues	63	14.001	15°C	78
Experiment 2	Proven	Horrues	64	14.001	15°C	98
Experiment 2	Proven	Horrues	65	14.001	15°C	115
Experiment 2	Proven	Horrues	66	14.001	15°C	101
Experiment 2	Proven	Horrues	67	14.001	15°C	93
Experiment 2	Proven	Horrues	68	14.001	15°C	121
Experiment 2	Proven	Horrues	69	14.001	15°C	98
Experiment 2	Proven	Horrues	70	14.001	15°C	118
Experiment 2	Proven	Horrues	71	14.001	15°C	71
Experiment 2	Proven	Horrues	72	14.001	15°C	120
Experiment 2	Proven	Horrues	73	14.001	15°C	93
Experiment 2	Proven	Horrues	74	14.001	15°C	130
Experiment 2	Proven	Horrues	75	14.001	15°C	114
Experiment 2	Proven	Horrues	76	14.001	15°C	151
Experiment 2	Proven	Horrues	77	14.001	15°C	92
Experiment 2	Proven	Horrues	78	14.001	15°C	72
Experiment 2	Proven	Horrues	79	14.001	15°C	122
Experiment 2	Proven	Horrues	80	14.001	15°C	78

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 2	Proven	Horrues	81	14.001	15°C	115
Experiment 2	Proven	Horrues	82	14.001	15°C	101
Experiment 2	Proven	Horrues	83	14.001	15°C	124
Experiment 2	Proven	Horrues	84	14.001	15°C	85
Experiment 2	Proven	Horrues	85	14.001	15°C	113
Experiment 2	Proven	Horrues	86	14.001	15°C	94
Experiment 2	Proven	Horrues	87	14.001	15°C	112
Experiment 2	Proven	Horrues	88	14.001	15°C	135
Experiment 2	Proven	Horrues	89	14.001	15°C	153
Experiment 2	Proven	Horrues	90	14.001	15°C	89
Experiment 2	Proven	Horrues	91	14.001	15°C	142
Experiment 2	Proven	Horrues	92	14.001	15°C	132
Experiment 2	Proven	Horrues	93	14.001	15°C	125
Experiment 2	Proven	Horrues	94	14.001	15°C	121
Experiment 2	Proven	Horrues	1	14.001	25°C	91
Experiment 2	Proven	Horrues	2	14.001	25°C	94
Experiment 2	Proven	Horrues	3	14.001	25°C	151
Experiment 2	Proven	Horrues	4	14.001	25°C	56
Experiment 2	Proven	Horrues	5	14.001	25°C	51
Experiment 2	Proven	Horrues	6	14.001	25°C	95
Experiment 2	Proven	Horrues	7	14.001	25°C	78
Experiment 2	Proven	Horrues	8	14.001	25°C	77
Experiment 2	Proven	Horrues	9	14.001	25°C	58
Experiment 2	Proven	Horrues	10	14.001	25°C	127
Experiment 2	Proven	Horrues	11	14.001	25°C	100
Experiment 2	Proven	Horrues	12	14.001	25°C	42
Experiment 2	Proven	Horrues	13	14.001	25°C	41
Experiment 2	Proven	Horrues	14	14.001	25°C	109
Experiment 2	Proven	Horrues	15	14.001	25°C	82
Experiment 2	Proven	Horrues	16	14.001	25°C	116
Experiment 2	Proven	Horrues	17	14.001	25°C	100
Experiment 2	Proven	Horrues	18	14.001	25°C	77
Experiment 2	Proven	Horrues	19	14.001	25°C	140
Experiment 2	Proven	Horrues	20	14.001	25°C	58
Experiment 2	Proven	Horrues	21	14.001	25°C	80
Experiment 2	Proven	Horrues	22	14.001	25°C	129
Experiment 2	Proven	Horrues	23	14.001	25°C	99
Experiment 2	Proven	Horrues	24	14.001	25°C	130
Experiment 2	Proven	Horrues	25	14.001	25°C	120
Experiment 2	Proven	Horrues	26	14.001	25°C	149
Experiment 2	Proven	Horrues	27	14.001	25°C	125
Experiment 2	Proven	Horrues	28	14.001	25°C	87
Experiment 2	Proven	Horrues	29	14.001	25°C	80
Experiment 2	Proven	Horrues	30	14.001	25°C	109
Experiment 2	Proven	Horrues	31	14.001	25°C	112
Experiment 2	Proven	Horrues	32	14.001	25°C	92

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 2	Proven	Horrues	33	14.001	25°C	106
Experiment 2	Proven	Horrues	34	14.001	25°C	140
Experiment 2	Proven	Horrues	35	14.001	25°C	95
Experiment 2	Proven	Horrues	36	14.001	25°C	115
Experiment 2	Proven	Horrues	37	14.001	25°C	91
Experiment 2	Proven	Horrues	38	14.001	25°C	102
Experiment 2	Proven	Horrues	39	14.001	25°C	99
Experiment 2	Proven	Horrues	40	14.001	25°C	91
Experiment 2	Proven	Horrues	41	14.001	25°C	77
Experiment 2	Proven	Horrues	42	14.001	25°C	135
Experiment 2	Proven	Horrues	43	14.001	25°C	136
Experiment 2	Proven	Horrues	44	14.001	25°C	120
Experiment 2	Proven	Horrues	45	14.001	25°C	134
Experiment 2	Proven	Horrues	46	14.001	25°C	140
Experiment 2	Proven	Horrues	47	14.001	25°C	57
Experiment 2	Proven	Horrues	48	14.001	25°C	76
Experiment 2	Proven	Horrues	49	14.001	25°C	69
Experiment 2	Proven	Horrues	95	14.001	15°C»25°C	113
Experiment 2	Proven	Horrues	96	14.001	15°C»25°C	131
Experiment 2	Proven	Horrues	97	14.001	15°C»25°C	112
Experiment 2	Proven	Horrues	98	14.001	15°C»25°C	80
Experiment 2	Proven	Horrues	99	14.001	15°C»25°C	100
Experiment 2	Proven	Horrues	100	14.001	15°C»25°C	102
Experiment 2	Proven	Horrues	101	14.001	15°C»25°C	110
Experiment 2	Proven	Horrues	102	14.001	15°C»25°C	83
Experiment 2	Proven	Horrues	103	14.001	15°C»25°C	72
Experiment 2	Proven	Horrues	104	14.001	15°C»25°C	158
Experiment 2	Proven	Horrues	105	14.001	15°C»25°C	44
Experiment 2	Proven	Horrues	106	14.001	15°C»25°C	145
Experiment 2	Proven	Horrues	107	14.001	15°C»25°C	104
Experiment 2	Proven	Horrues	108	14.001	15°C»25°C	66
Experiment 2	Proven	Horrues	109	14.001	15°C»25°C	110
Experiment 2	Proven	Horrues	110	14.001	15°C»25°C	105
Experiment 2	Proven	Horrues	111	14.001	15°C»25°C	123
Experiment 2	Proven	Horrues	112	14.001	15°C»25°C	131
Experiment 3	Meers	Elst	235	14.002	15°C	175
Experiment 3	Meers	Elst	236	14.002	15°C	139
Experiment 3	Meers	Elst	237	14.002	15°C	170
Experiment 3	Meers	Elst	238	14.002	15°C	85
Experiment 3	Meers	Elst	239	14.002	15°C	110
Experiment 3	Meers	Elst	240	14.002	15°C	142
Experiment 3	Meers	Elst	241	14.002	15°C	108
Experiment 3	Meers	Elst	242	14.002	15°C	118
Experiment 3	Meers	Elst	243	14.002	15°C	110
Experiment 3	Meers	Elst	244	14.002	15°C	75
Experiment 3	Meers	Elst	245	14.002	15°C	100
Experiment 3	Meers	Elst	246	14.002	15°C	90

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 3	Meers	Elst	247	14.002	15°C	126
Experiment 3	Meers	Elst	248	14.002	15°C	95
Experiment 3	Meers	Elst	249	14.002	15°C	62
Experiment 3	Meers	Elst	250	14.002	15°C	105
Experiment 3	Meers	Elst	251	14.002	15°C	133
Experiment 3	Meers	Elst	252	14.002	15°C	142
Experiment 3	Meers	Elst	253	14.002	15°C	102
Experiment 3	Meers	Elst	254	14.002	15°C	110
Experiment 3	Meers	Elst	255	14.002	15°C	123
Experiment 3	Meers	Elst	256	14.002	15°C	111
Experiment 3	Meers	Elst	257	14.002	15°C	108
Experiment 3	Meers	Elst	258	14.002	15°C	95
Experiment 3	Meers	Elst	259	14.002	15°C	129
Experiment 3	Meers	Elst	260	14.002	15°C	81
Experiment 3	Meers	Elst	261	14.002	15°C	133
Experiment 3	Meers	Elst	262	14.002	15°C	54
Experiment 3	Meers	Elst	263	14.002	15°C	95
Experiment 3	Meers	Elst	264	14.002	15°C	102
Experiment 3	Meers	Elst	265	14.002	15°C	79
Experiment 3	Meers	Elst	266	14.002	15°C	106
Experiment 3	Meers	Elst	267	14.002	15°C	116
Experiment 3	Meers	Elst	268	14.002	15°C	110
Experiment 3	Meers	Elst	269	14.002	15°C	59
Experiment 3	Meers	Elst	270	14.002	15°C	62
Experiment 3	Meers	Elst	271	14.002	15°C	92
Experiment 3	Meers	Elst	272	14.002	15°C	106
Experiment 3	Meers	Elst	273	14.002	15°C	74
Experiment 3	Meers	Elst	274	14.002	15°C	61
Experiment 3	Meers	Elst	275	14.002	15°C	59
Experiment 3	Meers	Elst	276	14.002	15°C	75
Experiment 3	Meers	Elst	277	14.002	15°C	90
Experiment 3	Meers	Elst	278	14.002	15°C	59
Experiment 3	Meers	Elst	279	14.002	15°C	111
Experiment 3	Oosterzele	Remincourt	421	14.003	15°C	102
Experiment 3	Oosterzele	Remincourt	422	14.003	15°C	82
Experiment 3	Oosterzele	Remincourt	423	14.003	15°C	71
Experiment 3	Oosterzele	Remincourt	424	14.003	15°C	80
Experiment 3	Oosterzele	Remincourt	425	14.003	15°C	60
Experiment 3	Oosterzele	Remincourt	426	14.003	15°C	48
Experiment 3	Oosterzele	Remincourt	427	14.003	15°C	131
Experiment 3	Oosterzele	Remincourt	428	14.003	15°C	41
Experiment 3	Oosterzele	Remincourt	429	14.003	15°C	85
Experiment 3	Oosterzele	Remincourt	430	14.003	15°C	55
Experiment 3	Oosterzele	Remincourt	431	14.003	15°C	90
Experiment 3	Oosterzele	Remincourt	432	14.003	15°C	44
Experiment 3	Oosterzele	Remincourt	433	14.003	15°C	106

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 3	Oosterzele	Remincourt	434	14.003	15°C	81
Experiment 3	Oosterzele	Remincourt	435	14.003	15°C	35
Experiment 3	Oosterzele	Remincourt	436	14.003	15°C	86
Experiment 3	Oosterzele	Remincourt	437	14.003	15°C	102
Experiment 3	Oosterzele	Remincourt	438	14.003	15°C	141
Experiment 3	Oosterzele	Remincourt	439	14.003	15°C	58
Experiment 3	Oosterzele	Remincourt	440	14.003	15°C	109
Experiment 3	Oosterzele	Remincourt	441	14.003	15°C	68
Experiment 3	Oosterzele	Remincourt	442	14.003	15°C	102
Experiment 3	Oosterzele	Remincourt	443	14.003	15°C	136
Experiment 3	Oosterzele	Remincourt	444	14.003	15°C	129
Experiment 3	Oosterzele	Remincourt	445	14.003	15°C	84
Experiment 3	Oosterzele	Remincourt	446	14.003	15°C	86
Experiment 3	Oosterzele	Remincourt	447	14.003	15°C	75
Experiment 3	Oosterzele	Remincourt	448	14.003	15°C	98
Experiment 3	Oosterzele	Remincourt	449	14.003	15°C	109
Experiment 3	Oosterzele	Remincourt	450	14.003	15°C	68
Experiment 3	Oosterzele	Remincourt	451	14.003	15°C	112
Experiment 3	Oosterzele	Remincourt	452	14.003	15°C	110
Experiment 3	Oosterzele	Remincourt	453	14.003	15°C	95
Experiment 3	Oosterzele	Remincourt	454	14.003	15°C	67
Experiment 3	Oosterzele	Remincourt	455	14.003	15°C	81
Experiment 3	Oosterzele	Remincourt	456	14.003	15°C	77
Experiment 3	Oosterzele	Remincourt	457	14.003	15°C	53
Experiment 3	Oosterzele	Remincourt	458	14.003	15°C	95
Experiment 3	Oosterzele	Remincourt	459	14.003	15°C	109
Experiment 3	Oosterzele	Remincourt	460	14.003	15°C	95
Experiment 3	Oosterzele	Remincourt	461	14.003	15°C	62
Experiment 3	Oosterzele	Remincourt	462	14.003	15°C	94
Experiment 3	Oosterzele	Remincourt	463	14.003	15°C	93
Experiment 3	Oosterzele	Remincourt	464	14.003	15°C	63
Experiment 3	Oosterzele	Remincourt	465	14.003	15°C	32
Experiment 3	Oosterzele	Remincourt	466	14.003	15°C	93
Experiment 3	Meers	Elst	189	14.002	25°C	120
Experiment 3	Meers	Elst	190	14.002	25°C	86
Experiment 3	Meers	Elst	191	14.002	25°C	115
Experiment 3	Meers	Elst	192	14.002	25°C	87
Experiment 3	Meers	Elst	193	14.002	25°C	112
Experiment 3	Meers	Elst	194	14.002	25°C	73
Experiment 3	Meers	Elst	195	14.002	25°C	63
Experiment 3	Meers	Elst	196	14.002	25°C	42
Experiment 3	Meers	Elst	197	14.002	25°C	99
Experiment 3	Meers	Elst	198	14.002	25°C	97
Experiment 3	Meers	Elst	199	14.002	25°C	65
Experiment 3	Meers	Elst	200	14.002	25°C	115
Experiment 3	Meers	Elst	201	14.002	25°C	45
Experiment 3	Meers	Elst	202	14.002	25°C	43

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 3	Meers	Elst	203	14.002	25°C	97
Experiment 3	Meers	Elst	204	14.002	25°C	52
Experiment 3	Meers	Elst	205	14.002	25°C	17
Experiment 3	Meers	Elst	206	14.002	25°C	104
Experiment 3	Meers	Elst	207	14.002	25°C	81
Experiment 3	Meers	Elst	208	14.002	25°C	112
Experiment 3	Meers	Elst	209	14.002	25°C	104
Experiment 3	Meers	Elst	210	14.002	25°C	107
Experiment 3	Meers	Elst	211	14.002	25°C	63
Experiment 3	Meers	Elst	212	14.002	25°C	136
Experiment 3	Meers	Elst	213	14.002	25°C	135
Experiment 3	Meers	Elst	214	14.002	25°C	61
Experiment 3	Meers	Elst	215	14.002	25°C	148
Experiment 3	Meers	Elst	216	14.002	25°C	88
Experiment 3	Meers	Elst	217	14.002	25°C	136
Experiment 3	Meers	Elst	218	14.002	25°C	146
Experiment 3	Meers	Elst	219	14.002	25°C	120
Experiment 3	Meers	Elst	220	14.002	25°C	106
Experiment 3	Meers	Elst	221	14.002	25°C	81
Experiment 3	Meers	Elst	222	14.002	25°C	121
Experiment 3	Meers	Elst	223	14.002	25°C	119
Experiment 3	Meers	Elst	224	14.002	25°C	105
Experiment 3	Meers	Elst	225	14.002	25°C	126
Experiment 3	Meers	Elst	226	14.002	25°C	102
Experiment 3	Meers	Elst	227	14.002	25°C	171
Experiment 3	Meers	Elst	228	14.002	25°C	126
Experiment 3	Meers	Elst	229	14.002	25°C	114
Experiment 3	Meers	Elst	230	14.002	25°C	172
Experiment 3	Meers	Elst	231	14.002	25°C	76
Experiment 3	Meers	Elst	232	14.002	25°C	37
Experiment 3	Meers	Elst	233	14.002	25°C	115
Experiment 3	Meers	Elst	234	14.002	25°C	72
Experiment 3	Oosterzele	Remincourt	417	14.003	25°C	116
Experiment 3	Oosterzele	Remincourt	418	14.003	25°C	45
Experiment 3	Oosterzele	Remincourt	419	14.003	25°C	36
Experiment 3	Oosterzele	Remincourt	420	14.003	25°C	66
Experiment 3	Meers	Elst	280	14.002	15°C»25°C	140
Experiment 3	Meers	Elst	281	14.002	15°C»25°C	93
Experiment 3	Meers	Elst	282	14.002	15°C»25°C	154
Experiment 3	Meers	Elst	283	14.002	15°C»25°C	83
Experiment 3	Meers	Elst	284	14.002	15°C»25°C	55
Experiment 3	Meers	Elst	285	14.002	15°C»25°C	92
Experiment 3	Meers	Elst	286	14.002	15°C»25°C	71
Experiment 3	Meers	Elst	287	14.002	15°C»25°C	0
Experiment 3	Meers	Elst	288	14.002	15°C»25°C	98
Experiment 3	Meers	Elst	289	14.002	15°C»25°C	102

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 3	Meers	Elst	290	14.002	15°C»25°C	77
Experiment 3	Meers	Elst	291	14.002	15°C»25°C	100
Experiment 3	Meers	Elst	292	14.002	15°C»25°C	136
Experiment 3	Meers	Elst	293	14.002	15°C»25°C	99
Experiment 3	Meers	Elst	294	14.002	15°C»25°C	89
Experiment 3	Meers	Elst	295	14.002	15°C»25°C	68
Experiment 3	Meers	Elst	296	14.002	15°C»25°C	84
Experiment 3	Meers	Elst	297	14.002	15°C»25°C	95
Experiment 3	Meers	Elst	298	14.002	15°C»25°C	35
Experiment 3	Meers	Elst	299	14.002	15°C»25°C	60
Experiment 3	Meers	Elst	300	14.002	15°C»25°C	120
Experiment 3	Meers	Elst	301	14.002	15°C»25°C	137
Experiment 3	Meers	Elst	302	14.002	15°C»25°C	93
Experiment 3	Meers	Elst	303	14.002	15°C»25°C	40
Experiment 3	Meers	Elst	304	14.002	15°C»25°C	122
Experiment 3	Meers	Elst	305	14.002	15°C»25°C	69
Experiment 3	Meers	Elst	306	14.002	15°C»25°C	132
Experiment 3	Meers	Elst	307	14.002	15°C»25°C	82
Experiment 3	Meers	Elst	308	14.002	15°C»25°C	71
Experiment 3	Meers	Elst	309	14.002	15°C»25°C	110
Experiment 3	Meers	Elst	310	14.002	15°C»25°C	75
Experiment 3	Meers	Elst	311	14.002	15°C»25°C	75
Experiment 3	Meers	Elst	312	14.002	15°C»25°C	74
Experiment 3	Meers	Elst	313	14.002	15°C»25°C	128
Experiment 3	Meers	Elst	314	14.002	15°C»25°C	95
Experiment 3	Meers	Elst	315	14.002	15°C»25°C	88
Experiment 3	Meers	Elst	316	14.002	15°C»25°C	122
Experiment 3	Meers	Elst	317	14.002	15°C»25°C	85
Experiment 3	Meers	Elst	318	14.002	15°C»25°C	43
Experiment 3	Meers	Elst	319	14.002	15°C»25°C	47
Experiment 3	Meers	Elst	320	14.002	15°C»25°C	138
Experiment 3	Meers	Elst	321	14.002	15°C»25°C	73
Experiment 3	Meers	Elst	322	14.002	15°C»25°C	85
Experiment 3	Meers	Elst	323	14.002	15°C»25°C	110
Experiment 3	Oosterzele	Remincourt	467	14.003	15°C»25°C	47
Experiment 3	Oosterzele	Remincourt	468	14.003	15°C»25°C	68
Experiment 3	Oosterzele	Remincourt	469	14.003	15°C»25°C	131
Experiment 3	Oosterzele	Remincourt	470	14.003	15°C»25°C	57
Experiment 3	Oosterzele	Remincourt	471	14.003	15°C»25°C	107
Experiment 3	Oosterzele	Remincourt	472	14.003	15°C»25°C	86
Experiment 3	Oosterzele	Remincourt	473	14.003	15°C»25°C	89
Experiment 3	Oosterzele	Remincourt	474	14.003	15°C»25°C	51
Experiment 3	Oosterzele	Remincourt	475	14.003	15°C»25°C	42
Experiment 3	Oosterzele	Remincourt	476	14.003	15°C»25°C	101
Experiment 3	Oosterzele	Remincourt	477	14.003	15°C»25°C	108
Experiment 3	Oosterzele	Remincourt	478	14.003	15°C»25°C	54
Experiment 3	Oosterzele	Remincourt	479	14.003	15°C»25°C	45

Experiment	Mother	Father	ID	Cross	Temperature	Height (cm)
Experiment 3	Oosterzele	Remincourt	480	14.003	15°C»25°C	92
Experiment 3	Oosterzele	Remincourt	481	14.003	15°C»25°C	97
Experiment 3	Oosterzele	Remincourt	482	14.003	15°C»25°C	57
Experiment 3	Oosterzele	Remincourt	483	14.003	15°C»25°C	64
Experiment 3	Oosterzele	Remincourt	484	14.003	15°C»25°C	94
Experiment 3	Oosterzele	Remincourt	485	14.003	15°C»25°C	115
Experiment 3	Oosterzele	Remincourt	486	14.003	15°C»25°C	105
Experiment 3	Oosterzele	Remincourt	487	14.003	15°C»25°C	74
Experiment 3	Oosterzele	Remincourt	488	14.003	15°C»25°C	111
Experiment 3	Oosterzele	Remincourt	489	14.003	15°C»25°C	90
Experiment 3	Oosterzele	Remincourt	490	14.003	15°C»25°C	102
Experiment 3	Oosterzele	Remincourt	491	14.003	15°C»25°C	110
Experiment 3	Oosterzele	Remincourt	492	14.003	15°C»25°C	108
Experiment 3	Oosterzele	Remincourt	493	14.003	15°C»25°C	130
Experiment 3	Oosterzele	Remincourt	494	14.003	15°C»25°C	91
Experiment 3	Oosterzele	Remincourt	495	14.003	15°C»25°C	12
Experiment 3	Oosterzele	Remincourt	496	14.003	15°C»25°C	25
Experiment 3	Oosterzele	Remincourt	497	14.003	15°C»25°C	67
Experiment 3	Oosterzele	Remincourt	498	14.003	15°C»25°C	79
Experiment 3	Oosterzele	Remincourt	499	14.003	15°C»25°C	37
Experiment 3	Oosterzele	Remincourt	500	14.003	15°C»25°C	70
Experiment 3	Oosterzele	Remincourt	501	14.003	15°C»25°C	87
Experiment 3	Oosterzele	Remincourt	502	14.003	15°C»25°C	44
Experiment 3	Oosterzele	Remincourt	503	14.003	15°C»25°C	126
Experiment 3	Oosterzele	Remincourt	504	14.003	15°C»25°C	25
Experiment 3	Oosterzele	Remincourt	505	14.003	15°C»25°C	65
Experiment 3	Oosterzele	Remincourt	506	14.003	15°C»25°C	93
Experiment 3	Oosterzele	Remincourt	507	14.003	15°C»25°C	79
Experiment 3	Oosterzele	Remincourt	508	14.003	15°C»25°C	102
Experiment 3	Oosterzele	Remincourt	509	14.003	15°C»25°C	113
Experiment 3	Oosterzele	Remincourt	510	14.003	15°C»25°C	102
Experiment 3	Oosterzele	Remincourt	511	14.003	15°C»25°C	80
Experiment 3	Oosterzele	Remincourt	512	14.003	15°C»25°C	103
Experiment 3	Oosterzele	Remincourt	513	14.003	15°C»25°C	85
Experiment 3	Oosterzele	Remincourt	514	14.003	15°C»25°C	84

Appendix D Transgenerational effects in asexually reproduced offspring of *Populus*

Appendix Table D.1 Description of the scoring systems of bud burst and bud set in poplar cuttings based on visual observations.

Bud burst score	**Description of visual evaluation**
0	Dormant bud; no sign of any physiological activity
1	Buds were slightly swollen and the bud scales reddishly coloured
2	Buds were fully swollen and turned towards a rounded shape, no sign of breakage of buds
3	Buds started breaking, wet and sticky, tip of reddish shoots appeared
4	Bud burst and reddish shoots turned towards a green colour, very young leaves could be observed
5	Green leaves started growing and venation of leaf could be observed
Bud set score	
3	More than two rolled-up leaves
2	Last leaf (partially) rolled-up, other leaves fully stretched
1	Bud well visible, bud scales predominantly green colour, all leaves are stretched
0	Apical bud reddish-brown colour

Appendix Table D.2 Number of individuals collected and monitored from respective genotpes and country for determination of DNA mathylation and bud phenology (bud burst and set). In total, we samples 54 leaf samples for DNA methylation. But we removed one sample due to the mismatch after verifying the identity of the genotype.

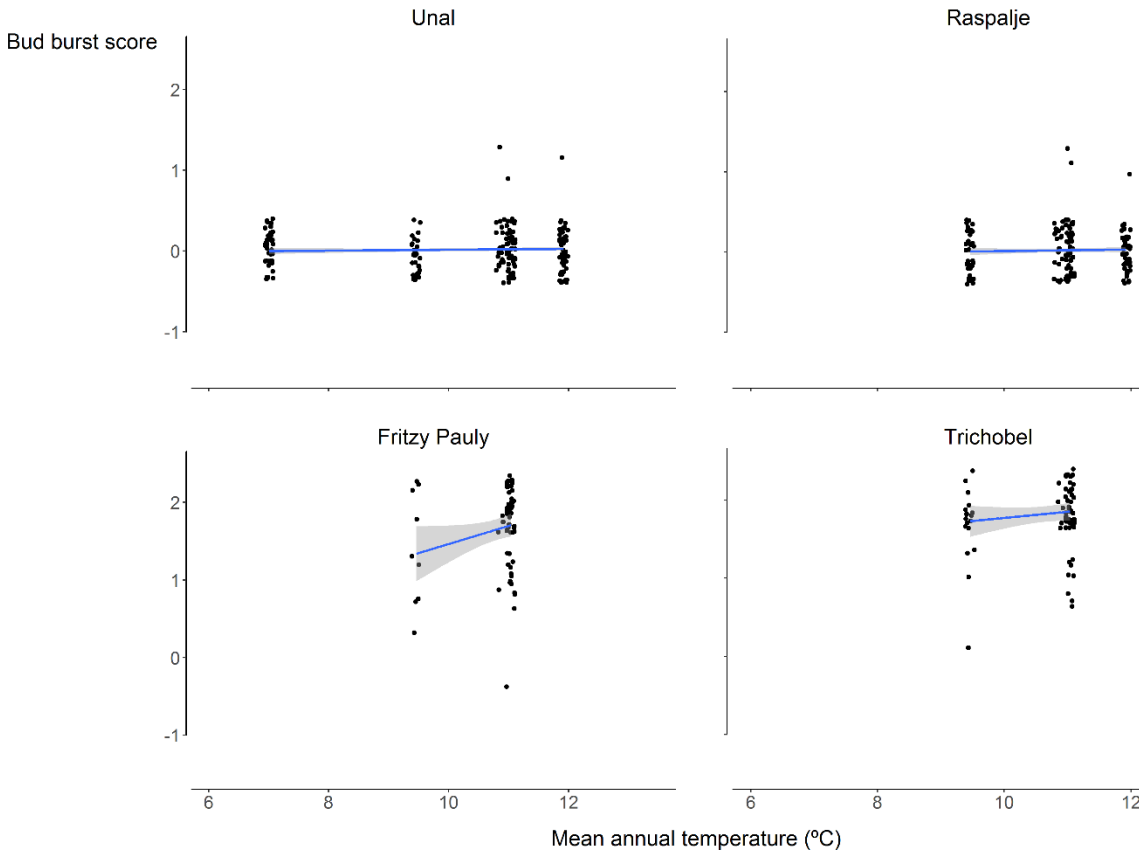
Country	**Name of the genotypes**	**No of individuals**			
		DNA methylation	**Bud burst 2015**	**Bud set 2014**	**Bud set 2015**
Belgium					
	Beaupré	2	18	19	18
	Fritzi Pauley		4	4	3
	Raspalje		20	20	19
	Trichobel	2	5	5	5
	Unal	3	20	20	19
France (Beuxes)					
	Beaupré	3	25	28	21
France (Gueméne Penfao)	Beaupré	4	49	48	50
	Fritzi Pauley	2	50	47	48
	Raspalje		49	48	49
	Trichobel	4	49	42	49
	Unal	4	50	50	48
France (Saint-Usage)					
	Beaupré	5	18	19	13
Italy					
	Beaupré	4	32	33	31
	Fritzi Pauley	2	9	9	7
	Raspalje	3	34	34	31
	Trichobel	2	18	18	17
	Unal	3	30	29	30
Spain					
	Beaupré	3	49	49	48
	Raspalje		46	47	42
	Unal	4	50	49	48
Sweden	Unal	3	39	38	38

Appendix Table D.3 List of the microsatellite markers used for the genotype identification. TA; annealing temperature, MP: multiplex

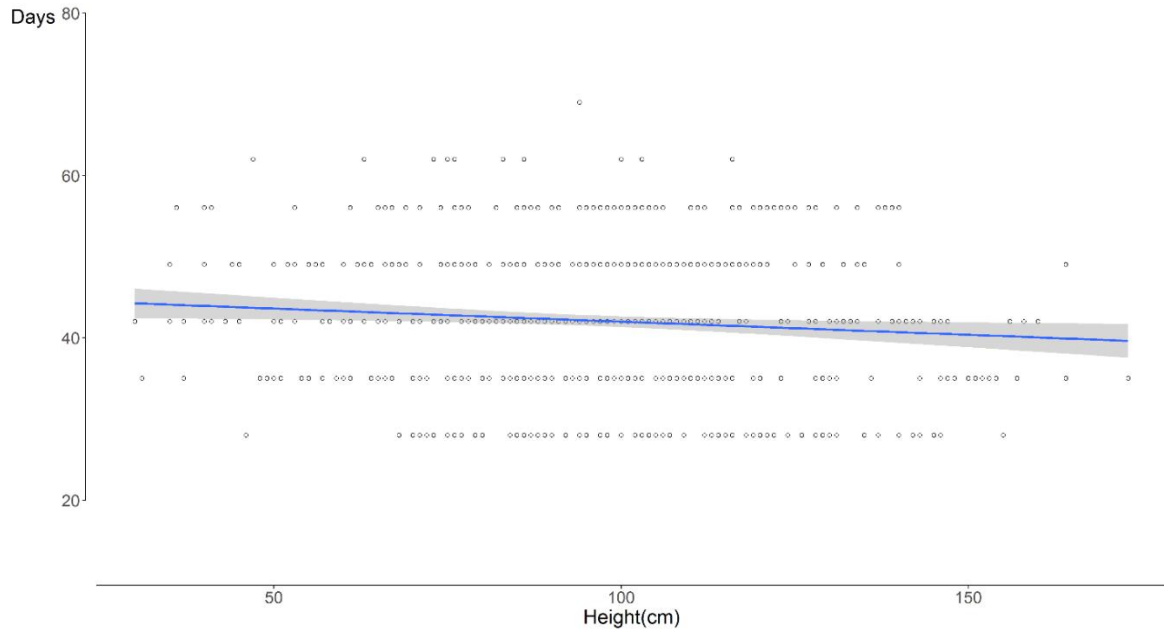
Sl. No.	Locus	LeftPrimer /forward (5′→3′)	RightPrimer/reverse (5′→3′)	Motif	Expected Product length	TA	MP	Dye
1	PMGC_14	TTCAGAATGTGCATGATGG	GTGATGATCTCACCGTTTG	CTT	179 - 227	52	1	FAM
2	PMGC_2163	CAATCGAAGGTAAGGTTAGTG	CGTTGGACATAGATCACACG	GA	198 - 220	52	1	NED
3	WPMS_05	TTCTTTTTCAACTGCCTAACTT	TGATCCAATAACAGACAGAACA	GT	263 - 291	52	1	VIC
4	WPMS_16	CTCGTACTATTTCCGATGATGACC	AGATTATTAGGTGGGCCAAGGACT	GTC	128-167	52	1	PET
5	ORPM 312	GTGGGGATCAATCCAAAAGA	CCCATATCAAACCATTTGAAAAA	CCT	189–201		2	FAM
6	WPMS_20	GTGCGCACATCTATGACTATCG	ATCTTGTAATTCTCCGGGCATCT	TTCTGG	224 - 242	57	2	NED
7	PTR2	AAGAAGAACTCGAAGATGAAGAACT	ACTGACAAAACCCCTAATCTAACAA	TGG	207 -228	57	2	VIC
8	PTR7	ATTTGATGCCTCTTCCTTCCAGT	TATTTTCATTTTCCCTTTGCTTT	(CT)5AT(CT)	230 -250	57	2	PET
9	WPMS_14	CAGCCGCAGCCACTGAGAAATC	GCCTGCTGAGAAGACTGCCTTGAC	CGT	221 - 304	57	3	FAM
10	WPMS_15	CAACAAACCATCAATGAAGAAGAC	AGAGGGTGTTGGGGGTGACTA	CCT	188 - 203	57	3	NED
11	WPMS_19	AGCCACAGCAAATTCAGATGATGC	CCTGCTGAGAAGACTGCCTTGACA	CAG	174-252	57	3	VIC
12	WPMS_22	ACATGCTACGTGTTTGGAATG	ATCGTATGGATGTAATTGTCTTA	TGA	100-135	57	3	FAM

Appendix Table D.4 Primer combinations used, number of loci and estimated genotyping error rates

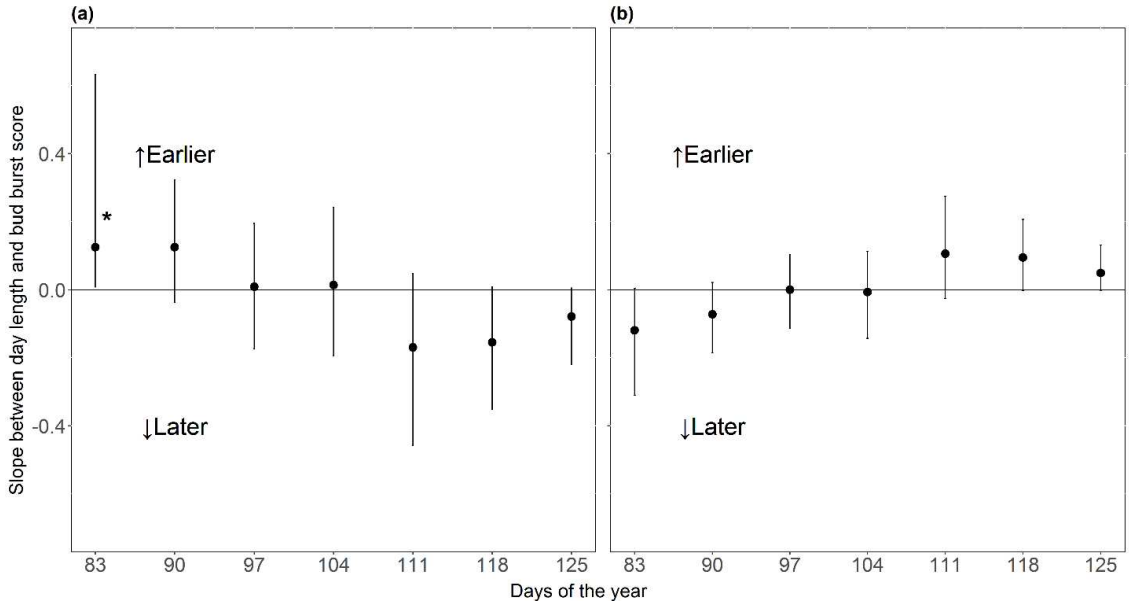
Primer combinations	Number of polymorphic fragments	Estimated genotyping error rate
*Eco*RI + ACC / *Hpa*II-*Msp*I + TAC	26	0.000
*Eco*RI + ACC / *Hpa*II-*Msp*I + TAG	38	0.025
*Eco*RI + AGC / *Hpa*II-*Msp*I + TCC	25	0.000
*Eco*RI + AGC / *Hpa*II-*Msp*I + TCT	33	0.029
*Eco*RI + AGC / *Hpa*II-*Msp*I + TCG	24	0.040
*Eco*RI + AGC / *Hpa*II-*Msp*I + TAA	61	0.024
*Eco*RI + ACT / *Hpa*II-*Msp*I + TAG	26	0.037
Total	*233*	
Mean		*0.022*



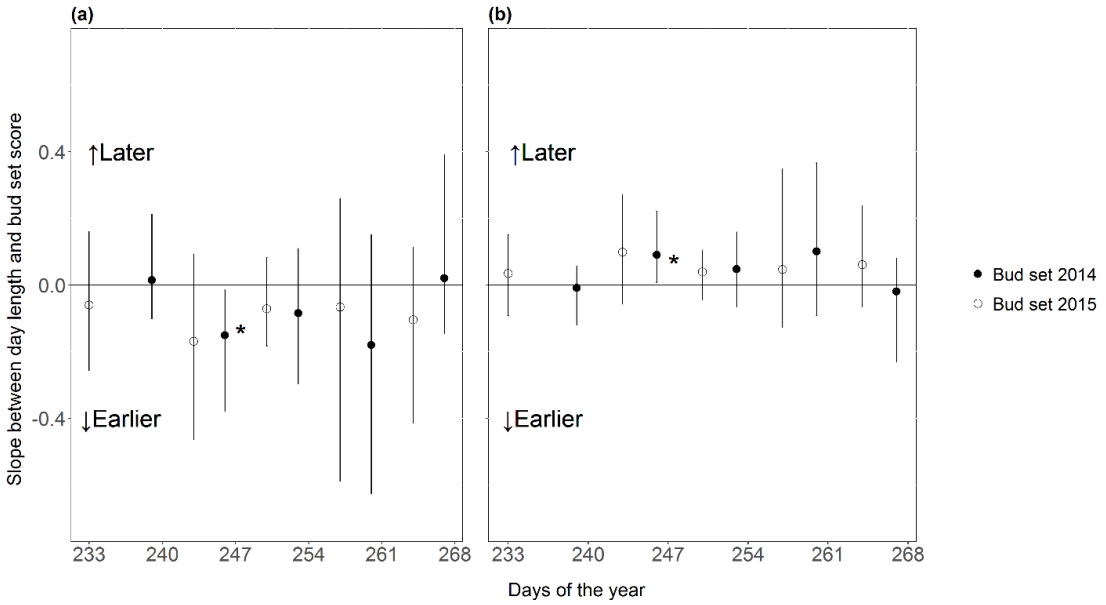
Appendix Figure D.1 The relationship between mean annual temperature (MAT) and mean bud burst score in the cuttings of four different clones on 2nd observation (83th day of the year-DOY) where a, b, c and d represents respectively for genotypes Unal, Raspalje, Fritzy Pauley and Trichobel. On 83 DOY the variance in bud burst score for clone Beaupré was o (zero).



Appendix Figure D.2 The relationship between number of days to bud burst in 2015 and the height of the seedlings during end of growing season in December 2014



Appendix Figure D.3 Mean weighted (bootstrapped) slopes of the relationship between the mean bud burst score in 2015 and day lengths on 1 May (a) and 1 January (b) experienced by the parent trees. Significances at the 95% level are denoted by *. “Earlier” means that buds set earlier with increasing temperature and “Later” means that buds set later with increasing temperature. Error bars denote 95% confidence interval (upper and lower) across the 500 bootstrapped values.



Appendix Figure D.4 Mean weighted (bootstrapped) slopes of the relationship between the mean bud set score in 2014 and 2015 and day lengths on 1 May (a) and 1 January (b) experienced by the parent trees. Error bars denote 95% confidence interval (upper and lower) across the 500 bootstrapped values. Significances at the 95% level are denoted by *. “Earlier” means that buds set earlier with increasing temperature and “Later” means that buds set later with increasing temperature.

Appendix Table D.5 The results from the linear mixed effect models (in response to temperature variables and stem diameter). NA means no data available due to 0 (zero) variance in response variable. We used *lmerTest* package to extract the p values from the linear mixed effects models (Kuznetsova et al., 2017).

Fixed effects															
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
Bud set 2014	Beaupre	239	Temperature	0.01	0.10	0.12	0.91	0.04	0.09	0.45	0.65	0.00	0.07	-0.06	0.96
		239	Dia_stem	0.03	0.01	2.99	0.00	0.03	0.01	2.99	0.00	0.03	0.01	2.99	0.00
	Fritzy P.	239	Temperature	0.16	0.12	1.40	0.16	-0.03	0.34	-0.08	0.94	0.12	0.09	1.36	0.17
		239	Dia_stem	-0.02	0.03	-0.53	0.59	-0.01	0.03	-0.21	0.84	-0.01	0.03	-0.49	0.63
	Raspalje	239	Temperature	-0.04	0.04	-1.19	0.24	-0.03	0.02	-1.31	0.19	-0.02	0.03	-0.52	0.60
		239	Dia_stem	0.02	0.01	1.45	0.15	0.02	0.01	1.60	0.11	0.03	0.01	2.16	0.03
	Trichobel	239	Temperature	0.00	0.09	0.04	0.97	0.05	0.27	0.18	0.86	0.00	0.07	0.01	0.99
		239	Dia_stem	0.03	0.04	0.78	0.44	0.03	0.03	0.83	0.41	0.03	0.04	0.80	0.42
	Unal	239	Temperature	0.04	0.02	2.29	0.02	0.04	0.02	2.22	0.03	0.04	0.02	1.96	0.05
		239	Dia_stem	0.01	0.01	1.18	0.24	0.01	0.01	0.87	0.38	0.02	0.01	1.42	0.16
	Beaupre	246	Temperature	-0.03	0.09	-0.34	0.73	0.00	0.08	-0.04	0.97	-0.02	0.06	-0.30	0.76
		246	Dia_stem	0.01	0.01	0.48	0.63	0.01	0.01	0.43	0.67	0.01	0.01	0.48	0.63
	Fritzy P.	246	Temperature	-0.18	0.16	-1.07	0.28	0.13	0.41	0.32	0.75	-0.14	0.12	-1.14	0.26
		246	Dia_stem	0.02	0.04	0.39	0.70	0.00	0.04	-0.05	0.96	0.02	0.04	0.40	0.69
	Raspalje	246	Temperature	0.02	0.06	0.36	0.72	0.04	0.03	1.23	0.22	-0.01	0.05	-0.26	0.79
		246	Dia_stem	0.04	0.02	1.92	0.05	0.04	0.02	2.37	0.02	0.03	0.02	1.79	0.07
	Trichobel	246	Temperature	-0.10	0.12	-0.86	0.39	-0.24	0.35	-0.70	0.48	-0.06	0.09	-0.71	0.48
		246	Dia_stem	-0.01	0.05	-0.16	0.87	-0.02	0.05	-0.44	0.66	-0.01	0.05	-0.25	0.80
	Unal	246	Temperature	0.04	0.02	1.92	0.05	0.04	0.03	1.35	0.18	0.03	0.02	1.84	0.07
		246	Dia_stem	0.00	0.01	0.38	0.71	0.00	0.01	-0.13	0.90	0.01	0.01	0.72	0.47
	Beaupre	253	Temperature	0.02	0.08	0.22	0.83	0.01	0.07	0.15	0.88	0.01	0.06	0.24	0.81
		253	Dia_stem	0.01	0.01	0.36	0.72	0.01	0.01	0.35	0.73	0.01	0.01	0.37	0.71

Fixed effects													
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
	Fritzy P.	253	Temperature	-0.07	0.17	-0.43	0.67	0.04	0.39	0.10	0.92	-0.06	0.12	-0.45	0.65
		253	Dia_stem	0.00	0.04	0.08	0.93	-0.01	0.04	-0.14	0.89	0.00	0.04	0.08	0.93
	Raspalje	253	Temperature	0.02	0.05	0.41	0.68	-0.01	0.03	-0.31	0.76	0.04	0.04	0.93	0.35
		253	Dia_stem	0.01	0.01	0.93	0.35	0.01	0.01	0.66	0.51	0.01	0.01	1.10	0.27
	Trichobel	253	Temperature	-0.15	0.13	-1.19	0.24	-0.05	0.39	-0.13	0.90	-0.10	0.09	-1.13	0.26
		253	Dia_stem	-0.02	0.05	-0.46	0.64	-0.04	0.05	-0.81	0.42	-0.03	0.05	-0.52	0.60
	Unal	253	Temperature	0.06	0.02	2.79	0.01	0.06	0.03	1.87	0.06	0.06	0.02	2.66	0.01
		253	Dia_stem	0.01	0.01	0.62	0.54	0.00	0.02	0.03	0.97	0.01	0.01	1.05	0.29
	Beaupre	260	Temperature	-0.03	0.04	-0.78	0.44	-0.06	0.02	-2.36	0.02	0.00	0.03	0.03	0.98
		260	Dia_stem	-0.01	0.01	-0.91	0.37	-0.01	0.01	-0.52	0.60	-0.01	0.01	-1.01	0.31
	Fritzy P.	260	Temperature	-0.23	0.17	-1.31	0.19	0.37	0.51	0.73	0.47	-0.19	0.13	-1.47	0.14
		260	Dia_stem	0.02	0.05	0.40	0.69	0.00	0.04	0.04	0.97	0.02	0.04	0.45	0.65
	Raspalje	260	Temperature	0.05	0.07	0.73	0.46	0.06	0.03	2.00	0.05	-0.01	0.07	-0.20	0.84
		260	Dia_stem	0.04	0.02	2.05	0.04	0.05	0.02	2.53	0.01	0.04	0.02	1.81	0.07
	Trichobel	260	Temperature	-0.27	0.09	-2.94	0.00	0.02	0.52	0.05	0.96	-0.19	0.07	-2.79	0.01
		260	Dia_stem	0.02	0.04	0.58	0.56	0.02	0.04	0.50	0.62	0.02	0.04	0.45	0.66
	Unal	260	Temperature	0.04	0.03	1.31	0.19	0.06	0.02	3.16	0.00	0.02	0.03	0.83	0.40
		260	Dia_stem	-0.01	0.01	-0.96	0.34	-0.02	0.01	-1.60	0.11	-0.01	0.01	-0.83	0.41
	Beaupre	267	Temperature	-0.03	0.03	-1.07	0.28	0.01	0.02	0.33	0.74	-0.03	0.02	-1.77	0.08
		267	Dia_stem	0.00	0.01	-0.27	0.78	0.00	0.01	-0.40	0.69	0.00	0.01	-0.20	0.84
	Fritzy P.	267	Temperature	0.06	0.37	0.16	0.87	-0.74	0.22	-3.36	0.00	0.10	0.23	0.44	0.66
		267	Dia_stem	0.02	0.02	0.79	0.43	0.01	0.02	0.64	0.52	0.02	0.02	0.76	0.45
	Raspalje	267	Temperature	0.04	0.04	0.99	0.32	0.01	0.02	0.59	0.55	0.02	0.03	0.70	0.49
		267	Dia_stem	0.01	0.01	0.67	0.50	0.01	0.01	0.40	0.69	0.00	0.01	0.39	0.70
	Trichobel	267	Temperature	-0.09	0.07	-1.30	0.20	-0.06	0.24	-0.24	0.81	-0.06	0.05	-1.20	0.23
		267	Dia_stem	0.00	0.03	-0.13	0.90	-0.01	0.03	-0.40	0.69	-0.01	0.03	-0.20	0.84

Fixed effects															
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
	Unal	267	Temperature	0.01	0.02	0.44	0.66	0.02	0.02	1.12	0.26	0.00	0.02	0.05	0.96
		267	Dia_stem	-0.02	0.01	-1.68	0.09	-0.02	0.01	-1.88	0.06	-0.02	0.01	-1.63	0.10
Bud set 2015	Beaupre	233	Temperature	0.08	0.13	0.62	0.53	0.10	0.10	0.95	0.34	0.03	0.10	0.33	0.74
		233	Dia_stem	0.06	0.02	3.06	0.00	0.06	0.02	3.12	0.00	0.06	0.02	3.03	0.00
	Fritzy P.	233	Temperature	-0.02	0.12	-0.15	0.88	-0.23	0.31	-0.73	0.46	0.00	0.09	0.02	0.99
		233	Dia_stem	-0.02	0.03	-0.51	0.61	-0.01	0.03	-0.49	0.62	-0.02	0.03	-0.57	0.57
	Raspalje	233	Temperature	-0.04	0.08	-0.50	0.62	-0.06	0.03	-1.78	0.08	0.01	0.08	0.15	0.88
		233	Dia_stem	0.01	0.02	0.52	0.60	0.01	0.02	0.30	0.76	0.01	0.02	0.77	0.44
	Trichobel	233	Temperature	0.03	0.14	0.22	0.83	0.33	0.24	1.38	0.17	0.00	0.10	-0.04	0.97
		233	Dia_stem	0.02	0.03	0.52	0.61	0.02	0.03	0.69	0.49	0.02	0.03	0.56	0.58
	Unal	233	Temperature	0.03	0.03	0.85	0.39	0.04	0.03	1.16	0.24	0.02	0.03	0.66	0.51
		233	Dia_stem	0.03	0.02	1.37	0.17	0.02	0.02	1.00	0.32	0.03	0.02	1.60	0.11
	Beaupre	243	Temperature	-0.10	0.14	-0.69	0.49	0.02	0.13	0.13	0.90	-0.09	0.10	-0.92	0.36
		243	Dia_stem	0.06	0.02	2.75	0.01	0.06	0.02	2.59	0.01	0.07	0.02	2.85	0.00
	Fritzy P.	243	Temperature	-0.04	0.15	-0.30	0.77	0.01	0.37	0.03	0.98	-0.03	0.11	-0.30	0.76
		243	Dia_stem	0.03	0.04	0.74	0.46	0.02	0.04	0.67	0.50	0.03	0.04	0.74	0.46
	Raspalje	243	Temperature	-0.12	0.08	-1.53	0.13	-0.08	0.04	-1.94	0.05	-0.05	0.09	-0.58	0.56
		243	Dia_stem	0.00	0.03	0.05	0.96	0.00	0.02	0.17	0.86	0.01	0.03	0.35	0.73
	Trichobel	243	Temperature	0.05	0.40	0.13	0.89	0.79	0.40	1.96	0.05	-0.03	0.27	-0.12	0.90
		243	Dia_stem	-0.03	0.05	-0.67	0.50	-0.02	0.05	-0.46	0.64	-0.03	0.05	-0.65	0.52
	Unal	243	Temperature	0.08	0.05	1.58	0.11	0.12	0.04	2.82	0.00	0.05	0.06	0.90	0.37
		243	Dia_stem	0.01	0.03	0.20	0.84	-0.01	0.03	-0.42	0.67	0.01	0.03	0.36	0.72
	Beaupre	250	Temperature	-0.02	0.09	-0.26	0.79	0.03	0.08	0.39	0.70	-0.03	0.06	-0.54	0.59
		250	Dia_stem	0.07	0.02	3.18	0.00	0.06	0.02	3.01	0.00	0.07	0.02	3.41	0.00
	Fritzy P.	250	Temperature	-0.19	0.19	-1.04	0.30	-0.41	0.47	-0.86	0.39	-0.12	0.14	-0.85	0.40
		250	Dia_stem	0.02	0.05	0.41	0.68	0.01	0.04	0.16	0.87	0.01	0.05	0.32	0.75

Fixed effects															
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
	Raspalje	250	Temperature	-0.06	0.12	-0.47	0.64	-0.06	0.06	-0.94	0.35	-0.01	0.12	-0.12	0.90
		250	Dia_stem	0.03	0.03	0.96	0.34	0.03	0.03	0.95	0.34	0.03	0.03	1.14	0.25
	Trichobel	250	Temperature	0.06	0.13	0.47	0.64	0.29	0.38	0.77	0.44	0.03	0.10	0.31	0.76
		250	Dia_stem	-0.01	0.05	-0.12	0.91	0.00	0.05	-0.06	0.95	0.00	0.05	-0.05	0.96
	Unal	250	Temperature	0.06	0.06	0.91	0.37	0.11	0.04	2.75	0.01	0.03	0.06	0.49	0.62
		250	Dia_stem	0.03	0.03	1.24	0.21	0.02	0.02	0.61	0.54	0.04	0.03	1.36	0.17
	Beaupre	257	Temperature	-0.04	0.04	-0.98	0.33	0.01	0.03	0.22	0.83	-0.05	0.03	-1.50	0.13
		257	Dia_stem	0.03	0.01	2.63	0.01	0.04	0.01	2.68	0.01	0.03	0.01	2.65	0.01
	Fritzy P.	257	Temperature	-0.22	0.20	-1.12	0.26	0.48	0.57	0.84	0.40	-0.19	0.15	-1.31	0.19
		257	Dia_stem	0.01	0.05	0.10	0.92	-0.01	0.05	-0.29	0.77	0.01	0.05	0.13	0.90
	Raspalje	257	Temperature	-0.04	0.10	-0.38	0.70	-0.07	0.05	-1.52	0.13	0.04	0.09	0.41	0.68
		257	Dia_stem	0.03	0.03	1.02	0.31	0.02	0.03	0.73	0.47	0.04	0.03	1.49	0.14
	Trichobel	257	Temperature	0.05	0.15	0.34	0.73	0.30	0.42	0.73	0.47	0.02	0.11	0.20	0.84
		257	Dia_stem	-0.02	0.05	-0.28	0.78	-0.01	0.05	-0.27	0.78	-0.01	0.05	-0.23	0.82
	Unal	257	Temperature	-0.02	0.04	-0.40	0.69	0.01	0.04	0.12	0.90	-0.02	0.04	-0.54	0.59
		257	Dia_stem	0.04	0.02	2.09	0.04	0.04	0.02	1.81	0.07	0.04	0.02	2.12	0.03
	Beaupre	264	Temperature	-0.03	0.02	-1.44	0.15	0.00	0.02	-0.25	0.80	-0.02	0.01	-1.69	0.09
		264	Dia_stem	0.01	0.01	1.87	0.06	0.01	0.01	1.91	0.06	0.01	0.01	1.87	0.06
	Fritzy P.	264	Temperature	-0.03	0.16	-0.22	0.83	0.26	0.41	0.63	0.53	-0.04	0.12	-0.36	0.72
		264	Dia_stem	-0.05	0.04	-1.22	0.22	-0.06	0.04	-1.47	0.14	-0.05	0.04	-1.19	0.23
	Raspalje	264	Temperature	-0.06	0.07	-0.85	0.40	-0.04	0.04	-1.16	0.24	-0.01	0.06	-0.11	0.91
		264	Dia_stem	0.02	0.02	0.69	0.49	0.02	0.02	0.71	0.48	0.03	0.02	1.29	0.20
	Trichobel	264	Temperature	-0.04	0.28	-0.13	0.90	0.60	0.36	1.69	0.09	-0.07	0.18	-0.38	0.71
		264	Dia_stem	0.02	0.05	0.38	0.70	0.02	0.04	0.40	0.69	0.02	0.05	0.42	0.67
	Unal	264	Temperature	0.01	0.03	0.33	0.74	0.01	0.03	0.34	0.74	0.01	0.03	0.38	0.70
		264	Dia_stem	0.00	0.01	0.34	0.73	0.00	0.01	0.24	0.81	0.01	0.01	0.39	0.69

Fixed effects															
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
Bud burst 2015	Beaupre	83	Temperature	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		83	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Fritzy P.	83	Temperature	-0.25	0.15	-1.71	0.09	-0.12	0.06	-1.85	0.07	0.28	0.16	1.82	0.07
		83	Dia_stem	-0.01	0.03	-0.18	0.86	-0.01	0.04	-0.31	0.75	-0.01	0.04	-0.39	0.70
	Raspalje	83	Temperature	-0.01	0.02	-0.28	0.80	0.00	0.01	-0.43	0.72	0.03	0.02	1.32	0.19
		83	Dia_stem	0.00	0.00	0.07	0.94	0.00	0.00	0.03	0.98	0.00	0.01	0.57	0.57
	Trichobel	83	Temperature	-0.13	0.09	-1.46	0.15	-0.05	0.04	-1.42	0.16	0.10	0.09	1.11	0.27
		83	Dia_stem	-0.02	0.03	-0.57	0.57	-0.02	0.03	-0.61	0.54	-0.02	0.03	-0.58	0.56
	Unal	83	Temperature	0.00	0.01	0.77	0.45	0.00	0.01	-0.14	0.89	0.00	0.00	1.16	0.25
		83	Dia_stem	0.00	0.00	-0.55	0.59	0.00	0.01	0.07	0.94	0.00	0.00	-0.60	0.55
	Beaupre	90	Temperature	0.00	0.01	0.27	0.79	0.00	0.00	0.83	0.40	0.00	0.01	0.20	0.85
		90	Dia_stem	0.00	0.00	0.62	0.54	0.00	0.00	0.55	0.58	0.00	0.00	0.73	0.47
	Fritzy P.	90	Temperature	-0.09	0.12	-0.79	0.44	-0.05	0.05	-0.92	0.36	0.14	0.13	1.10	0.28
		90	Dia_stem	0.06	0.03	2.11	0.04	0.06	0.03	1.97	0.05	0.05	0.03	1.81	0.08
	Raspalje	90	Temperature	-0.08	0.10	-0.76	0.53	-0.04	0.04	-0.89	0.48	-0.10	0.15	-0.64	0.59
		90	Dia_stem	0.00	0.02	-0.04	0.97	0.00	0.02	-0.06	0.95	-0.01	0.02	-0.31	0.76
	Trichobel	90	Temperature	-0.05	0.14	-0.34	0.78	-0.03	0.06	-0.58	0.67	0.16	0.10	1.66	0.10
		90	Dia_stem	0.06	0.03	1.70	0.09	0.06	0.03	1.69	0.10	0.05	0.03	1.57	0.12
	Unal	90	Temperature	0.02	0.02	0.84	0.45	0.02	0.03	0.66	0.53	0.01	0.01	1.00	0.32
		90	Dia_stem	-0.01	0.02	-0.64	0.52	-0.01	0.02	-0.59	0.55	-0.01	0.01	-0.58	0.56
	Beaupre	97	Temperature	0.03	0.03	1.11	0.27	0.00	0.01	0.21	0.85	0.04	0.02	1.72	0.09
		97	Dia_stem	0.00	0.01	0.42	0.68	0.00	0.01	0.81	0.43	0.00	0.00	0.74	0.46
	Fritzy P.	97	Temperature	0.06	0.20	0.29	0.81	0.01	0.09	0.13	0.92	0.13	0.26	0.52	0.71
		97	Dia_stem	0.00	0.02	0.14	0.89	0.00	0.02	0.11	0.91	0.00	0.02	0.02	0.98
	Raspalje	97	Temperature	-0.08	0.08	-0.90	0.37	-0.03	0.03	-0.85	0.40	-0.06	0.12	-0.50	0.62
		97	Dia_stem	0.03	0.02	1.45	0.15	0.03	0.02	1.35	0.18	0.03	0.03	1.04	0.30

Fixed effects															
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
	Trichobel	97	Temperature	0.02	0.13	0.13	0.91	0.00	0.06	-0.03	0.98	0.09	0.12	0.80	0.76
		97	Dia_stem	-0.02	0.03	-0.57	0.57	-0.02	0.03	-0.59	0.56	-0.02	0.03	-0.66	0.51
	Unal	97	Temperature	0.03	0.02	1.27	0.21	0.02	0.03	0.59	0.57	0.02	0.01	1.14	0.25
		97	Dia_stem	-0.02	0.02	-0.93	0.36	-0.01	0.02	-0.55	0.58	-0.01	0.02	-0.65	0.52
	Beaupre	104	Temperature	0.05	0.10	0.55	0.59	0.03	0.04	0.81	0.42	0.00	0.08	0.01	0.99
		104	Dia_stem	-0.01	0.02	-0.69	0.49	-0.01	0.02	-0.68	0.50	-0.01	0.02	-0.56	0.57
	Fritzy P.	104	Temperature	0.00	0.15	0.03	0.98	0.00	0.06	0.00	1.00	0.01	0.16	0.06	0.95
		104	Dia_stem	0.09	0.03	2.46	0.02	0.09	0.04	2.40	0.02	0.08	0.04	2.32	0.02
	Raspalje	104	Temperature	-0.14	0.12	-1.18	0.24	-0.05	0.05	-1.09	0.28	0.07	0.16	0.45	0.65
		104	Dia_stem	-0.01	0.03	-0.45	0.65	-0.02	0.03	-0.58	0.56	-0.01	0.04	-0.30	0.76
	Trichobel	104	Temperature	0.01	0.22	0.04	0.97	-0.01	0.10	-0.12	0.92	0.20	0.20	0.97	0.67
		104	Dia_stem	-0.03	0.04	-0.74	0.46	-0.03	0.04	-0.75	0.45	-0.03	0.04	-0.80	0.43
	Unal	104	Temperature	0.03	0.05	0.53	0.62	0.00	0.06	-0.07	0.94	0.02	0.03	0.71	0.52
		104	Dia_stem	-0.04	0.03	-1.13	0.26	-0.03	0.04	-0.74	0.46	-0.04	0.03	-1.20	0.24
	Beaupre	111	Temperature	0.17	0.17	1.01	0.31	0.09	0.07	1.30	0.20	-0.02	0.14	-0.17	0.86
		111	Dia_stem	-0.05	0.03	-1.40	0.16	-0.04	0.03	-1.35	0.18	-0.04	0.03	-1.16	0.25
	Fritzy P.	111	Temperature	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		111	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Raspalje	111	Temperature	-0.14	0.14	-0.97	0.33	-0.05	0.06	-0.89	0.37	0.22	0.19	1.13	0.26
		111	Dia_stem	-0.06	0.04	-1.58	0.12	-0.07	0.04	-1.69	0.09	-0.04	0.04	-1.02	0.31
	Trichobel	111	Temperature	0.00	0.02	0.07	0.94	0.00	0.01	0.12	0.91	-0.01	0.02	-0.21	0.83
		111	Dia_stem	-0.01	0.01	-1.18	0.24	-0.01	0.01	-1.15	0.25	-0.01	0.01	-1.08	0.28
	Unal	111	Temperature	0.02	0.08	0.24	0.82	-0.01	0.09	-0.15	0.89	0.03	0.06	0.44	0.69
		111	Dia_stem	-0.05	0.04	-1.15	0.25	-0.04	0.04	-0.92	0.36	-0.05	0.04	-1.23	0.22
	Beaupre	118	Temperature	0.07	0.16	0.47	0.67	0.08	0.06	1.26	0.21	-0.17	0.13	-1.31	0.19
		118	Dia_stem	-0.02	0.03	-0.78	0.44	-0.03	0.03	-0.86	0.39	-0.02	0.03	-0.66	0.51

Fixed effects															
				MAT				MjulyT				MjanT			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value	estimate	std. error	t value	p value
	Fritzy P.	118	Temperature	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		118	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Raspalje	118	Temperature	-0.06	0.07	-0.77	0.52	-0.02	0.03	-0.69	0.57	0.17	0.07	2.30	0.02
		118	Dia_stem	-0.03	0.02	-1.49	0.15	-0.03	0.02	-1.50	0.14	-0.02	0.02	-1.12	0.27
	Trichobel	118	Temperature	0.03	0.07	0.42	0.68	0.02	0.03	0.52	0.61	-0.05	0.08	-0.70	0.48
		118	Dia_stem	0.01	0.02	0.20	0.84	0.01	0.03	0.25	0.81	0.01	0.03	0.34	0.73
	Unal	118	Temperature	0.01	0.03	0.34	0.75	-0.01	0.04	-0.36	0.72	0.02	0.02	0.84	0.46
		118	Dia_stem	-0.03	0.02	-1.23	0.22	-0.02	0.03	-0.72	0.47	-0.03	0.02	-1.52	0.14
	Beaupre	125	Temperature	-0.01	0.11	-0.05	0.97	0.04	0.05	0.85	0.46	-0.12	0.09	-1.42	0.16
		125	Dia_stem	-0.01	0.02	-0.63	0.54	-0.02	0.02	-0.79	0.44	-0.01	0.02	-0.63	0.53
	Fritzy P.	125	Temperature	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		125	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Raspalje	125	Temperature	-0.01	0.01	-1.01	0.32	0.00	0.00	-1.24	0.22	0.00	0.02	0.19	0.87
		125	Dia_stem	-0.01	0.00	-3.64	0.00	-0.01	0.00	-3.78	0.00	-0.01	0.00	-3.33	0.00
	Trichobel	125	Temperature	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		125	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Unal	125	Temperature	0.00	0.01	0.15	0.89	0.00	0.01	-0.17	0.87	0.00	0.01	0.62	0.58
		125	Dia_stem	-0.01	0.01	-0.65	0.52	0.00	0.01	-0.41	0.68	-0.01	0.01	-0.90	0.38

Appendix Table D.6 The results from the linear mixed effect models (in response to day lengths and stem diameter). NA means no data available due to 0 (zero) variance in response variable. We used lmerTest package to extract the p values from the linear mixed effects models (Kuznetsova et al., 2017).

Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
Bud set 2014	Beaupre	239	Day length	-0.06	0.47	-0.14	0.89	0.04	0.28	0.13	0.90
		239	Dia_stem	0.03	0.01	2.99	0.00	0.03	0.01	2.99	0.00
	Fritzy P.	239	Day length	0.53	0.58	0.91	0.36	-0.31	0.34	-0.90	0.37
		239	Dia_stem	-0.01	0.03	-0.18	0.86	-0.01	0.03	-0.18	0.86
	Raspalje	239	Day length	0.10	0.10	0.94	0.35	-0.06	0.06	-0.93	0.35
		239	Dia_stem	0.02	0.01	2.18	0.03	0.02	0.01	2.19	0.03
	Trichobel	239	Day length	-0.04	0.49	-0.08	0.94	0.02	0.29	0.08	0.94
		239	Dia_stem	0.03	0.04	0.86	0.39	0.03	0.04	0.86	0.39
	Unal	239	Day length	-0.13	0.05	-2.59	0.01	0.07	0.03	2.58	0.01
		239	Dia_stem	0.00	0.01	0.24	0.81	0.00	0.01	0.27	0.79
	Beaupre	246	Day length	0.03	0.44	0.06	0.95	-0.02	0.26	-0.06	0.95
		246	Dia_stem	0.01	0.01	0.44	0.66	0.01	0.01	0.44	0.66
	Fritzy P.	246	Day length	-0.81	0.80	-1.00	0.32	0.47	0.47	1.00	0.32
		246	Dia_stem	0.01	0.04	0.19	0.85	0.01	0.04	0.18	0.86
	Raspalje	246	Day length	-0.18	0.15	-1.18	0.24	0.10	0.09	1.17	0.24
		246	Dia_stem	0.04	0.02	2.31	0.02	0.04	0.02	2.30	0.02
	Trichobel	246	Day length	-0.11	0.66	-0.17	0.87	0.06	0.39	0.16	0.87
		246	Dia_stem	-0.02	0.05	-0.43	0.67	-0.02	0.05	-0.43	0.67
	Unal	246	Day length	-0.13	0.06	-2.09	0.04	0.07	0.03	2.12	0.03

Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
		246	Dia_stem	-0.01	0.01	-0.41	0.68	-0.01	0.01	-0.40	0.69
	Beaupre	253	Day length	0.11	0.39	0.28	0.78	-0.07	0.23	-0.29	0.77
		253	Dia_stem	0.01	0.01	0.40	0.69	0.01	0.01	0.41	0.68
	Fritzy P.	253	Day length	-0.32	0.82	-0.39	0.70	0.19	0.48	0.39	0.70
		253	Dia_stem	0.00	0.04	-0.01	1.00	0.00	0.04	-0.01	0.99
	Raspalje	253	Day length	0.11	0.14	0.77	0.44	-0.07	0.08	-0.79	0.43
		253	Dia_stem	0.01	0.01	0.61	0.54	0.01	0.01	0.60	0.55
	Trichobel	253	Day length	-0.53	0.66	-0.79	0.43	0.31	0.39	0.79	0.43
		253	Dia_stem	-0.04	0.05	-0.79	0.43	-0.04	0.05	-0.79	0.43
	Unal	253	Day length	-0.22	0.07	-3.16	0.00	0.12	0.04	3.17	0.00
		253	Dia_stem	-0.01	0.02	-0.57	0.57	-0.01	0.02	-0.55	0.58
	Beaupre	260	Day length	0.33	0.14	2.35	0.02	-0.20	0.08	-2.35	0.02
		260	Dia_stem	-0.01	0.01	-0.49	0.62	-0.01	0.01	-0.49	0.62
	Fritzy P.	260	Day length	-1.27	0.85	-1.49	0.14	0.74	0.50	1.48	0.14
		260	Dia_stem	0.01	0.04	0.23	0.82	0.01	0.04	0.22	0.83
	Raspalje	260	Day length	-0.31	0.16	-1.96	0.05	0.18	0.09	1.95	0.05
		260	Dia_stem	0.04	0.02	2.32	0.02	0.04	0.02	2.31	0.02
	Trichobel	260	Day length	-0.63	0.83	-0.75	0.45	0.36	0.49	0.75	0.46
		260	Dia_stem	0.02	0.04	0.47	0.64	0.02	0.04	0.47	0.64
	Unal	260	Day length	-0.12	0.07	-1.61	0.11	0.06	0.04	1.54	0.12
		260	Dia_stem	-0.02	0.01	-1.31	0.19	-0.02	0.01	-1.28	0.20
	Beaupre	267	Day length	-0.08	0.13	-0.62	0.53	0.05	0.08	0.62	0.54
		267	Dia_stem	0.00	0.01	-0.37	0.71	0.00	0.01	-0.37	0.71
	Fritzy P.	267	Day length	1.06	0.92	1.15	0.25	-0.62	0.53	-1.16	0.24
		267	Dia_stem	0.02	0.02	0.67	0.50	0.02	0.02	0.67	0.50
	Raspalje	267	Day length	-0.04	0.10	-0.35	0.73	0.02	0.06	0.35	0.73

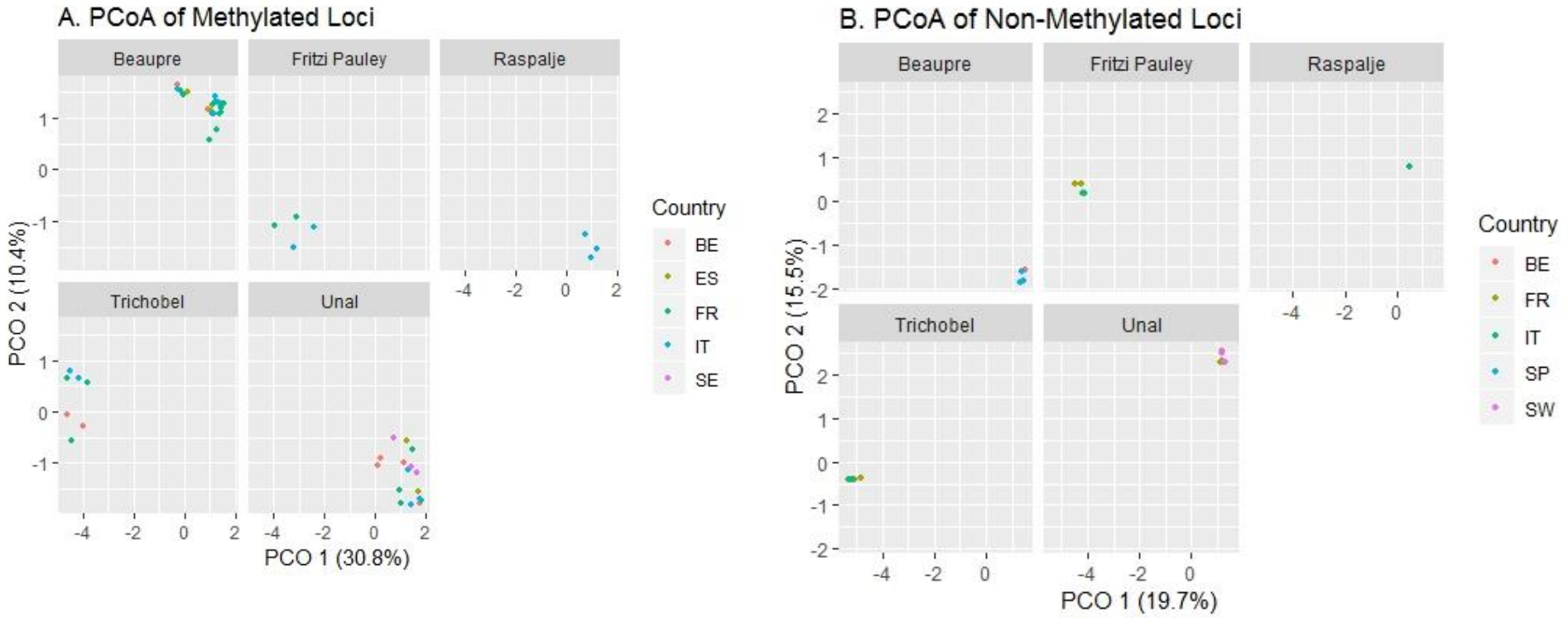
Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
		267	Dia_stem	0.00	0.01	0.22	0.83	0.00	0.01	0.22	0.83
	Trichobel	267	Day length	-0.27	0.40	-0.66	0.51	0.15	0.24	0.65	0.52
		267	Dia_stem	-0.01	0.03	-0.42	0.68	-0.01	0.03	-0.42	0.68
	Unal	267	Day length	-0.04	0.05	-0.82	0.41	0.02	0.03	0.79	0.43
		267	Dia_stem	-0.02	0.01	-1.77	0.08	-0.02	0.01	-1.76	0.08
Bud set 2015	Beaupre	233	Day length	-0.31	0.60	-0.52	0.60	0.18	0.35	0.51	0.61
		233	Dia_stem	0.06	0.02	3.05	0.00	0.06	0.02	3.05	0.00
	Fritzy P.	233	Day length	0.26	0.63	0.42	0.68	-0.16	0.37	-0.42	0.67
		233	Dia_stem	-0.02	0.03	-0.67	0.50	-0.02	0.03	-0.67	0.50
	Raspalje	233	Day length	0.27	0.17	1.58	0.11	-0.16	0.10	-1.56	0.12
		233	Dia_stem	0.01	0.02	0.70	0.49	0.01	0.02	0.70	0.48
	Trichobel	233	Day length	-0.28	0.56	-0.51	0.61	0.17	0.33	0.52	0.61
		233	Dia_stem	0.02	0.03	0.66	0.51	0.02	0.03	0.66	0.51
	Unal	233	Day length	-0.08	0.10	-0.78	0.44	0.04	0.06	0.75	0.45
		233	Dia_stem	0.02	0.02	0.93	0.35	0.02	0.02	0.96	0.34
	Beaupre	243	Day length	-0.22	0.70	-0.32	0.75	0.13	0.42	0.32	0.75
		243	Dia_stem	0.06	0.02	2.60	0.01	0.06	0.02	2.60	0.01
	Fritzy P.	243	Day length	-0.19	0.76	-0.25	0.80	0.11	0.45	0.25	0.80
		243	Dia_stem	0.03	0.04	0.71	0.48	0.03	0.04	0.71	0.48
	Raspalje	243	Day length	0.30	0.21	1.46	0.15	-0.18	0.12	-1.44	0.15
		243	Dia_stem	0.02	0.02	0.78	0.44	0.02	0.02	0.79	0.43
	Trichobel	243	Day length	-0.83	1.30	-0.64	0.53	0.49	0.76	0.64	0.52
		243	Dia_stem	-0.03	0.05	-0.60	0.55	-0.03	0.05	-0.60	0.55
	Unal	243	Day length	-0.22	0.15	-1.45	0.15	0.12	0.09	1.38	0.17
		243	Dia_stem	-0.01	0.03	-0.23	0.82	-0.01	0.03	-0.19	0.85
	Beaupre	250	Day length	-0.20	0.42	-0.48	0.63	0.12	0.25	0.48	0.63

Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
		250	Dia_stem	0.07	0.02	3.07	0.00	0.07	0.02	3.07	0.00
	Fritzy P.	250	Day length	-0.07	1.09	-0.06	0.95	0.03	0.64	0.05	0.96
		250	Dia_stem	0.01	0.05	0.23	0.82	0.01	0.05	0.23	0.82
	Raspalje	250	Day length	0.22	0.34	0.63	0.53	-0.13	0.20	-0.62	0.54
		250	Dia_stem	0.03	0.03	1.16	0.25	0.03	0.03	1.16	0.25
	Trichobel	250	Day length	-0.09	0.69	-0.13	0.90	0.05	0.41	0.13	0.89
		250	Dia_stem	0.00	0.05	0.08	0.93	0.00	0.05	0.08	0.93
	Unal	250	Day length	-0.15	0.17	-0.89	0.37	0.08	0.10	0.84	0.40
		250	Dia_stem	0.03	0.03	0.93	0.35	0.03	0.03	0.95	0.34
	Beaupre	257	Day length	-0.12	0.16	-0.72	0.47	0.07	0.10	0.73	0.46
		257	Dia_stem	0.03	0.01	2.66	0.01	0.03	0.01	2.66	0.01
	Fritzy P.	257	Day length	-1.51	1.03	-1.47	0.14	0.89	0.60	1.47	0.14
		257	Dia_stem	0.00	0.05	-0.04	0.97	0.00	0.05	-0.05	0.96
	Raspalje	257	Day length	0.37	0.24	1.53	0.12	-0.22	0.14	-1.53	0.13
		257	Dia_stem	0.03	0.03	1.14	0.25	0.03	0.03	1.15	0.25
	Trichobel	257	Day length	-0.15	0.77	-0.20	0.84	0.09	0.45	0.20	0.84
		257	Dia_stem	-0.01	0.05	-0.12	0.90	-0.01	0.05	-0.12	0.90
	Unal	257	Day length	0.10	0.12	0.83	0.40	-0.05	0.06	-0.87	0.39
		257	Dia_stem	0.05	0.02	2.18	0.03	0.05	0.02	2.19	0.03
	Beaupre	264	Day length	-0.05	0.09	-0.56	0.58	0.03	0.05	0.57	0.57
		264	Dia_stem	0.01	0.01	1.85	0.06	0.01	0.01	1.85	0.06
	Fritzy P.	264	Day length	-0.52	0.83	-0.62	0.53	0.30	0.49	0.63	0.53
		264	Dia_stem	-0.05	0.04	-1.25	0.21	-0.05	0.04	-1.25	0.21
	Raspalje	264	Day length	0.20	0.19	1.05	0.29	-0.12	0.11	-1.05	0.29
		264	Dia_stem	0.02	0.02	1.07	0.28	0.02	0.02	1.08	0.28
	Trichobel	264	Day length	-0.76	0.83	-0.92	0.36	0.45	0.48	0.93	0.35

Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
		264	Dia_stem	0.03	0.05	0.55	0.58	0.03	0.05	0.55	0.58
	Unal	264	Day length	0.01	0.08	0.10	0.92	0.00	0.04	-0.10	0.92
		264	Dia_stem	0.01	0.02	0.45	0.65	0.01	0.02	0.45	0.65
Bud burst 2015	Beaupre	83	Day length	NA	NA	NA	NA	NA	NA	NA	NA
		83	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA
	Fritzy P.	83	Day length	0.98	0.75	1.31	0.26	-0.57	0.44	-1.29	0.27
		83	Dia_stem	0.00	0.03	-0.10	0.92	0.00	0.03	-0.10	0.92
	Raspalje	83	Day length	-0.01	0.05	-0.11	0.92	0.00	0.03	0.12	0.92
		83	Dia_stem	0.00	0.01	0.12	0.90	0.00	0.01	0.12	0.90
	Trichobel	83	Day length	0.61	0.42	1.47	0.15	-0.36	0.25	-1.47	0.15
		83	Dia_stem	-0.01	0.03	-0.49	0.63	-0.01	0.03	-0.48	0.63
	Unal	83	Day length	-0.01	0.02	-0.81	0.42	0.01	0.01	0.83	0.41
		83	Dia_stem	0.00	0.00	-0.56	0.57	0.00	0.00	-0.57	0.57
	Beaupre	90	Day length	-0.03	0.02	-1.44	0.15	0.02	0.01	1.43	0.15
		90	Dia_stem	0.00	0.00	0.59	0.55	0.00	0.00	0.59	0.55
	Fritzy P.	90	Day length	0.33	0.56	0.59	0.56	-0.19	0.33	-0.58	0.57
		90	Dia_stem	0.06	0.03	2.29	0.03	0.06	0.03	2.29	0.03
	Raspalje	90	Day length	0.36	0.17	2.08	0.17	-0.21	0.10	-2.08	0.17
		90	Dia_stem	0.00	0.02	0.01	0.99	0.00	0.02	0.02	0.98
	Trichobel	90	Day length	0.08	0.66	0.13	0.91	-0.05	0.38	-0.12	0.92
		90	Dia_stem	0.06	0.03	1.71	0.09	0.06	0.03	1.71	0.09
	Unal	90	Day length	-0.08	0.07	-1.15	0.25	0.04	0.04	1.13	0.26
		90	Dia_stem	-0.01	0.02	-0.83	0.41	-0.01	0.02	-0.81	0.42
	Beaupre	97	Day length	-0.04	0.07	-0.60	0.61	0.03	0.04	0.61	0.61
		97	Dia_stem	0.00	0.01	0.78	0.44	0.00	0.01	0.78	0.44
	Fritzy P.	97	Day length	-0.40	0.84	-0.47	0.70	0.23	0.49	0.48	0.69

Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
		97	Dia_stem	0.00	0.02	0.17	0.87	0.00	0.02	0.17	0.87
	Raspalje	97	Day length	0.28	0.22	1.26	0.21	-0.17	0.13	-1.27	0.21
		97	Dia_stem	0.02	0.02	0.89	0.38	0.02	0.02	0.89	0.37
	Trichobel	97	Day length	-0.17	0.56	-0.30	0.79	0.10	0.33	0.31	0.78
		97	Dia_stem	-0.02	0.03	-0.55	0.58	-0.02	0.03	-0.55	0.58
	Unal	97	Day length	-0.06	0.07	-0.87	0.42	0.04	0.04	0.91	0.40
		97	Dia_stem	-0.01	0.02	-0.66	0.51	-0.01	0.02	-0.68	0.50
	Beaupre	104	Day length	-0.26	0.25	-1.06	0.29	0.15	0.15	1.06	0.29
		104	Dia_stem	-0.01	0.02	-0.64	0.53	-0.01	0.02	-0.64	0.53
	Fritzy P.	104	Day length	-0.04	0.69	-0.05	0.96	0.02	0.41	0.05	0.96
		104	Dia_stem	0.09	0.03	2.54	0.01	0.09	0.03	2.54	0.01
	Raspalje	104	Day length	0.32	0.31	1.03	0.30	-0.19	0.18	-1.04	0.30
		104	Dia_stem	-0.03	0.03	-0.86	0.39	-0.03	0.03	-0.86	0.39
	Trichobel	104	Day length	-0.21	0.97	-0.22	0.85	0.13	0.57	0.22	0.85
		104	Dia_stem	-0.03	0.04	-0.73	0.47	-0.03	0.04	-0.73	0.47
	Unal	104	Day length	-0.07	0.18	-0.38	0.72	0.04	0.10	0.40	0.71
		104	Dia_stem	-0.03	0.03	-1.06	0.29	-0.03	0.03	-1.07	0.29
	Beaupre	111	Day length	-0.64	0.42	-1.52	0.13	0.38	0.25	1.53	0.13
		111	Dia_stem	-0.04	0.03	-1.27	0.21	-0.04	0.03	-1.27	0.21
	Fritzy P.	111	Day length	NA	NA	NA	NA	NA	NA	NA	NA
		111	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA
	Raspalje	111	Day length	0.17	0.37	0.45	0.66	-0.10	0.22	-0.45	0.65
		111	Dia_stem	-0.07	0.04	-1.71	0.09	-0.07	0.04	-1.71	0.09
	Trichobel	111	Day length	0.00	0.12	-0.02	0.99	0.00	0.07	0.02	0.99
		111	Dia_stem	-0.01	0.01	-1.21	0.23	-0.01	0.01	-1.21	0.23
	Unal	111	Day length	-0.06	0.28	-0.22	0.83	0.04	0.16	0.23	0.83

Fixed effects											
				Day length1 May (DLMay)				Day length 1 January (DLJan)			
Response	Clone	Observation (Days of the year)	Variable	estimate	std. error	t value	p value	estimate	std. error	t value	p value
		111	Dia_stem	-0.05	0.04	-1.15	0.25	-0.05	0.04	-1.15	0.25
	Beaupre	118	Day length	-0.46	0.38	-1.19	0.24	0.27	0.23	1.20	0.23
		118	Dia_stem	-0.02	0.03	-0.75	0.46	-0.02	0.03	-0.75	0.45
	Fritzy P.	118	Day length	NA	NA	NA	NA	NA	NA	NA	NA
		118	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA
	Raspalje	118	Day length	-0.01	0.23	-0.06	0.96	0.01	0.14	0.06	0.96
		118	Dia_stem	-0.02	0.02	-1.31	0.20	-0.02	0.02	-1.31	0.20
	Trichobel	118	Day length	-0.10	0.36	-0.28	0.78	0.06	0.21	0.27	0.79
		118	Dia_stem	0.00	0.02	0.15	0.88	0.00	0.02	0.15	0.88
	Unal	118	Day length	-0.05	0.12	-0.40	0.71	0.03	0.06	0.41	0.70
		118	Dia_stem	-0.03	0.02	-1.26	0.21	-0.03	0.02	-1.28	0.21
	Beaupre	125	Day length	-0.25	0.29	-0.84	0.48	0.15	0.17	0.85	0.48
		125	Dia_stem	-0.02	0.02	-0.73	0.48	-0.02	0.02	-0.73	0.48
	Fritzy P.	125	Day length	NA	NA	NA	NA	NA	NA	NA	NA
		125	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA
	Raspalje	125	Day length	0.04	0.03	1.48	0.14	-0.02	0.02	-1.48	0.14
		125	Dia_stem	-0.01	0.00	-4.03	0.00	-0.01	0.00	-4.02	0.00
	Trichobel	125	Day length	NA	NA	NA	NA	NA	NA	NA	NA
		125	Dia_stem	NA	NA	NA	NA	NA	NA	NA	NA
	Unal	125	Day length	-0.02	0.04	-0.55	0.62	0.01	0.02	0.53	0.63
		125	Dia_stem	-0.01	0.01	-0.90	0.37	-0.01	0.01	-0.89	0.38



Appendix Figure D.5 Representation of Principal Coordinate Analysis (PCoA) for methylated (A) and non-methylated (B) loci. Samples are grouped per clone (countries are here BE= Belgium, FR=France, ES=Spain, IT=Italy and SE=Sweden). The first two coordinates (PCO1 and PCO2) are shown with the percentage of variance explained by them. Different point types represent individuals from different groups

Appendix E Captions of the photographs used between chapters

page 27 Experimental warming of seedlings (*Q. robur* and *F. sylvatica*) during end of January 2015 at Forest & Nature Lab (top), Seedlings of the same experiment in June 2016 (bottom)

page 46 Photograph showing one of selected mother trees in provenance trial of *Q. robur* in Nyskov, Denmark

page 63 Two years old seedlings of *Populus nigra* generated from control and warm (+10°C) maternal environment growing in a common garden at the Research Institute for Nature and Forest (INBO)

page 84 Vegetative cuttings of hybrid poplar growing in a common garden at the Research Institute for Nature and Forest

Curriculum vitae

Personal Information

Name	Sumitra DEWAN
Address	Bellemstraat 109, Aalter, Belgium
Date of birth	02 March 1981
Place of birth	Rangamati, Bangladesh
Nationality	Bangladeshi
Email	sumitra.dewan@ugent.be

Education

2007- 2009	**M.Sc. in Mountain Forestry,** University of Natural Resources and Life Sciences (BOKU), Vienna, Austria.
2006-2007	**M.Sc. (Thesis) in Forestry**, Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong, Bangladesh.
1999-2006	**B.Sc. (Honors) in Forestry,** Institute of Forestry and Environmental Sciences, University of Chittagong, Bangladesh

Work experience

05/2014-05/2018	PhD student, Title “Effects of maternal temperature on offspring performance of temperate forest trees”, Forest & Nature Lab, Department of Forest and Water Management, Ghent University, Belgium
02/2013-10/2013	Research assistant, Project title- ‘STARTCLIM 2012 C’, Institute of forest ecology, University of Natural Resources and Life Sciences, Vienna, Austria (part time).
07/2013-09/2013	Transitional Access to “INCREASE” infrastructure project in Bangor, UK to conduct the study on “Effect of drought and soil warming on mycorrhizal production in relation to decomposition and N mineralization”
01/01/2011-19/04/2012	Assistant Manager (Forest), Karnafully Paper Mills Limited (an organization of Bangladesh Chemical Industries Corporation) Chandraghona, Rangamati, Bangladesh.
01/08/2010-01/10/ 2010	Scientist, Agricultural feasibility survey for Chittagong Hill Tracts Rural Development Project II under Asian Development Bank-Bangladesh (TA 7432-BAN); Data collection, compiling data, analysis and report writing.
01/03/2010-01/02 2011	Project Officer, Itchari Community Reserve Forest Conservation Project, BIRAM-a non-government organization, Khagrachari Hill

District, Bangladesh works for community based forest management; a project funded by Arranyak Foundation, Dhaka (an USAID programme).

01/112009-01/03/2010 Research Assistant, project title "*Evaluating the role of Arbuscular mycorrhizal Fungi in Arsenic uptake by crops*" funded by USDA. Mycorrhiza Laboratory, Department of Botany, University of Chittagong, Chittagong.

01/01/2006-01/09/2007 Research Assistant, project title '*Introduction of mycorrhizal Technology in Hill Farming System*', Mycorrhiza Laboratory, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

01/06/2003-01/06/2005 Volunteer in *Taungya* a non-government organization works for Indegenous culture, Environment & Socio-economic Advancement, Rangamati, Bangladesh.

Publication List

Dewan S., Vander Mijnsbrugge K., De Frenne P., Steenackersb M., Michiels B. and Verheyen K., 2018: Maternal temperature during seed maturation affects seed germination and timing of bud set in seedlings of European black poplar, Forest Ecology and Management 410 :126–135 doi.org/10.1016/j.foreco.2018.01.002

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Mridha M. A. U., Jabbar F., Bhuiyan M. K., Rahman M., Akter, F. and **Dewan S**., 2007. The severity and cause of leaf spot disease of *Pongamia pinnata* L. and fungicidal control of the pathogen. *J. For. Res*., 18(3): 236-240 DOI: 10.1007/s11676-007-0048-2

Other scientific activities

Dewan, S., De Frenne, P., Vander Mijnsbrugge, K. & Verheyen, K. (2014) Effect of maternal temperatures on the performance of tree seedlings. Poster

presentation at the Joint Annual Meeting of the British Ecological Society (BES) and Société Française d'Ecologie (SFE), 09-12 December 2014, Lille, France.

Dewan, S., De Frenne, P., Vander Mijnsbrugge, K., Verheyen, K. (2015) Does phenology of beech and oak seedlings varies with the prevailing environment of seed development? Oral presentation at Seminar on Global Forestry Trends, 7-10 October 2015, Joensuu, Finland.

Dewan, S., De Frenne, P., Steenackers, M., Vander Mijnsbrugge, K., Verheyen, K. (2017) Temperature memory effects persistently alter the phenology of tree clones. Oral presentation at Starters in het Natuur- en Bosonderzoek, 24 March 2017, Ghent, Belgium.

Dewan, S., Vander Mijnsbrugge, K., De Frenne, P., Steenackers, M., Michiels, B., Verheyen, K. (2017) Maternal temperature affects the germination success and the phenology of tree seedlings. Oral presentation at the Conference on Restoring Forests, 12-14 September 2017, Lund, Sweden.

Grants and Fellowships

PhD fellowships

May 2014-May2018: FORBIO climate- *Adaptation potential of biodiverse forests in the face of climate change* funded by Belgian Science Policy Office (BELSPO) via the BRAIN-be programme.

Scholarship

October 2007- September 2009: 'One World Scholarship', Österreichische Orient-Gesellschaft (ÖOG); Funded by Development aid fund of Austrian Development Agency (ADA), Austria

Professional Trainings and workshop

17, 31 May, 7 June, 2017: Effective scientific communication taught by Jean-luc Doumont, Principiae, Belgium.

24-25 February, 2010: National workshop on Community Conserved Area in Bangladesh, organized by Wildlife Trust of Bangladesh (WTB) and United Nations Development Programme (UNDP), BIAM Foundation, New Eskaton, Dhaka, Bangladesh.

21st November 2009: National workshop on Climate Change impact on indigenous community in Bangladesh, Dhaka, Bangladesh

24-25 April, 2009: "Dynamics of Interpersonal Communication" trained by Robert M. Anderson, Ed.D.President, McDonald Anderson, Graz, Austria.

4-5 April, 2008: "Facilitation Skills" trained by Robert M. Anderson, Ed.D. President, McDonald Anderson, Graz, Austria.

07 June 2003-19 June 2003: " Field Engineering Course For Forestry And Environmental Sciences Students (FECFESS-5) at Engineer Center and School of Military Engineering, Quadirabad Cantonment, Natore, Bangladesh

Additional Skills

Language

Bangla	: Native speaker
English	: Fluent in speaking, reading and writing
Nederlands	: Independent user
German	: Basic knowledge