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B.J.H.M. Possen

Searching for traits behind growth and biomass:

A case study with silver birch

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Academic Dissertation
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To Sylvia, Marion and Marcel for allowing me to dream.

ABSTRACT

Knowledge of differences between genotypes within local populations is relevant, since these differences are important for the capacity of these populations to cope with environmental stress and climate change. General consensus is that the more differences there are between genotypes within a population, the better the chance a genotype exists that has the capacity to cope. Yet for trees, where differences between genotypes have added importance due to their sessile nature and longevity, the differences in traits related to growth and survival remain unexplored. Therefore, this thesis aims to contribute to the understanding of the magnitude and importance of differences between genotypes within local tree populations for the dominant, ecologically and economically most important broad-leaved species in Northern Europe, silver birch (*Betula pendula* Roth). To establish whether the traits found in silver birch are also important in other broadleaved species native to the boreal zone, the responses of aspen (*Populus tremula* L.) genotypes to water stress were also determined.

All silver birch genotypes included in this thesis were micropropagated from trees selected from a one hectare mixed silver and downy birch (*B. pubescens* Ehrh.) forest stand that regenerated naturally after logging in 1979. Thus, the material used represents a natural population. The aspen genotypes were selected from four populations on the same latitude.

To find traits underlying differences in growth between the genotypes a field experiment in Punkaharju, Finland, established in 1999 for long-term monitoring of within-stand genotypic differences in growth phenomena, was used. Two greenhouse experiments were established to study if and how traits with a high relative importance under field conditions in the current climate are involved in coping with environmental stresses projected to occur in a future climate, focussing on water availability and temperature.

In the field experiment, in addition to measurements of biomass and growth, 18 traits related to physiology (e.g. gas exchange, leaf pigments), leaf morphology (e.g. leaf size, leaf thickness) and phenology (e.g. bud burst, carbon sink-source transition) were examined during multiple growing seasons. In the greenhouse experiments the same suit of traits was measured, with the exception of phenology, in plants subjected to combinations of water stress (low, optimum and excess water) and increased temperature (ambient temperature and ambient +1 °C).

There were differences between genotypes in almost all measured traits, but the differences varied seemingly at random in relation to biomass. Differences in bud burst were generally small, but were greatly enhanced under conditions of variable temperature sum accumulation in spring (i.e. cold spells in spring). The same genotypes consistently showed early or late bud burst. Differences in bud burst were not carried over to the estimated period of carbon gain. Due to faster leaf expansion in genotypes with late bud burst and the lack of differences between genotypes in autumn senescence the estimated period of carbon gain was similar between genotypes. As a result the measured phenological traits could not be used to explain differences in growth between the genotypes. However, differences between genotypes in the timing of phenological events as well as the presence of genotypes with different climatic optima within a local population indicate possibilities for acclimation to a changing climate of such populations.

Both greenhouse experiments showed that the responses of genotypes to adverse conditions were mostly similar. High net photosynthesis and water potential in the leaves in combination with a higher investment in roots compared to leaves were important traits for superior growth (in terms of biomass and height) after the two year experiment, irrespective of treatment. However, exceptions were traits related to relative investment in leaves, specific leaf area and root length indicating that changes in allocation patterns as a result of changing environmental conditions may depend on genotype, even within a local population. The relative investment in leaves, both in terms of mass and area, was acclimated to the adverse environmental conditions during the first year of treatment only, while leaf morphological and physiological traits showed acclimation during both growing seasons. This indicates that acclimation of biomass allocation is an important mechanism in coping with changing environmental conditions, at least in younger trees.

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Betula pendula, Genotype-environment interaction, Acclimatization, Plants - Variation, Trees - Growth, Plant biomass, Plant phenology, Plant physiology

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While writing this final chapter, feelings of relief and responsibility alternate. Relief, because this chapter implies this thesis is almost finished. Responsibility, because of all the chapters in this thesis, this particular chapter will undoubtedly be read most. Allow me to recommend the other chapters; they were interesting enough to me for the past 4 years!

Before I quit my day-job to start work on this thesis a friend (Arend de Wilde) said "*A thesis is 1% inspiration and 99% sweat*". He was right. Field or laboratory work, no matter how exciting, becomes a string of repetitive, factory-like motions. Publishing becomes a desperate struggle over non-essential matters, aimed at pleasing the conveniently anonymous reviewer. Nonetheless, the momentary sense of achievement after the challenge of turning the data into something comprehensible and the feeling to have learned and contributed something, make it all well worthwhile! It was my dream to successfully complete a PhD thesis and I would do it all again, given the chance.

Yet, although this was *my* dream, a thesis has collateral effects on the lives of many people whose dream it never was, like loved ones and friends. They were involuntarily exposed to the random, often odd demands a thesis seems to make from time to time. Sylvia and I set out on this journey together and a mere "*thank you*" doesn't cut it for her endless support, her patience and understanding, her driving me to the finish! It wasn't always easy, but if anything, the experience has brought us closer together. I am also indebted to my parents for their unconditional support, for allowing me to dream and for raising me to always shoot for the stars. My brother is probably the best person in the world to put "*counting leaves*" in perspective from time to time, a rather useful trait!

I owe this thesis to the support, trust and hard work of my supervisors Elina Vapaavuori, Matti Rousi and Elina Oksanen. Without Matti's friendship and Elina Oksanen's trust, I would never have tried to undertake this journey and without Elina Vapaavuori offering me a place to do so, it wouldn't have been possible. Without their suggestions and tireless corrections, this thesis wouldn't be what it is today. Ronald Buskens and Hans van Poppel encouraged and enabled me to start this thesis while still working as a consultant. Mikko Anttonen thanks for the many discussions and your "*down to earth*" approach to this whole project! Further thanks go to all co-authors and collaborators for investing their time and expertise. Special thanks go to Jaakko Heinonen, for putting statistics in perspective and making them understandable and interesting. Thanks for your patience. With the comments and suggestions provided by Professor Koike and Dr. Mikola, who reviewed this thesis, I was able to further improve my thesis.

My life in Finland -and therefor this thesis- wouldn't have been possible without Egbert Beuker's friendship. Thanks for your friendship, introducing me to moose-hunting and for spending countless hours together trying to find that one rare bird! Life would have been a lot less interesting without the stimulating discussions on virtually any topic with Seppo Ruotsalainen. The friendship offered by Leena Ahonen, Wolfgang Berger, Heli Hakala, Anni Harju, Katriina Huttunen, Hanna Pitkänen, Jussi Pitkänen, Petteri Pulkkinen, Pasi Pulkkinen, Marko Sairanen, Hanni

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I am grateful.

B.J.H.M. Possen

"There is no substitute for careful and intensive field work if one wants to find out what is happening in natural populations" (Endler 1986)[1]

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[1] Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton.

LIST OF ABBREVIATIONS

[CO$_2$]	Carbon dioxide concentration
Car	Carotenoid content
Chl a	Chlorophyll a content
Chl b	Chlorophyll b content
Chl a / b	Ratio between Chl a and Chl b
DW	Dry weight
FW	Fresh weight
FWDW	Fresh-to-dry-weight ratio of the leaves
g_s	stomatal conductance
LA	Leaf area
LAR	Leaf area ratio (total leaf area / total plant biomass)
LMF	Leaf mass fraction (total leaf mass / total plant biomass)
LT	Thickness of the leaves
P_n	Light-saturated instantaneous net photosynthesis
P_{n_amb}/P_{n_sat} (or PC)	Carboxylation limitations for photosynthesis
RMF	Root mass fraction (total root mass / total plant biomass)
SLA	Specific leaf area
SMF	Stem mass fraction (stem mass / total plant biomass)
SRR	Shoot-to-root ratio ((leaf + stem dry mass) / root dry mass)
VWC	Volumetric water content of the soil
WP	Xylem water potential of the leaves
WUE	Water use efficiency (P_n/g_s)

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on data presented in the following articles:

I. Possen BJHM, Oksanen E, Rousi M, Ruhanen H, Ahonen V, Tervahauta A, Heinonen J, Heiskanen J, Kärenlampi S, Vapaavuori E (2011) Adaptability of birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) genotypes to different soil moisture conditions. Forest Ecology and Management 262:1387-1399.

II. Possen BJHM, Anttonen MJ, Oksanen E, Rousi M, Heinonen J, Kostiainen K, Kontunen-Soppela S, Heiskanen J, Vapaavuori EM (2014) Variation in 13 leaf morphological and physiological traits within a silver birch (*Betula pendula* Roth) stand and their relation to growth. Canadian Journal of Forest Research 44:1-9.

III. Possen BJHM, Rousi M, Silfver T, Anttonen MJ, Ruotsalainen S, Oksanen E, Vapaavuori E. Within-stand variation in silver birch (*Betula pendula* Roth) phenology. Submitted to Trees-Structure and Function.

IV. Possen BJHM, Anttonen M, Heinonen J, Rousi M, Kontunen-Soppela S, Oksanen E, Vapaavuori E. How do 10 silver birch (*Betula pendula* Roth) genotypes cloned from a single population respond to changing temperature and water regime? Manuscript.

Manuscripts are referred to by their Roman numerals in the remainder of the summary.

Author’s Contribution

For article I Boy Possen planned the experiment with his supervisors and had the primary responsibility for implementation, data collecting, data processing and writing of the article. The gene-expression work (I) was planned by Elina Oksanen and Sirpa Kärenlampi and the connected laboratory work was carried out by Viivi Ahonen and Arja Tervahauta. For articles II, III and IV the planning was done jointly by Boy Possen, Mikko Anttonen and the supervisors. Implementation and data collection for these experiments was shared with Mikko Anttonen and Boy Possen had the primary responsibility for data processing and writing the articles.

Contents

1 Introduction

1.1 TREES AND CLIMATE CHANGE

Global climate is changing. A rise in temperature, an increase in precipitation and a decrease in the extent of snow cover are being observed, especially in Northern latitudes (IPCC 2013). Furthermore, extreme weather events such as heat waves occur more frequently and their occurrence is expected to increase further (IPCC 2013). IPCC (2013) predictions consider average trends across biomes or continents, but similar results have been found for Finland specifically, with an increase in the occurrence of peak temperatures during the summer months, for example (Jylhä et al. 2009).

Climate warming affects many aspects of tree growth (Saxe et al. 2001, Way and Oren 2010, Peñuelas et al. 2013), but changes in plant phenology in Northern latitudes are especially well documented (Menzel et al. 2006). Not only are phenological traits easy to observe (Forrest and Miller-Rushing 2010), the correct timing of phenological events like bud burst in spring or growth cessation in autumn is critical for growth and survival of trees in the strongly seasonal environment found in Northern latitudes (Sarvas 1972, 1974). As a result of continuous warming, leaf flush in spring has advanced (Menzel 2000, Menzel et al. 2006) and the growing season has lengthened (Menzel 2000, Vitasse et al. 2009). The effect on autumn senescence on the other hand seems ambiguous (Menzel et al. 2006, Hänninen and Tanino 2011). These findings are confirmed by experimental warming studies including several tree species (e.g. Gunderson et al. 2012). The lengthening of the growing season observed on large geographical scales has led to the prediction of increased tree growth under climate change (Kramer et al. 2000, Briceño-Elizondo et al. 2006). Furthermore, studies on tree rings show evidence of increased growth as a result of increasing temperatures during the growing season (e.g. Jacoby et al. 2000, Kujansuu et al. 2007).

These studies cover large geographic areas, but have a clear emphasis on the temperate zone of Europe. Although advancement of bud burst, lengthening of the growing season and an ambiguous signal for autumn senescence have been observed for boreal conditions (Pudas et al. 2008, Linkosalo et al. 2009), ambiguous signals for spring phenology have been reported as well (Rousi and Heinonen 2007). Pudas et al. (2008) show that trends differ between the northern, central and southern boreal zone. They report that the timing of bud burst advanced least in the southern boreal zone (0.7 days year^{-1} compared to 1.4 days year^{-1} in the central and northern boreal zone), where Rousi and Heinonen (2007) conducted their experiment, working with a single stand. Since the study periods in Pudas et al. (2008) and Rousi and Heinonen (2007) are near identical (1997-2006 and 1997-2005, respectively), this suggests that trends found from studies covering large geographical areas may be confounded by differences in latitude and longitude between the places where observations were made. On the other hand, the variation in timing of bud burst across years may override the overall trend on small spatial scales, indicating that time-series longer than a few decades are needed to reliably estimate a trend in phenological observations (Rousi and Heinonen 2007).

However, temperature is not the only driver of plant growth and the final effects of climate change on tree growth depend on the interaction of many environmental variables, such as nutrient and water availability (Leuzinger et al. 2012). As an example, the temperature dependency of tree-ring width significantly weakened during the mid-20th century (e.g. Jacoby et al. 2000), which was probably due to limitations posed by other environmental variables, for example, water availability (D'Arrigo et al. 2008) or changes in winter precipitation (Vaganov et al. 1999).

Indeed, from the mid-20th century onwards widespread global drying was observed, increasing the occurrence of drought (Dai 2011). Drought in turn, reduces growth and ecosystem productivity (Bréda et al. 2006). Similarly, increased snow depth, resulting in delayed snow melt may reduce tree growth (Vaganov et al. 1999, Kujansuu et al. 2007). Moreover, the effect of a changing climate on tree growth depends on the environmental factors limiting growth in today's climate. For example, as a result of warming, growth is expected to decline in areas currently limited by water, whereas an increase in growth is expected in areas, like the boreal zone, where water is currently not limiting (Peñuelas et al. 2013). Modelling studies indeed show that tree growth in the boreal zone increases in response to temperature, irrespective of changes in precipitation (Briceño-Elizondo et al. 2006).

1.2 ACCLIMATION TO STRESSFUL CONDITIONS

Vulnerability to climate change is thought to depend on an organism's sensitivity, exposure, resilience and ability to acclimate to environmental change (Williams et al. 2008) and the capacity of a population to physiologically and morphologically adapt to these changes is key to its success under the new conditions (Bernardo et al. 2007). Molecular changes as a consequence of changes in gene-expression underlie the physiological and morphological plasticity needed to acclimate and ultimately adapt (Grishkevich and Yanai 2013, Peñuelas et al. 2013 and references therein). However, physiological and morphological acclimation is the result of changes in a complex of traits and underlying gene regulation is poorly understood (Howe et al. 2003), although attributes of genes exhibiting genotype x environment interactions (i.e. genes showing plasticity in their expression in response to the environment) are starting to be identified (Grishkevich and Yanai 2013).

It is clear that new, to some extent beneficial growing conditions as a consequence of a changing climate may also increase the occurrence of environmental conditions causing stress to plants. The loss of correlation between tree growth and temperature mid-20th century (Jacoby et al. 2000) indicates, for example, that temperature has surpassed a threshold above which limitations to growth occur, either directly as a result of increased temperature (Way and Oren 2010) or indirectly through correlation with other environmental variables like drought (Dai 2011). Currently, sustained drought and water logging are common stresses in forests (Niinemets 2010) and water availability, interacting with temperature, may create large-scale drought events limiting forest productivity and tree survival (Bréda et al. 2006). Moreover, in Northern Europe precipitation and temperature are changing and the occurrence of their extremes is predicted to increase (Jylhä et al. 2009, IPCC 2013). Therefore, temperature and water availability are relevant environmental variables when considering growth of silver birch in the boreal zone, not only to better understand tree growth under a changing climate, but also under current climatic conditions.

Responses of trees to differences in water availability and temperature are widely studied. All aspects of plant growth are acclimated to the environmental conditions at hand, optimizing the balance between, for example, water lost in maintaining photosynthesis and its availability in the soil (reviewed by Yordanov et al. 2000, Saxe et al. 2001, Kozlowski and Pallardy 2002, Chaves et al. 2003, Bréda et al. 2006, Way and Oren 2010, Poorter et al. 2012, Ashraf and Harris, 2013).

Under water stress or conditions of elevated temperature trees tend to minimize the loss of water, while maximizing its uptake. On a physiological level, water use efficiency (WUE; defined in this thesis as the ratio between net photosynthesis (P_n) and stomatal conductance (g_s)) is optimized. This is accomplished, among others, by reducing g_s through the closure of the stomata. This reduces transpiration of water from the leaves, allowing the xylem water potential in the leaves (WP) to remain high enough to prevent damage to the photosynthetic machinery. At the same time, however, reduction of g_s limits photosynthesis, reducing carbon gain. On a morphological level leaf area (LA) tends to be reduced, while the thickness (LT) and dry weight (DW) of the leaves tends to increase. This lowers the specific leaf area (SLA) and fresh-to-dry-weight ratio (FWDW) of the leaves. Such morphological and physiological responses are accompanied (but at the same time influenced) by changes in the investment in leaves, stem and roots relative to total biomass production. Reducing both leaf mass and leaf area relative to the total amount of plant biomass (leaf mass fraction (LMF) and leaf area ratio (LAR), respectively), while increasing the relative investment in roots (root mass fraction (RMF)), particularly fine roots (Koike et al. 2003) are efficient mechanisms to cope with low water availability. As a result, biomass allocated to the stem (SMF) as well the ratio between above and below ground parts (SRR) is reduced. Under conditions of excess soil moisture, availability of oxygen prevents the roots from functioning properly (Newsome et al. 1982, Kozlowski 1997), again reducing g_s and $P_{n,}$ limiting growth. As a consequence of limited root growth under such conditions RMF decreases, increasing SRR (Kozlowski 1997, Poorter et al. 2012).

Effects of temperature have been shown to differ between functional groups, i.e. between evergreen and deciduous trees, such that deciduous species tend to show larger responses to warming than do evergreen species (Way and Oren 2010). For example, in evergreen species, leaf mass and leaf area were less responsive to warming compared to deciduous species, but responses to temperature of traits related to photosynthesis were not different between both functional groups (Way and Oren 2010). These differences may be partly explained by wood structure (Hacke et al. 2001, Chave et al. 2009), indicating an interaction with water and nutrient transport (Hacke et al. 2001). In general, for deciduous species, elevated temperature brings about increased height growth, net photosynthesis, leaf mass, leaf area, and fine root length, while stomatal conductance and the shoot-to-root ratio decrease (Saxe et al. 2001, Way and Oren 2010).

Leaf pigments are also influenced by both temperature and water availability (Yordanov et al. 2000, Ashraf and Harris 2013 and references therein). Chlorophyll a (Chl a) and chlorophyll b (Chl b) content, both important pigments involved in light harvesting, have been shown to decrease in response to water availability and high temperature, but depending on plant species and genotype, an increase in response to drought has also been shown (Ashraf and Harris 2013). The concentration of carotenoids (Car), necessary for photoprotection, is less sensitive (Yordanov et al. 2000). In general, Chl a content decreases more rapidly compared to Chl b, decreasing the Chl a to Chl b ratio (Chl a/b). Although the photosynthetic machinery is comparatively resistant to changes in soil water availability (Cornic and Fresneau 2002), enzymes important for efficient photosynthesis, like ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) are also sensitive to both water availability and increased temperature, such that its functioning is usually decreased (Yordanov et al. 2000, Ashraf and Harris 2013). In this thesis the functioning of the mechanisms driving photosynthesis is approximated using the ratio between P_n under ambient and saturating CO_2 concentration [CO_2] (P_{n_amb} / P_{n_sat}). This ratio gives an indication of how much apparent photosynthesis was limited by [CO_2] under ambient conditions and may also reflect the influence of water availability on the photosynthetic machinery (Cornic and Fresneau 2002).

Water availability and temperature frequently interact in nature. When multiple stresses are superimposed, the optimal response changes, but the change is further affected by past stress history and ontogeny. Although responses to individual stresses are relatively well understood, responses to interacting stresses are not, especially so when ontogeny and whole-tree physiology are taken into account (Niinemets 2010). Therefore, data covering whole trees and different ontogenic stages are needed to advance our understanding of acclimation and adaptation of tree growth under stressful conditions.

1.3 THE IMPORTANCE OF VARIATION

Terrestrial ecosystems have been dominated by trees for more than 370 million years (Niklas 1997) and the plant communities making up forests today have existed for a few thousand years (Huntley and Webb 1989), developing sets of life-history traits that allow the species to coexist within those communities (Nakashizuka 2001). Thus, trees have a long history of adapting to changing environments and environmental stress of various magnitude, duration and origin and have shown to be capable of adapting (Jacobsen and Dieffenbacher-Krall 1995). Although the climate has been gradually warming since the last glacial period (Davis et al. 2003), current climate change goes beyond any changes experienced in the past, both in terms of magnitude and speed (Peñuelas et al. 2013), especially for the Northern latitudes (Benito-Garzón et al. 2014). Although trees combine life-history traits and levels of genetic diversity that may allow them to adapt relatively quickly to predicted environmental changes (Hamrick 2004, Petit and Hampe 2006), it is predicted that trees may not be able to keep up due to, for example, anthropogenic limitations to dispersal and gene-flow (Davis and Shaw 2001). This increases the risk of local extinction (Jump and Peñuelas 2005). Therefore, variation in traits relevant for growth and survival (like physiology and morphology and life-history traits such as phenology) among individuals in local populations is important for the long-term survival of these populations. The more unique individuals within a population, the higher the chance that an individual is present that has the ability to acclimate to the adverse or new conditions (Peñuelas et al. 2013).

The genotypic richness (i.e. the number of unique individuals) of local populations has been shown to determine its performance under adverse environmental conditions. Populations with a higher number of genotypes are able to cope better in terms of productivity (Drummond and Vellend 2012). Such studies have not been carried out for trees, but for trees a wealth of information on genetic variation among populations has been compiled over the last two centuries (Langlet 1971), mainly using provenance trials and common garden experiments. These trials typically include material covering large spatial scales. However, it has also been shown that for trees most of the genetic variation is found within populations (Rusanen et al. 2003, Järvinen 2004, Petit and Hampe 2006) and that studies including genetic material selected from large geographic areas may not accurately reflect the ability to adapt to adverse environmental conditions (Jump and Peñuelas 2005). Therefore, provenance trials are not particularly suited to study genetic variation within populations and despite the apparent importance this aspect remains mostly unstudied in trees. A notable exception is silver birch (*Betula pendula* Roth), where differences between genotypes within a local population have been shown for resistance to ozone and frost (Prozherina et al. 2003, Oksanen et al. 2005), insect herbivory (Silfver 2009), secondary chemistry (Laitinen 2003), drought tolerance (Possen et al. 2011) and phenology (Rousi and Heinonen 2007, Rousi et al. 2011). Without exception, these studies show differences between genotypes within a local population.

Despite the evidence for differences between genotypes within a local silver birch population, the magnitude of this variation for physiological, morphological and phenological traits and their connection to growth in both the current and a future climate has not been quantified. This is relevant, however, in the light of the persistence of local populations under adverse environmental conditions.

1.4 SILVER BIRCH AND ASPEN AS A STUDY SPECIES

The focus of this thesis is on silver birch, but the response of aspen (*Populus tremula* L.) to different levels of water availability was studied as well (I) to test if the same traits underlie superior growth in both species. Silver birch is the ecologically and economically most important broadleaved species in the boreal zone (Atkinson 1992, Hynynen et al. 2010). Currently, aspen mainly has ecological importance (Myking et al. 2011), but economic interest is increasing (Hynynen and Viherä-Aarnio 1999, MacKenzie 2010).

Both silver birch and aspen are typical, light demanding pioneer species with distribution ranges spanning the entire Eurasian continent (Atkinson 1992, Worrell 1995, Hynynen et al. 2010, MacKenzie 2010). Silver birch prefers lighter, more fertile soils and adequate soil moisture (Atkinson 1992). Aspen has only modest site requirements and may occur over a wider range of environmental conditions compared to silver birch (Niinemets and Valladares 2006), but grows best on well-drained, loamy soils rich in organic matter and nitrogen (MacKenzie 2010). However, both species are the climax species in sites unsuited for other species, particularly in frequently disturbed sites (Atkinson 1992, Worrel 1995). Furthermore, for both species northward range shifts are expected as a result of climate warming (Hemery et al. 2010, Bogaert et al. 2010).

There are key differences in reproductive strategies between silver birch and aspen, however. Silver birch is a monoecious, cross-pollinating, wind-pollinated species with efficient pollen dispersal (Hynynen et al. 2010). Reproduction through suckers is rare and only occurs after major disturbances (Atkinson 1992). Aspen, a dioecious, wind-pollinated species, almost exclusively regenerates through root suckers (Worrel 1995, Mackenzie 2010). Although seeds are produced, establishment from seeds is seldom accomplished (Worrel 1995).

These differences represent species-specific trade-offs in a life-history trait (Nakashizuka 2001). The near-exclusive dependence on regeneration through seeds that are efficiently spread by wind allows silver birch to quickly colonize new suitable sites (Atkinson 1992) and maintain high within-population genetic diversity (Rusanen et al. 2003, Järvinen 2004). However, a large proportion of the produced seeds may never germinate or establish successfully. Regeneration through root suckers allows aspen to successfully persist and regenerate in a certain site for long periods of time, but restricts the ability to colonize new, more distant sites. As a result, aspen typically occurs in small stands, containing only few genetically different individuals (MacKenzie 2010). Therefore, aspen may be more vulnerable to local extinction in the case of changing site conditions compared to silver birch, increasing the importance of the ability to acclimate to new environmental conditions.

1.5 AIM OF THE THESIS AND HYPOTHESES

The main aim of this doctoral thesis is to increase the understanding of the magnitude and importance of variation in traits relevant for growth and acclimation to changing temperature and water regimes among genotypes within a local population.

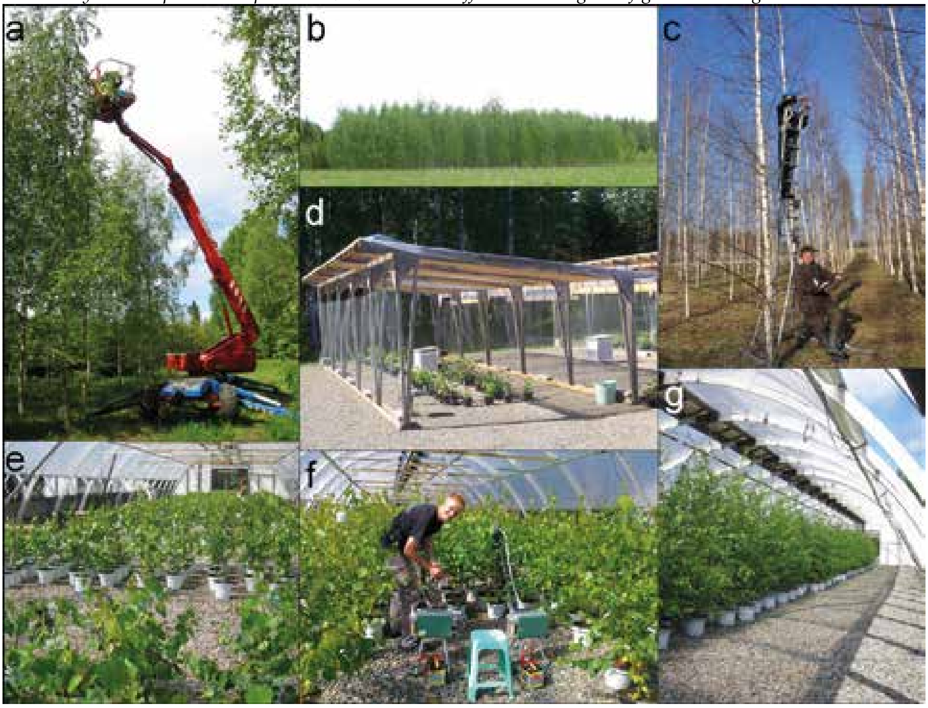
This thesis takes into account a suit of morphological (SLA, FWDW, LA, DW and LT) and physiological traits (P_n, g_s, WUE, P_{n_amb} / P_{n_sat} and WP) as well as traits related to the relative investment in plant biomass (LAR, LMF, SMF, RMF and SRR) relevant for acclimation to environmental conditions projected to change under a future climate (i.e. water availability and temperature). These traits were evaluated under field conditions to assess the importance of variation among genotypes in these traits for growth in the current climate (Figure 1a-c; II, III). The importance of variation in these traits for acclimation to possible future climatic conditions was evaluated by means of two controlled greenhouse experiment (Figure 1e-f; I, IV). Variation among genotypes was assessed for a set of 4 (I), 15 (II, III) or 10 (IV) genotypes, randomly selected from the same, single, naturally regenerated forest stand. To increase the understanding of molecular mechanisms underlying acclimation to drought gene-expression in response to water availability was studied in aspen (Figure 1d; I), using several genes that have previously been shown to be drought-inducible. Through measuring leaf morphological, physiological, phenological and biomass traits the whole tree was considered (I-V).

It was expected that differences between genotypes within a local population in relevant phenological (III), physiological (I, II, IV) and morphological (I, II, IV) traits could be used to explain differences in growth. Furthermore, it was expected that differences between genotypes in these traits could explain differences in acclimation to changing environmental conditions (I, IV), evaluated as differences in growth (IV). The questions addressed in the four articles included in this thesis and their accompanying hypotheses have been summarized in Table 1.

Table 1. Aims and hypotheses for the original articles included in this thesis

Article	
I	*Aim:* Assess the presence of differences in drought tolerance between genotypes within a local silver birch population and among aspen populations. *Hypothesis:* In response to changes in soil moisture content, there is large genotypic variation within birch and aspen populations which is expressed in growth as well as at the morphological and physiological level
II	*Aim:* 1. If present, how large are the differences between genotypes in leaf physiological and morphological traits within a local silver birch population? 2. Do these differences relate to differences in biomass among the genotypes after more than 10 years of growth under field conditions? *Hypothesis:* Differences between genotypes in these traits are present and can be used to explain variation in biomass within a local population
III	*Aim:* 1. Is the timing of phenological events different between genotypes within a local population? 2. If present, do these differences result in genotype-specific periods of carbon gain? *Hypothesis:* Significant differences between genotypes in both spring and autumn phenological events are present within a silver birch stand and lead to genotype-specific periods of carbon gain.
IV	*Aim:* 1. Do genotypes within a local population respond differently to adverse environmental conditions? 2. If present, do the responses to adverse environmental conditions change over time in a genotype-specifc manner? 3. Which traits underlie superior growth (in terms of biomass) under adverse environmental conditions? *Hypothesis:* Genotypes consistently respond differently to adverse environmental conditions and traits underlying superior growth under field conditions are also important in acclimation to adverse environmental conditions.

Figure 1: Photographs of the experiments studied in this thesis. a: Measuring leaf morphological and physiological traits up in the canopy in the field experiment (II), b: The field experiment from a distance (II, III), c: Observing spring phenology in the field experiment (III), d: One of the shelters with removable roof used in the drought experiment with silver birch and aspen (I), e: Overview (before campaign 1 in 2011) of one of the greenhouses used in the water availability and temperature experiment (IV), f: Measuring gas exchange in the water availability and temperature experiment (IV), g: Overview (campaign 2 2012) of one of the greenhouses used in the water availability and temperature experiment (IV). Note the difference in height in figure 1e and 1g.



2 Material and Methods

A summary of the material and methods for all four articles included in this thesis is given in Table 2 and photographs of the experiments are shown in Figure 1.

2.1 PLANT MATERIAL, EXPERIMENTS AND TREATMENTS

All silver birch genotypes were micropropagated from randomly selected trees growing in the same, one hectare mixed silver and downy birch (*Betula pubescens* Ehrh.) forest stand in Punkaharju, Finland (61°48′N, 29°18′E). The stand regenerated naturally after logging operations in 1979. The four aspen genotypes (I) were randomly selected from distant populations in southern Finland within the same latitude (61-62° N, 25-30° E).

The field experiment (II and III) was established in 1999 and consists of six blocks, each containing two replicates for 22 genotypes (Figures 1a-c). Based on biomass measurements after a thinning harvest in 2008, 15 genotypes (Table 2) covering the range of biomass in the field experiment were selected for further studies. One replicate tree for each genotype was selected from four randomly selected blocks, resulting in a total of 60 trees and four replicates for each genotype (II and III). Measurements of leaf morphology and physiology (II) lasted two growing seasons (Figure 1a), for phenological observations (III) three (Figure 1c). The time-series for bud burst was complemented with data available from the long-term monitoring efforts in the same field experiment (III).

The two greenhouse experiments (I and IV) focussed on drought, waterlogging and increased temperature. The experiment for article I, focussing on drought and waterlogging, was conducted in Punkaharju, Finland (61°48′N, 29°18′E) and lasted 50 days (2007). Four silver birch and four aspen genotypes (Table 2) were grown in two adjacent shelters, equipped with a removable roof, allowing for controlled soil moisture conditions, while retaining near ambient environmental conditions (Figure 1d). A split-split plot design consisting of 10 blocks and a sub-plot for each of the three contrasting watering treatments resulted in a total of 120 plantlets and 10 replicates for each genotype x treatment combination for both species.

The experiment for article IV, focussing on drought, waterlogging and increased temperature, was conducted in Suonenjoki, Finland (62°38′N, 27°03′E) and lasted two growing seasons (2011 and 2012). Ten genotypes (Table 2) were grown in two adjacent plastic greenhouses with open sides, allowing for controlled soil moisture conditions for the plantlets under close to ambient air temperature (Figures 1e-g). A split-split plot design consisting of 3 blocks and a sub-plot for each temperature x watering combination was used. Each sub-plot contained two replicates for each genotype in each year. Therefore, each year a total of 360 plantlets representing 3 replicates for each genotype x treatment combination was used.

The three watering treatments aimed to simulate excess, normal and limiting water in all greenhouse experiments (I and IV), equivalent to a volumetric water content (VWC) of >60%, 50% and <20%, respectively. The target VWC was maintained through daily manual watering and the amount of water needed was determined by weighting the pots. All plantlets started with normal VWC followed by the watering treatment that was applied for five to seven weeks (I, IV). Thereafter, the VWC in all pots was returned to normal for two weeks before a final measurement campaign was conducted to assess the recovery of the plantlets from the watering treatment (IV). In the experiment focussing on water availability (I), plantlets were harvested immediately after the watering treatment. The two temperature treatments (IV) aimed to maintain either ambient or ambient +1 °C at the top of the saplings. The ambient +1 °C treatment was applied using infrared heaters.

2.2 MEASUREMENTS

2.2.1 Phenology

In the field experiment phenological observations were conducted in the lower third of the canopy (III; Figure 1c). During 2010 to 2012 daily bud burst measurements were conducted for 2 branches, one facing north the other facing south, and 10 buds per branch (i.e. a total of 20 buds per tree). During 2011 and 2012, immediately after bud burst, leaf unfolding was monitored for a subset of 3 buds per branch (i.e. a total of 6 buds per tree) by measuring leaf length and width. In autumn (2011 and 2012), senescence was monitored by measuring the chlorophyll content of the leaves non-destructively, using a CCM-200 plus (Opti-Sciences Inc., Hudson, NH, USA). The chlorophyll content of all leaves emerging from the 20 buds selected in spring was measured. From these measurements, the start (50 % of final leaf size) and end (50 % chlorophyll loss) of carbon gain were estimated. The period of carbon gain was defined as the difference in days between the start and end of carbon gain. The time in days between bud burst and 50 % of final leaf size was used as a measure for leaf unfolding.

2.2.2 Leaf morphological traits

Leaf morphological traits were measured using fully expanded sun-exposed short-shoot leaves in the field experiment (II; Figure 1a) or the youngest fully expanded leaf on the main axis of the tree in the greenhouse experiments (I and IV). LA (using a Li-3000, Li-Cor Inc., Lincoln, NE, USA) and leaf fresh weight (FW) were determined in the field after which the leaf material was dried to constant dry weight (DW) at 60 °C. LA and DW were used to calculate SLA, FW and DW to calculate FWDW. LT was measured using a digital micro-meter (Mitutoyo absolute ID-C1012BS, Mitutoyo, Japan).

2.2.3 Physiological and pigment traits

Like leaf morphological traits, physiological traits were measured using fully expanded sun-exposed short-shoot leaves in the field experiment (II) or the youngest fully expanded leaf on the main axis of the tree in the greenhouse experiments (I and IV; Figure 1f). Leaf level gas exchange (P_n and g_s) were measured under saturating light (1000 (II), 1100 (I) and 1250 (IV) μmol m^{-2} s^{-1}) and both ambient (380 ppm) and saturating (950 (I) and 1000 (II, IV) ppm) [CO_2] using portable gas exchange equipment (LiCor 6400 and LiCor 6400XT, Li-COR Inc., Lincoln, NE, USA). P_n and g_s were used to calculate WUE and P_n under ambient and saturating [CO2] was used to estimate carboxylation limitations (P_{n_amb}/P_{n_sat}, denoted as PC in article I). WP was measured using the pressure bomb method (Scholander et al. 1965). In experiment I dark-respiration was measured under ambient [CO_2]. Leaf pigment concentrations (IV) were determined following the spectrophotometric method described by Wellburn (1994).

2.2.4 Biomass and biomass allocation

For the trees growing in the field experiment (II and III) total biomass (sum of roots, stem, branches and leaves) was estimated from annual height and diameter measurements using biomass equations developed for silver birch in Finland by Repola (2008). In the greenhouse experiments (I, IV) biomass was measured at the end of the growing season by means of destructive harvests of the plantlets, assessing fresh and dry weight of roots, stem, branches and leaves separately. Ratios between the different biomass fractions were used as a measure of biomass allocation and changes in tree architecture (IV).

Table 2. Overview of the experiments, materials and measurements reported in the four articles. Silver birch genotypes common to all articles are shown in bold.

Article	**I**		**II**	**III**	**IV**
Species	silver birch	aspen	silver birch	silver birch	silver birch
No. genotypes	4	4	15	15	10
Genotypes	**12**, **14** 15, **25**	6, 22 51, 52	2, 3, 4, 8, **12**, **14**, 16, 17, 18, 19, 22, 23, 24, **25**, 26	2, 3, 4, 8, **12**, **14**, 16, 17, 18, 19, 22, 23, 24, **25**, 26	2, 4, 8, **12**, **14**, 18, 19, 23, **25**, 26
Experiment	Shelter		Field	Field	Open greenhouse
Treatments	Watering		None	None	Watering and Temperature
Measurements and estimates	Biomass Leaf morphology Physiology Gene-expression[1]		Biomass Leaf morphology Physiology Leaf pigments	Bud burst Leaf unfolding Senescence Period of C-gain	Biomass Biomass allocation Leaf morphology Physiology Leaf pigments

[1] Gene-expression was studied only in aspen genotype 6

3 Results and Discussion

3.1 SUMMARY OF THE MAIN FINDINGS

The aim of this thesis was to determine the magnitude of differences between genotypes for relevant physiological, morphological and phenological traits and the importance of these differences for growth and acclimation.

For almost all traits measured, there were significant differences between genotypes (I-IV). Notable exceptions were the estimated period of carbon gain and its determinants (III) and P_n (I and II). Differences in bud burst in spring were large whenever cold spells interrupted bud burst (III). Leaf morphological traits, specifically SLA and FWDW, had most explanatory power with regards to variation in biomass in the field experiment, while phenological and physiological traits played a minor role (II, III). Changes in biomass allocation, specifically an increase in root mass at the expense of leaf mass and leaf area, and physiological traits were important in acclimating to the treatments under greenhouse conditions (I, IV).

The conclusions from each of the four articles included in this thesis are summarized in Table 3.

Table 3. Conclusions from the articles included in the thesis.

Article	
I	• Acclimation to soil moisture conditions varied among genotypes. • Aspen and silver birch utilized different strategies to cope with the soil moisture conditions. • Gene-expression in aspen was changed in response to soil moisture conditions.
II	• There was considerable variation among genotypes in leaf morphological and physiological traits and they could be used to explain the differences in growth between the genotypes observed after 10 years of growth under field conditions. • Of the measured traits, leaf morphological traits, specifically SLA and FWDW, had most explanatory power with regard to the variation in biomass among genotypes; genotypes with the lowest SLA and FWDW produced most biomass under field conditions.
III	• Differences between genotypes within a local population in the timing of phenological events were present, but did not result in genotype-specific periods of carbon gain. Therefore, the measured phenological traits could not be used to explain the differences in growth between the genotypes observed after 10 years of growth under field conditions. • Variation in the timing of bud burst among genotypes was compensated for through faster leaf development in genotypes with 'late' bud burst, resulting in an equal estimated period of carbon gain among genotypes. • Genotypes with 'late' bud burst were more sensitive to variable temperature sum accumulation during spring compared to genotypes with 'early' bud burst.
IV	• Genotypes generally showed similar acclimation responses to the treatments. Exceptions were traits related to the relative investment in leaves. • The investment in leaves was acclimated to the environmental conditions during the first year of treatment only, while leaf morphological and physiological traits showed acclimation during both growing seasons • Those plantlets that were able to maintain high P_n by maintaining high WP through increased investment in roots at the expense of leaves produced showed superior growth (in terms of biomass and height), irrespective of treatment.

3.2 DIFFERENCES BETWEEN GENOTYPES AND THEIR IMPORTANCE IN THE FIELD

3.2.1 Phenology

The magnitude of the differences between genotypes in the timing of bud burst were found to vary from year-to-year, but the same genotypes consistently showed 'early' or 'late' bud burst (III). Because temperature is the main environmental variable driving bud burst in pioneer species (Basler and Körner 2012), like silver birch (Rousi and Heinonen 2007), this indicates that even within a local population genotypes with different responses to temperature conditions in spring are retained. Such differences may be a consequence of the (micro) climate during seedling establishment (Kelly et al. 2003) or may reflect differences in the temperature regime at the sites from which the pollen originated (Rousi et al. 2011). Both may allow for the occurrence of individuals with different climatic optima in the same population, despite strong natural selection (Savolainen et al. 2007). However, the differences between genotypes may also represent normal local genotypic differences, since the chance of successful pollination by pollen transported over long distances is small (Rousi et al. 2011). Furthermore, when established, even individuals not optimally adapted to local environmental conditions may remain in a population of trees for a long time (Kuparinen et al. 2010). For this population the presence of genotypes with different climatic optima in a local population represents a possibility to acclimate and ultimately adapt to projected climate change.

Phenology of silver birch has been studied intensively, but there still is contradicting evidence as to what baseline temperature is best used for the prediction of bud burst. For the seven-year time series of bud burst included in this thesis the predictive power of baseline temperatures 0 and 5 °C was determined following Rousi and Pusenius (2005) and Rousi and Heinonen (2007) and the emerging pattern is inconsistent (Table 4). The error in the prediction was different between years and genotypes and the year x genotype interaction was significant (data not shown). The error between the predicted and the actual date of bud burst was greatest in genotype 17, as a result of the early bud burst in the cold spring of 2003 (Table 4).

The result is consistent with literature. Rousi and Heinonen (2007) found that a baseline temperature of 5 °C accurately predicted the date of bud burst in spring. Rousi and Pusenius (2005) found that from a range of baseline temperatures (+5 - -2 °C), temperatures between +2 and -1 °C were most accurate in predicting bud burst in silver birch. Sarvas (1972) on the other hand showed that bud development is possible even at 0 °C and Gunderson et al. (2012) concluded that temperature sum alone could not sufficiently explain the variation in date of bud burst. Myking and Heide (1995) and Heide (2003) found that high autumn temperatures delayed bud burst the following spring. Furthermore, in modelling studies different starting dates for temperature sum accumulation are frequently found (e.g. Linkosalo et al. 2008 and 2009).

The former clearly shows that there is much to learn about the way trees sense the environment in order to correctly time the transition from dormancy to active growth. The main issue seems to be that the correlation between growth initiation measured as visible bud burst and the shift from dormancy to active growth is rather loose (Howe et al. 2000). Bud burst is the final, but first visible stage in the shift from dormancy to active growth. Ultimately, the phenological cycle is controlled by meristem activity (Horvarth 2003, Russell et al. 2013) and mitotic activity is restarted well before any external signs of bud burst can be observed (Rinne et al. 2011, van der Schoot et al. 2014). Consequently, how the environment is sensed with regard to the timing of bud burst and what mechanisms regulate spring phenology is not well understood (Howe et al. 2003). Molecular mechanisms and hormone regulation of dormancy development are better understood (Russell et al. 2013, van der Schoot et al. 2014) and may provide clues to how dormancy is released. According to Russell et al. (2013), meristems remain sensitive to hormones promoting growth like gibberellic acid (GA) after they have entered dormancy. Short days inhibit the synthesis of GA and promote the synthesis of DELLA-proteins (growth inhibitors), maintaining dormancy. Lengthening of the days allows GA-synthesis to restart. GA then stimulates the breakdown of DELLA-proteins, allowing the resumption of meristem activity and growth (Russell et al. 2013).

This mechanism may also be at work in species that are thought to be irresponsive to photoperiod at the onset of bud burst, like silver birch. For example, in silver birch phytochromes, responsible for sensing light (Smith 2000), have been suggested to be involved in the regulation of the annual cycle (Linkosalo and Lechowicz 2006). As indicated previously, visible bud burst follows the re-initiation of meristem activity. Therefore, release of dormancy may depend on photoperiod, at least partly, after which temperature becomes the most important regulator of the further development of the buds. Development of vegetative tissues has been shown to be highly temperature dependent (Larcher 2003). Furthermore, it has been suggested that phytochrome regulation may be subject to high within-species variation (Smith 2000), allowing for the genetic variation in the regulation of spring phenology observed in article III.

The differences between genotypes in bud burst were compensated for through faster leaf development in genotypes with ‘late’ bud burst (III). Fast leaf development in genotypes with ’late’ bud burst is possible, because development of vegetative tissues is strongly temperature dependent (Larcher 2003). As spring progresses, average daily temperature increases continuously. Therefore, genotypes with late bud burst are likely to benefit more from higher average temperatures during leaf development, increasing the speed of leaf development in these genotypes compared to genotypes with early bud burst.

Some caution is needed, since a longer time-series, including years with more variable temperature development in spring (like 2003), could shed more light on the importance of the relationship between temperature sum at bud burst, leaf unfolding and the period of carbon gain.

Table 4: Mean day of the year (DoY) and temperature sums at the start of bud burst (7-year average) and the yearly deviation in days between the observed and predicted start of bud burst per genotype. The baseline temperatures for temperature sum calculations were 5 °C (BL5) and 0 °C (BL0). For example: for the whole 7-year time series, genotype 2 started bud burst at a BL5 of 16.0 (Mean BL5). In 2002 the mean BL5 of 16.0 was reached 1 day earlier compared to the mean date of bud burst (Mean date). Mean Y: average (absolute values) over years. Mean GT: average (absolute values) over genotypes. Letters represent significant different at P<0.05 for Mean Y. Data are the mean of 4 replicates per genotype per year, 28 replicates for Mean GT and 60 replicates for Mean Y.

Year	Genotype																
	2	3	4	8	12	14	16	17	18	19	22	23	24	25	26	**Mean Y**	
Mean DoY	123	121	123	123	124	124	123	121	123	120	123	125	125	125	124	**123.1**	
Mean BL5	16.0	14.3	18.2	18.1	19.8	20.3	18.6	15.5	17.3	11.1	18.3	24.7	24.6	24.7	19.5	**18.2**	
2002	-1	0	-2	-3	-2	-3	-1	-2	-2	0	-3	-2	-2	-1	-3	**2.0**	a
2003	2	-5	2	4	4	5	3	-9	4	-5	3	4	4	4	5	**5.0**	b
2004	2	2	1	2	1	1	1	1	1	2	2	1	2	2	2	**1.4**	a
2005	-1	-1	-1	-2	-3	-3	-1	-2	-1	2	-2	-4	-3	-3	-1	**2.1**	a
2010	-8	-8	-9	-9	-6	-9	-7	-7	-6	-10	-8	-7	-8	-8	-8	**7.9**	c
2011	2	1	1	2	2	2	1	2	1	1	1	1	0	1	1	**1.2**	a
2012	0	1	-1	-1	0	1	-2	4	0	0	0	0	-2	-1	-3	**1.7**	a
Mean GT	*2.3*	*3.3*	*3.1*	*3.3*	*2.5*	*3.5*	*2.3*	*4.1*	*2.4*	*3.1*	*3*	*3*	*3.2*	*2.7*	*3*	**3.0**	
Mean BL0	123.9	118.7	127.7	127.2	134.8	133.2	129.9	119.8	128.6	106.6	128.3	143.2	142.1	142.7	131.1	**129.3**	
2002	-1	-1	-2	-3	-2	-3	-1	-2	-3	0	-3	-2	-2	-1	-3	**2.1**	bc
2003	2	-5	1	3	3	4	3	-9	3	-5	2	4	4	4	5	**4.7**	d
2004	1	1	0	1	0	0	0	0	0	1	1	0	1	1	1	**0.5**	a
2005	5	6	5	4	3	3	5	5	5	8	4	3	4	4	5	**4.4**	d
2010	1	0	0	0	2	-1	2	1	2	1	1	-1	-1	-1	1	**1.4**	ab
2011	-1	-1	-2	-1	-2	-2	-2	-1	-2	-1	-2	-6	-6	-5	-2	**2.4**	c
2012	1	1	0	0	1	2	-1	4	1	-2	1	1	-1	0	-2	**1.7**	bc
Mean GT	*1.9*	*2.9*	*2.8*	*2.1*	*2.1*	*2.3*	*2.4*	*3.6*	*2.5*	*2.8*	*2*	*2.5*	*2.7*	*2.8*	*2.5*	**2.5**	

For this local population no differences in the timing of the end of the period of carbon gain were found (III). Decline of photosynthetic capacity and chlorophyll content are known to vary little among years (Morecroft et al. 2003, Keskitalo et al. 2005). Because of the importance of a correct timing of winter hardening and dormancy in the boreal zone (Sarvas 1974, Koski and Sievänen 1985), there may simply be little room for variation in the end of the period of carbon gain (Morecroft et al. 2003, Keskitalo et al. 2005), especially within local populations. Together with the near equal start of the period of carbon gain, this resulted in equal estimated periods of carbon gain among genotypes. Furthermore, although autumn senescence is thought to be under strong photoperiodic control (e.g. Kramer 1936, Viherä-Aarnio et al. 2006), growth cessation is likely to be modified by temperature (Hänninen and Tanino 2011 and references therein). For example, in a recent study Rousi et al. (2012) reported that Finnish silver birches readily acclimated to growth conditions in Canada where the photoperiod was continuously longer than those required for the initiation of dormancy in Finland; they outgrew the local Canadian birch species.

Accounting for differences between genotypes in the timing of phenological events is a hurdle that needs to be overcome in order to be able to develop generalizable phenological models (Richardson et al. 2013). The results in this thesis suggest that differences in the timing of phenological events, mainly spring phenology, between genotypes on small spatial scales exist and that genotypes with different climatic optima may be retained on this spatial scale. Although the genotypes were well adapted to the local climatic conditions (the differences in the timing of phenological events tended to be small and across the 7-year time-series no frost events occurred after bud burst was started), the differences were much more pronounced under conditions of variable temperature sum development in spring. Therefore, when considering climate change, such differences may be important for the persistence of local populations (Peñuelas et al. 2013).

3.2.2 Physiological and morphological traits

Regulation of growth under field conditions is complex, involving simultaneous acclimation of all aspects of tree growth to a multitude of environmental variables acting in concert (Niinemets 2010) and therefore is still poorly understood. This thesis, based on a suit of interacting traits determining growth, shows that variation among genotypes in most measured traits had little relative importance in explaining differences in biomass between genotypes (II). This is in line with previous studies showing that different morphological and physiological configurations can potentially lead to a similar outcome in terms of growth under field conditions (Niinemets 2010, Bertolli et al. 2013). Among the traits studied in this thesis, SLA and FWDW had most explanatory power with regard to the variation in biomass among genotypes under field conditions. Furthermore, these traits had a consistent negative relationship with mean genotype biomass (II). Thus, genotypes with high biomass tended to have lower mean values for SLA and FWDW.

The observed negative trend is not common in literature; in most cases SLA and FWDW tend to show a positive correlation with growth (Poorter and Remkes 1990; Cornelissen et al. 1996; Poorter and de Jong 1999; Poorter et al. 2012), although the *Betulaceae* family may be an exception (Cornelissen et al. 1996). However, on small spatial scales the correlation between SLA and growth tends to weaken due to larger within-species variation (Poorter and de Jong 1999), indicating that trade-offs with other functions of SLA become more important.

SLA and FWDW govern growth as part of a complex, poorly understood trait syndrome, including both morphological and physiological traits (Garnier and Laurent 1994, Castro-Díez et al. 2000, Wright et al. 2004, Poorter et al. 2012, Tholen et al. 2012). Traits belonging to this syndrome, among which SLA and FWDW, are also involved in other essential functions, like defence against herbivores (Coley 1988, Pérez-Harguindeguy et al. 2003, Wright et al. 2004). Leaves with a lower SLA and FWDW are more durable, with higher resistance against herbivores and physical damage and a longer leaf life-span (Coley 1988, Pérez-Harguindeguy et al. 2003, Wright et al. 2004).

One of the aspects of the ecological importance of silver birch is its specific association with a number of herbivorous insects (Atkinson 1992). Immature leaves are most nutritious to herbivores (Kursar and Coley 1991) and young leaves experience higher levels of herbivory (Coley 1980). Short-shoot leaves contribute most to tree growth in various species in the *Betula* genus (Kozlowski and Clausen 1966) and for some of these species (*B. platyphylla* Suk. and *B. ermanii* Cham.) greater investments in the defence of short-shoot leaves have indeed been reported (Matsuki et al. 2004). Thus, genotypes investing in more durable short-shoot leaves (i.e. lower SLA and FWDW), possibly resulting in less damage or lower maintenance costs during the growing season, may have an advantage in terms of long-term growth. It is likely therefore that a trade-off exists between the various functions of these traits (Herms and Mattson 1992).

Measurements in the field experiment concern short-shoot leaves at the top of the canopy: sampling short-shoot leaves, developed under similar environmental conditions in spring, ensured comparability of the measurements both within and across growing seasons. This protocol eliminated or reduced variation due to differences in leaf age and light conditions between genotypes within campaigns while retaining changes across the growing season, but does not consider differences between leaves in different canopy positions. At the top of the canopy higher irradiation and temperature and lower xylem water potentials of the leaves require more rigid (low SLA and low FWDW) leaves with lower osmotic potentials (reviewed by Niinemets and Valladares 2004). Therefore, more durable leaves may also reflect better acclimation to the microenvironment found in the upper canopy. However, in boreal spring conditions, short-shoot leaves develop under high light, but high temperature or drought conditions generally do not occur. Since the morphology (i.e. LA, SLA) of leaves is acclimated to the environmental conditions during which they develop (Niinemets 2007), it seems unlikely that more durable leaves in genotypes with higher biomass represent acclimation to the specific conditions experienced by the leaves measured in this thesis.

3.3 TRAITS IMPORTANT FOR ACCLIMATION TO ADVERSE CONDITIONS

Differences between genotypes within local populations are important with respect to acclimation to adverse environmental conditions or adaptation to a changing climate (Peñuelas et al. 2013). In a field experiment with older trees (II, III), it is not possible to accurately study the importance of a single environmental factor. To study the importance of differences between genotypes found under field conditions for acclimation to new environmental conditions this thesis includes two controlled greenhouse experiments focussing on two relevant environmental parameters, water availability (I, IV), temperature and their interaction (IV).

From the greenhouse experiments it became clear that the ability to maintain high P_n and WP through increased investment in root mass at the expense of leaf area and leaf mass resulted in superior growth in terms of biomass and height (IV), irrespective of treatment. In contrast, growth was not strongly related to physiological traits in the field experiment (II), confirming the complex regulation of growth in silver birch.

That different traits proved to be important in the field experiment compared to the greenhouse experiments may be partly due to ontogeny (Niinemets 2010). The trees in the field experiment were older and larger compared to the plantlets in both greenhouse experiments. Smaller trees, like the plantlets used in the greenhouse experiments, are much more dependent on the immediate carbon sequestered in the leaves, while more mature trees can utilize carbon reserves stored in their woody biomass (e.g. Niinemets 2010). Moreover, more mature, larger trees are less affected by mild or short-term drought due to their more extensive root system and its ability to reach deeper, more stable sources of water (Dawson 1996). As a result, acclimation of physiological traits related to the use of water (P_n, WP) and biomass allocation to leaves and roots are likely to be more important in younger trees, irrespective of treatments, indicating the importance of ontogeny in considering acclimation to a changing environment.

Allometric relationships were affected by water availability only during the first year of treatment (2011), while leaf morphological and physiological traits were affected in both years (2011-2012; IV). Furthermore, after the first growing season, differences between genotypes in biomass and height growth were modest, but increased after the second growing season (IV). Under moderate levels of stress, juvenile trees are known to be able to realize similar growth, irrespective of treatment (Padilla et al. 2009), but allocation patterns change (Poorter et al. 2012). Thus, the data (IV) suggest changes in allocation in response to the treatments during the first year primed the plantlets for the conditions to be encountered during the second year (Liu and Dickmann 1993). That the differences between treatments in terms of growth became more pronounced only after the second year (2012) indicates that in the long run reoccurring environmental stress, in concert with effects of secondary stressors, for example, herbivores (e.g. Pusenius et al. 2002, Prittinen et al. 2003, 2006), may determine the future structure of the population (Peñuelas et al. 2013).

However, where in the field experiment with older trees the genotypes clearly responded differently to the environment they encountered (II, III), this typically was not the case in the greenhouse experiments with younger plantlets (I, IV). This shows that extrapolating results from greenhouse experiments with young plants to older trees under field conditions remains a challenge (Wolkovich et al. 2012). Similarities can be found, however. In both the field and greenhouse experiments different responses by the genotypes (genotype x environment or treatment) were found for leaf-related traits (SLA (I) and LMF (IV)). Leaves are the main sites for photosynthesis in plants, providing them with the energy needed for growth and reproduction, while water is lost in the process (Larcher 2003, Lambers et al. 2006).

This illustrates the need and importance for properly balancing investments in leaves, their shape and size in response to adverse conditions.

For organisms with a complicated genetic structure, like trees, it is not known whether populations differ in their plasticity (Salmela 2014). The significant genotype x treatment interaction found for LMF (IV), indicates that within a small population the extent to which allocation patterns are changed in response to adverse environmental conditions may depend on genotype. This suggestion is further strengthened by the seemingly consistent occurrence of genotype x environment interactions in the same genotype; genotype 25 was the only genotype to show a genotype x treatment interaction in both greenhouse experiments (I, IV).

Genotype x treatment interactions are ultimately determined by gene-expression, but the mechanisms are still poorly understood (Nicotra et al. 2010, Grishkevich and Yanai 2013). Genes displaying genotype x environment interactions are thought to have unique regulatory mechanisms and to be affected by the sum of changes at all loci involved in the expression of a certain trait (Grishkevich and Yanai 2013). It has recently been shown for trees that differences in the environment (treatments) trigger different gene-expression patterns in genetically identical individuals (Jermstad et al. 2001, 2003, Pelgas et al. 2011), but the underlying mechanisms remain unclear (Nicotra et al. 2010, Grishkevich and Yanai 2013).

All patterns and correlations reported in article IV with respect to acclimation to adverse conditions are based on individual silver birch plantlets. None of the correlations were significant at the level of genotypes, making it difficult to draw conclusions on possible different strategies employed by individual genotypes in acclimating to adverse environmental conditions. Nevertheless, the results show that differences in plasticity to changing environmental conditions are possible within local populations. Such differences may be important in the acclimation and ultimately the adaptation of local populations to a changing environment. This clearly deserves further research.

3.4 DIFFERENCES BETWEEN ASPEN AND BIRCH

In this thesis the focus is on silver birch, but in article I, the acclimation responses of silver birch and aspen were compared. Although in article I results from aspen were confounded with infection by *Venturia* spp. (a common fungus affecting aspen, especially in years with high levels of precipitation in summer (Newcombe 1996, MacKenzie 2010)), there were differences in growth, leaf morphology and physiology between the two species. These differences were most pronounced in the dry treatment. Together the differences were indicative of different strategies for coping with adverse soil moisture conditions: aspen appeared to invest more in perennial parts, while silver birch seemed to maintain foliar processes more effectively.

These differences may be related to the life-history of both species. Aspen almost exclusively uses vegetative reproduction i.e. root suckers (Worrel 1995). This implies that newly established aspen plantlets can partly rely on resources obtained by an already established root system (Bärring 1988). Silver birch in contrast, almost exclusively establishes through seed (Atkinson 1992), implying that seedlings have to rely on their own capacity to acclimate to adverse environments from the beginning. Thus, in the presence of an already established root system, investment in perennial parts might be advantageous in terms of competition for light, drawing nutrients from this root system under conditions when resources become limiting for seedlings of species that cannot rely on an already established root system. This may be related to the ability of aspen to persist in the same site for long periods of time (MacKenzie 2010). In the absence of an already established root system, acclimation of foliar processes might be advantageous for maintenance of maximum height growth rate relative to the environmental conditions, ensuring high competitive ability in distant sites that may have become available for colonization only recently. This illustrates that traits underlying superior growth are likely to be species specific and are likely related to a species' life-history (Nakashizuka 2001).

4 Conclusions

Based on a large number of genotypes representing a single stand this thesis shows the complex regulation of growth in trees. To date, this regulation is poorly understood, especially when multiple environmental factors interact, like in field experiments. This may be a result of the tendency in science to compartmentalize, loosing track of the whole. In this thesis a broad spectrum of relevant traits was included, coming closer to a whole-tree approach.

Differences between genotypes in bud burst were usually small, but were greatly enhanced under conditions of variable temperature sum development in spring. Additionally, the presence of genotypes with consistent 'early' and 'late' bud burst shows that even within a local population, genotypes with different climatic optima are retained. This may be important for the acclimation of local populations to changing environmental conditions. Differences between genotypes in bud burst were compensated for through faster leaf expansion in genotypes with 'late' bud burst, resulting in an equal estimated period of carbon gain. As a result, together with a similar timing of the end of carbon gain among genotypes, the estimated period of carbon gain was similar among genotypes. Therefore, the measured phenological traits could not be used to explain the variation in growth observed among the genotypes. Nevertheless, differences between genotypes in the timing of phenological events and the presence of genotypes with different climatic optima provides possibilities for the acclimation (and ultimately the adaptation) of local population to a changing climate. In this light, the mechanisms involved in the transition from dormancy to active growth deserve further research, because the consequences for a correct timing of phenological events under a changing climate can only be accurately predicted once these mechanisms have been elucidated.

In both the field and greenhouse experiments differences between genotypes in morphological and physiological traits occurred as a rule. However, in all experiments differences between genotypes tended to vary over a rather narrow range for most of the measured traits. Under field conditions, leaf morphology, specifically SLA and FWDW, had most explanatory power with regard to the variation in estimated biomass observed in the field. Genotypes with low SLA and FWDW achieved superior growth in terms of biomass. This may be related to insect herbivory, since leaves with low SLA and FWDW are more resistant to herbivory, meaning that such leaves require less investments in terms of, for example, maintenance and repair. Thus, within a local silver birch population different morphological and physiological configurations may lead to a similar outcome in terms of biomass conditions, but SLA, FWDW and their variability stand out as important traits for growth.

Under greenhouse conditions, the relative investment in leaves, both in terms of mass and area, was acclimated to the adverse environmental conditions during the first year of treatment only, while leaf morphological and physiological traits showed adjustment during both growing seasons. Furthermore, the genotypes mostly responded in a similar way to the adverse conditions. Plants that were able to maintain high P_n by maintaining high WP through increased investment in root mass at the expense of investment in leaf area and leaf mass, produced most biomass and grew tallest, irrespective of treatment. Exceptions were traits related to relative investment in leaves (IV), SLA and root length (I) indicating that changes in allocation patterns as a result of adverse environmental conditions may depend on genotype, even within a local population. The different relative importance of the measured traits under field and greenhouse conditions shows the importance of taking ontogeny into account when considering acclimation and adaptation to projected climate change. Although no genotype-specific acclimation responses were observed, there were indications that differences in plasticity in response to changing environmental conditions are possible within local populations. This deserves further research, because such differences may be important in the acclimation and ultimately the adaptation of local populations to a changing environment.

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Searching for traits behind growth and biomass:

A case study with silver birch

How well a local population of trees is able to acclimate to changing environmental conditions depends on the differences between individual plants (genotypes) within such populations. In this thesis these differences are quantified under field conditions as well as under changing water and temperature regimes for a suit of physiological, morphological and phenological traits and traits related to biomass allocation. Subsequently the differences are related to the growth of the genotypes, improving the understanding of tree growth not only in the current, but also under a future climate.

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