GÖTTINGER ZENTRUM
FÜR BIODIVERSITÄTSFORSCHUNG UND ÖKOLOGIE

– GÖTTINGEN CENTRE FOR BIODIVERSITY AND ECOLOGY –

Variability of physiological traits and growth performance in aspen assemblages differing in genetic relatedness

Dissertation zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität Göttingen

vorgelegt von

Diplom-Biologin

Annika Müller

aus
Bad Oeynhausen

Göttingen, Januar, 2011

Referent: Prof. Christoph Leuschner

Korreferent: Prof. Christoph Kleinn

Tag der mündlichen Prüfung: 09.02.2011

There stood a Poplar, tall and straight
The fair, round Moon, uprisen late,
Made the long shadow on the grass
A ghostly bridge 'twixt heaven and me
But May, with slumbrous nights, must pass;
And blustering winds will strip the tree.
And I've no magic to express
The moment of that loveliness.

(Siegfried Sassoon)

Summary

Due to the increasing demand for wood and renewable energy sources, short-rotation forestry with its reliance on highly productive *Populus* species is in the focus of current ecological research. In order to optimize biomass gain, *Populus* research has in recent decades mainly centred on productivity and related traits for poplar species which could be proven to be high-yielding. Aspen (*Populus tremula* and *Populus tremuloides*), however, have for a long time been neglected, because their highest annual increment occurs later than for e.g. black poplar and balsam poplar and they therefore need longer rotation times. However, in this thesis we concentrated on aspen (*P. tremula* and *P. tremuloides*) as study objects because they cope better with drought on poor soils and have the lowest demand regarding habitat conditions when compared to other poplar species. These features make aspen a promising alternative for short-rotation forestry in the face of the future climate scenarios, which include increasing temperatures and decreasing summer precipitation.

Therefore, we established a common-garden experiment in 2008 with closely related aspen full-sib families (*P. tremula*: 2-30% genetic distance) as study objects. In the subsequent year, we established a field experiment with more distantly related aspen collectives (*P. tremula*: 20-40% genetic distance) originating from Central Europe as well as two different aspen species (*P. tremula* with German origin and *P. tremuloides* with American background differing 77% in their genetic distance) as study objects. Hence, we worked with three aspen assemblages along a gradient of genetic relatedness.

In both experiments we investigated more than 30 phenotypic traits with the aim 1) to identify from this pool of phenological, morphological and plant physiological traits, the best biomass predictors and controlling factors and 2) to reveal their contribution to successful plant growth for each study assemblage and 3) to determine their dependency on genetic constitution. The ultimate goal of this study was to use our results to provide advice for plant breeding and cultivation programmes in the context of short-rotation forestry.

The results of the common-garden experiment showed that despite genetic distances of 2 to 30%, the aspen full-sib families had no significant differences in photosynthesis related traits, even though productivity differed up to twofold between the families. Growth rate was related to several morphological traits, most closely to leaf number and total leaf area. The start of bud burst correlated with the leaf number (early-starting families produced more leaves), and was significantly influenced by the genetic constitution.

The more distantly related aspen collectives studied in the field experiment differed by more than 30% in productivity with a large genotype effect, while assimilation rate and most

photosynthetic and water status traits showed a relatively small intraspecific variation with no significant influence on productivity. The timing of the beginning of net leaf loss (leaf abscission > leaf production) in early and mid-summer differed between the studied aspen collectives and resulted in different maximum leaf areas and ratios of leaves lost to leaves produced, which were identified as key factors controlling productivity.

The comparison between the two aspen species showed a 20% higher productivity in American aspen than European aspen, which was nearly entirely caused by a larger mean leaf area of *P. tremuloides*, while mean assimilation rate and the length of the leafy period were of minor importance. Species-specific differences in the onset of leaf abscission in early autumn were identified as main determinants of the size of mean plant leaf area and thus of productivity.

This study showed that most plant physiological parameters were not suitable for selection or breeding programmes due to their low phenotypic variation, but should not be neglected in growth experiments because their impact on productivity might increase under non-optimal habitat conditions. Therefore we conclude that selection for high-yielding aspen genotypes should focus on leaf phenology and total leaf area associated traits, because they are stable and have a great impact on yield irrespective of the variability in the plant material.

Chapter 1

General Introduction

Short-rotation forestry

The global demand for wood in terms of biomass or wood-based energy is expected to grow 1.7% annually and hence will increase by 20% in the coming decades. At the same time, deforestation is reducing the world's forest cover by 9.4 million hectares per year (FAO 2008). Most of the world's wood is still taken from naturally grown forests, and only 12% of the total amount of wood consumed is provided by trees which were grown in plantations (Fenning and Gershenzon 2002). This ongoing destruction of forests will lead to an irreplaceable loss of habitat and the endangering of wildlife (Fox 2000). According to the afore mentioned mismatch between wood demand and actual stock, as well as the agreement of sustainable forestry as proclaimed by the United Nations Conference on Environment and Development (UNCED) (Sedjo et al. 1998), currently managed forest and plantations are not able to supply the actual wood demand on a long-term basis. Even more alarmingly, native forests resources will be rapidly exhausted if exploitation at current rates continues to cover wood demand (Fox 2000, Fenning and Gershenzon 2002).

A popular alternative to the exploitation of natural habitats are provided by high-yielding short-rotation plantation forests on former arable land. The idea of short-rotation forestry is not a new invention, and has in fact been a research subject of the applied sciences since the 70s. The current discussion on renewable energy demand and climate warming has, however, recently brought it into the spotlight (Karp and Shield 2008). Under commercial cultivation, these plantations can help to meet the requirements of future wood demand and can also sequester carbon as contribution to the reduction of greenhouse gases (Dickmann 2006). According to Kauter et al. (2001) short-rotation plantations are able to produce 10 to 12 Mg dry biomass ha^{-1} yr^{-1}, which is in agreement with the required rate of 8 to 10 Mg dry biomass ha^{-1} yr^{-1} needed to maintain renewable energy production on a long-term basis as projected by the US Department of Energy (English et al. 2006). Short-rotation forestry implies fast-growing tree species and rotation times of less than 30 years (Makeschin 1999), however the recommended tree density and the particularly rotation times depend on the individual objectives (Dickmann 2006). A high stem density for example is recommended for a maximum uptake of soil contaminants, whereas a lower density is favoured where a high wood-bark ratio is important. However, the global goal (cf. Kyoto protocol) is to replace fossil fuel by renewable energy sources (biomass) (Lasch et al. 2010) with trees as living store of available biomass (Hinchee et al. 2009). To meet the demand for wood, planted cultivations are mainly product-orientated in order to maximize yield gain; nevertheless some aspects of landscape ecology and intraspecific diversity are desirable. Therefore the choice of

the right plant material is of prime importance. The most important characteristics of plants used for short-rotation forestry are fast growth-rates (Zsuffa et al. 1996), high photosynthetic capacity (Barigah et al. 1994), efficient nitrogen storage (Pregitzer et al. 1990) and easy propagation (Yu 2001). *Populus* species fulfil these requirements, and in addition are globally distributed (Dickmann and Kuzovkina 2008). Consequently, *Populus* is the most used plant material for short-rotation forestry and by now the species are grown worldwide in plantations to obtain pulp, paper, lumber and energy (Bradshaw 2000).

The great interest in *Populus* species for short-rotation forestry could be further attributed to the fact that they have become a favourite subject in ecology conservation, environmental sciences, molecular studies, physiology and biotechnology due to their key role in several ecosystems and landscapes. *Populus* act as keystone species with a high level of phenotypic and genetic diversity, provide habitat for wildlife and are involved in complex community-level interactions, and are hence recognised for their ecosystem services (Turner et al. 2003, Cooke and Rood 2007, Rogers et al. 2007). The use of such a species for short-rotation forestry prevents wood plantations being biological deserts.

Populus - A genetic model tree and ecologically important tree species

In the context of short-rotation forestry, the research in recent decades has been focused on the identification of the most productive poplar clones and species in different environments and under different treatments (e.g. Rae et al. 2004, Monclus et al. 2005, Marron et al. 2006, 2007).

Further research aimed at the improvement of growth performance by controlled plant breeding. The 30 *Populus* species that are native to the northern hemisphere (Eckenwalder 1996) provide an enormous gene bank and consequently a large amount of material for selective breeding to enhance quality and quantity of growth (Bunn et al. 2004). The overall challenge is to generate varieties that grow fast, across a wide range of different environments and are able to cope with abiotic stressors either by specific breeding of successfully proven hybrids or direct modification of genes (Hinchee et al. 2009). The intense molecular research is a consequence of the fact that the *Populus* genome was the first tree genome which was completely sequenced in 2004 (Tuskan et al. 2006) and since this breakthrough, the species act as a model tree in forest genetics. The high level of natural genetic variation caused by the wide distribution range and several existing hybrids, as well as the small genome size, influenced the decision to select *Populus* as the model tree for future research in terms of understanding tree growth development and wood formation (Taylor 2002, Boerjan 2005).

The first molecular genetic maps were identified by Bradshaw et al. (1994) for an F_2 progeny of *P. nigra* x *P. deltoides* to locate quantitative trait loci (QTL) for yield related traits. At present the QTL mapping and the assessment of the underlying candidate genes is in process for several poplar species and their hybrids that have been proven to be the most successful for high-yielding plantations (e.g. *P. nigra*, *P. deltoides*, *and P. tremuloides*). A large variety of phenotyping approaches (i.e. assessing trait values of an individual) are available to characterize the most suitable traits associated with successful yield gain for different genotypes, however the most interest is in QTL, which are involved in the regulation of leaf phenology, bud phenology or branchiness.

The ultimate objective is to know which particular gene or genes control yield component traits and how they work together (Nelson and Johnsen 2008) in order to develop a poplar ideotype for high yield across different environments (Taylor 2002). The concept of the poplar ideotype is described in Dickmann and Keathley (1996) and included seven major attributes: growth and physiology, ecological characteristics, morphology, stem and wood properties and roots, with a further subdivision of every attribute e.g. a high rate of leaf-photosynthesis or high foliage density on branching. Due to the intense poplar research of the last decade the concept is being constantly extended, because plant growth performance is not only a result of genetic constitution, but also a response to environment or the interaction of both. Therefore, plant ideotypes have to be adapted to the specific environmental conditions they are growing in (Whitham et al. 2006). In general, plant ideotypes serve as bridges between phenotyping and genotyping (determining the alleles of an individual) (Nelson and Johnsen 2008). Even if genetic engineering can help to optimize seed stock in terms of productivity, vulnerability to diseases or climatic stressors and the related reduction of rotation times, what is essential for meeting the world's wood demand (Fenning and Gershenzon 2002, Hinchee et al. 2009), it cannot replace the analysis of plant morphological and physiological traits (Karp et al. 1997).

However, it should not be forgotten that next to the undeniably important role in forest genetics, *Populus* species play a further important role from the ecological point of view. Poplars have an enormous distribution range spanning entire continents. They are distributed over temperate, boreal, montane as well as tropical latitudes (Dickmann and Kuzovkina 2008) and include about 30 species in the northern hemisphere (Eckenwalder 1996). They reproduce asexually via root suckers as well as sexually and occupy a variety of ecological habitats. Poplars are pioneer species and common plant invaders in the early succession on disturbed sites, whereas their performance could be in accordance to pure, monotypic stands or to

mixed forests with other hardwood and conifer trees (Dickmann and Kuzovkina 2008). The impact of *Populus* species on ecosystem functioning could be demonstrated on various levels: ecosystem level, population level or gene level. The development of riparian forest for example is mainly influenced by poplar species (Monde et al. 2008, Smulders et al. 2008), and when poplar forests are old and established they provide habitat for endangered species (invertebrates, fungi, small mammals) (Kouki et al. 2004). Furthermore, *Populus* forests have a high level of genetic diversity at the population level (David et al. 2001), which is linked with the variation in belowground processes (Madritch et al. 2007, 2009) and thus plays an important role in evolutionary processes in natural forests (Whitham et al. 2006). Even when poplars are not the dominant species in the forest community they exert influence on ecosystem functioning. Campbell et al. (2010) showed that the presence of *P. tremula* with surrounding conifer trees facilitates the diversity and growth performance of cyanolichens and could further enhance the productivity of black spruce by altering the nutrient cycle (Legare et al. 2005). The examples show that *Populus* is in many aspects a keystone species and endorsing its role for ecosystem services and functioning, and point to the importance of poplar research either commercially or ecologically motivated concerns.

Genotype x environment interaction

Tree growth and related traits are fundamental components of planted forests but also of survival and productivity in natural undomesticated forests (Grattapaglia et al. 2009). The detailed recording of morphological and physiological plant functioning is in particular of interest because of the plant's ability to express different phenotypes as a response to changing environments, known as `phenotypic plasticity´ (Agrawal 2001). Phenotypic plasticity is considered to maximise fitness in variable environments (Coleman et al. 1994) and has a genetic basis (Tuskan et al. 2006), making it an important component for breeding approaches (Wu 2000). The consideration of changing environments and the respective reactions in the phenotype based on genotypic trait expression (genotype x environment interaction) plays an important role in several fields of forest management and includes abiotic (e.g. drought, CO_2 enrichment, elevated ozone) as well as biotic changes such as plant-fungi interaction or plant defence against parasites. Especially the latter is of prime importance, because there is no doubt that plantation forestry has to face the problems of pest management as well as agricultural systems. Attacks from insect herbivores, which rarely cause destruction in natural habitats, might damage poplar plantations and cause economic loss (Coyle et al. 2002). The trade-off between defence (e.g. chemical defence in terms of

phenolics) and growth is frequently discussed in the literature. In the absence of herbivores, defence costs can reduce plant growth or reproduction (Simms and Rausher 1987), whereas a reduction in defence cost can decrease fitness and growth under the presence of herbivores (Philippe and Bohlmann 2007). Therefore, the partitioning of phenotypic variation into environmental, genetic and genetic x environment components is involved in the most of the poplar growth experiments in order to improve selection and breeding programmes (Nelson and Johnsen 2008). A traditional method for the partitioning is the so called "quantitative genetics", which has the objective to identify biomass associated traits with a high genetic variation (coefficient of genetic variation, CV_G) because they are mostly related to high fitness and are hence more suited for breeding programmes (Houle 1992). A trait which is less responsive to environmental changes (nonplasticity) is more suitable for breeding approaches than traits whose variation is mostly controlled by environment (non-genetic variance). The genetic variation corresponds to broad-sense heritability (proportion of total phenotypic variation that is genetically based). The estimation of variance components can be obtained from phenotypic trait measurements of a single population including several families, whereas the variation within a family could be attributed to the environmental variation (CV_E). The variation among families is thereby equated to the genetic variation (Falconer and Mackay 1996).

Molecular ecology

The modern technology of molecular markers has facilitated great progress in terms of quantitative genetics, because individual genes are the basis for determining the genetic variation at the molecular level (Bradshaw 1996). Molecular markers are defined as sections of the genome, which are treated as single loci, irrespective of the function as a functional gene. The degree of polymorphism at these loci among species or genotypes is used to quantify genetic structures and dynamics of the genome in order to relate it to phenotypic expression (Bachmann 1994). Several techniques have been developed to record this polymorphism. One of the first methods was restriction fragment length polymorphism (RFLP) which is based on the variation in the size of fragment length after digestion with restriction enzymes (Rafalski and Tingey 1993). However, the breakthrough was the discovery of the polymerase chain reaction (PCR), which is able to copy small amounts of DNA in an exponential way in short time and hence overcomes the need for large amounts of DNA (Mullis et al. 1986). A further important contribution was the discovery of microsatellites, which are highly polymorph caused by different numbers of repeats of a small

tandemly repeated DNA sequence (2-4 nucleotides). With the help of primers which are complementary to certain DNA flanking regions of the microsatellite and a subsequent PCR it is possible to identify the alleles at each locus. Microsatellite markers became the method of choice, because in some cases the same microsatellite loci can be used in several species (Beebee and Rowe 2005). Other techniques, like randomly amplified polymorphic DNA (RAPD), or the related amplified fragment-length polymorphism (AFLP), do not need specific primers or knowledge of sequenced DNA because it uses random sequenced primers, but cannot distinguish between homozygotes and heterozygotes (Vos et al. 1995).

The application of molecular markers has become an important component in ecological research and can help to understand ecosystem functioning not least because of the genetic diversity aspect, because markers were also used to determine the genetic distances or similarities among species or genotypes. Particularly in regard of short-rotation forestry, the increase in plant productivity caused by the increase in varietal diversity is an important topic in current ecological research. Several studies stated an influence of genetic diversity on ecosystem functioning, whereas the most approaches involve productivity (Hughes et al. 2008). Phenotypic variation within species may be smaller than the variation among species but the impact on ecological processes can exceed the effect of species diversity (Bangert et al. 2005, Shuster et al. 2006, Schweitzer et al. 2008).

References

Agrawal, A. A. 2001. Ecology - Phenotypic plasticity in the interactions and evolution of species. Science **294**:321-326.

Bachmann, K. 1994. Molecular markers in plant ecology. New Phytologist **126**:403-418.

Bangert, R. K., R. J. Turek, G. D. Martinsen, G. M. Wimp, J. K. Bailey, and T. G. Whitham. 2005. Benefits of conservation of plant genetic diversity to arthropod diversity. Conservation Biology **19**:379-390.

Barigah, T. S., B. Saugier, M. Mousseau, J. Guittet, and R. Ceulemans. 1994. Photosynthesis, leaf-area and productivity of 5 poplar clones during their establishment year. Annals of Forest Sciences **51**:613-625.

Beebee, T. J. C., and G. Rowe. 2005. An introduction to molecular ecology. Oxford University Press, New York, US.

Boerjan, W. 2005. Biotechnology and the domestication of forest trees. Current Opinion in Biotechnology **16**:159-166.

Bradshaw, H. D. 1996. Molecular genetics of *Populus*. *in*: R. F. Stettler, H. D. Bradshaw, P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Ontario, Canada, pp. 183-199.

Bradshaw, H. D., R. Ceulemans, J. Davis, and R. Stettler. 2000. Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulation **19**:306-313.

Bradshaw, H. D., M. Villar, B. D. Watson, K. G. Otto, S. Stewart and, R. F. Stettler. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of a hybrid poplar composed of RFLP, STS, and RAPD markers. Theoretical and Applied Genetics **89**:167-178.

Bunn, S. M., A. M. Rae, C. S. Herbert, and G. Taylor. 2004. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. Forestry **77**:307-323.

Campbell, J., G. E. Bradfield, C. E. Prescott, and A. L. Fredeen. 2010. The influence of overstorey *Populus* on epiphytic lichens in subboreal spruce forests of British Columbia. Canadian Journal of Forest Research **40**:143-154.

Coleman, J. S., K. D. M. Mcconnaughay, and D. D. Ackerly. 1994. Interpreting phenotypic variation in plants. Trends in Ecology & Evolution **9**:187-191.

Cooke, E. K., and S. B. Rood. 2007. Trees of the people: the growing science of poplars in Canada and worldwide. Canadian Journal of Botany **85**:1103-1110.

Coyle, D. R., J. D. McMilin, R. B. Hall and E. R. Hart. 2002. Deployment of tree resistance to insects in short-rotation *Populus* plantations. *in*: M. R. Wagner, K. M. Clancy, T. D. Paine, and F. Lieutier, editors. Mechanisms and deployment of resistance in trees to insects. Kluwer Academic, New York, US, pp. 189-215.

David, A. J., J. C. Zasada, D. W. Gilmore, and S. M. Landhäusser. 2001. Current trends in the management of aspen and mixed aspen forests for sustainable production. Forestry Chronicle **77**:525-532.

Dickmann, D. I. 2006. Silviculture and biology of short-rotation woody crops in temperate regions: Then and now. Biomass & Bioenergy **30**:696-705.

Dickmann, D. I., and D. E. Keathly. 1996. Linking physiology, molecular genetics and the *Populus* ideotype. *in:* R. F. Stettler, H. D. Bradshaw, P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Ontario, Canada, pp. 491-511.

Dickmann, D. I., and J. Kuzovkina. 2008. Poplars and willows of the world, with emphasis on silviculturally important species. *in*: Poplars and willows in the world. Rome, Italy. FAO Forest Management Division Working Paper IPC/9-2.

Eckenwalder, J. E. 1996. Systematics and evolution of *Populus*. *in*: R. F. Stettler, H. D. Bradshaw, P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Ontario, Canada, pp. 7-30.

English, B. C., D. G. De La Torre Ugarte, K. Jensen, C. Hellwinckel, J. Menard, B. Wilson, R. Roberts, and M. Walsh. 2006. 25% renewable energy for the United States by 2025: Agricultural and economic impacts. University of Tennessee Agricultural Economics. Published online at: http://www.agpolicy.org/ppap/REPORT%2025x25.pdf.

Food and Agricultural Organisation. 2008. Forests and Energy. FAO Forestry Paper **154**, Rome, Italy. Published online at: ftp://ftp.fao.org/docrep/fao/010/i0139e/i0139e00.pdf.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Prentice Hall, Harlow, England.

Fenning, T. M., and J. Gershenzon. 2002. Where will the wood come from? Plantation forests and the role of biotechnology. Trends in Biotechnology **20**:291-296.

Fox, T. R. 2000. Sustained productivity in intensively managed forest plantations. Forest Ecology and Management **138**:187-202.

Grattapaglia, D., C. Plomion, M. Kirst, and R. R. Sederoff. 2009. Genomics of growth traits in forest trees. Current Opinion in Plant Biology **12**:148-156.

Hinchee, M., W. Rottmann, L. Mullinax, C. S. Zhang, S. J. Chang, M. Cunningham, L. Pearson, and N. Nehra. 2009. Short-rotation woody crops for bioenergy and biofuels applications. In Vitro Cellular & Developmental Biology-Plant **45**:619-629.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics **130**:195-204.

Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecology Letters **11**:609-623.

Karp, A., S. Kresovich, K. V. Bhat, W. G. Ayad and T. Hodgkin. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. International Plant Genetic Resource Institute, Rome, Italy. Published online at: http://pdf.usaid.gov/pdf_docs/PNACB166.pdf.

Karp, A., and I. Shield. 2008. Bioenergy from plants and the sustainable yield challenge. New Phytologist **179**:15-32.

Kauter, D., I. Lewandowski, and W. Claupein. 2001. Pappeln in Kurzumtriebswirtschaft: Eigenschaften und Qualitätsmanagement bei der Festbrennstoffbereitstellung – Ein Überblick. Pflanzenbauwissenschaften **5**:64-74.

Kouki, J., A. K. Arnold, and P. Martikainen. 2004. Long-term persistence of aspen – a key host for many threatened species – is endangered in old-growth conservation areas in Finland. Journal for Nature Conservation **12**:41-52.

Lasch, P., C. Kollas, J. Rock, and F. Suckow. 2010. Potentials and impacts of short-rotation coppice plantation with aspen in Eastern Germany under conditions of climate change. Regional Environmental Change **10**:83-94.

Legare, S., D. Pare, and Y. Bergeron. 2005. Influence of aspen on forest floor properties in black spruce-dominated stands. Plant and Soil **275**:207-220.

Madritch, M. D., J. R. Donaldson, and R. L. Lindroth. 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. Soil Biology & Biochemistry **39**:1192-1201.

Madritch, M. D., S. L. Greene, and R. L. Lindroth. 2009. Genetic mosaics of ecosystem functioning across aspen-dominated landscapes. Oecologia **160**:119-127.

Makeschin, F. 1999. Short rotation forestry in Central and Northern Europe - introduction and conclusions. Forest Ecology and Management **121**:1-7.

Marron, N., C. Bastien, M. Sabatti, G. Taylor, and R. Ceulemans. 2006. Plasticity of growth and sylleptic branchiness in two poplar families grown at three sites across Europe. Tree Physiology **26**:935-946.

Marron, N., S. Y. Dillen, and R. Ceulemans. 2007. Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. Environmental and Experimental Botany **61**:103-116.

Monclus, R., E. Dreyer, F. M. Delmotte, M. Villar, D. Delay, E. Boudouresque, J. M. Petit, N. Marron, C. Brechet, and F. Brignolas. 2005. Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides* x *P. nigra* clones. New Phytologist **167**:53-62.

Monde, Y., N. Miki, and K. Yoshikawa. 2008. Stand structure and regeneration of *Populus euphratica* forest in the lower reaches of the Heihe River, NW China. Landscape and Ecological Engineering **4**:115-124.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. Specific enzymatic amplification of DNA in vitro - the polymerase chain-reaction. Cold Spring Harbor Symposia on Quantitative Biology **51**:263-273.

Nelson, C. D., and K. H. Johnsen. 2008. Genomic and physiological approaches to advancing forest tree improvement. Tree Physiology **28**:1135-1143.

Philippe, R. N., and J. Bohlmann. 2007. Poplar defense against insect herbivores. Canadian Journal of Botany **85**:1111-1126.

Pregitzer, K. S., D. I. Dickmann, R. Hendrick, and P. V. Nguyen. 1990. Whole-Tree carbon and nitrogen partitioning in young hybrid poplars. Tree Physiology **7**:79-93.

Rae, A. M., K. M. Robinson, N. R. Street, and G. Taylor. 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. Canadian Journal of Forest Research **34**:1488-1498.

Rafalski, J. A., and S. V. Tingey. 1993. Genetic diagnostics in plant-breeding - RAPDs, microsatellites and machines. Trends in Genetics **9**:275-280.

Rogers, P. C., W. D. Shepperd, and D. L. Bartos. 2007. Aspen in the Sierra Nevada: Regional conservation of a continental species. Natural Areas Journal **27**:183-193.

Schweitzer, J. A., J. K. Bailey, D. G. Fischer, C. J. Leroy, E. V. Lonsdorf, T. G. Whitham, and S. C. Hart. 2008. Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. Ecology **89**:773-781.

Sedjo, R. A., A. Goetzl, and S. O. Moffat. 1998. History of evolution of sustainable forestry concepts. *in*: Sedjo, R. A., A. Goetzl, and S. O. Moffat, editors. Sustainability of temperate forests. Resources for the future. Washington, US, pp. 5-16.

Shuster, S. M., E. V. Lonsdorf, G. M. Wimp, J. K. Bailey, and T. G. Whitham. 2006. Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. Evolution **60**:991-1003.

Simms, E. L., and M. D. Rausher. 1987. Costs and benefits of plant-resistance to herbivory. American Naturalist **130**:570-581.

Smulders, M. J. M., J. E. Cottrell, F. Lefèvre, J. van der Schoot, P. Arens, B. Vosman, H. E. Tabbener, F. Grassi, T. Fossati, S. Castiglione, V. Krystufek, S. Fluch, K. Burg, B. Vornam, A. Pohl, K. Gebhardt, N. Alba, D. Agúndez, C. Maestro, E. Notivol, R. Volosyanchuk, M. Pospiskova, S. Bordács, J. Bovenschen, B. C. van Dam, H. P. Koelewijn, D. Halfmaerten, B. Ivens, J. van Slycken, A. Vanden Broeck, V. Storme,

and W. Boerjan. 2007. Structure of the genetic diversity in black poplar (*Populus nigra* L.) populations across European river systems: Consequences for conservation and restoration. Forest Ecology and Management **255**:1388-1399.

Taylor, G. 2002. *Populus*: Arabidopsis for forestry. Do we need a model tree? Annals of Botany **90**:681-689.

Turner, M. G., W. H. Romme, R. A. Reed, and G. A. Tuskan. 2003. Post-fire aspen seedling recruitment across the Yellowstone (USA) landscape. Landscape Ecology **18**:127-140.

Tuskan, G. A., S. DiFazio, S. Jansson, J. Bohlmann, I. Grigoriev, U. Hellsten, N. Putnam, S. Ralph, S. Rombauts, A. Salamov, J. Schein, L. Sterck, A. Aerts, R. R. Bhalerao, R. P. Bhalerao, D. Blaudez, W. Boerjan, A. Brun, A. Brunner, V. Busov, M. Campbell, J. Carlson, M. Chalot, J. Chapman, G. L. Chen, D. Cooper, P. M. Coutinho, J. Couturier, S. Covert, Q. Cronk, R. Cunningham, J. Davis, S. Degroeve, A. Dejardin, C. Depamphilis, J. Detter, B. Dirks, I. Dubchak, S. Duplessis, J. Ehlting, B. Ellis, K. Gendler, D. Goodstein, M. Gribskov, J. Grimwood, A. Groover, L. Gunter, B. Hamberger, B. Heinze, Y. Helariutta, B. Henrissat, D. Holligan, R. Holt, W. Huang, N. Islam-Faridi, S. Jones, M. Jones-Rhoades, R. Jorgensen, C. Joshi, J. Kangasjarvi, J. Karlsson, C. Kelleher, R. Kirkpatrick, M. Kirst, A. Kohler, U. Kalluri, F. Larimer, J. Leebens-Mack, J. C. Leple, P. Locascio, Y. Lou, S. Lucas, F. Martin, B. Montanini, C. Napoli, D. R. Nelson, C. Nelson, K. Nieminen, O. Nilsson, V. Pereda, G. Peter, R. Philippe, G. Pilate, A. Poliakov, J. Razumovskaya, P. Richardson, C. Rinaldi, K. Ritland, P. Rouze, D. Ryaboy, J. Schmutz, J. Schrader, B. Segerman, H. Shin, A. Siddiqui, F. Sterky, A. Terry, C. J. Tsai, E. Uberbacher, P. Unneberg, J. Vahala, K. Wall, S. Wessler, G. Yang, T. Yin, C. Douglas, M. Marra, G. Sandberg, Y. Van de Peer, and D. Rokhsar. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science **313**:1596-1604.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP - A new technique for DNA-fingerprinting. Nucleic Acids Research **23**:4407-4414.

Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. Leroy, E. V. Lonsdorf, G. J. Allan, S. P. DiFazio, B. M. Potts, D. G. Fischer, C. A. Gehring, R. L. Lindroth, J. C. Marks, S. C. Hart, G. M. Wimp, and S. C. Wooley. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics **7**:510-523.

Wu, R. L. 2000. Quantitative genetic variation of leaf size and shape in a mixed diploid and triploid population of *Populus*. Genetical Research **75**:215-222.

Yu, Q. 2001. Selection and propagation of hybrid aspen clones for growth and fibre quality. 2001. Dissertation University of Helsinki. Published online at: http://ethesis.helsinki.fi/julkaisut/maa/sbiol/vk/yu/.

Zsuffa, L., E. Giordano, L. D. Pryor, and R. F. Stettler. 1996. Trends in poplar culture: some global and regional perspectives. *in*: R. F. Stettler, H. D. Bradshaw, P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Ontario, Canada, pp. 515-539.

Chapter 2

The Göttingen poplar diversity project

The Göttingen poplar diversity project

The Göttingen poplar diversity project is associated with the Göttingen cluster of excellence "Functional Biodiversity Research". The research cluster was established in 2008 at the University of Göttingen with the overall topic of biodiversity and ecosystem functioning, including experiments in grasslands and in the soil as well as historical studies in terms of biodiversity alterations.

The poplar diversity project is concentrated on the functional role of intraspecific diversity in woody plants. Therefore a common-garden experiment was established in 2008 and a field trial in the subsequent year. In particular, both experiments had the common aim to quantify the variability in tree growth performance and related traits using poplar as model species and with a special interest in short-rotation forestry. *Populus tremula* L., which is native to Europe, and the American equivalent *Populus tremuloides* Michx. were used as study species. A detailed description of both experiments is given in the study design section. The project involved several working groups in order to address the aim from different point of views. A molecular analysis of the studied plant material was performed by the Department of Forest Genetics and Forest Tree Breeding, whereas the questions of how plant-insect and plant-fungi interactions affect growth performance were investigated by the Department of Agroecology and the Department of Forest Botany and Tree Physiology, respectively. My own thesis aimed to analyse the most decisive parameters for successful growth performance from a pool of phenological, morphological and physiological traits.

Study species (*Populus tremula* L. & *Populus tremuloides* Michx.)

Most poplar research which is product-orientated in terms of biomass gain is mainly focused on the species *P. nigra*, *P. trichocarpa* or *P. deltoides*, as a result of their outstanding growth rates in comparison to other poplar species. Hence, the question is why is this project focussing on aspen? It can be assumed that the popularity of aspen (*P. tremula*, *P. tremuloides*) for growth experiments aiming to clarify their suitability for short-rotation forestry will increase. This is due to the fact that aspen can reach considerable growth rates even on poor soils and under unfavourable habitat conditions (Hofman 1998) and are less sensitive to drought like e.g. *P. trichocarpa*. This feature will be a benefit under future climate scenarios, which predict an increase in drought periods and decrease in summer precipitation. Under the assumption that aspen will increase in their popularity for short-rotation forestry, it is of prime importance to know the intraspecific differences in their growth performance in order to obtain the yield potential and give advice for future studies

and aspen plantation management. Beyond this, cultivation programmes in Central Europe should further address the question of utilizing native seed stock (*P. tremula*) or the introduction of non-native (*P. tremuloides*) seed stock. Planting introduced tree species bears the risk that the exotic species will become invasive with unpredictable effects on native ecosystems, underlining the significance of essential knowledge of the strengths and weaknesses of the two species in terms of cultivation.

Furthermore, next to commercial and product-orientated interests; aspen have an ecologically relevant aspect. Aspen have an extensive distribution range and belong to the most widespread species in the world (Bradshaw et al. 2000). Natural grown aspen forests provide habitat and food for endangered species (Kouki et al. 2004), insects and pathogens (David et al. 2001) and in North America they are significantly embedded in the carbon cycle at the national scale caused by their high rates of carbon sequestration (Kurz and Apps 1999). Aspen are pioneer species and colonize on open sites and disturbed soils. They reproduce via root suckers as well as via sexual reproduction and can overcome long distances by wind dispersal of pollen and seeds. Hence, established aspen populations consist of several aspen genotypes caused by lots of possible crossing constellations of the parent trees in the given surroundings and present genetically rich ecosystems. These high level of genetic diversity could be significantly linked with soil processes through interactions with canopy herbivores and create different patches of belowground activity (Madritch et al. 2007, 2009). The current stated dieback (Hogg et al. 2008, Worrall et al. 2008) and reduced growth of aspen forests caused by pests, diseases and increasing drought periods (Worrall et al. 2008), supports the importance of aspen research and requires knowledge of the characteristics of aspen forests differing in their genetic constitution and place of origin.

Study design: common-garden experiment

In 2008 a common-garden experiment with *P. tremula* as study species was performed in the outdoor area of the Department of Forest Botany and Tree Physiology at the University of Göttingen (51°32´N, 9°56´O). Eight full-sib families with German origin were selected and planted in a randomized design in two blocks. Each block contained four plots and each plot included three aspen saplings of each full-sib family (Fig. 1). Four plots were treated monthly with a systematic fungicide in order to analyse the impact on plants growth and associated organisms. The other four blocks were treated with water and act as control plots. All trees were planted in nutrient-rich humus soil. The progenies of each full-sib family were bred by controlled crossings in 2000, from parent tree material originating from 30-year-old trees

selected in the district of Geismar in Göttingen. We used three pollen donors and five mother plants. The progenies of each full-sib family were characterized by the same mother and father tree, whereas the relatedness between the full-sib families was either characterized by a half-sib relationship (common father or mother) or no relationship (no common father or mother). We tried to simulate a population, which could be the result of a natural pair-crossing from a small founder aspen population. The crossings were performed under laboratory conditions by the group members of Forest Genetics and Tree Breeding at the University of Göttingen, who also performed the genetic analysis of the described plant material in order to quantify the genetic distances among families and to confirm the assumption of closely relatedness. Genetic distance is defined as genetic relatedness between species or populations. The aim of the common-garden experiment was to study the variability in this closely related aspen assemblage due to their growth performance, physiology and interactions with associated organisms. In this experiment the following parameters were investigated:

- Plant biomass and the partitioning in roots, leaves and shoots
- Morphological parameters of the leaves (number of leaves, total leaf area, leaf size, specific leaf area, leaf mass ratio, leaf area ratio)
- Morphological parameters of the root (specific fine root area, root mass ratio)
- Morphological parameters of the shoot (diameter, height and side branch increment, shoot mass ratio)
- Phenological parameters of the leaves (time of leaf flushing)
- Phytochemical components and elements of the leaves
- Seasonal changes in photosynthesis related traits using light-response curves, internal CO_2response curves and measurements of chlorophyll-fluorescence
- Seasonal changes in plant water-household related traits using leaf water potential and leaf conductance
- Aspen associated organisms (mycorrhiza, endophytes and insects)

The results are described in Chapter 3 and 4. Chapter 3 (Relating genetic variation of ecologically important tree traits to associated organisms in full-sib aspen families) is mainly focussed on the variation in growth and leaf traits in the full-sib families in relation to interacting organisms (mycorrhiza, endophytes and insects), whereas Chapter 4 (Physiological vs. morphological traits controlling the productivity of six aspen full-sib

families) is focused on leaf-level traits and physiological parameters which control the productivity.

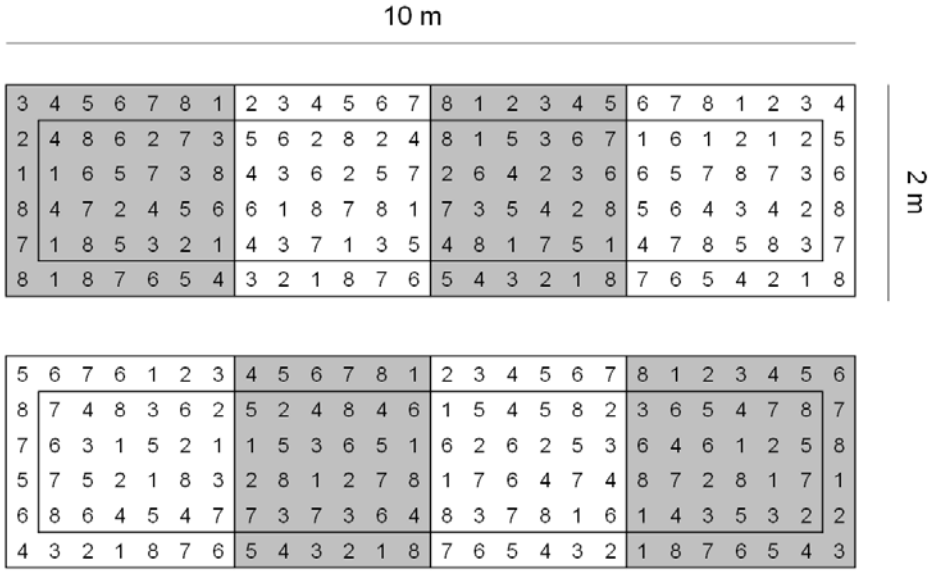

Figure 1 Design of the poplar common-garden experiment started in 2008 at the Department of Forest Botany and Tree Physiology at the University of Göttingen. 24 progenies of eight (1-8) full-sib families were planted in 8 plots, of which four (grey) were treated monthly with a systematic fungicide and four were used as control. Figure design by F. Kleemann.

Study design: field experiment

In 2009 a poplar diversity experiment (POPDIV) was established at the Relliehausen Experimental Farm near Silberborn (51°44´56´´N, 9°32´28´´O) about 60 km west of Göttingen in the Solling Mountains, which is a forest rich low mountain range in Central Germany (Lower Saxony). The study area is described by a montane elevation of 500 m a.s.l. and a mean annual temperature of 6.9°C and an annual precipitation of 1030 mm.

POPDIV was established with the specific objective to analyse ecosystem services according to intraspecific diversity with poplar as model tree and *P. tremula* and *P. tremuloides* as specific study species. In order to answer the question if a reduction in intraspecific diversity has a negative impact on ecosystem functioning with a special interest in productivity, eight aspen collectives (seven *P. tremula* and one *P. tremuloides*) were arranged in a complex experimental design along a gradient starting with monocultures and increasing the number of cultures (Fig. 2). The plant material (either seeds or small plants) was derived from Germany (3 collectives), Poland, Switzerland, Austria, Sweden and the USA provided by a tree nursery or other institutes (detailed information is given in the material and methods section in Chapter 5 and 6). Small plants with Polish (Bialystok) and German (Holstein) origin were

provided by a tree nursery (Bunk Pflanzen, Elmshorn), whereas the plants of the Swedish collective were collected in Sweden (Edsvalla). The German, Swiss, Austrian and US collective were derived from seeds gained directly from the mother plant in the respective habitat (Germany: Göttingen; Switzerland: Birmensdorf; Austria: Vienna Woods, USA: Sandwich) and hence, including one or more pollen donors. Seed propagation was performed by the Department of Forest Botany and Tree Physiology at the University of Göttingen. When the plants had reached a height of about 20 cm they were, together with the plants provided by the nursery and the small plants collected in Sweden, planted at the study area. The plantation of the selected aspen collectives was established on unfertilized, moderately poor soil (22% sand, 67% siltstone, 12% loam and 8% humus soil) which was previously used for extensive pasture.

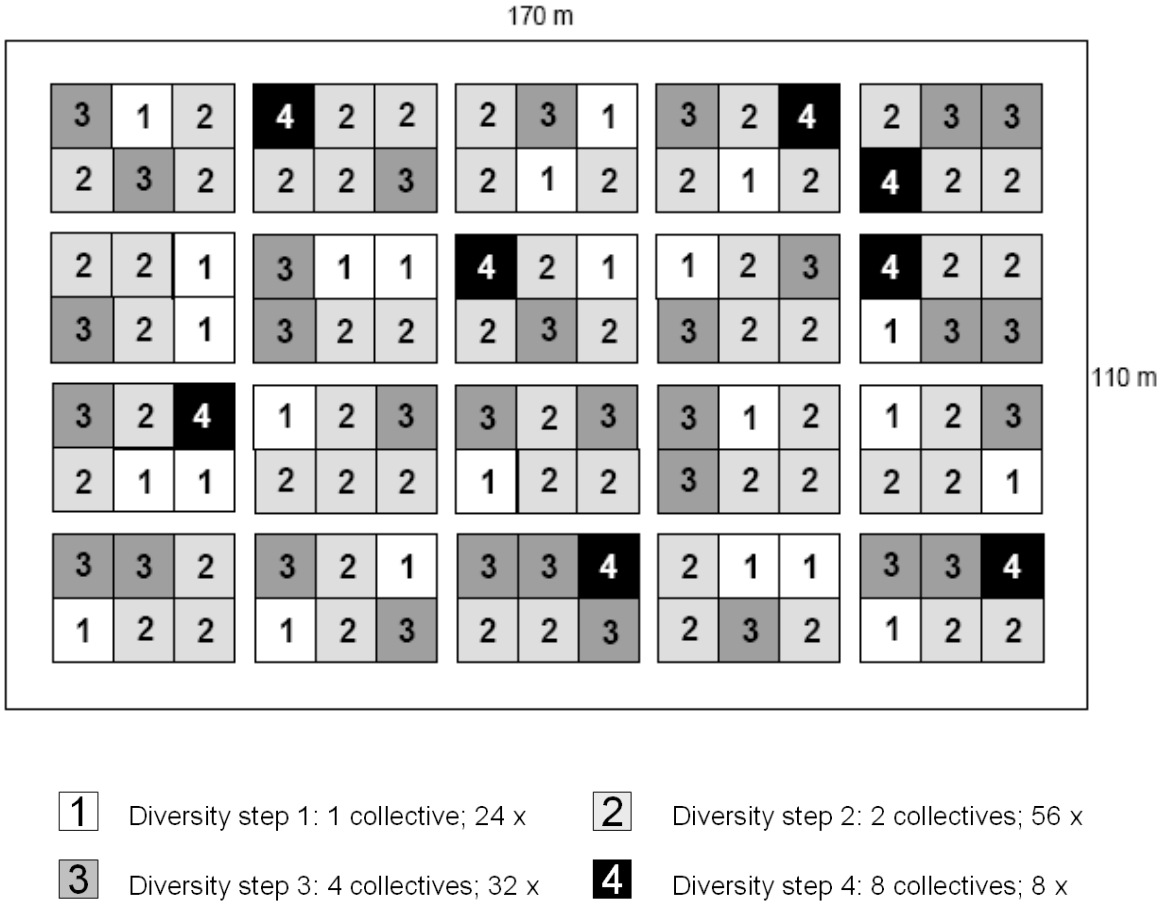

Figure 2 Design of the Göttingen poplar diversity experiment located at the Relliehausen Experimental Farm near Silberborn. Eight poplar collectives (7 x *P. tremula* and 1 x *P. tremuloides*) were planted in four diversity steps. Figure design by F. Kleemann.

In comparison to the common-garden experiment, where we used defined plant material (closely related full-sib families), here we tried to increase the genetic richness within each

collective by an increase in pollen donors and further we tried to increase the genetic distances among them by an increase in geographic distance. Each group was defined as collective and simulated the genetic constellation of a population, which could be the result of a natural pair-crossing from a small founder aspen population. The Department of Forest Genetics and Tree Breeding at the University of Göttingen performed again the molecular analysis to reveal the genetic distances among the studied collectives. The American collective (*P. tremuloides*) was used as outlier in order to obtain a high value of genetic distance in relation to the European aspen and to study the growth performance of a native aspen species in comparison to a non-native species.

In the first phase of the experiment, the involved working groups were mainly focused on the characterization of the individual collectives. Plant-plant interactions and the associated effects on ecosystem functioning were not expected during the first year of growth. The aspen collectives were characterized according to their genetic structure, plant physiological and morphological traits. Hence, the results of the experiment will be able to give a great contribution to a better understanding of ecosystem functioning and the importance of genetic resources with a special interest for short-rotation forestry and the associated yield gain. In this experiment the following parameters were investigated:

- Canopy carbon gain
- Morphological parameters of the leaves (number of leaves, total leaf area, leaf size, leaf area increment rate, ratio of leaves lost to leaves produced, specific leaf area)
- Morphological parameters of the shoot (diameter, height increment, number and increment of side branches)
- Phenological parameters of the leaves (time of leaf flushing and leaf abscission)
- Seasonal changes in photosynthesis related traits using light-response curves and internal CO_2-response curves
- Seasonal changes in plant water-household related traits using leaf water potential, leaf conductance and photosynthetic water-use-efficiency

Chapter 5 (Different growth strategies determine the carbon gain and productivity of aspen collectives to be used in short-rotation plantations) gives the results of the comparison of four *P. tremula* collectives due to their growth performance and the decisive parameters, whereas Chapter 6 (Comparing native and non-native aspen species (*Populus tremula* vs. *P. tremuloides*) for their suitability in short-rotation forestry: photosynthetic performance and

growth analysis) is focused on the differences between a European *P. tremula* collective and the American *P. tremuloides* collective.

Study objectives and Chapter outline

This thesis was conducted in the framework of the Göttingen poplar diversity project. The general scope of the thesis was to characterize trait variability in aspen assemblages, with an emphasis on productivity, along a gradient of genetic relatedness and places of origin:

- in a study group of closely related aspen full-sib families with German origin (Chapter 3 & 4)
- in a study group of distantly related aspen collectives with Central European origin (Chapter 5)
- in a group of two aspen species originating from Europe and the USA (Chapter 6)

Our specific objectives were:

1) to characterize the variability of phenological, physiological and morphological traits along this gradient (Chapters 3 - 6) and to partition the trait variability in environmental and genetic variation (Chapters 4 & 5)

2) to identify the best biomass predictors and controlling traits from a pool of phenological, morphological and plant physiological traits and to reveal their contribution to successful plant growth for each study assemblage (Chapters 3 - 6)

3) to relate genetic distance with phenotypic trait variation (Chapter 3)

4) to record aspen associated organism and their impact on growth performance (Chapter 3)

In order to fulfil the objectives of this study I investigated productivity and several morphological and physiological traits in each aspen assemblage and applied a detailed growth analysis based on a preceding and detailed phenotyping. Further, we used the methods of molecular and quantitative genetics to estimate the degree of genetic impact on the variation in the studied traits and related genetic variance with tree performance in order to provide advice for breeding programmes and short-rotation forestry (Chapter 3 - 5). Chapter 6 included the American aspen species and examined the use of native or non-native seed stock in short-rotation forestry from an ecological point of view.

The synthesis of Chapters 3 to 6 allowed us to compare the degree of within-species variability (in a closely and distantly related aspen assemblage) with the between-species

variability, what will support the understanding of the functioning of genetically different aspen assemblages with regard to natural populations.

References

Bradshaw, H. D., R. Ceulemans, J. Davis, and R. Stettler. 2000. Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulation **19**:306-313.

David, A. J., J. C. Zasada, D. W. Gilmore, and S. M. Landhäusser. 2001. Current trends in the management of aspen and mixed aspen forests for sustainable production. Forestry Chronicle **77**:525-532.

Hofman, M. 1998. Bewirtschaftung schnellwachsende Baumarten auf landwirtschaftlichen Flächen im Kurzumtrieb. Forschungsinstitut für schnellwachsende Baumarten. Merkblatt 11. Hann. Münden, Germany.

Hogg, E. H., J. P. Brandt, and M. Michaellian. 2008. Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Canadian Journal of Forest Research **38**:1373-1384.

Kouki, J., A. K. Arnold, and P. Martikainen. 2004. Long-term persistence of aspen – a key host for many threatened species – is endangered in old-growth conservation areas in Finland. Journal for Nature Conservation **12**:41-52.

Kurz, W. A., and M. J. Apps. 1999. A 70-year retrospective analysis of carbon fluxes in the Canadian forest sector. Journal of Applied Ecology **9**:526-547.

Madritch, M. D., J. R. Donaldson, and R. L. Lindroth. 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. Soil Biology & Biochemistry **39**:1192-1201.

Madritch, M. D., S. L. Greene, and R. L. Lindroth. 2009. Genetic mosaics of ecosystem functioning across aspen-dominated landscapes. Oecologia **160**:119-127.

Worrall, J. J., L. Egeland, T. Eager, R. A. Mask, E. W. Johnson, P. A. Kemp, and W. D. Shepperd. 2008. Rapid mortality of *Populus tremuloides* in southwestern Colorado, USA. Forest Ecology and Management **255**:686-696.

Chapter 3

Relating genetic variation of ecologically important tree traits to associated organisms in full-sib aspen families

(European Journal of Forest Research, in press)

Relating genetic variation of ecologically important tree traits to associated organisms in full-sib aspen families

Frauke Kleemann[1], Maximilian von Fragstein und Niemsdorff[2], Barbara Vornam[3], Annika Müller[4], Christoph Leuschner[4], Andrea Holzschuh[2], Teja Tscharntke[2], Reiner Finkeldey[3], and Andrea Polle[1]

[1]Forest Botany and Tree Physiology, Büsgen-Institute, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[2]Agroecology, Department of Crop Science, University of Göttingen, Grisebachstraße 6, 37077 Göttingen, Germany

[3]Forest Genetics and Forest Tree Breeding, Büsgen-Institute, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[4]Plantecology, Albrecht von Haller Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany

Correspondence:

A. Polle, Email: apolle@gwdg.de, Fax: +49 (0)551 3922705, Phone: + 49 (0)551 393480

Abstract

Knowledge on phenological, morphometric, and phytochemical variation of local progenies of European aspen (*Populus tremula*, L.) is limited. The goal of this study was to characterize variation in growth and ecologically important leaf properties in aspen full-sib families in relation to interacting organisms (mycorrhiza, endophytes and insects) and to determine if these interactions were affected by soil-application of a systemic fungicide. In local progenies, within family variation of neutral molecular genetic markers (nuclear microsatellites) was higher than between families. Significant variation in growth, production of phenolic defensive compounds and other phytochemical leaf traits was found between families. Phenolic compounds showed clear negative correlation with generalist herbivores, but did not result in negative trade-off with biomass production. Differences in mycorrhizal colonization were not found among full-sib families and application of a systemic fungicide

suppressed neither mycorrhizal colonization nor infestation with insects. However, a strong suppression of endophytes occurred, whose long-term consequences may require attention when fungicides are used in agro-forestry plantations.

Keywords: agro-forestry, molecular marker, nitrogen, nutrition, phenolic compounds, *Populus.*

1. Introduction

European and American aspen (*Populus tremula*, *P. tremuloides* Michx.) are among the most wide-spread tree species in circumpolar boreal and temperate forest regions (Hultén and Fries, 1986; Dickmann and Kuzovkina 2008). They are pioneering species with low nutrient demand that colonize disturbed and shallow soils (Dimpfelmeier 1963; Tamm 2006). In contrast to other poplar species that are typically found in alluvial, riparian and wetland ecosystems, aspens are relatively drought tolerant and form distinct forest communities. In past European silvicultural practices aspens have usually been removed to avoid competition in even aged, traditionally managed coniferous forests (DeChantal et al. 2009). However, it has recently been recognized that aspen create habitats for specific fauna including many endangered species and therefore provide important ecosystem services (Kouki et al. 2004). To date, aspens are increasingly valued because of their ecological functions as well as a possible resource for biomass production on marginal soils.

Traits of ecological and economic value have mainly been studied in American aspen (*P. tremuloides*) or in hybrids of *P. tremuloides* with *P. tremula* (Li and Wu 1997; Liesebach et al. 1999), whereas less information is available on its close relative, European aspen. American aspen show significant genetic variation in the phytochemistry of defence compounds such as phenolic glucosides and condensed tannins, whereas other foliar traits such as nitrogen content respond mainly to environment (Osier and Lindroth 2006; Donaldson and Lindroth 2007). Phenolic compounds protect against *Venturia* shoot blight infection (Holeski et al. 2009) and influence feeding behaviour of herbivorous insects on Salicaceae (Rowell-Rahier 1984; Donaldson & Lindroth 2007). Among herbivores, generalists such as the chrysomelid beetles *Phratora vitellinae*, *Phratora vulgatissima* and *Galerucella lineola* prefer leaves with low concentrations of phenol glucosides (Kendall et al. 1996; Orians et al. 1997; Glynn et al. 2004). In contrast, specialized chrysomelid beetles, for example, *Chrysomela populi* and *Gonioctena decemnotata,* prefer salicaceous species with relatively high concentrations of phenol glycosides in the leaves (Orians et al. 1997; Ikonen, 2002)

because their larvae sequester plant-derived allelochemicals such as salicylaldehyde for defence (Pasteels et al. 1983).

Trade-off between allocation to defence compounds and growth has been reported, at least under limiting nutrient resources (Donaldson et al. 2006). Therefore, differences in growth and biomass production among *P. tremula* progenies may have consequences for phytochemical traits, in particular for allocation of defence compounds, and for interactions with associated organisms such as mycorrhizae, endophytes or leaf feeding insects. Since economically and ecologically sustainable biomass production requires cultivation of trees adapted to regional climate with optimum growth and stress tolerance, we investigated variation in phenological, morphological and ecophysiological traits in a common-garden experiment with seven full-sib families generated by crossing of local *P. tremula* parent trees (Göttingen, Central Germany). It is often required to apply phyto-protective agents to prevent spreading of diseases in plantations. Therefore, we also studied the influence of a systemic fungicide on plant performance and biotic interactions. The following hypotheses were tested: (i) full-sib aspen families show significant variation in growth and ecophysiological leaf traits that are related to parenthood and modulate interactions with associated organisms; (ii) defensive compounds and growth are negatively related providing evidence for an energetic trade-off; (iii) application of a systemic fungicide has negative effects on associated organisms such as endophytes, mycorrhiza, and leaf-feeding insects.

2. Materials and methods

2.1 Plant materials and experimental set-up

The parent *P. tremula* trees were located close to Göttingen (Geismar, 51° 31′ N, 9° 57′E). In the year 2000, shoots with male and female flowers were transported to a greenhouse and used for controlled crossing of male trees number 1, 3 and 5 with female trees number 2, 4, 7, 8, and 9, respectively, resulting in the following crossings: C1 (4x5), C2 (9x5), C3 (8x5), C4 (2x5), C5 (2x3), C6 (9x3), and C7 (7x1). Seeds were germinated on moist Vermiculite (grain size 3 to 8 mm, Deutsche Vermiculite Dämmstoff GmbH, Sprockhövel, Germany). Seedlings were planted in pots (Fruhsdorfer soil, type N, Fruhsdorf, Germany), cultivated outdoors and watered as necessary. In spring 2008, 8-yrs-old trees were out-planted according to a randomized block design with 8 blocks (4 blocks treated monthly with 75 l Amistar Opti [25µl L-1, Syngenta, Maintal, Germany], 4 control blocks treated with water). Each block contained 24 plants; i.e. 3 trees of each of the 7 full-sib families in addition to 3 plants of a further crossing which was however contaminated and therefore not included in further

analyses. A bed contained four blocks of alternating control and fungicide treated blocks, which were separated by plastic barriers and surrounded by a row of additional trees to avoid edge effects. The experiment consisted of two beds. The trees were cultivated for one growth phase and watered as necessary. The mean ambient air temperature was 15°C.

2.2 Phenological and morphometric measurements

Before bud break, diameter at the bottom (root collar) and height of the main shoot of each tree were measured. Bud break at the apex of the leader shoot was scored regularly. The Julian days were recorded until the first leaf was fully expanded (according to the scores described by UPOV 1981). The trees were harvested in the first week of September 2008. At harvest, root collar diameter, height of main shoot, number of side branches, lengths of side branches, number of leaves, fresh mass of leaves, stems, fine and coarse roots were determined. Leaf mass was determined for 5 fully expanded leaves collected at the top of the leader shoot of each tree and their areas were measured using ImageJ (http://rsbweb.nih.gov/ij/). These data were used to convert leaf mass per tree to leaf area per tree. Aliquots of plant tissues were shock-frozen in liquid nitrogen and stored at -80°C for biochemical analysis. Aliquots of roots were used for mycorrhizal assessment. Other plant tissues were dried at 60°C to determine dry mass and the relative water content [fresh mass – dry mass)*100/fresh mass].

2.3 Endophyte colonization

Two fully expanded, healthy top leaves of 20 plants per treatment of C3 and of 18 plants per treatment of C4 were harvested (21th Aug. 2008), cut into quarters and surface-sterilized for 1 min in 96% EtOH, 3 min in 4% NaOCl and 30 s in 96% EtOH. The four leaf pieces were placed upside down in a Petri dish on antibiotics containing water-agar (15 g L^{-1} agar with 15 mg tetracycline, 100 ampicilline, 50 mg kanamycine, and 0.1 mg streptomycine) and were incubated for 7 days at 20°C in darkness (Petrini 1986). Subsequently hyphal outgrowth of leaf pieces was scored as absent or present on each leaf piece yielding a scale from 0%, 25%, 50%, 75% and 100% endophyte presence, respectively, per leaf in a Petri dish.

2.4 Insect sampling

Insects were captured by using a sweep net and an exhauster or were identified directly on the trees. Aphids, leaf beetle larvae and galls were quantified visually on each tree. Counting was done four times (monthly) from May to the middle of August. We recorded three leaf beetle

(*Phratora vitellinae*, *Crepidodera aurata* and *Crepidodera aurea*) and one aphid species (*Chaitophorus populi*). The identification of adult insects was done in the laboratory. Due to negligible abundances, miners, galls, Homoptera and different predators were not included in statistical analysis. Leaf beetles and their larvae and other chewing insects like Symphyta larvae and caterpillars were pooled and denominated as "sum of chewing insects" and aphids and cicada were pooled as "sum of sucking insects".

2.5 Mycorrhizal colonization

To determine colonization with ectomycorrhizal fungi fine roots were cut into small pieces and mixed. Aliquots of the mixtures were spread under a dissecting microscope (Zeiss, Stemi 2000-C) and the presence or absence of typical ectomycorrhizal mantle structures was recorded on 100 root tips per sample. To measure colonization with arbuscular mycorrhizal fungi, root samples were placed immediately after harvest in 80% EtOH. The samples were subsequently stained with trypane-blue in lactophenol, destained and mycorrhizae detected by the presence of hyphae, arbuscules or vesicles in root tissue whose abundance was recorded by the gridline intersection method as reported previously (Ducic et al. 2009).

2.6 Genetic analysis

To control the crossing experiment the DNA of the parental trees and their offspring was analysed using 5 nuclear encoded microsatellite markers. Total DNA was extracted from young leaves using the DNeasy Plant Minikit (Qiagen, Hilden, Germany). The amount and the quality of the DNA were analyzed by 0.8% agarose gel electrophoresis with 1 x TAE as running buffer (Sambrook et al. 1989). DNA was stained with ethidium bromide and visualized by UV illumination.

For microsatellite analysis the primers PMS14, PMS16 (Van der Schoot et al. 2000), PTR2, PTR4 (Dayanandan et al. 1998), and PTR5 (Rahman et al. 2000) were used. The PCR reactions were carried out as described above with the exception that primers were labelled with the fluorescent dyes 6-carboxyfluorescein (6-FAM) or hexachloro-fluorescein phosphoramidite (HEX). Fragments were separated on the ABI Genetic Analyser in a multiplex analysis. The microsatellite alleles were recognized using the software packages Genescan 3.7 and Genotyper 3.7 from Applied Biosystems.

Microsatellite loci were scored for the analysis of genetic parameters by using the computer program GENALEX (Peakall and Smouse 2001). The analysis confirmed seven of initially eight full-sib families. Genetic variances within and between full-sib families were calculated

with Molecular Analysis of Variance (MAMOVA, www.biosis.ac.uk/smart/unix/mamova) using 99 permutations.

2.7 Biochemical analysis

The biochemical analyses were conducted as described previously (Luo et al. 2006; Luo et al. 2008) and are therefore recorded here only briefly. Frozen leaves were ground to a fine powder in a ball mill cooled with liquid nitrogen (Retsch, Haan, Germany). Material of three plants of a full-sib family in each block was pooled. Glucose, fructose, sucrose and starch were extracted in DMSO/HCl and their concentrations were determined after enzymatic conversion at a wavelength of 340nm (Schopfer 1989). Soluble proteins were extracted in phosphate buffer and measured spectrophotometrically at a wavelength of 562nm using the bicinchoninic acid kit (BCA assay, Uptima, Montlucon, France). Bovine serum albumin served as the standard. Soluble phenolics were extracted twice in 50% methanol and measured spectrophotometrically after incubation with Folin-Ciocalteus phenol reagent at 765nm. Catechin was used for calibration. Leaf pigments were extracted in 80% acetone and measured at wavelengths of 646nm (chlorophyll b), 663nm (chlorophyll a) and 470nm (carotenoids). Their concentrations were calculated using the extinction coefficients determined by Lichtenthaler and Wellenburn (1983).

2.8 Element analysis

Dry leaves were milled to a fine powder in a ball mill (Retsch, Haan, Germany). The powder was wet-ashed in 65% HNO_3 at 170°C for 12h. The filtrate was used for ICP-OES analysis of P, S, K, Ca, Mg, Mn, and Fe (Spectro Analytic Instruments, Kleve, Germany) after Heinrichs et al. (1986). For analysis of nitrogen and carbon contents leaf powder was weighed into 5 x 9 mm tin cartouches (Hekatech, Wegberg, Germany) and analysed in a CHNS-O element analyzer EA1108 (Carlo Erba Instruments, Milan, Italy). Acetanilide was used as the standard.

2.9 Data analysis

Data were tested for normality with the Shapiro-Wilk's test. If required, data were log-transformed to meet the assumption of normality of residuals. For data analysis herbivore data of all sampling dates were pooled. Differences between parameter means were considered significant when the *P* value of the ANOVA *F* test was less than 0.05. Univariate or multivariate analysis of variance, principle component analysis, linear mixed effects models,

simple regression and graphics were carried out using the software R 2.10.0 (R Development Core Team 2009). The experimental design with two beds divided into four blocks each required statistical analysis with linear mixed effects models. We fitted linear mixed-effects models ("lme"-function in package "nlme"; Pinheiro and Bates 2000) using maximum likelihood with genotype, soluble phenolics and relative leaf water content plus their two-way interactions as fixed factors. To account for non-independence of different plot sizes, we used the following sequence of random effects: bed, block and genotype. To account for heteroscedasticity we inspected the residuals for constant variance and normality and used variance functions (Pinheiro and Bates 2000). For model simplification we performed stepwise backwards model selection by using the Akaike Information Criterion (AIC) (Crawley 2007; "stepAIC"-function within the "MASS"-package, Venables and Ripley 2002). The minimal adequate model was the one with the lowest AIC (Burnham and Anderson 2002). Multiple comparisons among factors having a significant effect in the minimal model were calculated using Tukey contrasts with *P*-values adjusted by single-step method ("multcomp"-package, Hsu 1996). The figures were generated with the software Origin 7.0 (Origin Lab Corp., Northampton, USA).

3. Results

3.1 Phenotypic and genetic differences between full-sib families of P. tremula

Bud break, a trait under strong genetic control, revealed distinct differences among *P. tremula* crossings (Fig. 1A). Bud break was completed 8 days earlier in C7 than in C6. The other full-sib families showed intermediate behaviour. The full-sib families furthermore differed significantly in growth (Fig. 1B) as well as in many other morphometric parameters (for details, see Supplement 1) such as the number of side shoots ($P < 0.001$), cumulative lengths of side branches per tree ($P = 0.048$), relative leaf water content ($P = 0.003$), relative height growth ($P = 0.002$), stem diameter ($P = 0.007$), stem height ($P < 0.001$), stem biomass ($P < 0.001$), leaf biomass ($P < 0.001$), below-ground biomass ($P = 0.033$), and whole plant fresh ($P < 0.001$) and dry mass ($P < 0.001$). Significant differences among full-sib families were also found for the concentrations of some leaf nutrients and for phytochemical traits [Ca ($P < 0.001$), N ($P = 0.004$), Mg ($P = 0.004$), Mn ($P < 0.001$), P ($P = 0.002$), K ($P = 0.009$), soluble phenolic compounds ($P = 0.005$), glucose ($P < 0.001$), fructose ($P = 0.009$)], whereas C, S, Fe, starch, chlorophyll, carotenoids, and soluble protein ($P > 0.05$) were unaffected by genetic differences between the full-sib families. Fungicide treatment had no significant influence on morphometric or phytochemical parameters in aspen (Supplement 1).

To classify full-sib aspen families according to their morpho- and chemometric characteristics, principle component analysis of growth and phytochemical parameters was conducted. Three components were extracted that contributed 39.7% (component 1), 22% (component 2) and 21% (component 3) of the variability. The performance of C1, C2, and C4 was strongly influenced by shoot biomass and side shoots numbers and that of C3, C5 and C7 by relative growth and Mn concentrations (1st component, Fig. 2). Only C6 was strongly affected by component 2 that was mainly defined by bud break and soluble phenolics (Fig. 2). To investigate relationships between genetic variance of the neutral markers and two parameter sets for tree performance, i.e., green leaf chemistry (mineral nutrients, phenolic compounds, carbohydrates, pigments and protein) and tree morphology (biomass of leaves, stem, and roots, leaf area, stem height increment, stem diameter, leaf numbers, whole-plant leaf area, relative leaf water content, number of side shoots, cumulative lengths of side shoots), Mantel tests were conducted (Table 1). However, neither leaf chemistry nor tree morphology showed significant relations with the genetic variance of the five neutral markers applied here. Furthermore, the neutral markers showed significantly higher molecular variance within a full-sib family than between families (Among families: DF = 6, variation 39%, within families: DF = 108, variation 69 %, PhiPT 0.3941, P = 0.010).

Table 1 Results of a Mantel test conducted for the relationship between genetic variance and tree performance.

Parameter group	$P_{(\text{Genetic Variance})}$*
Green leaf chemistry	0.3816
Plant morphology	0.9013
All parameters	0.8957

* Genetic variances were calculated on the basis of the five neutral markers used to test the populations. Performance parameters were leaf chemistry (mineral nutrients, phenolic compounds, carbohydrates, pigments and protein), tree morphology (biomass of leaves, stem, and roots, leaf area, stem height increment, stem diameter, leaf numbers, whole-plant leaf area, relative leaf water content, number of side shoots, cumulative length of side shoots) or all plant parameters analysed.

To investigate whether plant traits differed more strongly between families without common parents than between those with a common parent, the trees were combined in a matrix

showing 7 combinations for common fatherhood, 2 combinations for common motherhood and 12 combinations without common parents (Supplement 2). The differences between tree traits were calculated for each combination and compared by ANOVA.

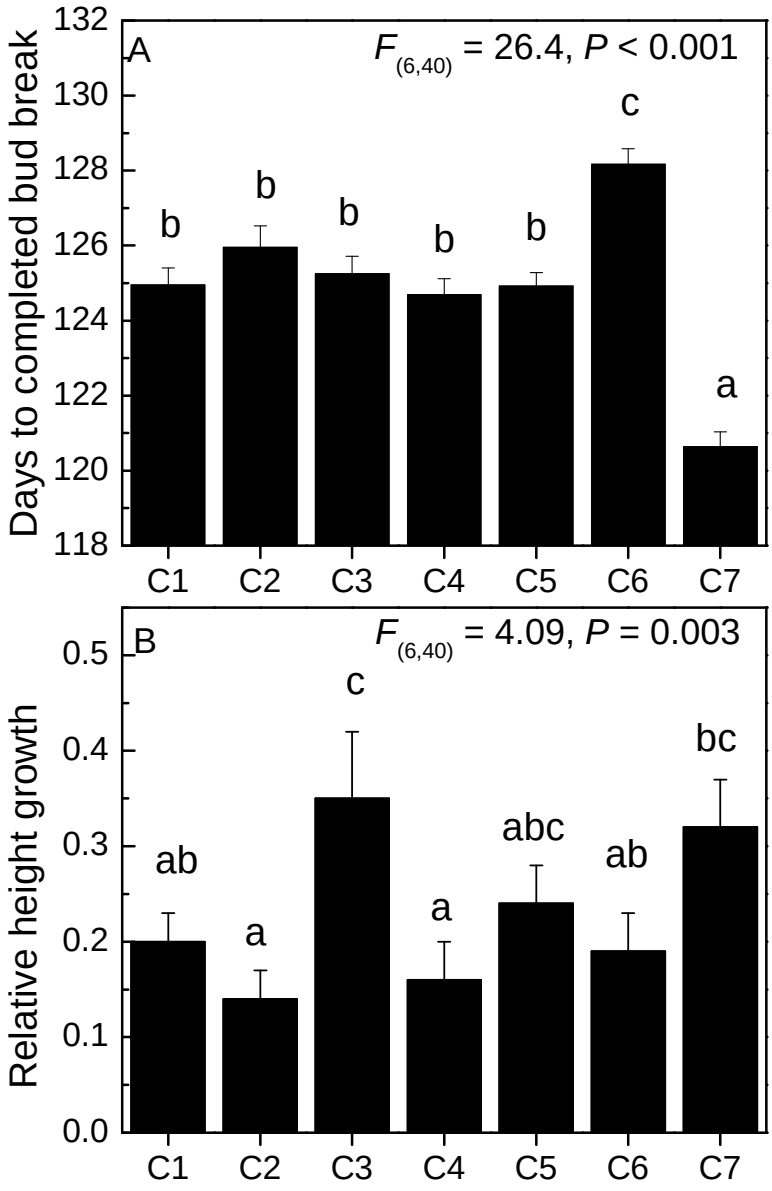

Figure 1 Bud break (A) and relative growth rate (B) of seven full-sib families of aspen (*P. tremula*). (A) Bud break was measured as Julian days to the first fully expanded leaf on the leader shoot. (B) Relative growth rate was determined as annual increment in shoot height/shoot height before bud break. Data indicate means (n = 24 to 28, ± SE). Different lower-case letters indicate significant pairwise differences between respective means at $P \leq 0.05$.

Among 30 traits tested 26% (annual stem diameter increment, number of side shoots, fructose, protein, carbon, calcium, potassium, and manganese) showed significant differences according to parenthood (Table 2). However, only half of them (annual stem increment, number of side shoots, fructose and protein) showed the expected stronger difference in progenies without than in those with common parents and suggests a strong paternal influence on these parameters.

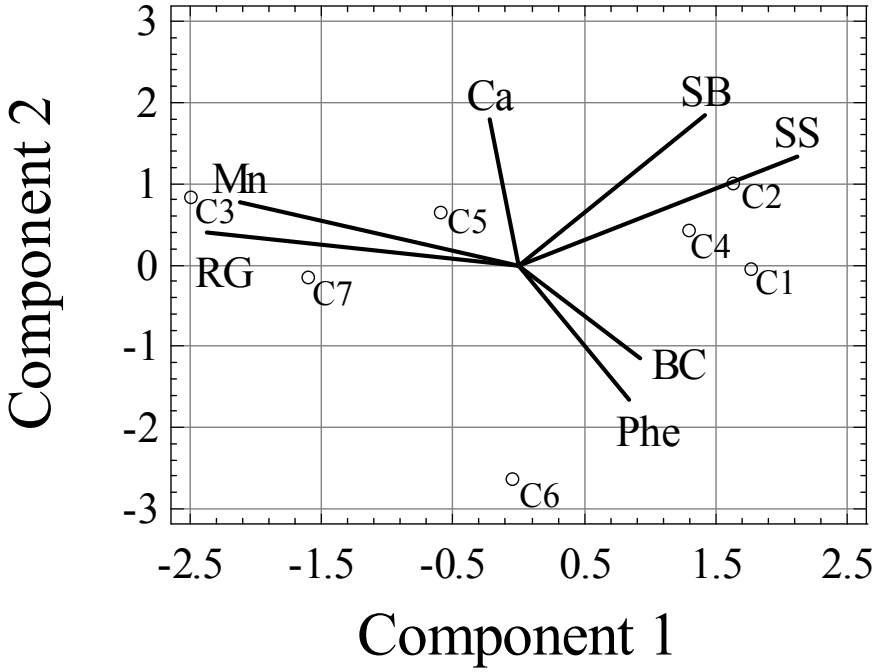

Figure 2 Principal component analysis. The analysis was based on the following parameters: SB = stem biomass, BC = time until bud break was completed, SS = number of side shoots, RG = relative growth rate, and foliar concentrations of Phe = soluble phenolics, Mn = manganese, and Ca = calcium.

3.2 Performance of full-sib families in relation to growth and defence compounds

Since growth-related parameters and soluble phenolic concentrations showed significant differences between the full-sib families, the relationship between these features was further explored. We expected that high production of phenolic compounds would consume carbon and energy, which would, thus, be unavailable for biomass production of stem and root tissues.

Table 2 Differences of plant traits between different parenthoods.

Parameter	----------Differences* for ---------- common father	common mother	no common parent	*P*
Annual stem				
Increment (mm)	-0.06 ± 0.27a	0.30 ± 0.37ab	1.02 ± 0.26b	0.042
Number of side shoots	1.32 ± 2.80a	11.17 ± 1.37b	8.97 ± 1.71b	0.004
Fructose (mg g^{-1} DM)	-0.09 ± 0.03a	0.25 ± 0.09b	0.20 ± 0.04ab	0.001
Protein (mg g^{-1} DM)	0.64 ± 0.072a	-2.87 ± 1.02b	-1.27 ± 0.57b	0.052
Carbon (%)	-0.29 ± 0.13a	0.33 ± 0.45b	0.09 ± 0.08b	0.037
Calcium (mg g^{-1} DM)	-2.15 ± 0.50a	-0.81 ± 0.17a	1.62 ± 0.24b	0.001
Potassium (mg g^{-1} DM)	-1.65 ± 0.22a	-1.12 ± 0.15ab	1.34 ± 0.48b	0.001
Manganese (mg g^{-1} DM)	-0.02 ± 0.00a	-0.01 ± 0.00a	0.01 ± 0.00b	0.000

*Differences were calculated for means for the combinations shown in Supplement 2. Data were tested with the factors: no common parents (0), common father (1), common mother (2). Data show means (± SE). Different lower-case letters indicate significant respective pairwise differences at $P \leq 0.05$. Parameters that showed no significant differences are not shown.

Instead of negative trade-off, we found that the total amount of phenolics in leaves was strictly positively correlated with total plant stem + root biomass (= non-green tissue, Fig. 3, open symbols). The same was true if the relationship between the amount of phenolics and stem biomass was considered ($R = 0.911$, $P = 0.004$). We further argued that if there was a trade-off between the production of non-green tissue and phenolics in leaves, a negative relationship between the concentration of phenolics per unit of leaf tissue and the amount of non-green tissue per green tissue must be expected. However, this was not observed (Fig. 3, closed symbols). Similarly, the concentration of phenolic compounds and the relative annual growth rate were unrelated ($R = 0.449$, $P = 0.311$).

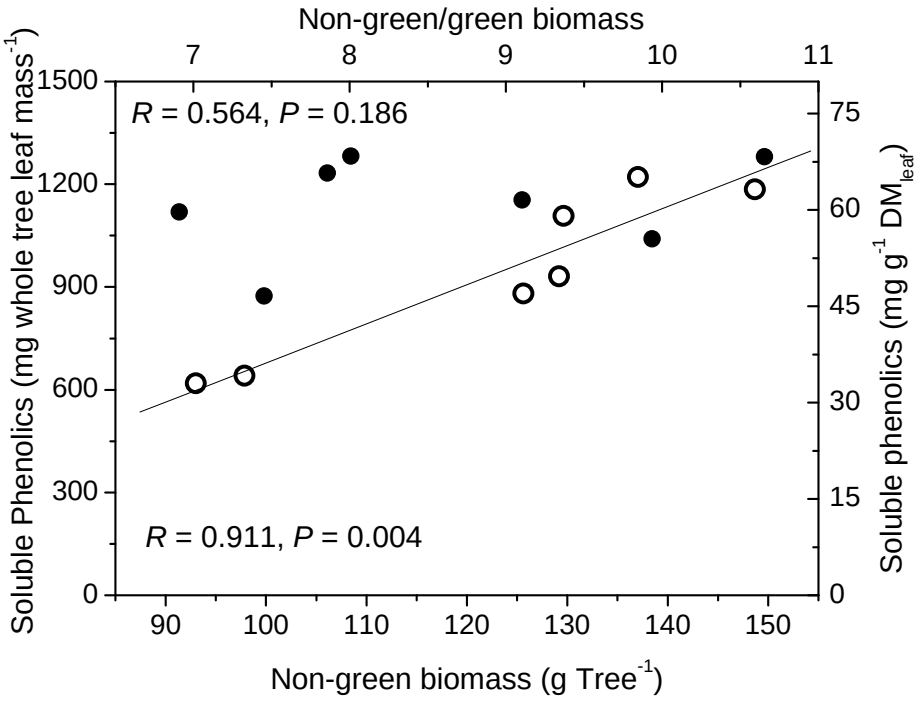

Figure 3 Relationship between total amount of soluble phenolics in leaves and non-green (= stem + root) biomass (left and lower axis, black symbols) and between the concentration of soluble phenolics and the ratio of non-green tissue-to-green tissue (right and upper axis, white symbols).

3.3 Relationships of full-sib aspen families with interacting biota and influence of fungicide treatment

The roots of all full-sib families were colonized by arbuscular (27%) and ectomycorrhizal fungi (16%). Spearman correlation revealed a marginally significant negative correlation between the abundance of arbuscular and ectomycorrhizal fungi (R = -0.741, P = 0.056). Significant effects of full-sib families or of fungicide treatment on mycorrhizal abundance were not found (Supplement 1).

Endophyte colonization was only scored in two full-sib families, C3 and C4, respectively, which were characterized by a stark contrast in the concentrations of phenolic compounds (20.9 versus 30.8 mg g^{-1} leaf fresh mass). Between these two families no significant differences for endophyte colonization were detected (score of leaf colonization: 43 ± 8%, P = 0.948). However, treatment with the fungicide Amistar, which was applied by soil drench, resulted in a significant decrease in endophyte colonization of leaves of both families (Table 3). The effect was specific for this fungal life style because mycorrhizal colonization was unaffected by the fungicide (Table 3, Supplement 1). The full-sib families also differed in herbivorous insect infestation since leaf beetle larvae were significantly less abundant on leaves of the families C2 and C6 than on those of C3 (Fig. 4).

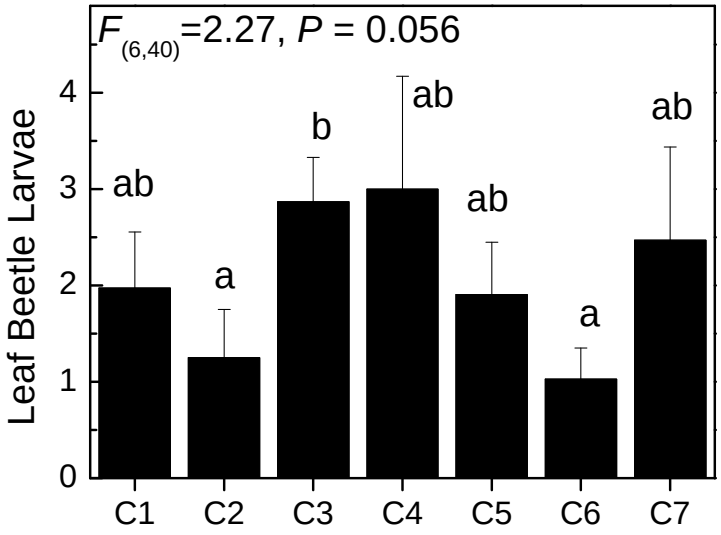

Figure 4 Abundance of chrysomelid larvae on leaves of seven full-sib families of aspen (*P. tremula*). Data indicate means (n = 24 to 28, ± SE). Different lower-case letters indicate significant pairwise differences between respective means at $P \leq 0.05$.

Table 3 Influence of fungicide treatment on endophytes and mycorrhizas.

	Control	+Fungicide*	*P*
Endophyte (% of leaf pieces)	65 ± 6	24 ± 5	< 0.001
Arbuscular mycorrhiza (% of root cells)	25 ± 3	27 ± 3	0.634
Ectomycorrhiza (% of root tips)	14 ± 1	18 ± 1	0.010

*The fungicide Amistar© was applied once a month from April to August. Colonization by endophytic fungi was scored on leaf pieces, colonization by ecto- and arbuscular mycorrhizal fungi was scored on roots. Data show means (± SE) for families C3 and C4.

There were no effects of different full-sib families on aphids and the sum of sucking insect abundance (Supplement 1). A linear mixed-effect model of foliar phenolics was significant for the abundance leaf beetle larvae ($F_{(1,19)}$ = 7.22, P = 0.014) as well as for the sum of

chewing insects (Fig. 5), whereas no significant effects were found for the abundance of aphids ($F_{(1,19)} = 0.37$, $P = 0.545$) or the sum of sucking insects ($F_{(1,19)} = 0.28$, $P = 0.602$).

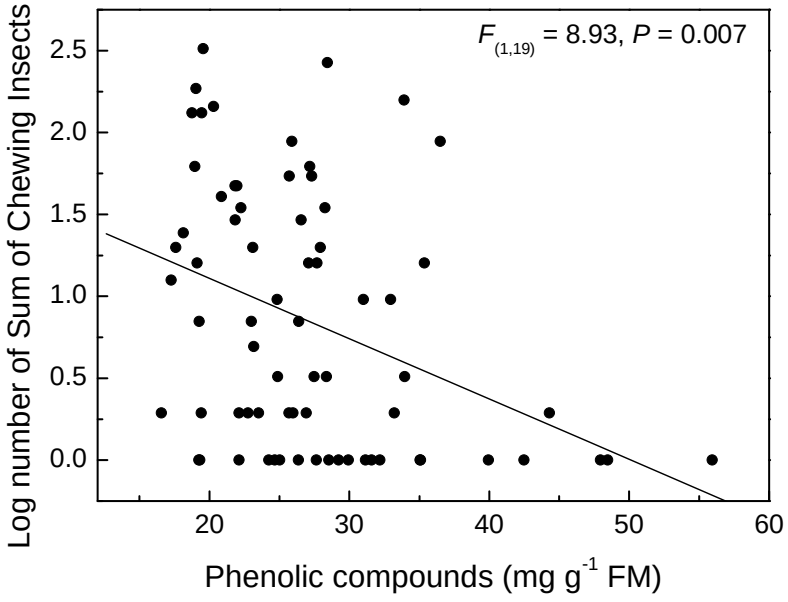

Figure 5 Relationship between the concentration of soluble phenolics in leaves and the abundance of chewing insects (note log-scale).

4. Discussion

In this study we included a large range of morphometric and chemometric measures for phenotyping of aspen. We found significant variation in these quantitative traits in the progenies of local parental trees but correspondence between variance for neutral molecular genetic markers and phenotypic characteristics was not found. This was not unexpected since attempts to correlate genetic information based on neutral markers with traits of ecophysiological significance, e.g. timing of bud break, growth, or other quantitative traits have frequently been unsuccessful (McKay and Latta 2002; Bekessy et al. 2003). Similarly, meta-analysis revealed only a very weak correlation between quantitative traits and molecular measures for genetic variation (mainly isozyme-based analyses, Reed and Frankham 2001). A comparison of neutral markers, SNPs in candidate genes and quantitative phenological parameters such as bud break, bud set, seasonal increase in tree height and stem diameters in *P. tremula* genotypes from a clinal gradient also failed to detect significant correlations between molecular and phenological measures (Hall et al. 2007). In contrast to those studies, Madritch et al. (2009) found a significant correlation between genetic distance and green leaf

chemistry for *P. tremuloides* clones. However, our data do not support such a relationship in *P. tremula*, probably, because the genetic variance within a family of siblings was higher than between different families. Nevertheless, some morphological and phytochemical traits showed significant parental influence. The reason for this apparent contradiction is probably that genetic variance was measured with neutral markers, whereas phenotypic characteristics are the integrative result of many functional genes.

In *P. tremula* the concentration of phenolics was under genetic control such as in *P. tremuloides* (Osier and Lindroth 2006; Donaldson and Lindroth 2007). Interestingly, allocation of a range of nutritional elements such as N, P, Ca, Mg and Mn was also under genetic control in *P. tremula*. N and P are major compounds in biogeochemical cycles. The elements Ca and Mg are important in ecological nutrient cycles stabilizing or counteracting decreases in soil pH (Guckland et al. 2009). Litter input of fast-degrading leaves therefore has profound effects on ecosystem processes. Whether the observed differences in leaf quality and quantities would be sufficient to influence ecosystem functions, for example, in plantations remains to be seen. With respect to Mn, genetic differences in uptake and root-to-shoot allocation have been reported for the interior and coastal race of Douglas fir (Ducic et al. 2006). The interior Douglas fir showed better performance on poor soils than the coastal provenience (Ducic et al. 2009), but in some locations its inability to limit Mn translocation to above-ground plant tissues caused severe bark diseases resulting in significant economic loss (Schöne 1992). Our findings underline that it will be worthwhile to investigate the genetic basis of mineral nutrient allocation, especially if aspens were used for agro-forestry systems.

Leaf concentrations of phenolic compounds are important factors shaping interactions with other biota (Orians et al. 1997; Glynn et al. 2004; Whitham et al. 2006). Their concentrations vary strongly between different *Populus* species and their hybrids and these variations are inversely correlated with infestation with leaf galls and arthropods (Glynn et al. 2004; Whitham et al. 2006; Holeski et al. 2009). In greenhouse experiments with *P. tremuloides* phenolic glycoside concentrations were the best predictor for gypsy moth larval performance (Donaldson et al. 2006; Donaldson and Lindroth 2007). Our data indicate that variation in leaf phenolics of *P. tremula* mediated interactions with leaf beetle larvae, belonging to the genus *Phratora* and *Crepidodera*. These chrysomelid species are generalists on salicaceous plants and not specifically adapted to utilization of phenolic glucosides like some other *Chrysomela* species (Denno et al. 1990; Ikonen 2002; Glynn et al. 2004). High concentrations of leaf phenolic compounds provide a protection from generalist herbivores as indicated by the negative relationship between phenolics and leaf beetle larvae abundance. Our data further

suggest that the costs incurred by this constitutive protection are too small to result in significant trade-off for growth or biomass production.

In this study the influence of Amistar Opti, a strobilurin-based antifungal compound, was also studied. The active agent has initially been isolated from *Strobilurus tenacelus,* a saprophytic fungus growing on pine cones (Anke et al. 1975). Although this fungicide acts against a broad number of fungal species from different classes (ascomycota, basidiomycota, oomycetes), we showed that it does not suppress mycorrhiza formation. This has also been reported for other modern fungicides (Feldman 2003; Watson 2006). Mycorrhizal colonization of aspens in our study was similar to that found in other field studies (Baum and Makeschin 2000). In fact, ectomycorrhizas even tended to be more abundant in fungicide-treated trees, which may be the result of reduced growth of potential competitors. Endophytic fungi, which colonize the apoplastic space of plant tissues and feed on plant carbohydrates, may be such competitors. They were strongly suppressed by Amistar Opti. Although endophytes often increase plant performance (Clay 1996; Morse et al. 2002; Bailey et al. 2005; but see Feath and Sullivan 2003), we did not find negative effects of their suppression on plant nutrition, growth or insect feeding in this short term study. Since trees are cultivated for several years before harvest, it will be important for future investigations to assess if reduction of endophytes has long-term negative effects.

Conclusions

We showed that full-sib aspen families exhibit significant intra-specific variation in growth and ecophysiological leaf traits and that some of these traits are clearly related to parenthood. In contrast to our expectation, production of phenolic compounds, which act as defence against generalist herbivores, did not show negative trade-off with growth. Probably, the concentrations of these compounds were too low compared with lignin production or other energy consuming processes to influence growth behaviour. Application of a systemic fungicide did neither suppress mycorrhizal colonization nor affected infestation with insects. However, a strong suppression of endophytes was found, whose long-term consequences may require attention when fungicides are used in agro-forestry plantations.

Acknowledgements

We are grateful to G. Langer-Kettner, C. Kettner, M. Smiatacz for help with installation of the common-garden experiment and plant harvest and to M. Franke-Klein for help with the photometric assays. We thank the Niedersächsisches Ministerium für Wissenschaft und

Kultur and the "Niedersächsisches Vorab" for funding „Functional Ecology Research“. The authors declare that they have no conflict of interest.

References

Anke T, Oberwinkler F, Steglich W, Schramm G (1977) The strobilurins – new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (PERS. Ex FR.). J Antibiot 30:806-810.

Bailey JK, Deckert R, Schweitzer JA, Rehill BJ, Lindroth RL, Gehring C, Whitham T (2005) Host plant genetics affect hidden ecological players: links among *Populus,* condensed tannins, and fungal endophyte infection. Can J Bot 83:356-361.

Baum C, Makeschin F (2000) Effects of nitrogen and phosphorus fertilization on mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* x *tremuloides*). J Plant Nutr Soil Sci 163:491-497.

Bekessy SA, Ennos RA, Burgman MA, Newton AC, Ades PK (2003) Neutral DNA markers fail to detect genetic divergence in an ecologically important trait. Biol Conserv 110:267-275.

Burnham KP, Anderson DR (2002) Model selection and multimodel interference. A practical information-Theoretic approach. Springer Science & Business Media, New York.

Clay K (1996) Interactions among fungal endophytes, grasses and herbivores. Res Popul Ecol 38:191-201.

Crawley MJ (2007) *The R Book*. Wiley, New York.

Dayanandan S, Rajora OP, Bawa KS (1998) Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theor Appl Genet 96:950-956.

DeChantal M, Lindberg H, Kallonen S (2009) The condition of *Populus tremula* and other deciduous saplings in a moose winter foraging area in southern Finland. Ann Bot Fennici 46:280-290.

Denno RF, Larsson S, Olmstead KL (1990) Role of enemy-free space and plant quality in host-plant selection by willow beetles. Ecol 71:124-137.

Dickmann DI, Kuzovkina J (2008) Poplars and willows of the world, with emphasis on silviculturally important species. Rome, Italy: FAO Forest Management Division Working Paper IPC/9-2. 129 p.

Dimpfelmeier R (1963) Ergebnisse fünfjähriger Kombinationszüchtungsversuche mit *Populus tremula* L. Forstwiss Centralbl 82:295-304.

Donaldson JR, Kruger EL, Lindroth RL (2006) Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*) New Phytol 169:561-570.

Donaldson JR, Lindroth RL (2007) Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. Ecol 88:729-739.

Dučić T, Berthold D, Langenfeld-Heyser R, Beese F, Polle A (2009) Mycorrhizal communities in relation to biomass production and nutrient use efficiency in two varieties of Douglas fir (*Pseudotsuga menziesii* var. *menziesii* and var. *glauca*) in different forest soils. Soil Biol Biochem 41:742-753.

Dučić T, Leinemann L, Finkeldey R, Polle A (2006) Uptake and translocation of manganese in seedlings of two races of Douglas fir (*Pseudotsuga menziesii* var. *viridis* and *glauca*). New Phytol 170:11-20.

Faeth S, Sullivan T (2003) Mutualistic asexual endophytes in a native grass are usually parasitic. American Nat 161:310-325.

Feldmann F (2003) Nebenwirkungen von Pflanzenschutzmitteln auf arbuskuläre Mykorrhizapilze als Nichtzielorganismen, Fachtagung, 4.11.2003, BBA Braunschweig.

Glynn C, Rönnberg-Wästljung AC, Julkunen-Tiitto R, Weih M (2004) Willow genotype, but not drought treatment, affects foliar phenolic conentrations and leaf beetle resistance. Entomol Exp Appl 113:1-14.

Guckland A, Jacob M, Flessa H, Thomas FM, Leuschner C (2009) Acidity, nutrient stocks, and organic-matter content in soils of a temperate deciduous forest with different abundance of European beech (*Fagus sylvatica* L.) J Plant Nutr Soil Sci 172:500–511.

Hall D, Luquez V, Garcia VM, St Onge KR, Jansson S, Ingvarsson PK (2007) Adaptive population differentiation in phenology across latitudinal gradient in European aspen (*Populus tremula,* L.): a comparison of neutral markers, candidate genes and phenotypic traits. Evol 61:2849–2860.

Heinrichs H, Brumsack HJ, Loftfield N, Konig N, (1986) Improved pressure digestion system for biological and anorganic materials. Z Pflanzenernähr Bodenkd 149:350–353.

Holeski LM, Vogelzang A, Stanosz G, Lindroth RL (2009) Incidence of *Venturia* shoot blight in aspen (*Populus tremuloides* Michx.) varies with tree chemistry and genotype. Biochem Syst Ecol 37:139-145.

Hsu JC (1996) *Multiple Comparisons: Theory and Methods*. Chapman and Hall, London.

Hultén E, Fries M (1986) Atlas of North European Vascular Plants, North of the Tropic of Cancer. Konigstein. V. 1-3:1172.

Ikonen A (2002) Preferences of six leaf beetle species among qualitatively different leaf age classes of three Salicaceous host species. Chemoecol 12:23-28.

Kendall DA, Hunter T, Arnold GM, Liggit J, Morris T, Wiltshire CW (1996) Susceptibility of willow clones (*Salix* spp.) to herbivory by *Phyllodecta vulgatissima* (L.) and *Galerucella lineola* (Fab.) (Coleoptera, Chrysomelidae). Ann Appl Biol 129: 379-390.

Kouki J, Arnold K, Martikainen P (2004) Long-term persistence of aspen – a key host for many threatened species – is endangered in old-growth conservation areas in Finland. J Nature Conserv 12:41–52.

Li B, Wu R (1997) Heterosis and genotype x environment interactions of juvenile aspens in two contrasting sites. Can J For Res 27:1525-1537.

Lichtenthaler HK, Wellenburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans 11:591-592.

Liesebach M, von Wuehlisch G, Muhs HJ (1999) Aspen for short-rotation coppice plantations on agricultural sites in Germany: Effects of spacing and rotation time on growth and biomass production of aspen progenies. For Ecol Manag 121:25-39.

Luo ZB, Calfapietra C, Libberloo M, Scarascia-Mugnozza G, Polle A (2006) Carbon partitioning to mobile and structural fractions in poplar wood under elevated CO_2 (EUROFACE) and N fertilization. Global Change Biol 12: 272-283.

Luo ZB, Calfapietra C, Scarascia-Mugnozza G, Liberloo M, Polle A (2008) Carbon-based secondary metabolites and internal nitrogen pools in *Populus nigra* under free air CO_2 enrichment (FACE) and nitrogen fertilisation. Plant Soil 304:45-57.

Madritch MD, Greene SL, Lindroth RL (2009) Genetic mosaics of ecosystem functioning across aspen-dominated landscapes. Oecol 160:119-127.

McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. Trends Ecol Evol 17:285-291.

Morse LJ, Day TA, Faeth SH (2002) Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. Environ Exp Bot 48:257-268.

Orians CM, Huang CH, Wild A, Dorfman KA, Zee P, Dao MTT, Fritz RS (1997) Willow hybridization differentially affects preference and performance of herbivorous beetles. Entomol Exp Appl 83:285-294.

Osier TL, Lindroth RL (2006) Genotype and environment determine allocation to and costs of resistance in quaking aspen. Oecol 148:293-303.

Pasteels JM, Rowell-Rahier M, Braekman JC, Dupont A (1983) Salicin from host plant as precursor of salicylaldehyde in defensive secretion of chrysomeline larvae. Physiol Entomol 8:307-314.

Peakall R, Smouse PE (2001) GENALEX V5.04: Genetic Analysis in EXCEL. Population Genetic Software for Teaching and Research. Australian National University, Canberra, Australia, available at http://www.anm.edu.au/ BoZo/GcnAlEX/.

Petrini O (1986) Taxonomy of endophytic fungi of aerial plant tissues, In: Fokkema N. J., van den Heuvel J. (eds.) Microbiology of the Phyllosphere, Cambridge University Press, Cambridge, pp. 175–187.

Pinheiro JC, Bates DM (2000) *Mixed-effects models in S and S-PLUS*. Springer, New York.

R Development Core Team 2007. R: A language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria, http://www.R-project.org

Rahman MH, Dayanandan S, Rajora OP (2000) Microsatellite DNA markers in *Populus tremuloides*. Genome 43:293–297.

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103.

Rowell-Rahier M (1984) The presence or absence of phenolglycosides in *Salix* (Salicaceae) leaves and the level of dietary specialisation of some of their herbivorous insects. Oecol 62: 26-30.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, New York.

Schöne D (1992) Site-rain and acid-rain induced nutritional disorders of Douglas-fir in Southwestern Germany. Allg Forst Jagdztg 163:53–59.

Schopfer P (1989) Experimentelle Pflanzenphysiologie, Band 2, Einführung in die Anwendung, Springer-Verlag, Berlin, Heidelberg, New York, pp. 39-50.

Tamm Ü (2006) *Populus tremula*, in Enzyklopädie der Laubbäume, Nikolai, Hamburg, pp. 405–414.

UPOV (1981) Guidelines for the conduct of tests for distinctness, homogeneity and stability: poplar (*Populus* L.), UPOV, TG 21/7, pp. 20.

Van Der Schoot M, Pospiškova M, Vosman B, Smulders MJM (2000) Development and characterization of microsatellite markers in black poplar (*Populus nigra* L.). Theor Appl Genet 101:317-322.

Venables WN, Ripley BD (2002) *Modern applied statistics with S, fourth ed*. Springer, New York.

Watson GW (2006) The effect of paclobutrazol treatment on starch content, mycorrhizal colonization, and fine root density of white oaks (*Quercus alba* L.). Arboricult Urb For 32:114-117.

Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks J, Hart SC, Wimp GM, Wooley SC (2006) A framework for community and ecosystem genetics: from genes to ecosystems. Nature Rev Gen 7:510-523.

Supplement 1

Results of linear mixed-effects models for different response variables in seven full-sib aspen families (C1, C2, C3, C4, C5, C6, and C7), describing the effects of full-sib families (Genotype) and of fungicide treatment. NumDF = numerator degrees of freedom, DenDF = denominator degrees of freedom. Bold font indicates significant *P*-values.

Group of traits	parameter	units	Genotype num DF	DenDF	*F*-value
herbivory	Chrysomelidae larvae	number	6	40	2.27
	sum of chewing insects	number	6	40	2.06
	Aphidae	number	6	40	1.40
	sum of sucking insects	number	6	40	1.59
mycorrhiza	EM	% colonization	6	40	0.56
	AM	% colonization	6	25	1.41
phytochemical leaf traits	soluble phenolic compounds	mg g^{-1} fresh mass	6	37	17.26
	glucose	mg g^{-1} fresh mass	6	37	6.90
	fructose	mg g^{-1} fresh mass	6	37	3.39
	starch	mg g^{-1} fresh mass	6	37	1.03
	chlorophyll a+b	mg g^{-1} dry mass	6	35	0.86
	carotinoid	mg g^{-1} dry mass	6	37	2.30
	soluble protein	mg g^{-1} dry mass	6	35	0.96
leaf nutrients	Ca	mg g^{-1} dry mass	6	37	92.65
	Mn	mg g^{-1} dry mass	6	37	7.04
	N	%	6	37	3.83
	C	%	6	37	1.00
	P	mg g^{-1} dry mass	6	37	4.24
	K	mg g^{-1} dry mass	6	37	3.39
	S	mg g^{-1} dry mass	6	37	0.41
	Fe	mg g^{-1} dry mass	6	37	2.05
morphometric parameter	number of side shoots	number	6	40	4.87
	cumulative lenghts of side shoots	cm	6	40	2.44
	leaf number per tree	number	6	40	5.43
	total leaf biomass	g	6	40	4.81
	leaf area per tree	cm^2	6	40	2.87
	relative leaf water content	% of fresh mass	6	40	3.95
	relative height growth		6	40	4.09
	stem diameter	mm	6	40	3.53
	stem height	cm	6	40	9.33
	stem biomass	g	6	40	6.32
	below ground biomass	g	6	40	2.58
	plant fresh biomass (above ground)	g	6	40	5.72
	whole plant dry biomass	g	6	40	5.06
	days to completed bud break	days	6	40	26.40

Group of traits	parameter	fungicide treatment				
		P-value	num DF	DenDF	*F*-value	*P*-value
herbivory	Chrysomelidae larvae	0.0561 (*)	1	5	0.1765	0.6919
	sum of chewing insects	0.0800 (*)	1	5	0.0937	0.7718
	Aphidae	0.2370	1	5	3.3125	0.1284
	sum of sucking insects	0.1756	1	5	3.5309	0.1190
mycorrhiza	EM	0.7604	1	5	2.7835	0.1561
	AM	0.2510	1	5	0.9100	0.3839
phytochemical leaf traits	soluble phenolic compounds	**< 0.0001**	1	5	0.0920	0.7736
	glucose	**0.0001**	1	5	0.9350	0.2229
	fructose	**0.0091**	1	5	0.0110	0.9207
	starch	0.4328	1	5	4.0342	0.1008
	chlorophyll a+b	0.5323	1	5	0.0373	0.8545
	carotinoid	0.0546 (*)	1	5	7.2800	**0.0429**
	soluble protein	0.4644	1	5	0.4422	0.5355
leaf nutrients	Ca	**< 0.0001**	1	5	0.3508	0.5794
	Mn	**< 0.0001**	1	5	0.3760	0.5666
	N	**0.0045**	1	5	1.8490	0.2320
	C	0.2179	1	5	0.0000	0.8229
	P	**0.0024**	1	5	1.9620	0.2203
	K	**0.0092**	1	5	1.9140	0.2251
	S	0.8665	1	5	0.3880	0.5608
	Fe	0.0835 (*)	1	5	5.7100	0.0625
morphometric parameter	number of side shoots	**0.0008**	1	5	0.3800	0.5648
	cumulative lenghts of side shoots	**0.0483**	1	5	1.1344	0.3655
	leaf number per tree	**0.0003**	1	5	0.3740	0.5675
	total leaf biomass	**0.0009**	1	5	2.4540	0.1780
	leaf area per tree	**0.0203**	1	5	0.0090	0.9288
	relative leaf water content	**0.0034**	1	5	0.9800	0.3669
	relative height growth	**0.0027**	1	5	0.7783	0.4180
	stem diameter	**0.0068**	1	5	0.3300	0.5897
	stem height	**< 0.0001**	1	5	0.0600	0.8183
	stem biomass	**0.0001**	1	5	1.6620	0.2537
	below ground biomass	**0.0333**	1	5	0.7990	0.4123
	plant fresh biomass (above ground)	**0.0002**	1	5	0.3060	0.6040
	whole plant dry biomass	**0.0006**	1	5	0.5370	0.4964
	days to completed bud break	**< 0.0001**	not tested			

Group of traits	parameter	C1		C2	
		mean	se	mean	se
herbivory	Chrysomelidae larvae	1.97	0.60	1.25	0.51
	sum of chewing insects	2.22	0.59	1.36	0.51
	Aphidae	4.13	1.00	4.94	1.70
	sum of sucking insects	4.25	1.02	5.11	1.71
mycorrhiza	EM	15.96	1.86	14.42	1.68
	AM	26.27	2.65	37.64	3.24
phytochemical leaf traits	soluble phenolic compounds	26.35	1.13	25.69	1.23
	glucose	17.79	0.39	17.67	1.28
	fructose	0.60	0.02	0.54	0.03
	starch	1.65	0.39	1.72	0.31
	chlorophyll a+b	3.54	0.28	3.78	0.28
	carotinoid	443.05	29.81	488.56	23.00
	soluble protein	34.35	1.46	34.89	2.46
leaf nutrients	Ca	12.06	0.50	16.36	0.34
	Mn	49.52	1.72	48.62	2.37
	N	2.25	0.05	2.03	0.04
	C	47.22	0.19	47.00	0.15
	P	1.85	0.05	1.79	0.07
	K	12.42	0.51	8.23	0.42
	S	1.90	0.04	1.84	0.06
	Fe	85.95	2.20	84.15	4.51
morphometric parameter	number of side shoots	53.48	7.22	53.50	4.56
	cumulative lenghts of side shoots	55.57	10.68	33.55	10.36
	leaf number per tree	310.90	31.48	237.96	28.91
	total leaf biomass	43.99	4.91	32.96	5.37
	leaf area per tree	3504.61	410.33	2795.96	641.54
	relative leaf water content	47.23	0.69	51.55	1.37
	relative height growth	0.21	0.02	0.15	0.03
	stem diameter	15.29	0.44	14.66	0.48
	stem height	223.29	6.72	204.04	7.01
	stem biomass	172.96	16.91	140.88	12.42
	below ground biomass	57.88	6.39	46.89	4.26
	plant fresh biomass (above ground)	233.40	22.31	191.73	18.28
	whole plant dry biomass	171.18	68.76	141.23	60.54
	days to completed bud break	124.95	0.45	125.96	0.57

Group of traits	parameter	C3		C4	
		mean	se	mean	se
herbivory	Chrysomelidae larvae	2.87	0.47	3.00	1.19
	sum of chewing insects	3.13	0.52	3.15	1.20
	Aphidae	18.45	8.86	2.43	1.68
	sum of sucking insects	18.67	8.84	2.56	1.69
mycorrhiza	EM	13.71	2.03	16.75	2.23
	AM	27.75	4.24	25.72	2.91
phytochemical leaf traits	soluble phenolic compounds	20.98	0.65	30.84	1.90
	glucose	21.57	1.91	17.96	1.40
	fructose	0.68	0.03	0.66	0.02
	starch	2.17	0.61	1.82	0.47
	chlorophyll a+b	3.28	0.42	3.28	0.26
	carotinoid	665.64	78.55	425.39	29.91
	soluble protein	32.68	1.89	32.75	2.38
leaf nutrients	Ca	14.70	0.55	14.19	0.52
	Mn	79.43	3.15	63.36	2.30
	N	2.17	0.04	2.11	0.04
	C	47.21	0.29	47.76	0.29
	P	1.87	0.07	1.78	0.05
	K	9.73	0.50	10.09	0.33
	S	1.92	0.06	1.95	0.06
	Fe	95.72	3.30	80.45	2.09
morphometric parameter	number of side shoots	43.75	3.07	55.08	3.13
	cumulative lenghts of side shoots	50.78	10.90	77.59	16.25
	leaf number per tree	230.33	19.30	303.42	25.68
	total leaf biomass	32.25	3.73	45.61	5.22
	leaf area per tree	2615.82	317.86	3255.67	408.08
	relative leaf water content	47.68	0.86	47.47	0.87
	relative height growth	0.41	0.07	0.19	0.03
	stem diameter	13.67	0.48	14.28	0.28
	stem height	160.88	8.84	201.75	7.63
	stem biomass	113.41	10.93	159.25	13.30
	below ground biomass	38.79	2.96	54.19	3.45
	plant fresh biomass (above ground)	163.34	15.25	220.98	18.86
	whole plant dry biomass	112.11	46.30	156.69	50.71
	days to completed bud break	125.04	0.41	124.38	0.42

Group of traits	parameter	C5		C6	
		mean	se	mean	se
herbivory	Chrysomelidae larvae	1.98	0.59	1.03	0.34
	sum of chewing insects	2.17	0.63	1.18	0.34
	Aphidae	4.25	2.10	6.53	3.02
	sum of sucking insects	4.40	2.11	6.71	3.03
mycorrhiza	EM	15.97	1.88	18.09	2.18
	AM	31.65	4.13	15.78	1.58
phytochemical leaf traits	soluble phenolic compounds	27.61	1.65	30.76	1.59
	glucose	20.25	2.02	18.75	3.10
	fructose	0.48	0.04	0.81	0.18
	starch	1.54	0.45	1.91	0.41
	chlorophyll a+b	4.06	0.23	3.21	0.18
	carotinoid	503.26	25.07	418.77	23.52
	soluble protein	40.02	2.43	33.49	1.85
leaf nutrients	Ca	14.44	0.46	13.04	0.47
	Mn	65.00	3.61	55.22	1.77
	N	2.12	0.03	2.08	0.04
	C	46.96	0.09	47.12	0.08
	P	1.60	0.04	1.83	0.11
	K	9.28	0.43	10.41	0.88
	S	1.98	0.11	1.85	0.06
	Fe	90.05	2.65	92.67	5.71
morphometric parameter	number of side shoots	46.04	3.35	40.96	3.60
	cumulative lenghts of side shoots	63.34	15.02	21.39	4.85
	leaf number per tree	216.33	16.87	162.00	16.89
	total leaf biomass	39.58	3.86	22.60	2.73
	leaf area per tree	2435.54	275.82	1587.80	208.51
	relative leaf water content	48.72	0.89	50.56	1.01
	relative height growth	0.24	0.04	0.20	0.05
	stem diameter	15.50	0.40	13.42	0.36
	stem height	210.63	7.82	156.04	7.71
	stem biomass	144.15	9.24	91.16	8.79
	below ground biomass	56.52	4.13	44.51	3.17
	plant fresh biomass (above ground)	200.12	12.53	124.87	11.76
	whole plant dry biomass	146.35	45.55	103.10	37.66
	days to completed bud break	124.79	0.45	128.17	0.37

Group of traits	parameter	C7 mean	se
herbivory	Chrysomelidae larvae	2.47	0.97
	sum of chewing insects	2.67	1.00
	Aphidae	3.71	1.02
	sum of sucking insects	3.85	1.01
mycorrhiza	EM	17.24	2.19
	AM	22.49	1.96
phytochemical leaf traits	soluble phenolic compounds	33.80	1.43
	glucose	28.31	2.46
	fructose	0.44	0.03
	starch	5.25	0.87
	chlorophyll a+b	2.72	0.13
	carotinoid	369.10	11.92
	soluble protein	30.09	1.19
leaf nutrients	Ca	15.70	0.39
	Mn	64.83	3.10
	N	1.84	0.02
	C	47.05	0.06
	P	1.54	0.02
	K	7.65	0.20
	S	1.64	0.03
	Fe	83.55	1.49
morphometric parameter	number of side shoots	33.32	2.04
	cumulative lenghts of side shoots	79.14	15.34
	leaf number per tree	231.50	15.69
	total leaf biomass	45.55	4.27
	leaf area per tree	2805.98	291.38
	relative leaf water content	49.26	0.77
	relative height growth	0.32	0.05
	stem diameter	15.37	0.58
	stem height	212.59	13.63
	stem biomass	139.03	13.78
	below ground biomass	54.23	4.08
	plant fresh biomass (above ground)	197.13	18.42
	whole plant dry biomass	147.51	51.56
	days to completed bud break	120.43	0.44

Supplement 2

Matrix showing combination of common fathers (1), common mothers (2) and no common parents (0) for the full-sib aspen families.

Parent	C1 4x5	C2 9x5	C3 8x5	C4 2x5	C5 2x3	C6 9x3	C7 7x1
C1 4x5							
C2 9x5	1						
C3 8x5	1	1					
C4 2x5	1	1	1				
C5 2x3	0	0	0	2			
C6 9x3	0	2	0	0	1		
C7 7x1	0	0	0	0	0	0	

Chapter 4

Physiological vs. morphological traits controlling the productivity of six aspen full-sib families

(submitted)

Physiological vs. morphological traits controlling the productivity of six aspen full-sib families

Annika Müller [1,*], Viviana Horna [1], Frauke Kleemann [2], Barbara Vornam [3] and Christoph Leuschner [1]

[1]Plant Ecology, Albrecht von Haller Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany, vhorna@gwdg.de; cleusch@gwdg.de

[2]Forest Office Rottweil, Königstraße 36, 78628 Rottweil, Germany, frauke.kleemann@landkreis-rottweil.de

[3]Forest Genetics and Forest Tree Breeding, Büsgen Institute, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany, bvornam1@gwdg.de

[*]Corresponding author: Email: amuelle3@gwdg.de, Fax: +49 (0) 551-395701, phone: +49 (0) 551-3922088

Abstract

For investigating genotypic differences in the production potential of *Populus tremula* L., we grew poplar plants of six full-sib families under optimal water and nutrient conditions and analysed more than 20 physiological and morphological traits with a potential impact on productivity. The six families were produced from controlled crossings of two male and four female trees. Despite genetic distances of 2 to 28%, the families showed no significant differences in photosynthetic and leaf water status parameters (photosynthetic capacity, leaf water potential and others), even though productivity differed up to twofold between the families. Hence, growth rate was not related to photosynthetic activity but showed a close association with several morphological traits, most closely with the leaf number (L) and total leaf area. Variation in L explained 70% of the growth variation across the six families, and the start of bud burst (BB) correlated with the leaf number (early-starting families produced more leaves). The between-family variation in growth-related morphological traits was much larger than that in physiological traits (coefficient of genetic variation 4-29% vs. 0-4%). Even though the genetic constitution had a significant effect on eight morphological (leaf and root-related) traits, we found no relation between the genetic differences between any two families and the corresponding growth differences. We conclude that the timing of bud burst and the

resulting total number of leaves developed are the determinants of growth in *P. tremula*. Selection programmes should focus on the considerable intraspecific variation in L and BB in order to increase yield.

Keywords: genetic distance, growth analysis, leaf phenology, photosynthetic capacity, *Populus tremula*, relative growth rate.

1. Introduction

The interplay of genetic variation and productivity is of prime importance for forest industries, because it offers the potential to increase biomass gain for global renewable energy needs. There is an urgent need to improve the properties and increase the productivity of high-yielding woody plantation systems (e.g. short-rotation coppice) in order to satisfy the market demand on a long-term basis [1]. At present, bioenergy and fibre production within short-rotation forestry in Europe and North America is based mainly on poplar species and their cultivars [2-4]. A recent focus of ecological research is to disentangle the relationship between biodiversity and ecosystem functioning [5-7] and to understand the conditions under which plant species diversity has a positive effect on productivity e.g. [8,9]. In contrast, the role of plant genotype diversity for ecosystem functioning is not well studied and probably underestimated. Even though the variation between genotypes may be smaller than the variation between species, the impact on the productivity of the populations may be large enough to affect ecosystem structures and functions. In the recent past, empirical evidence for plant genotypic diversity increasing productivity has become available [10], but a deeper understanding of the functional role of intraspecific genetic diversity is still lacking.

In this study, we focus on *Populus tremula* L. plantings in short-rotation coppice, one of the important tree species for the energy wood industries. Many selection programmes have been developed in order to screen for the most promising poplar genotypes in terms of productivity [11,12]. Yet, the contribution of intraspecific genetic variation to differences in productivity is not sufficiently understood [13]. For estimating the production potential, detailed knowledge of a plant's yield components together with the genetic variation in the available plants is required [14]. Aspen are successful pioneer species with a large geographic range [15]. Because of their high fecundity and wind dispersed pollen and seeds, aspen species have a high level of inter- and intra-population diversity [16]. As typical colonizer species, they also reproduce clonally via root suckers. Hence, newly established aspen populations at open sites and on barren land typically consist of numerous closely related genetic individuals [17]. We

tried to simulate this situation by investigating several closely related progenies (full-sibs) which are the offspring derived by pair-crossing from a few colonising mother and father trees.

In the current study, we characterize six different full-sib families of *P. tremula* by a broad set of morphological and physiological traits in order to understand the potential of intraspecific variation for growth promotion in poplar and to identify traits associated with high-yielding. We attempted to minimise the influence of a variable environment setting up an experiment under optimal growing conditions (enough water supply and fertile soil substrate) with plants of defined genetic constitution. This allows us to relate the productivity performance to physiological and morphological traits inherent to each full-sib family. The study's specific objectives were (1) to investigate the variability of yield in a group of aspen progenies with closely related genetic constitution, (2) to identify physiological and morphological key traits that contribute to aspen productivity, and (3) to assess the role of genetic variation for yield and the traits controlling it.

2. Materials and methods

2.1 Aspen full-sib families

The plants used in this study belong to six full-sib families of trembling aspen (*P. tremula*) bred by controlled crossing. The parent tree material originates from 30-year-old trees selected in Göttingen-Geismar, Central Germany (51°32´N, 9°56´E). Two male trees were used as pollen donors (Geismar #3 and 5) and four served as mother plants (Geismar # 2, 4, 8, 9). The crossings 2x3, 2x5, 4x5, 8x5, 9x3 and 9x5 (full-sib families) were carried out under laboratory conditions, and the offspring was raised in ten-litre pots by the group of Forest Genetics and Tree Breeding at the University of Göttingen in 2000. Twenty-four progenies per family were selected for the experiment.

2.2 Experimental design

The experiment was conducted in the outdoor area of the Department of Forest Botany and Tree Physiology at the University of Göttingen. In April 2008, progenies from the six full-sib families were transplanted in two blocks of 10 m x 2 m, with each block being composed of four plots. Each plot included three aspen saplings of each family which were randomly placed at a distance of 50 cm. The eight plots were treated as replicates, because ANOVA revealed no significant plot effects. To provide optimal growing conditions, the used soil substrate was nutrient-rich humus, and the trees were regularly watered. Every plot was

bordered by a single row of trees that were not used for the physiological and morphological measurements. This row served as a buffer zone to avoid the potential impact of edge effects on the target plants. Due to inherent differences in relative growth rate between the six families, the plants had reached different tree heights, total twig lengths and shoot diameters at the beginning of the experiment. To account for these size differences, initial tree height was used as a covariable in the statistical analyses (ANCOVA).

2.3 Molecular analyses

In order to characterize the genetic differences between the six full-sib families, DNA of the progenies was analysed using five nuclear encoded microsatellite markers. The total DNA from young leaves was extracted using the DNeasy Plant Minikit (Qiagen, Hilden, Germany). For microsatellite analyses the primers PMS14, PMS16 [18], PTR2, PTR4 [19] and PTR5 [20] were used. PCR amplification was performed in a 12.5 µl volume containing 10 ng template DNA, 10 mMTris/HCl pH 9.0, 0.2 mM of each dNTP, 1.5 mMMg Cl2, 50 mM KCI, 0.2 µM each of forward and reverse primers and 1 U of Tag polymerase (Qiagen, Hot Star Master Mix, Hilden, Germany). All amplifications were performed in a Peltier Thermal Cycler (PTC-0200 version 4.0, MJ Research) with a heated lid under the conditions described for each primer pair (see above). Primer pairs were preliminarily tested by running PCR products on 2% agarose gels in 1 x TAE buffer (see above). Microsatellite alleles were analysed on an automatic sequencer (ABI 3100 Genetic Analyser). The PCR reactions were carried out with one primer (the forward primer) labelled with the fluorescent dyes 6-FAM (blue) or HEX (green). Fragments were separated on the ABI Genetic Analyser in a multiplex analysis. GS 500 ROX (fluorescent dye ROX) was used as internal standard (Applied Biosystems, California, US). The microsatellite alleles were recognized using the software packages Genescan 3.7 and Genotyper 3.7 from Applied Biosystems. Microsatellite loci were scored for the analyses of genetic parameters by using the computer program GENALEX (Genetic Analysis in EXCEL). The genetic distance between the different full-sib families was calculated according to Nei [21].

2.4 Plant growth and allocation

Starting two weeks after planting in April 2008, bud flush was monitored at frequent time intervals (1-2 days) during the duration of the experiment according to UPOV [22]. In September, before leaf abscission occurred, all trees were harvested. Every tree was excavated individually with a soil corer of 25 cm radius. Above- and below-ground biomass were

separated and the entire root system was carefully washed out to remove soil residues. Coarse (Ø > 2 mm in diameter) and fine roots (Ø ≤ 2 mm) were separated, dried and weighed. A small sub-sample of fresh fine roots was taken apart for determination of root surface area and specific root area (SRA: $cm^2\ g^{-1}$ dry weight (DW)) using scanned images of the roots that were analysed with the software WinRhizo (Régent Instruments Inc., Quebec, Canada). Subsequently, these sub-samples were also dried and weighed. The main stem and the branches were separated from the leaf biomass, dried at 70°C for at least 48h and weighed to determine above-ground dry biomass. Total dry weight per plant was calculated by adding all above- and belowground fractions.

Stem height and stem diameter at a height of 22 cm above the ground and the total length of all twigs were recorded. The stem volume index was calculated based on plant height and stem diameter (SVI = H x D^2 (cm^3)) according to Pontailler et al. [23]. All leaves were counted to obtain the total number of leaves (L) produced during the experimental period. Mean plant growth over the vegetation period was estimated from the difference between initial and total twig length at harvest. Based on the relationship between final biomass and total twig length at the date of harvest, the initial biomass of the plants at the beginning of the experiment was recalculated using the twig length at this date, assuming that the twig length-biomass relationship was constant throughout the vegetation period. The biomass increase was then calculated as the difference of initial and final biomass, relative growth rate (RGR, in $mg\ g^{-1}\ d^{-1}$) by relating biomass increase to the duration of the experiment (150 days). Stem height increment was found to be a less suitable estimator of growth because a group of trees showed a negligible increase of main shoot height but had considerable length growth in the twigs.

Each ten leaves per tree were randomly selected to determine mean leaf size (*Ls*) through scanning and image analysis with the software WinFolia (Régent Instruments Inc., Quebec, Canada). Total leaf area (tLA) per tree was estimated from mean leaf size and the total number of leaves counted at harvest. tLA is equivalent to the total leaf area produced over the experimental period. Leaf area ratio (LAR: $cm^2\ g^{-1}$ DW) was calculated as the ratio of total leaf area and total dry mass of the plant. The proportion of leaf, shoot or root mass in total dry mass was expressed by leaf mass ratio (LMR), shoot mass ratio (SMR), or root mass ratio (RMR) (all in $g\ g^{-1}$ DW or % of plant mass). Root-to-shoot ratio (R/S) relates total root mass to shoot mass.

2.5 Gas exchange measurements

After full leaf expansion in June 2008, leaf gas exchange parameters were recorded monthly using a LI-6400 portable photosynthesis system (LI-Cor, Lincoln, NE, USA) until August. All measurements were made on fully expanded and sun exposed leaves between 900 and 1700 h solar time. Light-response curves were recorded for single leaves of eight to ten trees per family that were measured alternately over a week. The levels of photosynthetically active radiation (PAR) were adjusted to 1500, 1000, 750, 500, 300, 200, 100, 50, 20, and 0 µmol photons m^{-2} s^{-1} using a red and blue LED light source supplied by the manufacturer. All measurements were conducted at constant CO_2 concentration (370 ppm), constant leaf temperature (20-22°C) and constant vapour pressure deficit (VPD = 1kPa). Parameters obtained were light-saturated net photosynthesis rate (A_{max}), leaf dark respiration (DR), apparent quantum yield (Q_e), light compensation point (LCP), light saturation point (LSP) and stomatal conductance (g_s) at 1500 µmol m^{-2} s^{-1}. Furthermore, CO_2-response curves (50, 100, 150, 370, 600, 1200, 2000 µmol CO_2 mol air^{-1}) were established for all families at 1000 µmol m^{-2} s^{-1} PAR to calculate carboxylation efficiency (CE). The data points from the light- and CO_2-response curves were approximated to non-rectangular hyperbola according to Meir et al. [24] and evaluated with the Software SigmaPlot (Systat Software Inc., Version 10.0, California, USA). After gas exchange measurement, the fresh leaves were sampled to determine leaf surface area (WinFolia, Régent Instruments Inc., Quebec, Canada) and subsequently dried and weighed to calculate specific leaf area (SLA: cm^2 g^{-1} DW).

2.6 Chlorophyll-fluorescence

Chlorophyll-fluorescence was measured on fully expanded leaves of eight trees per family with a portable Pulse Amplitude Modulation Fluorometer (PAM 2000, Heinz Walz GmbH, Effeltrich, Germany) on three occasions in June, July and August. The chlorophyll-fluorescence was then expressed in terms of maximum quantum efficiency of photosystem II ($(F_m - F_o)/F_m = F_v/F_m$) at predawn (400 to 530 h solar time) and around midday (1200 to 1330 h solar time), and effective quantum yield of PS II ($(F_m' - F_t)/F_m' = \Phi_{PSII}$) at two light regimes (200 and 1500 µmol m^{-2} s^{-1} PAR). In order to detect the fluorescence in the absence of light, F_v/F_m was recorded after keeping the leaves in complete darkness (F_o) for 20 min, followed by the application of a sat pulse to achieve maximum fluorescence (F_m). To determine Φ_{PSII}, the leaves were re-adapted to the low and high irradiation levels used in the measurements. After reaching steady state in F_t (fluorescence in the light), a flash pulse was applied to the leaves and the maximum fluorescence in the light (F_m) could be measured.

2.7 Leaf water potential

Leaf water potential (Ψ) was measured with a pressure chamber (PMS Instruments, Corvallis, OR) on sunny days at midday between 1200 and 1330 h solar time on three occasions in June, July and August with the aim to record daily minima (ψ_{min}). Single fully expanded and sun-exposed leaves of eight trees per family were cut off and immediately measured.

2.8 Statistical analyses

Statistical analyses were performed with the Statistical Analysis System (Version 9.1, SAS Institute Inc., USA). All data were tested for normality using the Shapiro and Wilk's test ($P \leq 0.05$). In cases of non-normality, data were transformed to meet requirements of parametric tests. Time series were not specifically analysed, but all calculations with repeated measurements used means (and standard errors: SE) calculated over the vegetation period. Traits were correlated with each other using Spearman rank correlation. Data were analysed using ANOVA (general linear models, proc glm) with analyses for genetic effects and plot as block factor. Due to the differences in initial plant height, height was treated as a covariable (ANCOVA). Reported *P*-values were based on type III sum of squares estimates. To reveal significant differences in the analysed trait means between families, a Tukey *post hoc* test ($P \leq 0.05$) was applied. In order to identify traits most closely correlated with productivity, the relative growth rate was used as response variable in a multiple regression analysis (backward variable selection); explanatory variables were derived based on a Principal Components Analysis (PCA). In general, only variables were used in the PCA that were not related to each other and were not derived from each other (an exception was made for total leaf area and the number of leaves which were identified as key plant traits in other analyses). The morphological traits of leaves, shoots and roots, which exhibited a significant Spearman correlation to relative growth rate, were included in the PCA and subsequently in the multiple regression analysis. All included parameters were standardized (zero mean / unit variance) and constructed on the two main axes (PC1 and PC2) additionally to the allocation of the families in the orthogonal plane. PCA and multiple regression analysis were performed using STATISTICA software (StatSoft, USA).

2.9 Estimates of genetic parameters

When there was a significant effect of family on traits, we calculated broad-sense heritability (H^2) for the respected trait. H^2 was calculated from the genetic variance component (σ^2_G) related to the total phenotypic variance ($\sigma^2_p = \sigma^2_G + \sigma^2_\epsilon$) which comprises the genetic variance

and residual variance (σ^2_ϵ) component for each trait. Standard errors of H^2 were generated by the equation SE (H^2) = (1-H^2) [1+ (b-1) H^2] [2/ (bf)] $^{1/2}$ according to Singh et al. [25]. The coefficient of genetic variation (CV_G) was calculated from the genetic variance component and the trait mean: $CV_G = \frac{\sqrt{\sigma^2_g} \times 100}{mean}$. According to this expression, the coefficient of environmental variation was calculated with the respective residual variance component: $CV_\epsilon = \frac{\sqrt{\sigma^2 \varepsilon} \times 100}{mean}$ [26]. When the mean square of the residuals exceeded the mean square of the family, the genetic variance component and CV_G was set to zero.

3. Results

3.1 Family variation in plant physiological traits

Light-saturated net photosynthesis (A_{max}) showed an only very small variation with means ranging from 12.47 to 13.87 µmol CO_2 m^{-2} s^1 and no significant differences between the six families (Table 1). We found a significant correlation between A_{max} and carboxylation efficiency (mean of all families: 0.25 mol air m^{-2} s^{-1}, r: 0.6, P<0.001), stomatal conductance (0.16 mol H_2O m^{-2} s^{-1}, r: 0.41, P<0.01), the effective quantum yield at 1500 µmol m^{-2} s^{-1} (0.404, r: 0.45, P<0.001) and the light saturation point (805 µmol m^{-2} s^{-1}, r: 0.8, P<0.001). The variables related to plant water status (g_s and midday water potential ψ_{min} with means from -1.73 to -2.03 MPa) were not significantly related to each other or to any parameters associated with carbon assimilation. None of the gas exchange and water status parameter showed significant differences between the families. A significant seasonal trend during the experimental period was also lacking. Especially maximum quantum efficiency F_v/F_m was very similar between the six families and varied only slightly between 0.790 and 0.813 at predawn, while midday values were somewhat lower (Table 2). Leaf dark respiration, light compensation point, and apparent and effective quantum yield showed very little variation as well and consequently no significant differences between the six families (Tables 1 and 2).

3.2 Family variation in relative growth rate and biomass production

The means of relative growth rate (RGR) differed by up to 30 percent between the six families and thus were much more variable than the photosynthesis parameters, but the RGR differences were not significant due to considerable variation between the individuals of a family. Nevertheless, the families 2x5 and 4x5 showed the highest RGR means (3.85 mg g^{-1} d^{-1}) while family 9x3 reached a mean of only 3.02 mg g^{-1} d^{-1}, and the other three families

ranged between these extremes (Table 3). Moreover, the growth differences were significant between the families, when the absolute biomass increase in the 150 d-experimental period was considered instead of RGR in the analysis of variance with initial height as a covariable. As for RGR, the 2x5 and 4x5 families were the most productive ones (about 100 g biomass increase) whereas families 9x3 and 8x5 produced only 51 and 65 g, respectively. The other two families showed intermediate productivity (up to 80 g).

3.3 Plant growth analysis and biomass allocation patterns

Of all morphological parameters, the number of leaves and total leaf area correlated best with relative growth rate, while the relationships to other leaf-related traits (*Ls*, LAR, SLA and LMR) was less tight. A negative relation was found to RMR, root-to-shoot ratio and the timing of bud burst (Table 4, Fig. 1).

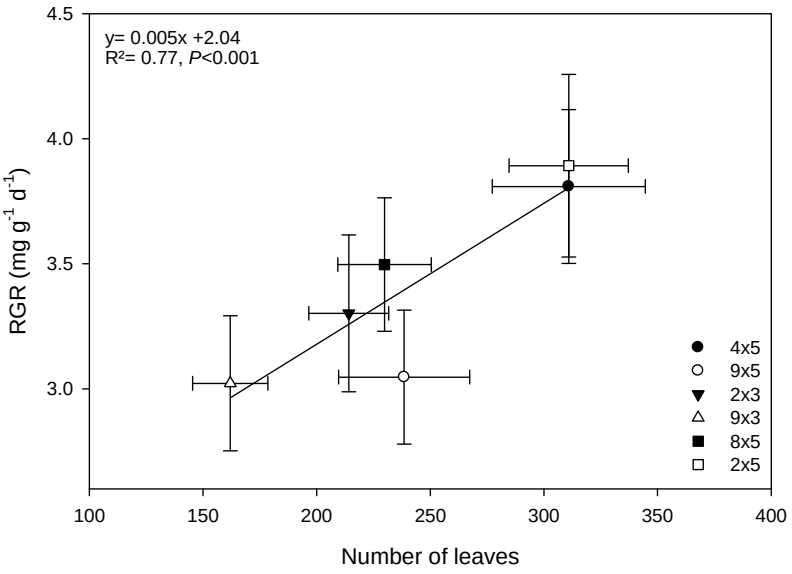

Figure 1 Relation between relative growth rate and the number of leaves produced during the experiment in the six aspen families (means and standard errors of 16-20 individuals per family).

A multiple regression analysis with backward variable selection confirmed the prominent role of leaf number with 70 % (R^2: 0.73, $P<0.001$) of the RGR variation between the families being explained by the variation in the number of leaves. How many leaves were formed during the experimental period was significantly influenced by the date when bud flushing started in spring (Fig. 2). The timing of leaf bud burst correlated negatively with most leaf-related traits, but most closely with leaf number, while RMR and R/S showed a positive relation to the onset of leaf flushing (Table 4). Due to the lack of variation in physiological

traits, none of them correlated with RGR. Consequently, they were not used in the multivariate analysis.

Table 1 Gas exchange parameters: light-saturated net photosynthesis rate (A_{max}), carboxylation efficiency (CE), apparent quantum yield (Q_e), light compensation point (LCP), light saturation point (LSP) and leaf dark respiration (DR) of the six aspen families. None of the differences between the families were significant at $P<0.05$ (means and standard errors of 24 measurements per family were conducted between June and August 2008).

	Gas exchange parameters					
Aspen families	A_{max} (μmol CO_2 m^{-2} s^{-1})	CE (mol air m^{-2} s^{-1})	Q_e (μmol CO_2 μmol photons^{-1})	LCP (μmol photons m^{-2} s^{-1})	LSP (μmol photons m^{-2} s^{-1})	DR (μmol CO_2 m^{-2} s^{-1})
2x3	13.78 ± 0.81	0.28 ± 0.02	0.091 ± 0.003	21.80 ± 1.62	836.75 ± 20.91	-1.76 ± 0.09
2x5	12.31 ± 0.99	0.25 ± 0.01	0.093 ± 0.003	21.94 ± 1.63	794.30 ± 29.41	-1.75 ± 0.12
4x5	13.02 ± 0.81	0.24 ± 0.01	0.091 ± 0.002	22.42 ± 2.00	820.65 ± 26.38	-1.79 ± 0.12
8x5	12.47 ± 0.76	0.23 ± 0.02	0.099 ± 0.002	20.92 ± 1.01	778.01 ± 20.41	-1.83 ± 0.07
9x3	12.87 ± 0.72	0.26 ± 0.02	0.102 ± 0.003	20.86 ± 1.13	778.63 ± 16.43	-1.91 ± 0.15
9x5	13.87 ± 0.79	0.28 ± 0.02	0.095 ± 0.004	20.72 ± 1.54	821.81 ± 26.88	-1.76 ± 0.12

Table 2 Leaf water status and chlorophyll fluorescence of the six studied aspen families (means and standard errors of 24 measurements per family were conducted between June and August 2008). Leaf water potential (Ψ_{min}) was recorded at midday hours (12:00-13:30) on sunny days; stomatal conductance (g_s) was measured at 1500 µmol photons m^{-2} s^{-1}. Chlorophyll fluorescence is expressed by the maximum quantum efficiency (F_v/F_m) and by the effective quantum yield (Φ_{PSII}) recorded at two light regimes (200 and 1500 µmol m^{-2} s^{-1}). Differences between the families were not significant at $P<0.05$.

	Leaf water status		Chlorophyll fluorescence			
Aspen families	Ψ_{min} (MPa)	g_s (mol H_2O m^{-2} s^{-1})	F_v/F_m predawn	F_v/F_m midday	Φ_{PSII} (at 200 µmol m^{-2} s^{-1})	Φ_{PSII} (at 1500 µmol m^{-2} s^{-1})
2x3	- 1.73 ± 0.12	0.168 ± 0.007	0.813 ± 0.002	0.733 ± 0.007	0.664 ± 0.011	0.417 ± 0.012
2x5	- 2.03 ± 0.13	0.178 ± 0.016	0.808 ± 0.005	0.734 ± 0.010	0.674 ± 0.010	0.403 ± 0.011
4x5	- 1.75 ± 0.13	0.169 ± 0.017	0.801 ± 0.004	0.748 ± 0.010	0.663 ± 0.012	0.392 ± 0.012
8x5	- 1.77 ± 0.10	0.161 ± 0.019	0.800 ± 0.007	0.739 ± 0.009	0.670 ± 0.010	0.382 ± 0.016
9x3	- 1.77 ± 0.15	0.172 ± 0.022	0.790 ± 0.007	0.741 ± 0.010	0.658 ± 0.011	0.394 ± 0.012
9x5	- 1.95 ± 0.11	0.153 ± 0.016	0.807 ± 0.004	0.731 ± 0.074	0.648 ± 0.010	0.433 ± 0.012

Table 3 Several growth-related morphological properties of the six aspen families studied: relative growth rate (RGR), stem volume index (SVI), total leaf area (tLA), root-to-shoot ratio (R/S), specific root area (SRA) and specific leaf area (SLA) (means and standard errors of 16-24 trees per family). Different small letters indicate significant differences (ANCOVA, $P \leq 0.05$) between the families.

Aspen families	RGR ($mg\ g^{-1}d^{-1}$)	SVI (cm^3)	tLA (cm^2)	R/S	SRA ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)
2x3	3.30 ± 0.32	280.28 ± 22.92 a, d	2414.95 ± 319.74 a, b	0.80 ± 0.07 a	286.69 ± 25.17 b	126.44 ± 4.21
2x5	3.89 ± 0.27	265.23 ± 21.02 a, d	3341.23 ± 425.73 a	0.71 ± 0.03 a	230.66 ± 13.38 a, b	134.84 ± 4.21
4x5	3.81 ± 0.33	331.54 ± 33.05 a	3504.61 ± 438.66 a	0.67 ± 0.05 a	240.80 ± 16.42 a, b	135.80 ± 5.26
8x5	3.49 ± 0.39	170.77 ± 18.11 b, c	2647.40 ± 337.23 a, b	0.75 ± 0.03 a	237.56 ± 19.68 a, b	137.71 ± 5.04
9x3	3.02 ± 0.29	146.38 ± 14.08 c	1587.79 ± 204.11 b	1.13 ± 0.09 b	240.77 ± 13.87 a, b	127.85 ± 4.69
9x5	3.04 ± 0.32	241.63 ± 17.65 b, d	2795.95 ± 641.54 a, b	0.66 ± 0.02 a	209.88 ± 13.34 a	124.43 ± 4.89

Table 4 Spearman correlation coefficients, for the relationship between RGR and timing of bud burst with several morphological traits in the poplar families (n= 16-24 trees per family). Asterisks indicate the level of significance $P<0.05$*, $P<0.01$**; $P<0.001$***. Abbreviations: number of leaves (L), leaf size (*Ls*), total leaf area (tLA), leaf area ratio (LAR), specific leaf area (SLA), leaf mass ratio (LMR), shoot mass ratio (SMR), root mass ratio (RMR), root-to-shoot ratio (R/S), specific root area (SRA) and stem volume index (SVI).

	L	*Ls*	tLA	LAR	SLA	LMR	SMR	RMR	R/S	SRA	SVI	BB
RGR	0.77	0.37	0.74	0.54	0.51	0.58	ns	-0.34	-0.26	ns	0.48	-0.19
	***	***	***	***	***	***		**	**		***	*
BB	-0.31	ns	-0.23	ns	-0.31	-0.17	-0.17	0.23	0.22	ns	-0.27	
	***		**		*	*	*	**	**		**	

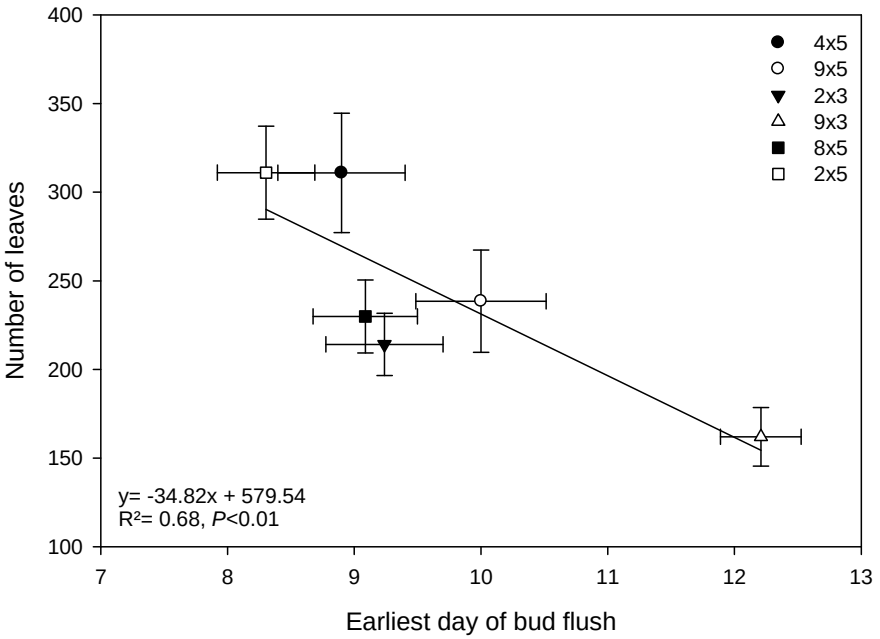

Figure 2 Relation between the onset of bud flushing (days after April 25th) and the total number of leaves produced in the 150-d experiment for the six aspen families (means and standard errors of 16-22 individuals per family).

The two main planes of the PCA with the traits leaf number, total leaf area, specific leaf area, root-to-shoot ratio, root mass ratio, stem volume index and timing of bud burst explained around 70% of the variability in all investigated traits between the six full-sib families (PC1: 50.6%, PC2: 18.1%) (Fig. 3a). Axis PC1 was mainly defined by the leaf and stem traits which were all strongly correlated with each other and the phenological timing of bud burst, whereas the root traits (RMR, R/S) captured PC1 and PC2 almost in equal parts.

If A_{max} was added, the scores along axis 1 remained similar but now axis 2 captured the variation in A_{max} (PC1: 44.6%, PC2: 17.9%) (Fig. 3b). Hence, there was no association between assimilation rate and the biomass-related parameters.

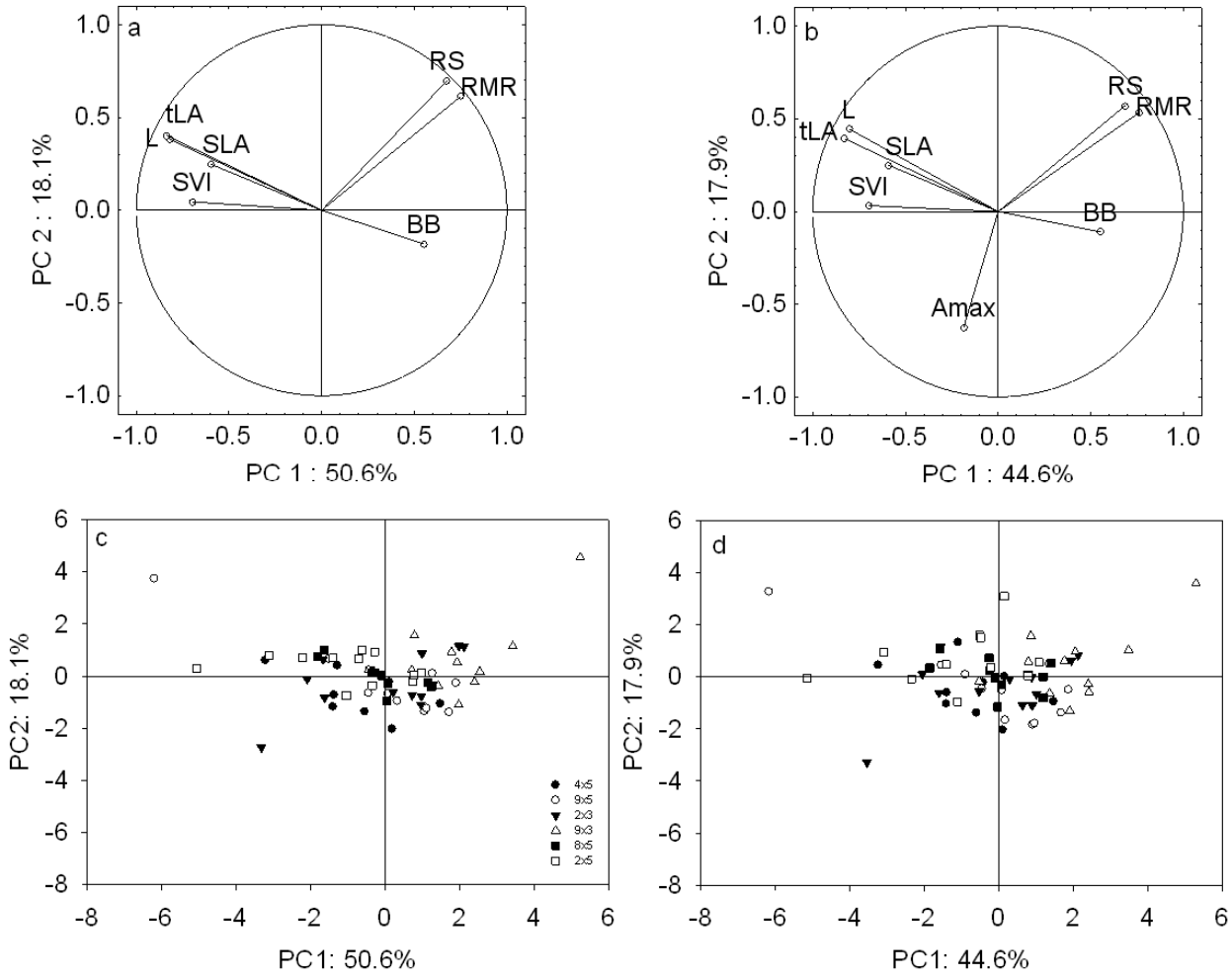

Figure 3 Distribution of the morphological traits (a) and the morphological traits in combination with the light-saturated net photosynthesis (A_{max}) (b) in the orthogonal plane (PC1x PC2) of a principal components analysis for the six studied full-sib families (c, d). L= number of leaves, tLA= total leaf area, SLA= specific leaf area, SVI= stem volume index, RMR= root mass ratio, R/S= root-to-shoot ratio, BB= timing of bud burst.

The location of the families in the ordination space (Fig. 3c, d) indicated that family 9x3 invested more into root biomass in relation to the total and above-ground biomass than the other five families, whereas families 4x5 and 2x5 tended to have higher biomass values for leaf and stem traits. The families 9x5 and 2x3 played an intermediate role. A subsequent ANCOVA confirmed these results.

Family 9x3 was the one with the lowest productivity, but it invested significantly more carbon in below-ground growth (R/S= 1.1, $P \leq 0.05$) which was reflected in a significantly higher root mass ratio than in the other families (Fig. 4). The root-to-shoot ratio varied between 0.66 and

0.80 among the families (Table 3), revealing no significant differences among the families except for family 9x3. This family exhibited also the lowest stem volume index (145 cm^3, difference to other families significant) and had the lowest number of leaves produced and the smallest leaf area development, with about five leaves and 300 cm^2 of leaf area produced per month on average. The families 4x5 and 2x5 reached stem volume indices of 265 to 330 cm^3, respectively, developed 25 new leaves $month^{-1}$ during the study period and showed a leaf area expansion of 700 cm^2 $month^{-1}$. In contrast, significant differences in specific root area (SRA) or specific leaf area (SLA) between the families did not exist, except for a significant difference in SRA between the families 9x5 and 2x3. Nevertheless, both parameters were relatively uniform among the families with means ranging from 210 to 290 cm^2 g^{-1} (SRA) and from 125 to 140 cm^2 g^{-1} (SLA) (Table 3).

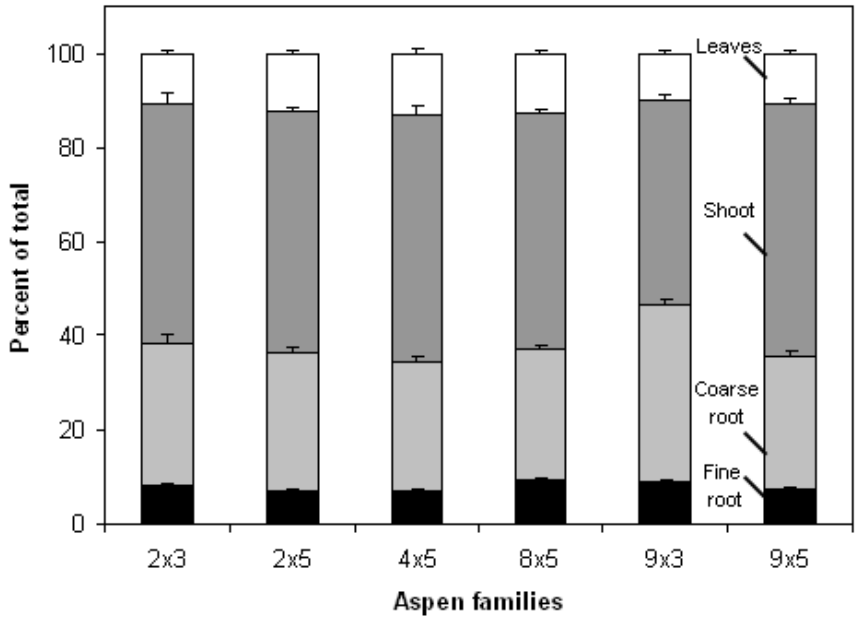

Figure 4 Partitioning of biomass to leaf, shoot, coarse root and fine root fractions in the six aspen families (means and standard errors of 20-24 individuals per family).

3.4 Influence of the genetic constitution on growth

According to the microsatellite analysis of the genomes, the largest genetic distance existed between the families 2x3, 8x5 and 9x5, while the highest genetic similarity was observed for the families 9x3 and 9x5 (Table 5). Half of the differences that were found in growth rate or morphological parameters between the families could, however, not be related to this genetic background, with the exception of the number of leaves, leaf size, leaf area ratio, the timing of bud flush, the plant mass ratios and root-to-shoot ratio. The genetic constitution (between-family variation) had no significant influence on any of the physiological parameters (Table

6). Therefore, we were able to calculate broad-sense heritability (H^2) for the variables leaf number (H^2= 0.24 ± 0.09), *L*s (H^2= 0.14 ± 0.07), LAR (H^2= 0.27 ± 0.09), LMR (H^2= 0.22 ± 0.09), SMR (H^2= 0.27 ± 0.09), RMR (H^2= 0.40 ± 0.10), R/S (H^2= 0.37 ± 0.10) and timing of bud flush (H^2= 0.48 ± 0.10) indicating that the genetic constitution explained 14% to 48% of the variation of the respective variables. Most of the morphological traits had rather high coefficients of variation for the between-family variation (CV_G: 4% up to 29.0%) but low CV_G values in the case of the physiological traits (0.71 - 4.3%). A similar difference was found for the coefficient of environmental variation (CV_E) between morphological (13 to 65%, mean: 32%) and physiological traits (2 to 35 %, mean: 16%) (Table 6). The remarkably small genetically-determined variation in the physiological traits is supported by the absence of a family effect in the covariance analysis.

Table 5 Matrix of genetic distances (% according to Nei [21]) between the six aspen families based on microsatellite analysis. For further details see the material and methods section.

Aspen families	2x3	2x5	4x5	8x5	9x3	9x5
2x3	0.0					
2x5	14.1	0.0				
4x5	13.2	3.9	0.0			
8x5	28.3	8.1	6.6	0.0		
9x3	6.3	16.0	12.7	22.0	0.0	
9x5	25.6	4.2	5.4	3.9	1.8	0.0

Table 6 Results of a covariance analysis (ANCOVA) on the effects of genetic constitution (family) on various morphological and physiological traits of the aspen families using plant height as a covariable. Given are the *F*-values (type III SS) and the coefficient of variation for the between-family (CV_G, genetic variation) and within-family (CV_ϵ, environmental variation) effects. Asterisks indicate a significant ($P<0.001$) influence of the family on the trait. Abbreviations: RGR= relative growth rate, L= number of leaves produced, *Ls*= leaf size, tLA= total leaf area, LAR= leaf area ratio, LMR= leaf mass ratio, SLA= specific leaf area, SVI= stem volume index, SMR= shoot mass ratio, RMR= root mass ratio, R/S= root-to-shoot ratio, BB= timing of bud burst, A_{max}= light-saturated net photosynthesis, CE= carboxylation efficiency, Q_e= apparent quantum yield, LCP= light compensation point, LSP= light saturation point, DR= leaf dark respiration, Ψ_{min}= midday leaf water potential, g_s= stomatal conductance at 1500 µmol photons m^{-2} s^{-1}, F_v/F_m= maximum quantum efficiency, Φ_{PSII} = effective quantum yield recorded at two light regimes (200, 1500 µmol m^{-2} s^{-1}).

	Family	CV_G (%)	CV_ϵ (%)		Family	CV_G (%)	CV_ϵ (%)
Trait	*F*-value			Trait	*F*-value		
RGR	1.39	9.77	39.39	A_{max}	0.48	0.00	19.62
L	3.48*	25.08	43.81	CE	0.41	0.00	32.12
Ls	2.40*	14.92	35.71	Q_e	0.97	2.30	11.69
tLA	2.03	23.03	65.55	LCP	0.10	0.00	22.06
LAR	4.07*	28.98	46.80	LSP	0.44	0.00	8.59
LMR	3.28*	15.61	29.23	DR	0.34	0.00	21.58
SLA	1.43	3.86	16.73	Ψ_{min}	1.22	4.34	22.43
SVI	1.55	7.37	26.13	g_s	0.38	0.00	34.29
SMR	4.01*	8.08	13.16	$F_v/F_{m\ predawn}$	1.59	0.71	4.13
RMR	6.23*	14.20	17.20	$F_v/F_{m\ midday}$	0.34	0.00	2.26
R/S	5.90*	26.70	34.61	Φ_{PSII} (200)	0.75	0.00	5.59
SRA	2.08	12.40	33.71	Φ_{PSII} (1500)	1.83	3.85	10.34
BB	8.55*	21.01	21.62				

4. Discussion

4.1 Intraspecific variation in poplar productivity and growth-related traits

The six poplar families derived from crossings of two father and four mother trees showed a remarkable uniformity in physiological traits related to photosynthesis and water relations. None of the 12 investigated parameters had coefficients of genetic variation (CV_G = variation among the 6 families / mean) greater than 4.3%, even though the six parent trees differed

considerably in their genetic constitution as evidenced by genetic distance analysis (13 - 28% relative distance between sib-families with no common father or mother). This indicates that the investigated physiological traits are either being inherited in a conservative manner, or that the very similar environment of the six parent trees in the past has promoted converging adaptive changes in the genes related to photosynthesis and water relations traits. In contrast, genetic variation was much higher in all morphological and phenological traits (CV_G: 4% - 29.0%). The largest genetically determined differences between the six families existed for traits related to leaf area, leaf phenology, and the relative size of the root and shoot system: leaf number, leaf size, leaf area ratio, the timing of bud burst, leaf, shoot and root mass ratio, and the root-to-shoot ratio were significantly influenced by family identity. Numerous studies have investigated the genetically determined variation in morphological and physiological traits in woody plant populations by analysing the variety of genotypes being present. These comparative investigations typically found a greater genetic variation in morphological than in physiological traits e.g. [27,28], which matches with our results.

4.2 Plant traits contributing to productivity

Leaf number per plant and the timing of bud burst were identified as key factors controlling the relative growth rate of the *P. tremula* families in our experiment. Surprisingly, leaf number was even more influential than total leaf area at the time of harvest. This is probably due to the fact that annual photosynthetic carbon gain is more closely related to leaf area duration (i.e. the product of mean leaf area and the length of the leafy period) than to leaf area itself with a strong influence of the duration of the assimilation period on annual C gain. Because the number of leaves produced during summer was related to the date of bud burst in spring, the timing of leaf flushing appeared as an influential factor for poplar productivity in the analysis. This is in agreement with Yu et al. [29] who reported a strong correlation between biomass production of *P. tremula* and the length of the growth period. According to Howe et al. [30], bud phenology of hybrid poplars is largely under genetic control. Therefore, these traits could be used for predicting the productivity of high-yielding species, as shown for *Salix* by Weih [31]. This is supported by the work of DeQuiang et al. [32] who were able to locate the genomic regions, that control leaf morphology and bud flush, using quantitative trait loci (QTL) analyses. Other leaf-related traits such as leaf area ratio, leaf mass ratio, leaf area index and specific leaf area have also been found to be reliable predictors of poplar productivity [33,34] mostly because they correlate closely with plant leaf area or leaf area duration. However, next to positive correlations with LAR, LMR and SLA, we also found a

negative correlation of RGR with root mass ratio. Consequently the family with the highest RMR (and highest R/S) and smallest values for leaf area-associated traits (family 9x3) exhibited the smallest RGR. This family combined all traits that were found to be associated with low productivity in this study, i.e. high RMR and R/S, small leaf number, low LAR and LMR and late onset of bud burst.

Both, a simple correlation analysis and a Principal Components Analysis revealed the insignificance of photosynthetic capacity (A_{max}) for RGR, thereby confirming earlier reports that productivity differences between poplar genotypes are best explained by morphological traits but not by photosynthetic characteristics [35]. Neither A_{max} nor quantum yield or carboxylation efficiency were suitable predictors of productivity. Similarly, Poorter and Remkes [36] and Garnier [37] demonstrated for non-woody plants that differences in RGR were most tightly related to differences in leaf morphology such as SLA, while the effect of photosynthetic capacity was insignificant. It appears that productivity differences between species of the same functional group, or between closely related species, and between genotypes of the same species are mostly caused by differences in morphological traits, while differences in physiological activity (including processes leading to biosynthesis of primary and secondary compounds) are mainly relevant when comparing different plant functional groups. Nevertheless, in many tree species, there are differences in trade-off traits, i.e. carbon allocation to defence compounds such as phenolics or antioxidants that may strongly affect productivity. Further, it should be kept in mind that the key predictors of high-yielding can differ between poplar families and may change with growth performance [38]. According to these authors, leaf area was a better indicator of productivity in poplar families with large leaves and higher leaf longevity, while leaf production rate was more useful in poplar plants with small leaves.

4.3 Genotypic vs. environmental control of yield

Our experiment with poplar families of defined genetic distance grown in a more or less uniform environment allowed us to distinguish between genetic and environmental effects on important growth-related plant traits. The genetic constitution had a significant influence on seven important traits associated with RGR (timing of bud burst, leaf number, leaf size, leaf area ratio, leaf and root mass ratio and the ratio between above and below-ground biomass); nevertheless, these properties were subject to a larger environmental (within-family) than genetic variation. Moreover, all other morphological and physiological traits investigated showed much higher CV_E than CV_G coefficients emphasizing the dominant environmental

control of productivity in *P. tremula* in our experiment. This is astonishing because the soil and climate conditions were highly uniform across all plots of the experiment. Further, this result seems to contradict other studies with poplar genotypes which indicate a moderate to strong genetic control of productivity [28,39]. We explain the rather small genetic influence on productivity in our experiment by the fact that the eight replicate plots per family contained genetically similar (same parent trees), but not identical, poplar individuals. A comparison of clonal individuals probably would have led to a greater influence of the genome on RGR. However, the detected genetic differences between any two families in our study did not correspond to the productivity differences observed between these families which may raise some doubt on the role of genetic constitution in controlling RGR. Moreover, our experiment was conducted under optimal light, soil moisture and nutrient conditions with significant stress periods lacking which may contrast with the field situation.

References

[1] Fenning TM, Gershenzon J. Where will the wood come from? Plantation forests and the role of biotechnology. Trends Biotechnol 2002;20:291-6.

[2] Hansen EA. Poplar woody biomass yields - A look to the future. Biomass and Bioenerg 1991;1:1-7.

[3] Heilman PE. Planted forests: poplars. New Forests;17:89-93.

[4] Makeschin F. Short rotation forestry in Central and Northern Europe - introduction and conclusions. Forest Ecol Manag 1999;121:1-7.

[5] Cianciaruso MV, Batalha MA, Gaston KJ, Petchey OL. Including intraspecific variability in functional diversity. Ecology 2009;90:81-9.

[6] Hughes AR, Stachowicz JJ. Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. Ecology 2009;90:1412-9.

[7] Loreau M. Linking biodiversity and ecosystems: towards a unifying ecological theory. Philos T R Soc B 2010;365:49-60.

[8] Tilman D, Wedin D, Knops J. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 1996;379:718-720.

[9] Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS et al. Impacts of plant diversity on biomass production increase through time because of species complementarity. P Natl Acad Sci USA 2007;104:18123-8.

[10] Kotowska AM, Cahill JF, Keddie BA. Plant genetic diversity yields increased plant productivity and herbivore performance. J Ecol 2010;98:237-245.

[11] Dickmann DI, Keathly DE. Linking physiology, molecular genetics and the *Populus* ideotype. In: Stettler RF, Bradshaw HD, Heilman PE, Hinckley TM, editors. Biology of *Populus* and its implications for management and conservation, Ottawa: NRC Research Press; 1996, p. 491-511.

[12] Marron N, Dillen SY, Ceulemans R. Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. Environ Exp Bot 2007;61:103-116.

[13] Crutsinger GM, Collins MD, Fordyce JA, Gompert Z, Nice CC, Sanders NJ. Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 2006;313:966-8.

[14] Stettler RF, Ceulemans R. Clonal material as a focus for genetic and physiological research in forest trees. In: Ahuja MR, Libby WJ, editors. Clonal Forestry. Genetics and biotechnology. New York: Springer Verlag; 1993, p.68-86.

[15] Dickmann DI, Kuzovkina J. Poplars and willows of the world, with emphasis on silviculturally important species. In: Poplars and willows in the world, International Poplar Commission Thematic Papers, 2008.

[16] Jelinski DE, Cheliak WM. Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. Am J Bot 1992;79:728-736.

[17] Tuskan GA, Francis KE, Russ SL, Romme WH, Turner MG. RAPD markers reveal diversity within and among clonal and seedling stands of aspen in Yellowstone National Park, USA. Can J For Res 1996;26:2088-2098.

[18] Van der Schoot J, Pospiskova M, Vosman B, Smulders MJM. Development and characterization of microsatellite markers in black poplar (*Populus nigra* L.). Theor Appl Genet 2000;101:317-322.

[19] Dayanandan S, Rajora OP, Bawa KS. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theor Appl Genet 1998;96:950-6.

[20] Rahman MH, Dayanandan S, Rajora OP. Microsatellite DNA markers in *Populus tremuloides*. Genome 2000;43:293-7.

[21] Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 1978;89:583-590.

[22] UPOV: International Union for the Protection of New Varieties of Plants. Guidelines for the conduct of tests for distinctness, homogeneity and stability, [TG/21/7], 1981.

[23] Pontailler JY, Ceulemans R, Guittet J, Mau F. Linear and non-linear functions of volume index to estimate woody biomass in high density young poplar stands. Ann Sci For 1997;54:335-345.

[24] Meir P, Levy PE, Grace J, Jarvis PG. Photosynthetic parameters from two contrasting woody vegetation types in West Africa. Plant Ecol 2007;192:277-287.

[25] Singh M, Ceccarelli S, Hamblin J. Estimation of heritability from varietal trials data. Theor Appl Genet 1993;86:437-441.

[26] Pliura A, Zhang SY, MacKay J, Bousquet J. Genotypic variation in wood density and growth traits of poplar hybrids at four clonal trials. Forest Ecol Manag 2007;238:92-106.

[27] Rowland DL. Diversity in physiological and morphological characteristics of four cottonwood (*Populus deltoides* var. *wislizenii*) populations in New Mexico: evidence for a genetic component of variation. Can J Forest Res 2001;31:845-853.

[28] Kanaga MK, Ryel RJ, Mock KE, Pfrender ME. Quantitative-genetic variation in morphological and physiological traits within a quaking aspen (*Populus tremuloides*) population. Can J Forest Res 2008;38:1690-4

[29] Yu Q, Pulkkinen P, Rautio M, Haapanen M, Alen R, Stener LG et al. Genetic control of wood physicochemical properties, growth, and phenology in hybrid aspen clones. Can J Forest Res 2001;31:1348-1356.

[30] Howe GT, Saruul P, Davis J, Chen THH. Quantitative genetics of bud phenology, frost damage, and winter survival in an F-2 family of hybrid poplars. Theor Appl Genet 2000;101:632-642.

[31] Weih M. Genetic and environmental variation in spring and autumn phenology of biomass willows (*Salix* spp.): effects on shoot growth and nitrogen economy. Tree Physiol 2009;29:1479-1490.

[32] DeQiang Z, ZhiYi Z, Kai Y, BaiLian L. QTL analysis of leaf morphology and spring bud flush in (*Populus tomentosa* × *P. bolleana*) × *P. tomentosa*. Sci Sil Sinica 2005;41:42-8.

[33] Barigah TS, Saugier B, Mousseau M, Guittet J, Ceulemans R. Photosynthesis, leaf-area and productivity of 5 poplar clones during their establishment year. Ann Sci For 1994;51:613-625.

[34] Marron N, Villar M, Dreyer E, Delay D, Boudouresque E, Petit J.M et al. Diversity of leaf traits related to productivity in 31 *Populus deltoides* x *Populus nigra* clones. Tree Physiol 2005;25:425-435.

[35] Bunn SM, Rae AM, Herbert CS, Taylor G. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. Forestry 2004;77:307-323.

[36] Poorter H, Remkes C. Leaf-area ratio and net assimilation rate of 24 wild-species differing in relative growth-rate. Oecologia 1990;83:553-9.

[37] Garnier E. Growth analysis of congeneric annual and perennial grass species. J Ecol 1992;80:665-675.

[38] Dillen SY, Marron N, Sabatti M, Ceulemans R, Bastien C. Relationships among productivity determinants in two hybrid poplar families grown during three years at two contrasting sites. Tree Physiol 2009;29:975-987

[39] Lojewski NR, Fischer DG, Bailey JK, Schweitzer JA, Whitham TG, Hart SC. Genetic basis of aboveground productivity in two native *Populus* species and their hybrids. Tree Physiol 2009;29:1133-1142.

.

Chapter 5

Different growth strategies determine the carbon gain and productivity of aspen collectives to be used in short-rotation plantations

(submitted)

Different growth strategies determine the carbon gain and productivity of aspen collectives to be used in short-rotation plantations

Annika Müller[1,3], Viviana Horna[1], Chunxia Zhang[2] and Christoph Leuschner[1]

[1]Plant Ecology, Albrecht von Haller Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, 37073 Göttingen

[2]Forest Genetics and Forest Tree Breeding, Büsgen Institute, University of Göttingen, Büsgenweg 2, 37077 Göttingen

[3]Corresponding author: amuelle3@gwdg.de, Tel.: +49551-3922088, Fax: +49551-395701

Abstract

Aspen (*Populus tremula*) is a favoured tree species in short-rotation forestry with a recognised large intraspecific variation in productivity. We compared the growth potential of 1-yr-old saplings of four Central European aspen collectives with different climate adaptation on a low-fertility site and searched for growth-determining physiological and morphological traits and their dependence on genetic constitution. Among the 35 investigated traits were photosynthetic capacity and mean assimilation rate, quantum yield and carboxylation efficiency, maximum rates of carboxylation and electron transport, leaf water potential maxima and minima, leaf phenology and the ratio of leaves lost to leaves produced (LP ratio), leaf size and total leaf area, plant height and axes length growth, and canopy carbon gain in the vegetation period as an estimate of total (above- and belowground) productivity. The collectives differed by more than 30% in cumulative carbon gain with a large genotype effect, while mean assimilation rate and most photosynthetic and water status traits showed a relatively small intraspecific variation with no significant influence on the variation in C gain. The timing of the beginning of net leaf loss (leaf abscission > leaf production) in early and mid August differed between the four collectives and resulted in different maximum leaf areas and LP ratios, which were identified as key factors controlling C gain. Mean assimilation rate, though not related to cumulative C gain, was positively correlated with the light, CO_2 and water-use-efficiencies of photosynthesis and with predawn leaf water potential, but not with the maximum rates of carboxylation and electron transport. We conclude that genotype selection for high-yielding aspen in short-rotation forestry at low-fertility sites should focus

on the parameters leaf phenology, LP ratio at the end of the growing season, and the resulting total leaf area as key traits.

Keywords: carbon gain, genotypic variation, growth performance, leaf phenology, photosynthesis, *Populus tremula*.

1. Introduction

Poplar (*Populus*) species are preferred plants in Europe and North America for short-rotation forestry to produce fibre and bioenergy for a growing demand [1-3]. Major advantages are high productivity, tolerance of a broad range of soil conditions, and only moderate to low sensitivity to abiotic and biotic stressors [4]. One possible species is *P. tremula* L., a wide-spread light-demanding pioneer tree in Eurasia and Africa with an impressive tolerance of acidic, dry, nutrient-poor and cold soils, which makes this species an attractive choice for short-rotation plantations on poor soils and in mountainous regions [5]. Various studies have shown that the capacity for high-yielding differs among the genotypes of *P. tremula* and of other *Populus* species [6,7], offering a considerable potential for plant breeding programmes in search for the ´optimal´ genotype or deme. Here, we report about a growth trial with four genetically differentiated collectives of *P. tremula* with different origin from Central Europe that were grown at montane elevation (about 500 m a.s.l.) on unfertilized moderately poor soil with the objective to identify yield differences among the collectives and to relate productivity to important morphological and physiological traits by means of growth analysis. The four collectives originated from locations in Central Europe that differed with respect to summer and winter temperatures and precipitation (sub-continental, summer-warm and semi-dry to sub-oceanic, summer-cool and moist climates) to represent a broad spectrum of adaptation histories in the plant material tested. The specific study aims were 1) to identify the collective with highest yield on this rather unfavourable soil, 2) to relate differences in canopy carbon gain and growth rate to each other, and 3) to quantify the heritability of the traits controlling productivity in order to assess the potential for yield increases through genotype selection and breeding programmes directed to optimize *P. tremula* plantations at low-productivity sites.

2. Materials and methods

2.1 Plant material

Sixty plants each of four European *P. tremula* collectives (Table 1) were propagated from seeds in March 2008. The seedlings were planted individually in 10 litre pots containing nutrient-rich soil and were grown for eight months until November when they were transplanted to the experimental field site.

2.2 Experimental design

The experiment was conducted in a low mountain range in Central Germany at lower montane elevation (Solling 51° 44′56′′N, 9° 32′28′′O at 485 m a.s.l.) at the Relliehausen experimental farm near Silberborn, about 60 km west of Göttingen. The area is characterized by a mean annual temperature of 6.9°C and 1030 mm annual precipitation.

In November 2008, the progenies of the four collectives were planted on a former grassland area on a relatively nutrient-poor acidic soil (haplic Cambisol). Each collective was planted in monoculture blocks with 1.50 m distance between the plants. Every block contained 30 plants of the same collective arranged in 6x5 rows with the blocks being replicated twice. To avoid edge effects, only the 12 central plants were used for the growth analysis and plant physiological measurements, while the 18 peripheral plants served as buffer zone.

2.3 Genetic analyses

Total genomic DNA was isolated from leaves sampled from the saplings of each collective with the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). The microsatellite primers WPMS14, WPMS16 [8], PTR2, PTR4 [9], PTR5, PTR6, PTR8, PTR14 [10,11] were used for PCR amplification. PCR reactions were carried out as described above with the exception that primers were labelled with the fluorescent dyes 6-carboxyfluorescein (6-FAM) or hexachloro-fluorescein phosphoramidite (HEX). The amplified products were resolved electrophoretically on an ABI Genetic Analyzer 3100 together with the internal size standard GenScan 500 ROX from Applified Biosystems. Nei's genetic distances [12] were calculated by the software GENALEX 6.2 [13].

2.4 Photosynthetic traits

After full leaf expansion in June 2009, leaf gas exchange was recorded monthly until August using a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). All measurements were made on fully expanded and sun-exposed leaves between 900 and 1700 h

solar time. Light-response curves were recorded for a single leaf of ten trees per collective. The levels of photosynthetically active radiation (PAR) were adjusted to 1500, 1000, 750, 500, 300, 200, 100, 50, 20, and 0 µmol photons m^{-2} s^{-1} using a red and blue LED light source supplied by the manufacturer. All measurements were conducted at constant CO_2 concentration (370 ppm), constant leaf temperature (25°C) and constant vapour pressure deficit (VPD = 1kPa). Light-saturated net photosynthesis rate (A_{max}), apparent quantum efficiency (Q_e), stomatal conductance (G_s) and transpiration rate (E) at 1500 µmol m^{-2} s^{-1} PAR, 25°C and VPD = 1kPa, and the photosynthetic water-use-efficiency (WUE = A_{max}/E) were calculated. Further, CO_2-response curves (50, 100, 150, 370, 600, 1200, 2000 µmol CO_2 mol air^{-1}) were established at 1500 µmol photons m^{-2} s^{-1} PAR and used to calculate carboxylation efficiency (CE), maximum carboxylation rate (V_{cmax}) and the maximum rate of electron transport (J_{max}). The data points from the light and CO_2-response curves were fitted with a non-rectangular hyperbola of the form $y = \frac{a \cdot bx}{(a + bx) - y0}$ following Meir et al. [14] and evaluated with the Software SigmaPlot (Systat Software Inc. Version 10.0, California, USA). J_{max} and V_{cmax} were calculated with the equations given by Bernacchi et al. [15] and Lenz et al. [16].

2.5 Leaf water potential

Leaf water potential (Ψ) was measured with a pressure chamber (PMS Instruments, Corvallis, OR) on sunny days before dawn between 330 and 500 h solar time and at midday between 1200 and 1330 h solar time on three occasions in June, July and August with the aim to record daily potential maxima (Ψ_{max}) and minima (Ψ_{min}), respectively. Single fully expanded and sun-exposed leaves of ten trees per collective were cut off and immediately measured. Table 2 gives the symbols and definitions of the physiological variables studied.

2.6 Leaf area development and stem increment

The measurements of leaf morphology and plant growth started in spring 2009 with initiation of bud break and ended at the end of August. Leaf flushing was monitored at frequent time intervals (every 1-3 days) between the time when the first buds started breaking and the date of complete leaf unfolding. The bud status of 10 plants per collective was analysed by using the bud scale given by the International Union for the Protection of New Varieties of Plants [17]. The time span between leaf flushing and complete leaf abscission in September was taken as the length of the growing period (VP) of a plant. Due to the fact that poplars are

forming new leaves continuously throughout the summer, the process of leaf area increment was investigated in detail in mid-summer (July and August) for 10 progenies per collective. Three to five buds per plant were marked and the length of the developing leaves was measured with a digital calliper every 1 to 2 days, until the leaves had attained their mature size and no further increment was recorded. Leaf area was estimated from the allometric relationship with leaf length established at a sample of 10 leaves per collective. Leaf area increment rate (LA_{inc}, $mm^2\ d^{-1}$) was calculated from the difference between the estimated final mature leaf size and the initial leaf size over the number of days needed to complete leaf expansion. Further, the change in total plant height of 10-11 plants per collective was recorded weekly. On the same plants, the total number of produced twigs was recorded and 6-9 twigs were marked for weekly measurement of twig length increment.

The 10-11 sample plants per collective were also used to monitor the total number of leaves (NL) at weekly intervals in order to analyse the seasonal development of leaf production. Based on detailed information about the number of leaves being present during the experiment, the number of leaves lost (NLL) was calculated as the difference between the maximum number of leaves (NL_{max}) and the leaf number at the end of August (NL_{min}). The date when NL_{max} was reached, was registered as t (NL_{max}). We calculated a leaf production rate by dividing NL_{max} by the time interval between leaf flushing and the date when t (NL_{max}) occurred, whereas leaf loss rate was calculated by relating NLL to the time span between t (NL_{max}) and the end of the experiment. From the leaf production and leaf loss rate we calculated the ratio of leaves lost to leaves produced per day (LP ratio, unit: d^{-1}). Values of the LP ratio > 1 indicate that more leaves were lost than were produced per time, values < 1 stand for the opposite.

Every month, leaf samples of 10 plants per collective were individually harvested and scanned with a flat bed scanner to determine the mean leaf size (LS) of a collective. The leaf silhouettes were analysed with the software WinFolia (Régent Instruments Inc., Quebec, Canada). After scanning, the fresh leaf samples were dried at 70°C for at least 48 h. Specific leaf area (SLA in $cm^2\ g^{-1}$) was calculated from leaf size (cm^2) and the corresponding leaf dry weight (g). The total leaf area (TLA) of a plant was calculated every month from the total number of leaves present and mean leaf size. Subsequently, mean (TLA_{mean}) and maximum total leaf area (TLA_{max}) per plant and collective were calculated.

2.7 Relative growth rate and carbon gain

Tree harvests for obtaining mass-based biomass data were not possible in our experiment, because this study is part of a long-term investigation, which will continue for several years. Therefore, we used two indirect approaches (canopy carbon gain and axes length increment) to approximate the relative growth rate and productivity of the four poplar collectives. Canopy carbon gain was derived from the photosynthetic light-response curves, daily mean values of photosynthetically active radiation, a plant's total leaf area and the length of the growing season, providing estimates of the amount of C fixed per plant per vegetation period minus leaf respiration during the daylight hours. Canopy C gain may be a better measure of plant productivity than aboveground biomass increase because this variable does not neglect belowground production as does the latter. The second approach based on the length increment of all axes (stem and twigs) to calculate the relative growth rate (RGR) of the plants during the growing season and to relate it to estimated canopy carbon gain. Our approach with two independent non-destructive growth determination methods appeared to be justified because we were interested in relative, rather than absolute, values of growth and productivity of the four collectives, and the growth parameters measured were also part of the growth analysis. Table 2 gives the symbols and definitions of the leaf-area and growth-related variables studied.

2.8 Statistical analyses

Statistical analyses were performed with the Statistical Analysis System (SAS Version 9.1, SAS Institute Inc., Cary, NC, USA). All data were tested for normal distribution using the Shapiro and Wilk´s test. In case of non-normality, the data were log-transformed to meet the requirements of parametric tests. Spearman rank correlation analysis was performed with carbon gain and assimilation rate as dependent variables. In case of significant correlation ($P \leq 0.05$), the traits were used for a multiple regression analysis with backward variable selection. ANOVAs (general linear models, proc glm) were employed to assess the impact of collective origin (genetic effect) on the studied phenotypic traits. To reveal significant differences in the analysed trait means between the collectives, a Tukey *post hoc* test ($P \leq 0.05$) was applied. When there was a significant effect of collective type on a trait, we calculated the broad-sense heritability (H^2) for that trait. H^2 was calculated from the genetic variance (between-collective variation) component (σ^2_G) related to the total phenotypic variance ($\sigma^2_p = \sigma^2_G + \sigma^2_e$). σ^2_p comprises the genetic variance and the residual variance (within-collective variation) component (σ^2_e) of a trait. Standard errors of H^2 were obtained from the equation

SE (H^2) = (1-H^2) [1+ (b-1) H^2] [2/ (bf)] $^{1/2}$ (f = degree of freedom of error term, b-1 = degree of freedom of the block term) according to Singh et al. [18]. The coefficient of genetic variation (CV_G) was calculated from the genetic variance component and the trait mean: $CV_G = \frac{\sqrt{\sigma^2_G} \times 100}{mean}$. Based on this expression, the coefficient of phenotypic and residual (environmental) variation was calculated with the respective phenotypic and residual variance component: $CV_p = \frac{\sqrt{\sigma^2_p} \times 100}{mean}$ and $CV_e = \frac{\sqrt{\sigma^2_e} \times 100}{mean}$ [19]. When the mean square of the residual component exceeded the mean square of the collective component, CV_G was set to zero.

Table 1 Acronym and origin of the four studied *P. tremula* collectives.

Collective (acronym)	Place of origin	Climate at place of origin	Mean annual temperature and precipitation	Source of plant material
Germany 1 (Gm_1)	Göttingen-Geismar, Germany (51° 14´N, 10° 10´O, 150 m a.s.l.)	mild winters and moderately warm summers	9.0 °C, 628 mm	Dept. of Forest Botany and Tree Physiology, Göttingen, Germany
Germany 2 (Gm_2)	Göttingen-Geismar, Germany (51° 14´N, 10° 10´O, 150 m a.s.l.)	mild winters and moderately warm summers	9.0 °C, 628 mm	Dept. of Forest Botany and Tree Physiology, Göttingen, Germany
Switzerland (CH)	Birmensdorf, Switzerland (47° 21´N, 8° 26´O, 518 m a.s.l.)	moderately cold winters and moderately warm summers	7.9 °C, 1123 mm	Swiss Federal Institute for Forest, Snow and Landscape Research, Switzerland
Austria (AU)	Vienna woods, Austria (48° 5´ N, 15° 55´O, 893 m a.s.l.)	moderately cold winters and warm summers	9.1 °C, 687 mm	Austrian group for Federal Research and Training centre for Forests, Natural Hazards and Landscape

Table 2 Traits associated with the foliage, growth and physiology of the poplar plants (symbols, units, definitions).

Symbols	Unit	Definition
Leaf area-related traits		
Number of leaves (NL)		Total leaf number per plant
Mean number of leaves (NL_{mean})		Mean number of leaves during the experiment
Maximum number of leaves (NL_{max})		Maximum number of leaves reached during the experiment
Minimum number of leaves (NL_{min})		Number of leaves at the end of the experiment
t (NL_{max})		Date when NL_{max} was reached
Leaves lost (NLL)		NL_{max} - NL_{min}
Leaf production rate ($NL_{max}/\Delta t$)	d^{-1}	NL_{max}/ time span between leaf flushing and t (NL_{max})
Leaf loss rate ($NLL/\Delta t$)	d^{-1}	NLL/ time span between t (NL_{max}) and the end of the experiment
Ratio of leaves lost to leaves produced (LP ratio)	d^{-1}	$(NLL/\Delta t)/ (NL_{max}/\Delta t)$
Leaf size (LS)	cm^2	
Mean leaf size (LS_{mean})	cm^2	Mean leaf size per plant
Total leaf area (TLA)	cm^2	LS* NL
Mean total leaf area (TLA_{mean})	cm^2	Mean TLA during the experiment
Maximum total leaf area (TLA_{max})	cm^2	Maximum TLA present during the experiment
Leaf area increment rate (LA_{inc})	$mm^2\ d^{-1}$	Leaf area increment per leaf and day
Specific leaf area (SLA)	$cm^2\ g^{-1}$	LS/ leaf dry weight
Growth-related traits		
Plant height increment (H)	cm	Plant height increment between May and the end of the experiment
Final number of twigs (NT)		Number of lateral twigs at the end of the experiment
Twig increment (T)	cm	Twig length increment between May and the end of the experiment (average over all marked twigs)
Relative growth rate, based on axes increment (RGR)	$cm\ cm^{-1}\ month^{-1}$	(H+T*TN)/ (initial plant height + initial twig length*NT)/ no. of months
t (LF)		Time of leaf flushing
t (LD)		Time of complete leaf abscission
Length of the vegetation period (VP)	d	Time span between t (LF) and t (LD)
Assimilation rate (A)	$g\ C\ m^{-2}\ d^{-1}$	Cumulative photosynthetic carbon gain per square meter leaf area and day*
Carbon gain per plant and vegetation period	$g\ C\ plant^{-1}\ VP^{-1}$	A*TLA*VP
Physiological traits		
Light-saturated net photosynthesis (A_{max})	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	Photosynthesis rate recorded at 1500 $\mu mol\ m^{-2}\ s^{-1}$ PAR, 25°C and VPD = 1kPA
Transpiration (E)	$mmol\ H_2O\ m^{-2}\ s^{-1}$	Leaf transpiration recorded at 1500 $\mu mol\ m^{-2}\ s^{-1}$ PAR, 25°C and VPD = 1kPA
Stomatal conductance (G_s)	$mol\ H_2O\ m^{-2}\ s^{-1}$	Stomatal conductance recorded at 1500 $\mu mol\ m^{-2}\ s^{-1}$ PAR, 25°C and VPD = 1kPA
Apparent quantum efficiency (Q_e)	$\mu mol\ CO_2\ \mu mol\ photons^{-1}$	
Carboxylation efficiency (CE)	$mol\ air\ m^{-2}\ s^{-1}$	
Leaf water potential (ψ_{max})	MPa	Leaf water potential recorded predawn
Leaf water potential (ψ_{min})	MPa	Leaf water potential recorded at midday
Water-use-efficiency (WUE)	$\mu mol\ CO_2\ mmol\ H_2O^{-1}$	A_{max}/E
Maximum rate of electron transport (J_{max})	$\mu mol\ electrons\ m^{-2}\ s^{-1}$	
Maximum rate of carboxylation (V_{cmax})	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	

* Minus leaf respiration during the daylight hours

3. Results

According to the nuclear microsatellite analysis, the four investigated aspen collectives are characterized by moderately high genetic distances to each other with Nei´s [12] genetic distance values ranging between 17% (Gm_1 vs. AU) and 40% (Gm_2 vs. CH) (Table 3).
Despite these genetic distances, the contribution of genetic constitution to trait variation was relatively small: a significant effect of collective origin was visible only in 8 of 23 investigated morphological and physiological traits including cumulative canopy carbon gain (Table 4), which was estimated from daily means of photosynthetically active radiation, light-response curves of photosynthesis, leaf area in its seasonal variation, and the length of the leafy period in each collective. With > 140 g of C assimilated per plant, the collectives AU and Gm_1 achieved a significantly higher cumulative C gain than the plants of Gm_2 and CH (Table 4). C gain showed a relatively high total (phenotypic) variation (CV_p: 63%) among the 40 plants of the four collectives tested. The influence of the collective was significant for this trait with a broad-sense heritability H^2 of 0.7. The component traits, on which canopy C gain depended (vegetation period length VP, leaf area, photosynthetic rate A at variable irradiances), showed a significant effect of the collective only in the case of leaf area (H^2 = 0.64 or 0.65 for mean or maximum plant leaf area), while VP and A were very similar between the four collectives (no significant collective effect, coefficient of genetic variation CV_G = 2 and 5%, Table 4).

Table 3 Matrix of genetic distances (% according to Nei [12]) between the four aspen collectives based on microsatellite analysis. For further details see the material and methods section.

Aspen collective	Gm_1	Gm_2	CH	AU
Gm_1	0.0			
Gm_2	22.9	0.0		
CH	25.7	41.6	0.0	
AU	17.0	17.6	18.4	0.0

Only the collective Gm_2 had a significantly smaller mean assimilation rate, but the difference to the collective with highest A (CH) was only 11% (Table 4). Hence, differences among the collectives in mean and maximum leaf area were responsible for the differences in carbon gain, and not differences in assimilation rate or the length of the leafy period. The leaf area differences were either caused by a particularly low mean leaf size (as in the collective Gm_2, Fig. 1a) or by differences in the number of leaves present (as in the collective CH, Fig. 2).

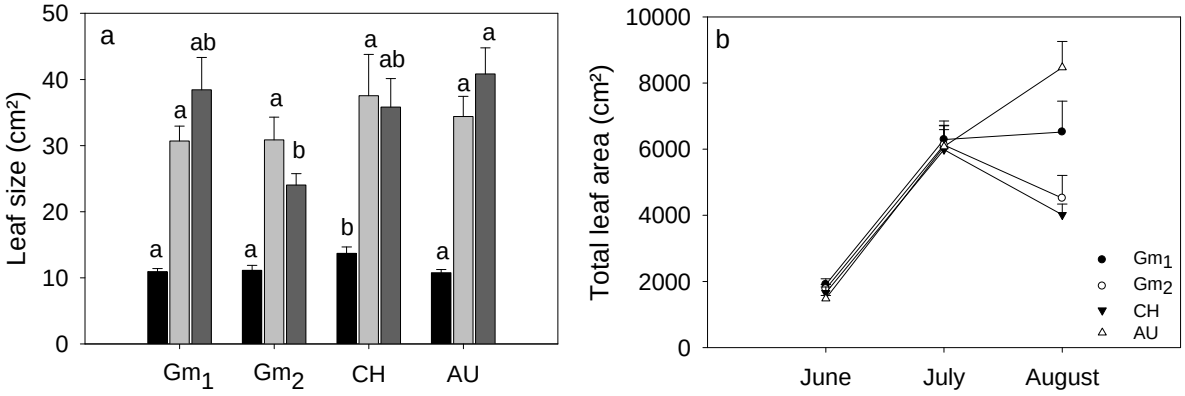

Figure 1 Mean leaf size (a) per plant of the four studied aspen collectives in June (black bars), July (light-grey bars) and August (dark-grey bars). Different letters indicate significant differences between the collectives for each month at $P<0.05$. The seasonal development of total leaf area is shown in Figure (b). The calculation of total leaf area was based on the monthly measurement of leaf size and the respective total number of leaves present at the same time (means and standard errors of 10-11 plants per collective).

In fact, leaf dynamics had a marked influence on canopy carbon gain: we observed a continuous increase in the number of leaves present until the end of July in all collectives, while leaf loss rates increased in August. The correlation analysis showed a highly significant positive influence of leaf production rate from May to August (positive effect) on canopy C gain and a negative effect of the ratio of leaves lost to leaves produced (LP ratio) (Table 5). While the collective CH showed a marked drop in the number of leaves present in August, Gm_1 and Gm_2 decreased their leaf numbers only slightly and AU revealed no net loss of leaves in this late summer period. Thus, the four collectives differed by about 2 weeks with respect to the timing of the seasonal culmination of total leaf number and the start of leaf net losses. Maximum leaf number was reached on average on August 3-5 (t (NL_{max})) in the CH collective, but on August 14 in Gm_1 and Gm_2, while AU maintained its peak leaf number for an extended period from August 14 till the end of the month (Fig. 2).

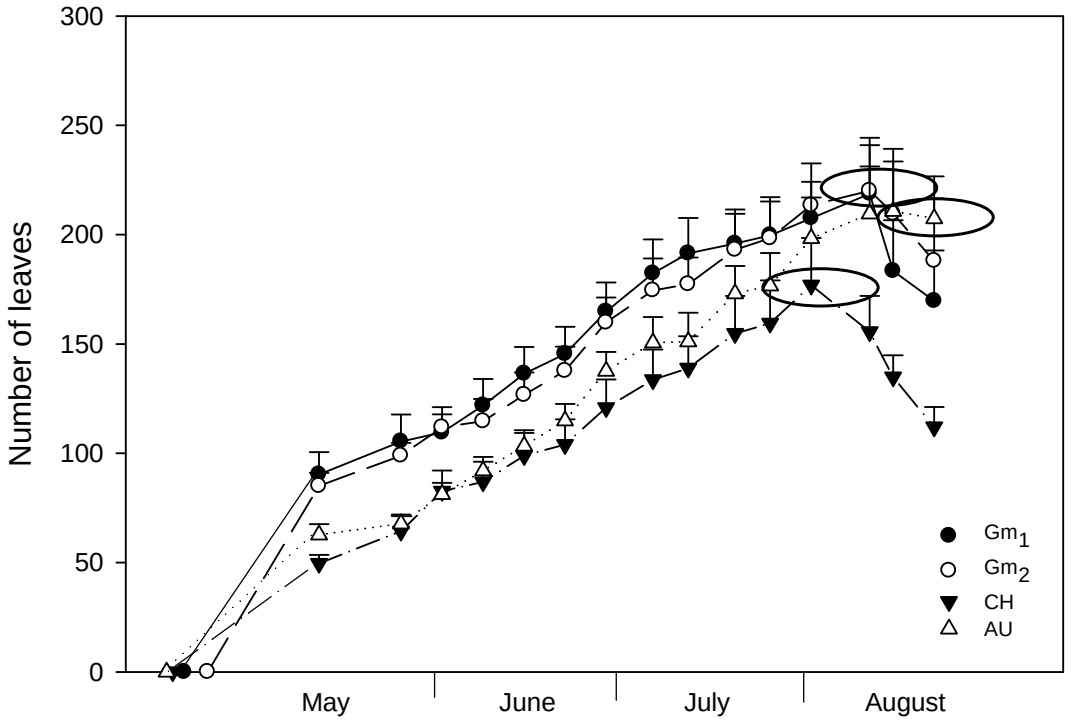

Figure 2 Seasonal changes in the mean leaf number per plant from May to August (means and standard errors of 10-11 trees). The ellipses indicate the time intervals when maximum leaf number was reached in the CH collective (left ellipse), in the two German collectives (middle ellipse) and in the AU collective (right ellipse). The first dots of the four curves indicate the mean time of leaf flushing in April (Gm_1: April 19, Gm_2: April 23, CH: April 17, AU: April 16).

The later t (NL_{max}) occurred in the season, the less leaves were lost in the subsequent early leaf abscission phase, the higher was the total leaf area in August, and the larger was canopy carbon gain in the growing season. From the leaf phenological data in Fig. 2, it is obvious that contrasts in the LP ratio in August are determining total leaf area and thus cumulative canopy carbon gain in the four collectives. Only plants with a low LP ratio in mid-summer were able to maintain, or even increase, their leaf area in August (as visible in many plants of the collective AU und Gm_1, Fig. 1b), while a high LP ratio resulted in a rapid decline of leaf area (as in CH and Gm_2, Fig. 3). The time of leaf flushing was in plants of the Gm_2 collective significantly later than in plants of the other collectives (Fig. 2), but was not related to canopy carbon gain or leaf area-related traits.

Table 4 Means and standard errors of various leaf area-, growth- and physiology-related traits (n = 10-30 plants per collective) of the four studied aspen collectives. Different small letters indicate significant differences between the collectives for a given trait at $P<0.05$. If no small letters are present, the respective trait means did not differ significantly among collectives. The asterisks indicate a significant impact of the collective origin on the related trait at $P<0.05$. If a significant impact of collective origin existed, we calculated broad-sense heritability (H^2). In addition, the coefficient of genetic variation (CV_G: between-collective variation), environmental variation (CV_e: within-collective variation) and phenotypic variation (CV_p: total variation) are presented for every trait.

Traits	Gm_1	Gm_2	CH	AU	P $H^2 \pm SE$	CV_G (%)	CV_e (%)	CV_p (%)
Leaf area-related traits								
Max. leaf number	224.2 ± 18.1	235.0 ± 24.4	182.7 ± 20.9	218.0 ± 21.0		24.7	30.1	54.8
Leaf production rate (d^{-1})	1.5 ± 0.1	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.2		11.5	35.0	46.5
Ratio of leaves lost to leaves produced (d^{-1})	1.8 ± 0.4 a	1.6 ± 0.4 a, b	2.5 ± 0.5 a	0.7 ± 0.3 b	* 0.49 ± 0.15	79.9	81.2	161.1
Mean leaf size (cm^2)	32.1 ± 3.9	24.5 ± 1.9	32.8 ± 3.4	35.0 ± 3.1		14.6	32.2	46.8
Mean total leaf area (cm^2)	4905.7 ± 487.9	4140.1 ± 432.4	3884.6 ± 382.2	5342.2 ± 451.5	* 0.64 ± 0.12	39.0	29.2	68.2
Max. total leaf area (cm^2)	7286.6 ± 692.9	6211.8 ± 580.4	6042.3 ± 723.6	8469.2 ± 786.9	* 0.65 ± 0.12	40.3	29.2	69.5
Leaf area increment rate ($mm^2\ d^{-1}$)	4.3 ± 0.5	6.2 ± 0.5	4.8 ± 0.4	5.9 ± 0.8		0.00	19.9	19.9
Specific leaf area ($cm^2\ g^{-1}$)	151.1 ± 3.4	155.1 ± 4.4	148.5 ± 5.1	144.7 ± 3.2		10.9	15.0	25.9
Growth-related traits								
Height increment (cm)	63.3 ± 4.3 a, b	60.8 ± 4.1 a	76.6 ± 4.0 a, b	82.8 ± 4.9 b		0.00	28.9	28.9
Twig number	14.0 ± 1.2 a	14.1 ± 0.9 a	9.2 ± 0.8 b	12.2 ± 0.7 a, b	* 0.47 ± 0.15	23.6	24.7	48.4
Twig increment (cm)	29.6 ± 3.1	27.2 ± 3.4	37.7 ± 3.4	32.7 ± 3.5		0.00	32.9	32.9
Relative axes growth rate (cm cm^{-1} $month^{-1}$)	3.4 ± 0.4	4.1 ± 0.7	2.8 ± 0.3	3.6 ± 0.4		0.00	45.1	45.1
Length of vegetation period (days)	151.4 ± 1.8 a	145.4 ± 1.2 b	151.1 ± 1.5 a	155.3 ± 1.3 a		2.0	3.1	5.1
Mean assimilation rate (g C $m^{-2}\ d^{-1}$)	1.91± 0.04 a	1.75 ± 0.03b	1.95 ± 0.03 a	1.82 ± 0.03 a, b		5.4	7.9	13.3
Canopy carbon gain (g C $plant^{-1}\ VP^{-1}$)	141.8 ± 13.2 a	102.3 ± 7.3 b	104.9 ± 6.2 b	149.0 ± 6.4 a	* 0.69 ± 0.11	38.2	25.2	63.4
Physiological traits								
Stomatal conductance (mol $H_2O\ m^{-2}\ s^{-1}$)	0.38 ± 0.02	0.33 ± 0.01	0.38 ± 0.02	0.31 ± 0.01		7.4	20.4	27.8
Apparent quantum yield (µmol CO_2 µmol $photons^{-1}$)	0.080 ± 0.001	0.073 ± 0.002	0.077 ± 0.002	0.077 ± 0.002		0.00	12.2	12.2
Carboxylation efficiency (mol air $m^{-2}\ s^{-1}$)	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	* 0.62 ± 0.12	24.7	19.4	44.1
Leaf water potential, predawn (MPa)	-0.14 ± 0.02	-0.10 ± 0.01	-0.13 ± 0.02	-0.12 ± 0.01		0.00	41.0	41.0
Leaf water potential, midday (MPa)	-1.02 ± 0.06	-0.84 ± 0.05	-0.90 ± 0.04	-0.96 ± 0.04		0.00	23.4	23.4
Water-use-efficiency (µmol CO_2 mmol H_2O^{-1})	3.47 ± 0.13	3.76 ± 0.13	3.39 ± 0.11	3.62 ± 0.12		0.00	16.7	16.7
Max. rate of electron transport (µmol electrons $m^{-2}\ s^{-1}$)	145.6 ± 5.0	146.2 ± 6.1	148.0 ± 5.3	150.6 ± 4.9	* 0.51± 0.13	14.8	14.4	29.2
Max. rate of carboxylation (µmol $CO_2\ m^{-2}\ s^{-1}$)	41.1 ± 1.3	41.4 ± 1.6	41.9 ± 1.3	42.6 ± 1.2	* 0.54 ± 0.13	13.8	12.8	26.6

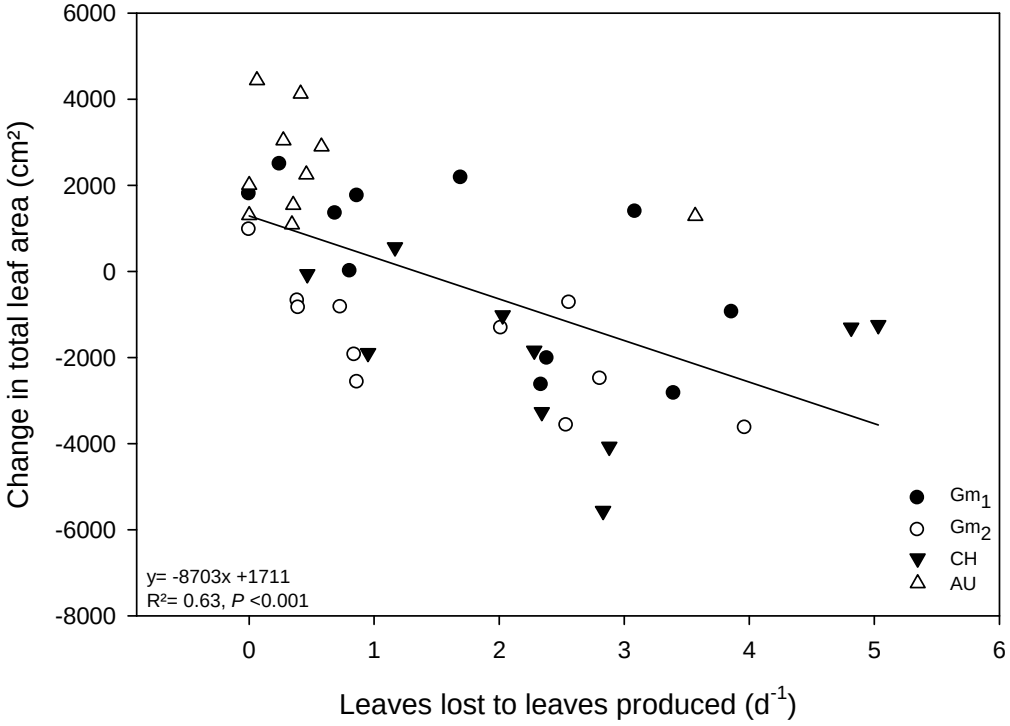

Figure 3 Increase (positive values) or decrease (negative values) in total leaf area between July 1 and August 25 in relation to the ratio of leaves lost to leaves produced (LP ratio) per day in plants of the four collectives (i.e. 1 = per daily produced leaf is one leaf lost, the leaf production rate is than equal to the leaf loss rate) (n = 10-11 plants per collective). Only plants and collectives with a low LP ratio were able to increase their total leaf area in mid-summer.

We found a fairly close correlation between our estimates of canopy carbon gain in the vegetation period and several simple morphological growth-related parameters, including the relative growth rate of axes length, plant height and twig length growth, and the number of side branches (Table 5). The results of a multiple regression analysis (backward variable selection) with carbon gain as dependent trait and the significantly correlated traits as explanatory variables showed that plant height increment (partial R^2: 0.04), twig number (partial R^2: 0.33), twig length increment (partial R^2: 0.12) and the LP ratio (partial R^2: 0.08) explained in combination almost 60% of the variation in canopy carbon gain across the 40 trees (Table 5). However, these proxies of plant productivity were not accurate enough to indicate significant differences in growth between the four collectives (except for the number of side branches, Table 4).

In contrast to the morphological traits, the bulk of traits characterizing photosynthetic efficiency or leaf water status were not related to canopy carbon gain, including J_{max}, V_{cmax}, WUE, CE and G_s.

Table 5 Spearman correlation coefficients (R) for the relationship between calculated canopy carbon gain and assimilation rate with several morphological and physiological traits in the sample of four poplar collectives (n = 10-11 plants per collective). Asterisks indicate the level of significance $P<0.05$*, $P<0.01$**, $P<0.001$***. Relative axes growth rate is relative stem and twig length increment.
Traits which revealed a significant correlation were used for a multiple regression analysis with backward variable selection and either with carbon gain or assimilation rate as dependent trait. The values in parentheses represent the partial and significant ($P<0.05$) coefficients of determination (R^2) of the multiple regression analysis.

	Canopy carbon gain	Assimilation rate
Relative axes growth rate	0.34*	ns
Height increment	0.45 * (0.04)	ns
Twig number	0.58 *** (0.33)	ns
Twig increment	0.42 * (0.12)	ns
Leaf production rate	0.67 ***	ns
Ratio of leaves lost to leaves produced	-0.41 * (0.08)	ns
Leaf area increment rate	ns	ns
Specific leaf area	ns	ns
Date of leaf flushing	ns	ns
Stomatal conductance	ns	0.52 *** (0.26)
Apparent quantum yield	0.31 *	0.34 **
Carboxylation efficiency	ns	0.59 *** (0.04)
Leaf water potential (predawn)	ns	-0.47 * (0.04)
Leaf water potential (midday)	ns	ns
Water-use-efficiency	ns	-0.34** (0.17)
Max. rate of electron transport	ns	ns
Max. rate of carboxylation	ns	ns

However, G_s, Q_e, CE, Ψ_{max} and WUE exhibited a significant positive correlation with the mean assimilation rate A (Table 5) and a multiple regression analysis with backward variable selection revealed G_s (partial R^2: 0.26), CE (partial R^2: 0.04), Ψ_{max} (partial R^2: 0.04) and WUE (partial R^2: 0.17) as the most important physiological factors influencing A; these variables explained 50% of the variation in mean assimilation rate. Accordingly, the use efficiencies of radiation, CO_2 and water were closer related to mean assimilation rate across the 40 trees than the maximum rates of electron transport and carboxylation. For only three of the analysed physiological traits (J_{max}, V_{cmax}, CE), we could prove significant differences among the collectives. The rather small influence of genetic constitution is also mirrored in the generally low values of CV_G in the physiological traits. In contrast, high degrees of genetic variation were detected for total leaf area and the LP ratio (Table 4), which relates to the prominent influence of these traits on canopy C gain.

4. Discussion

By monitoring important growth-related traits (seasonal leaf area variation, ratio of leaves lost to leaves produced, total leaf number, leaf size, twig length growth and plant height) and estimating cumulative canopy carbon gain in the vegetation period from photosynthesis and light measurements, we detected significant differences in productivity among the four aspen collectives with the most productive collective (AU) having a more than 30% larger C gain than the least productive ones (Gm_2 and CH). This finding is in accordance with many other reports about a strong influence of genetic constitution on the productivity of *Populus* species [20,21]. Our studied plant material is characterized by genetic distances between the collectives in the rage of 20% to 40%. Aspen collectives from neighbouring localities (Gm_1 and Gm_2) were genetically not closer related than collectives from more distant seed origins (Nei´s genetic distance: 23 vs. 17%), what is concurring with the fact that the growth performance of the four collectives was also quite variable but this variation could not be related to differences in genetic distance.

However, we were able to relate the differences in growth performance in a uniform environment to different genetically determined growth strategies in terms of foliage structure and leaf dynamics, and the height and length growth of stem and branches. The collective CH with little C gain showed by far the greatest length growth of its side branches and a remarkably strong height growth of the apical shoot while it possessed the smallest total number of leaves and the smallest total leaf area of the four collectives. A different carbon allocation strategy existed in Gm_2 with many, yet short side branches, poor height growth, but a very high number of small leaves. Though contrasting in many aspects, the growth strategies inherent to the collectives CH and Gm_2 were similar in leading to a relatively small plant leaf area and thus a rather small canopy carbon gain as compared to the two other collectives Gm_1 and AU. Crucial traits that lead to a small leaf area are either a small mean leaf size (Gm_2) or alternatively a reduced number of leaves (CH). The collectives with high canopy C gain were productive due to a large average leaf size and a fairly high number of leaves, but this resulted not necessarily in rapid height growth as visible in Gm_1. However, not only mean or maximum leaf area in the vegetation period were relevant for carbon gain, but the phenological development of leaf unfolding and leaf abscission as well, in particular in mid-to late summer (August) when the four collectives showed largely different patterns of leaf loss in the course of the first phase of autumn leaf fall. In conclusion, differences in leaf number, average leaf size and leaf longevity were identified as the key determinants of

canopy carbon gain, while mean assimilation rate or height growth and branching patterns were of minor importance in these one-yr-old *P. tremula* plants.

Extended growing season length has also been found in other studies to be a key factor increasing the productivity of *P. tremula* [6] and of other species used in short-rotation forestry such as *Salix* spp. [22]. In our experiment, the length of the leafy period differed by not more than 10 days between the collectives (i.e. by about 6%) which cannot explain the more than 30% difference in cumulative C gain. In fact, our detailed monitoring of leaf production and leaf loss showed that the absolute length of the period between leaf flushing and the time when complete leaf abscission was observed may be less important than the timing of the first phase of leaf abscission in mid-summer and early autumn in the different collectives. This trait seems to be mostly under genetic control as indicated by the high indices of heritability (H^2: 0.49).

The mean assimilation rate per day of the four collectives showed a significant influence of the resource use efficiencies (radiation, CO_2, water) in photosynthesis while the maximum capacities of the light and dark reactions of photosynthesis were not important. This is interpreted as a consequence of the fact that the photosynthesis of trees in humid Central Europe operates most of the time at irradiances below light saturation [23] where the performance under optimal conditions is less relevant than the carbon gain under resource-limited conditions. The impact of genetic constitution on photosynthetic variables was only significant for traits associated with carboxylation (CE and V_{cmax}) and electron transport. However, they were not identified as explanatory variables for the variation in canopy carbon gain and thus are unsuitable as predictors of productivity in aspen. This leads to the important conclusion that the genetic variation in morphological traits was much higher than that in physiological traits. Several other studies that compared the genotypic variation of morphological and physiological traits of poplar also evidenced the greater variability of the former [24,25].

In a growth trial (pot experiment) with six *P. tremula* populations, that were – in contrast to the recent study – closely related full-sib families, we identified different leaf numbers and different dates of leaf flushing as main causes of higher or lower productivity [26]. Both traits were found to be partly determined by the genetic constitution. In the current experiment, the time of leaf flushing could not be related with the genetic constitution, what might be due to the fact that the measuring intervals chosen were too long to detect differences in leaf flushing among the collectives. It may also be that the rough climate condition at the montane field site has reduced the impact of genetic constitution on leaf flushing. Nevertheless, the comparison

of the two growth experiments with either closely related (full-sibs) or genetically distant aspen collectives (this study) shows that leaf phenology and leaf and foliage morphology are strongly under genetic control in *P. tremula* irrespective of the variation in environment represented in the plant material. On the other hand, Müller et al. [26] found no relationship between the sib-family differences in growth-relevant leaf traits or productivity and the genetic distance between the families, which makes the identification of high-yielding aspen genotypes more complicated.

Conclusion

The four aspen collectives with adaptation histories to a rather broad range of temperate sub-oceanic to sub-continental climates and mutual genetic distances between 20% to 40% showed a more than 30% difference in canopy carbon gain in the growing season when cultivated at a low-productivity site in a summer-cool climate. Canopy carbon gain as a measure of total (above- and belowground) productivity was to a high degree under genetic control, which offers a considerable potential for genotype selection and breeding programmes. Main determinants of C gain were leaf phenology and the ratio of leaves lost to leaves produced in mid- to late summer, which were significantly influenced by the collective. These traits may serve as robust indicators for elevated yield and should be favoured over more classical traits like plant height growth, while differences among the collectives in physiological traits (photosynthetic performance and leaf water status) and mean assimilation rate were mostly small and insignificant. Our study further showed that a remarkable plant height increase can be a misleading measure of productivity, if it is linked to a small total leaf area. The timing of leaf abscission in late summer was identified as a key determinant of plant productivity in *P. tremula* and thus needs further investigation.

References

[1] Stanturf JA, van Oosten C, Netzer DA, Coleman MD, Portwood CJ. Ecology and silviculture of poplar plantations. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar Culture in North America, Ottawa, NRC Research Press, Ontario, 2001, p. 153–206.

[2] Hansen EA. Poplar woody biomass yields - A look to the future. Biomass Bioenerg 1991;1:1-7.

[3] Makeschin, F. Short rotation forestry in Central and Northern Europe - introduction and conclusions. Forest Ecol Manag 1999;21:1-7.

[4] Dickmann DI, Kuzovkina J. Poplars and willows of the world, with emphasis on silviculturally important species. In: Poplars and willows in the world, International Poplar Commission Thematic Papers, 2008.

[5] Liesebach M, von Wuehlisch G, Muhs HJ. Aspen for short-rotation coppice plantations on agricultural sites in Germany: Effects of spacing and rotation time on growth and biomass production of aspen progenies. Forest Ecol Manag 1999;121:25-39.

[6] Yu QB, Tigerstedt PMA, Haapanen M. Growth and phenology of hybrid aspen clones (*Populus tremula* L. x *Populus tremuloides* Michx.). Silva Fenn 2001;35:15-25.

[7] Marron N, Dillen SY, Ceulemans R. Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. Environ Exp Bot 2007;61:103-116.

[8] Smulders MJM, van der Schoot J, Arens P, Vosman B. Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). Mol Ecol Notes 2001;1:188–190.

[9] Dayanandan S, Rajora OP, Bawa KS. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theor Appl Genet 1998;96:950-6.

[10] Rahman MH, Rajora OP. Microsatellite DNA fingerprinting, differentiation, and genetic relationships of clones, cultivars, and varieties of six poplar species from three sections of the genus *Populus*. Genome 2002;45:1083–1094.

[11] Suvanto LI, Latva-Karjanmaa TB. Clone identification and clonal structure of the European aspen (*Populus tremula*). Mol Ecol 2005;14:2851–2860.

[12] Nei M. Genetic distance between populations. Am Nat 1972;106:283-292.

[13] Peakall R, Smouse PE. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 2006;6:288–295.

[14] Meir P, Levy PE, Grace J, Jarvis PG. Photosynthetic parameters from two contrasting woody vegetation types in West Africa. Plant Ecol 2007;192:277-287.

[15] Bernacchi CJ, Pimentel C, Long SP. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. Plant Cell Environ 2003; 26:1419-1430.

[16] Lenz KE, Host GE, Roskoski K, Noormets A, Sober A, Karnosky DF. Analysis of a Farquhar-von Caemmerer-Berry leaf-level photosynthetic rate model for *Populus tremuloides* in the context of modeling and measurement limitations. Environ Pollut 2010;158:1015-1022.

[17] UPOV: International Union for the Protection of New Varieties of Plants. Guidelines for the conduct of tests for distinctness, homogeneity and stability, [TG/21/7], 1981, Rome, Italy.

[18] Singh M, Ceccarelli S, Hamblin J. Estimation of heritability from varietal trials data. Theor Appl Genet 1993;86:437-441.

[19] Pliura AS, Zhang Y, MacKay J, Bousquet J. Genotypic variation in wood density and growth traits of poplar hybrids at four clonal trials. Forest Ecol Manag 2007;238:92-106.

[20] Lojewski NR, Fischer DG, Bailey JK, Schweitzer JA, Whitham TG, Hart SC. Genetic basis of aboveground productivity in two native *Populus* species and their hybrids. Tree Physiol 2009;29:1133-1142.

[21] Rae AM, Street NR, Robinson KM, Harris N, Taylor G. Five QTL hotspots for yield in short rotation coppice bioenergy poplar: The poplar biomass loci. BMC Plant Biol 2009;9:23-35.

[22] Weih, M. Genetic and environmental variation in spring and autumn phenology of biomass willows (*Salix* spp.): effects on shoot growth and nitrogen economy. Tree Physiol 2009;29:1479-1490.

[23] Schulze ED, Beck E, Müller-Hohenstein K. Plant Ecology. Spektrum Akademischer Verlag, Heidelberg, 2002.

[24] Rowland DL. Diversity in physiological and morphological characteristics of four cottonwood (*Populus deltoides* var. *wislizenii*) populations in New Mexico: evidence for a genetic component of variation. Can J Forest Res 2001;31:845-853.

[25] Kanaga MK, Ryel RJ, Mock KE, Pfreder ME. Quantitative-genetic variation in morphological and physiological traits within a quaking aspen (*Populus tremuloides*) population. Can J Forest Res 2008;38:1690-4.

[26] Müller A, Horna V, Kleemann F, Vornam B, Leuschner C. Physiological vs. morphological traits controlling the productivity of six aspen full-sib families. Unpublished results.

Chapter 6

Comparing native and non-native aspen species (*Populus tremula* vs. *P. tremuloides*) for their suitability in short-rotation forestry: photosynthetic performance and growth analysis

(submitted)

Comparing native and non-native aspen species (*Populus tremula* vs. *P. tremuloides*) for their suitability in short-rotation forestry: photosynthetic performance and growth analysis

Annika Müller[1], Christoph Leuschner[1,3], Viviana Horna[1], Chunxia Zhang[2]

[1]Plant Ecology, Albrecht von Haller Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, 37073 Göttingen

[2]Forest Genetics and Forest Tree Breeding, Büsgen Institute, University of Göttingen, Büsgenweg 2, 37077 Göttingen

[3]Corresponding author: cleusch@gwdg.de, Tel.: +49551-395718, Fax: +49551-395701

Abstract

Short-rotation forestry in Europe uses both native and non-native poplar species in an attempt to maximise yield. However, the planting of introduced tree species in large areas bears the risks, that the species may become invasive with harmful effects on native ecosystems, and that herbivores and pathogens will reduce the exotic species´ vitality in the future. We investigated the growth potential of the native European aspen (*Populus tremula* L.) and the non-native North American quaking aspen (*Populus tremuloides* Michx.) in a replicated growth trial with 1-year-old saplings and compared various indices of productivity (canopy carbon gain in the vegetation period, mean assimilation rate, twig length growth) between the two species in order to test for an assumed growth superiority of the exotic aspen species. By analysing the genetic relatedness and investigating about 30 morphological (leaf size, leaf area, leaf numbers, leaf growth, leaf phenology and the ratio of leaves lost to leaves produced) and physiological traits (A_{max}, quantum yield, carboxylation efficiency, maximum rates of carboxylation and electron transport, leaf dark respiration, leaf conductance, leaf water potential and WUE), we searched for key determinants of productivity, which could provide a possible basis for selection and breeding programmes. American aspen showed a by 20% higher canopy carbon gain than European aspen which was nearly entirely caused by a larger mean leaf area of *P. tremuloides*, while differences in mean assimilation rate and the length of the leafy period were of minor importance. Species-specific differences in the onset of leaf

abscission in early autumn were identified as main determinants of the size of mean plant leaf area and thus of canopy carbon gain. American aspen with a larger leaf area and C gain possessed by far fewer leaves and side branches than European aspen, but had considerably larger leaves. These morphological and phenological contrasts support the view that the two aspen collectives are indeed different species, what has been questioned recently. However, the large majority of physiological traits was not significantly different between the two species and was much less variable among the individuals of a species than the morphological traits. Exceptions were mean leaf conductance, that was smaller, and photosynthetic WUE, that was higher in *P. tremuloides*. We conclude that the non-native *P. tremuloides* exhibited a higher production potential than the native *P. tremula*, mostly due to delayed leaf abscission in early autumn. Selection of high-yielding poplar species should focus on leaf phenology and total leaf area rather than on more traditional parameters such as height and twig length growth.

Keywords: carbon gain, leaf area, leaf number, phenology, water-use-efficiency.

1. Introduction

Populus species are preferred research objects among woody plants because the genome is sequenced (Tuskan et al. 2006), they are easily propagated and fast-growing, and tolerant to a variety of abiotic and biotic stressors. Due to their rapid juvenile growth (Heilman 1999, Stanturf et al. 2001), poplars and willows are often favoured in short-rotation forestry systems (Karp and Shield 2008).

Since more than a century, poplar species from North America, or hybrids of European x American species, have widely been planted in Central Europe where they partly have replaced the local *Populus* species (e.g. *P. x canadensis* = *P. deltoides* x *P. nigra*). The forester's decision to use non-native species bases in most cases on the growth performance at a given site, or a higher tolerance against biotic and abiotic stressors. However, the growth performance and vitality of native and non-native *Populus* species has been found to vary considerably among different genotypes and with site conditions (Pellis et al. 2004, Zhang et al. 2004, Dillen et al. 2007), which requires growth trials under the specific growth conditions for deciding if non-native species should be preferred over native *Populus* species. The expanding short-rotation forestry in Central Europe is seeking for more productive genotypes and species, and does not hesitate to introduce American or Asian *Populus* species, which may bear a certain risk, because their long-term vitality in the new environment is not well

known and in case the non-native species would become invasive in Central Europe's landscapes. European common aspen (*Populus tremula* L.) and the North American quaking aspen (*Populus tremuloides* Michx.) both have very large distribution ranges and are of special commercial importance because they are highly productive even on poor soils (Dickmann and Kuzovkina 2008). Some authors emphasize the remarkable similarities in morphological traits between the two aspen species and thus propose to treat the whole complex as a single species (Eckenwalder 1996, Cervera et al. 2005).

In this study, we analysed the growth performance and aboveground biomass production of the native European common aspen (*P. tremula*) and the non-native North American quaking aspen (*P. tremuloides*) on a relatively unfertile soil in a cool montane climate, i.e. under suboptimal growing conditions. An increasing demand for cropland may compete with expanding short-rotation forestry plantations urging to use less optimal production sites as well. Both poplar species originate from similar climate conditions in Europe and America and are adapted to harsh environments which might recommend both species for short-rotation forestry on poor soils. Climate change scenarios predict increasing temperatures and decreasing summer precipitation for several regions of Germany and Central Europe (Saxe et al. 2001), suggesting to prefer species with adaptations to drought and low nutrient availability.

By analysing the genetic relatedness between the two species and measuring about 30 physiological and morphological and growth-related traits, we aimed at comparing the vitality and productivity of the two species and analysed the underlying growth-related physiological and morphological properties. The objective was to identify the strengths and weaknesses of the native and the non-native species in terms of cultivation on a poor soil in order to give advice for plant breeding and cultivation programmes in Central Europe in the context of short-rotation forestry. We further searched for evidence in support, for or against, the one-species hypothesis in aspen. We specifically addressed the questions, (1) whether the American species is indeed more productive at this site than the native species (which would suggest a preference of the non-native species), and (2) which physiological or morphological traits are most influential in controlling productivity, thereby providing a starting point for selection and breeding programmes. Finally, (3) our physiological and morphological data should help to clarify the relatedness of the two species from a phenotypic perspective.

2. Materials and methods

2.1 Plant material

Sixty plants each of European (*P. tremula*) and North American aspen (*P. tremuloides*) were propagated from seeds in March 2008. Germination took place in well-watered nursery soil. When the plants had reached a height of about 10 cm, the seedlings were transplanted in 10 litre pots containing a nutrient-rich mixture of loamy soil and humus. The pots were placed outdoors and watered as necessary until autumn. The seeds of the European aspen species originated from a single tree in Germany (Göttingen-Geismar 51°30´N, 9°57´E) and were provided by the Dept. of Forest Botany and Tree Physiology at the University of Göttingen. The climate at the origin is characterized by moderately mild winters and moderately warm summers (mean annual temperature of 9.0°C and annual precipitation of 628 mm). The seeds of the American aspen species originated from a single tree in Boston (MA) (41°22´N, 71°02´W) selected by the Institute F.W. Schumacher Co. Tree and Shrub Seeds located in Sandwich (MA). The local climate is similarly characterized by moderately mild winters and moderately warm summers, but somewhat higher rainfall (mean annual temperature 10.7°C and annual precipitation 1052 mm).

2.2 Experimental design

In autumn 2008, the progenies of the two aspen species were planted at lower montane elevation on unfertilized moderately-poor soil (haplic Cambisol) in Central Germany (Solling 51°44'56"N, 9°32'28"O, 485 m a.s.l) at the Relliehausen Experimental Farm near Silberborn in a fenced 4 ha-experimental plot that was used for decades as extensive pasture. Each species was planted in twofold replication in monoculture blocks of 30 trees in 6 x 5 rows with a tree distance of 1.5 m. To avoid edge effects, only the 12 central trees were used for the morphological and ecophysiological measurements, while the 18 more distal trees served as buffer zone.

2.3 Molecular Analyses

Total genomic DNA was isolated from leaves sampled from the European and American aspen seedlings with the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). The microsatellite primers WPMS14, WPMS16 (Smulders et al. 2001), PTR2, PTR4 (Dayanandan et al. 1998), PTR5, PTR6, PTR8, and PTR14 (Rahman et al. 2000, Suvanto et al. 2005) were used for PCR amplification. PCR reactions were carried out with the exception that the primers were labelled with the fluorescent dyes 6-carboxyfluorescein (6-FAM) or hexachloro-

fluorescein phosphoramidite (HEX). The amplified products were resolved electrophoretically on an ABI Genetic Analyzer 3100 together with the internal size standard GenScan 500 ROX from Applied Biosystems. Nei's standard genetic distance (Nei 1972) was calculated for the two species by the software GENALEX 6.2 (Peakall and Smouse 2006).

2.4 Gas exchange measurements

After full leaf expansion in June, leaf gas exchange was recorded monthly with a LI-COR 6400 portable IRGA system (LI-COR, Lincoln, NE, USA) until August. The measurements were always conducted on the fourth or fifth fully expanded and sun-exposed leaf counted from the terminal bud of a twig. Light-response curves were recorded between 900 and 1700 h solar time for a single leaf each of ten randomly selected trees per species. The flux density of photosynthetically active radiation (PAR) was adjusted to 1500, 1000, 750, 500, 250, 100, 50, 20, and 0 µmol photons m^{-2} s^{-1} using a red and blue LED light source supplied by the manufacturer. All measurements were conducted at a constant CO_2 concentration of 370 ppm, a constant leaf temperature of 25°C and a constant vapour pressure deficit (VPD) of 1kPa. From these relationships, light-saturated net photosynthesis rate (A_{max}), leaf dark respiration (DR at 25°C and zero quantum flux), apparent quantum efficiency (Q_e), light compensation point (LCP), light saturation point (LSP), stomatal conductance (G_s) at 1500 µmol m^{-2} s^{-1} and VPD = 1kPa, leaf transpiration rate (E) at 1500 µmol m^{-2} s^{-1} and VPD = 1kPa, and photosynthetic water-use-efficiency (WUE = A_{max}/E) were calculated. In addition, CO_2-response curves were established at 50, 100, 150, 370, 600, 1200, 2000 µmol CO_2 mol^{-1} air and 1500 µmol m^{-2} s^{-1} PAR and used to calculate the net assimilation rate at CO_2 and light-saturation (A_{CO2}), carboxylation efficiency (CE, i.e. the initial slope of the A/c_i relationship), CO_2 compensation point (CO_{2comp}), CO_2 saturation point (CO_{2sat}), maximum carboxylation rate (V_{cmax}) and the maximum rate of electron transport (J_{max}). The data points from the light and CO_2-response curves were fitted with a non-rectangular hyperbola of the form $y = \frac{a \cdot bx}{(a + bx) - y0}$ following Meir et al. (2007) and evaluated with the Software SigmaPlot (Systat Software Inc. Version 10.0, California, USA). J_{max} and V_{cmax} were calculated with the equations given by Bernacchi et al. (2003) and Lenz et al. (2010). After gas exchange measurement, the fresh leaves were scanned to determine leaf size (LS) and the obtained images were analysed with the Software WinFolia (Régent Instruments Inc., Quebec,

Canada). The leaves were subsequently dried and weighed to calculate specific leaf area (SLA: $cm^2\ g^{-1}$).

2.5 Leaf water potential

Leaf water potential (Ψ) was measured with a pressure chamber (PMS Instruments, Corvallis, OR, resolution: 0.01 MPa) on sunny days before dawn between 330 and 500 h solar time and at midday between 1200 and 1330 h solar time on three occasions in June, July and August in order to record daily potential maxima (Ψ_{max}) and minima (Ψ_{min}), respectively. Single fully expanded and sun-exposed leaves of ten trees per species were cut off and immediately measured.

2.6 Morphology

Continuous morphological measurements for monitoring plant growth and leaf phenology recording started with the initiation of bud break in April 2009 and were conducted until the end of August when the physiological measurements were terminated. In order to determine the time of first leaf flush (t (LF)), the bud status was monitored every 1-3 days during the process of bud breaking and complete leaf unfolding. The bud status was categorized based on the protocol issued by the International Union for the Protection of New Varieties of Plants (UPOV 1981). The time period between first leaf flushing and the last leaf lost in September was taken as the length of the leafy period of a species (VP). Because new leaves were enfolded continuously during summer, leaf area increment was recorded in detail in mid-summer (July and August) in 10 plants per species. This was done by marking 3-5 buds per plant and measuring the length of the growing leaves with a digital calliper every 1 to 2 days until the leaves had attained their mature size. Allometric relationships between leaf length and leaf area were established at samples of 10 leaves per species. They were used to calculate the relative leaf area increment rate (LA_{inc}) from the change in leaf length over time, related to initial leaf length.

From early June to the end of August, the height increment (H), mean twig increment (T), and the number of twigs (NT) per tree were measured regularly at weekly intervals on 10-11 trees per species. Two twig categories were distinguished: short (length: <5 cm) and long twigs (NT_{long}: length: >5 cm). This data was used to calculate the relative height and twig increment rate and the total length increment of all axes per tree (H + (T*NT)). On the same 10-11 trees per species, the leaf number (NL) was counted at weekly intervals to detect the time (t (NL_{max})) when maximum leaf number (NL_{max}) was reached. Leaf production rate ($NL_{max}/\Delta t$)

relates the increase in leaf number between leaf unfolding and t (NL_{max}) to the time elapsed. The number of leaves lost (NLL) was calculated as the difference between NL_{max} and the leaf number present at the end of August. Leaf loss rate (NLL/Δt) was calculated by dividing the number of leaves lost by the time span between t (NL_{max}) and the end of the experiment. Further, we calculated the LP ratio, which relates the number of leaves lost to the number of leaves produced per day. Because leaves produced during the second and third leaf flushing events were substantially larger than the first leaves produced in June, the mean leaf size (LS_{mean}) is given here for the two species for July and August. Based on mean leaf size (LS) and total leaf number, we calculated maximum leaf area (TLA_{max}) and mean leaf area during the vegetation period (TLA_{mean}) per tree and per species.

2.7 Carbon gain and relative growth rate

The direct measurement of mass-based biomass data by harvesting was not possible in our experiment, because this study is part of a long-term investigation, which will continue for several years and includes several working groups. In order to approximate the relative growth rate and productivity of the two aspen species, we applied two indirect non-destructive approaches (canopy carbon gain and relative length increment of axes). Canopy carbon gain (g C $plant^{-1}$ VP^{-1}) was derived from the photosynthetic light-response curves, daily mean values of photosynthetically active radiation, plant total leaf area and the length of the leafy period, and provided estimates of the amount of C fixed per plant per vegetation period minus leaf respiration during the daylight hours. Based on the assumption that about 50% of the assimilated carbon is invested in aboveground non-green tissue and that about 50% of ash free dry mass is carbon (Edwards et al. 1980), we extrapolated our carbon gain data to a very rough estimate of harvestable woody biomass for a plantation with moderate stem density (8500 plants ha^{-1}). The second growth measurement approach based on the length increment of all axes (stem and twigs) to calculate the relative length growth rate in cm cm^{-1} $month^{-1}$: ((H+T*TN)/ (initial plant height + initial twig length*NT)/ no. of months) of the plants during the growing season and to relate it to estimated canopy carbon gain. This approach with two independent non-destructive growth determination methods appeared to be justified because we were interested in relative, rather than absolute, values of growth and productivity of the two species. The growth parameters measured were also used in the growth analysis.

2.8 Statistical analyses

Statistical analyses were performed with the Statistical Analyses System (SAS Version 9.1 Institute Inc., Cary, NC, USA). All data were tested for normality of distribution using the Shapiro and Wilk´s test (P<0.05). In case of non-Gaussian distribution, the data were log-transformed to meet the requirements of parametric tests. Plant traits were correlated using Spearman rank correlation. In case of significance (P<0.05), linear regression analyses were also performed. Carbon gain was used as dependent variable in a multiple regression analysis with the studied leaf-level traits (t (NL_{max}), LA_{inc}, $NL_{max}/\Delta t$, $NLL/\Delta t$, LP ratio) as explanatory variables and backward variable selection conducted for both species. The parameters assimilation rate (A), length of the leafy period (VP) and total leaf area were not included in the multiple regression analysis, because they were previously used for calculating carbon gain. Analysis of variance (ANOVA) in combination with a Tukey *post hoc* test was used to reveal significant differences in the trait means between the two species for each month. In order to characterize the magnitude of variation of a trait in the investigated collectives of the two species, we calculated the coefficient of variation within and between species ($CV = \frac{\mathrm{SD}}{mean} \times 100\,;\,\%$).

3. Results

3.1 Genetic distance of the two aspen collectives

With nuclear microsatellite analysis, we found a genetic distance of 77% (according to Nei 1972) between the collectives of *P. tremula* and *P. tremuloides*.

3.2 Traits related to leaf area and leaf phenology

A striking difference between the European and the North American poplar species was that *P. tremula* possessed leaves with on average 30% smaller size than *P. tremuloides* (51.0 vs. 32.1 cm², Table 1), while total leaf number was significantly larger in the former. The time of leaf flushing was significantly different between the two species (mean date of leaf flushing: April 16 vs. April 19 in *P. tremuloides* and *P. tremula*, respectively), but the absolute time difference was small and not related to the total leaf area of a species. The larger mean leaf size in *P. tremuloides* was associated with a significantly larger relative leaf growth rate in July and August in the American species (26 vs. 14% d^{-1}, Table 1). Despite the larger average leaf size, specific leaf area was not different between the two species (157 vs. 155 $cm^2\ g^{-1}$). Total leaf area per plant tended to be larger in *P. tremuloides*, when the mean over the

vegetation period (5853 vs. 4906 cm²) and also the peak value (8918 vs. 7287 cm²) are considered, but these differences were not significant. Thus, the larger average leaf size in *P. tremuloides* more than compensated for the smaller total leaf number. While the two species had a very similar leaf area in June (1884 vs. 1907 cm² in *P. tremuloides* and *P. tremula*, respectively), the difference between the two species increased towards August due to a much faster leaf expansion growth in mid-summer of *P. tremuloides* (in August: 8773 vs. 6519 cm², difference not significant) (Fig. 1a). Our calculation of cumulative canopy carbon gain using data of plant leaf area, mean assimilation rate per unit leaf area (A), and the species-specific length of the leafy period (VP) showed a by 20% larger carbon gain in *P. tremuloides* than in the European species.

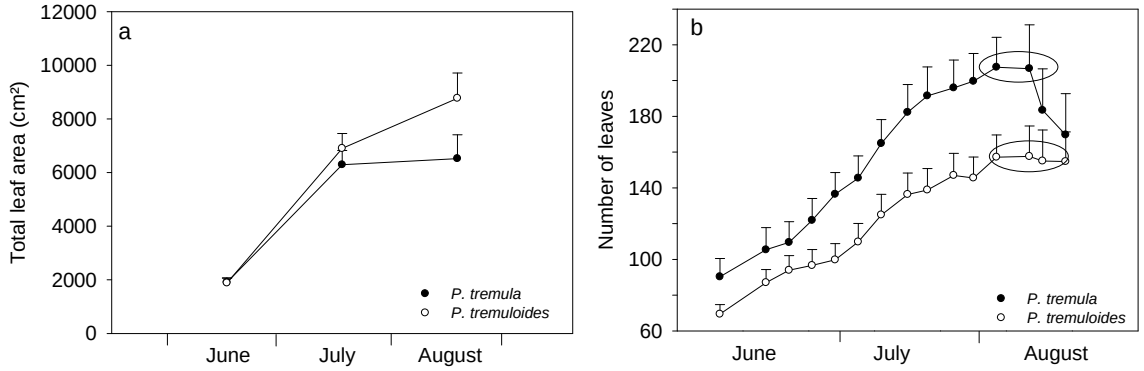

Figure 1 Seasonal changes in total leaf area (a) and leaf number per plant (b) in *P. tremula* and *P. tremuloides* (means ± SE, 10-11 trees per species). The calculation of total leaf area was based on the monthly measurement of leaf size and the respective total number of leaves present at the same time. Maximum leaf number, indicated by the ellipses, was reached for both species around August 14, but the phase of maximum leaf number persisted for 20 days in *P. tremuloides* as compared to 10 days in *P. tremula*.

Estimated wood production in a plantation of 8500 plants ha^{-1} was about 1.2 and 1.4 Mg DM ha^{-1} $year^{-1}$ for European and American aspen, respectively. Differences neither in mean assimilation rate nor in the length of the leafy period can explain the higher C gain of *P. tremuloides*; both variables showed an only small variation between the two species (CV_{inter} for A: 1.7%, and for VP: 4.3%; CV_{intra} for A: 7.1% and for VP: 2.1%, Table 2).

The higher carbon gain on *P. tremuloides* coincides well with the larger mean and maximum leaf area of this species. Furthermore, the two species differed with respect to leaf phenology which had also an effect on C gain. While the time of peak leaf number t (NL_{max}) was reached simultaneously by the plants of *P. tremula* and *P. tremuloides* (average date when the plateau of peak leaf number is reached: August 14), the American aspen plants maintained this peak longer until mid August and showed a later beginning of net leaf loss than the sister species

(Fig. 1b). The date when peak leaf number was reached was positively correlated with mean total leaf area and consequently with cumulative C gain in *P. tremuloides* (Fig. 2a, b), but not in *P. tremula*. In *P. tremuloides* the longer maintenance of peak leaf number (Fig. 1b) was related to a longer continuation of leaf area increment in August as compared to *P. tremula* (Fig. 1a), which increased cumulative C gain.

Table 1 Means and standard error (n = 8-13 trees per species) of 14 analysed morphological and growth-related traits in *P. tremula* and *P. tremuloides*: relative growth rate of all axes (stem and twigs), relative growth rate of twigs, relative height growth rate, total axes length increment, total number of twigs (NT), number of long twigs (>5 cm, NT_{long}), mean specific leaf area (SLA), maximum leaf number (NL_{max}), mean leaf production rate ($NL_{max}/\Delta t$), maximum total leaf area per plant (TLA_{max}), seasonal mean of total leaf area per plant (TLA_{mean}), relative leaf area increment rate (LA_{inc}), mean leaf size (LS_{mean}) and ratio of leaves lost to leaves produced per day (LP ratio) with the associated coefficients of variation within a species (CV_{intra}) and between the species (CV_{inter}, %). Different letters indicate significant differences in the means of a trait between *P. tremula* and *P. tremuloides* ($P<0.05$).

	P. tremula		*P. tremuloides*		
	Mean ± SE	CV_{intra}	Mean ± SE	CV_{intra}	CV_{inter}
Rel. growth rate of axes (cm cm^{-1} $month^{-1}$)	3.4 ± 0.4 a	43.8	2.5 ± 0.2 a	28.6	33.3
Rel. growth rate of twigs (cm cm^{-1} $month^{-1}$)	9.0 ± 2.1 a	79.3	3.8 ± 0.4 b	33.4	94.7
Rel. height growth rate (cm cm^{-1} $month^{-1}$)	0.9 ± 0.1 a	27.8	0.8 ± 0.1 a	21.4	0.0
Total axes increment (cm $month^{-1}$)	165.3 ± 24.2 a	48.6	177.0 ± 19.0 a	34.0	0.0
NT	14.0 ± 1.2 a	28.8	11.0 ± 1.0 a	28.8	27.0
NT_{long}	11.6 ± 3.5 a	30.3	6.0 ± 1.7 b	28.3	79.5
SLA (cm^2 g^{-1})	151.1 ± 3.4 a	9.0	155.1 ± 6.9 a	12.6	0.0
NL_{max}	224.2 ± 18.1 a	26.8	166.1 ± 15.1 b	28.8	35.4
$NL_{max}/\Delta t$ (d^{-1})	1.5 ± 0.1 a	31.3	1.0 ± 0.1 b	32.5	47.6
TLA_{max} (cm^2)	7286.6 ± 692.9 a	31.5	8918.3 ± 883.1 a	31.3	19.2
TLA_{mean} (cm^2)	4905.7 ± 487.9 a	33.0	5853.0 ± 537.5 a	29.0	14.9
LA_{inc} (% $leaf^{-1}$ d^{-1})	14.4 ± 2.4 a	57.0	26.1 ± 2.3 b	28.3	75.9
LS_{mean} (cm^2)	32.1 ± 3.9 a	34.3	51.0 ± 6.3 b	34.9	47.1
LP ratio (d^{-1})	1.8 ± 0.4 a	75.9	0.9 ± 0.3 a	91.1	67.1

Table 2 Means and standard error (n = 10 trees per species) of cumulative carbon gain, mean light-saturated CO_2 assimilation rate (A), length of the leafy period (VP) and maximum total leaf area per plant (TLA_{max}) for *P. tremula* and *P. tremuloides* with the associated coefficients of variation within a species (CV_{intra}) and between the species (CV_{inter}, %). Different letters indicate significantly different means of a trait between *P. tremula* and *P. tremuloides* (P<0.05). For details on the calculation of canopy carbon gain see Methods section.

	P. tremula		*P. tremuloides*		
	Mean ± SE	CV_{intra}	Mean ± SE	CV_{intra}	CV_{inter}
Carbon gain (g C plant^{-1} VP^{-1})	141.8 ± 13.2 a	33.4	172.5 ± 19.2 a	31.4	17.7
A (g C m^{-2} d^{-1})	1.91 ± 0.04 a	8.2	1.98 ± 0.01 a	7.1	1.7
VP (days)	151.6 ± 1.8 a	3.9	156.8 ± 1.1 b	2.1	4.3
TLA_{max} (cm²)	7286.6 ± 692.9 a	31.5	8918.3 ± 883.1 a	31.3	19.2

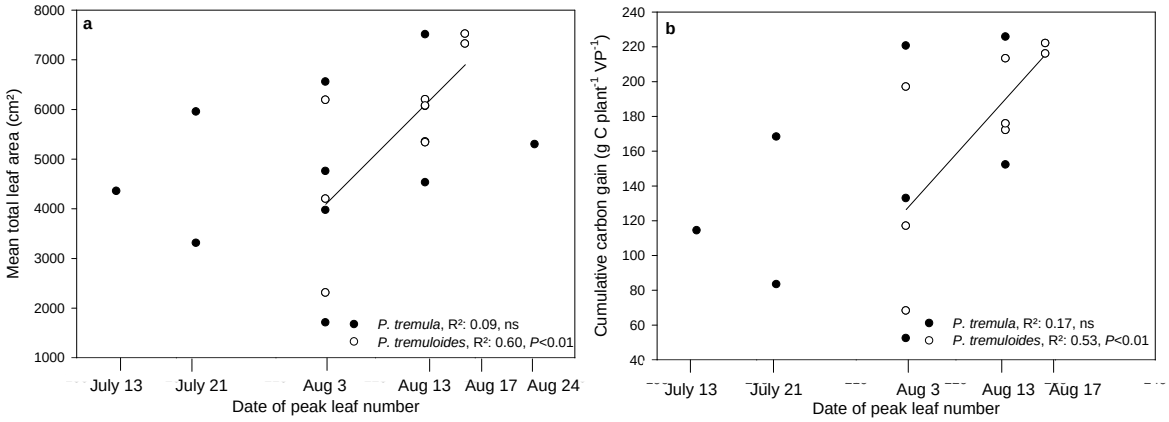

Figure 2 Influence of the date of peak leaf number on mean total leaf area (a), and on cumulative carbon gain (b) in *P. tremula* and *P. tremuloides*; n = 8-11 plants per species.

A multiple regression analysis with carbon gain as dependent trait and the associated leaf-level traits (date of peak leaf number, relative leaf area increment rate, leaf production rate, leaf loss rate, ratio of leaves lost to leaves produced) as explanatory variables with backward variable selection conducted for each species separately showed that the date of peak leaf number (partial R^2: 0.57) and the leaf area increment rate (partial R^2: 0.40) were the most important leaf-level traits influencing the variation in C gain in *P. tremula*. This was different in *P. tremuloides*, where leaf production rate (partial R^2: 0.87) was identified as only significant leaf trait influencing carbon gain (Table 3).

While the total number of twigs (NT) did not differ between the two species, *P. tremuloides* plants developed a smaller number of long twigs (NT_{long}). Even though *P. tremula* showed significantly higher relative growth rates of twig length and total axis length (stem + twigs),

the total increment of all axes was similar for the two species because *P. tremuloides* had a greater mean twig length which compensated the lower relative growth rate (Table 1).

Table 3 Coefficients of determination (R^2) for the dependency of carbon gain on associated leaf-level traits in the aspen species. Carbon gain was used as dependent variable in a multiple regression analysis with the leaf-level traits as explanatory variables and backward variable selection conducted for both species. All variables indicated with an asterisks are significant at the $P<0.05$ level, whereas the leaf-level traits without asterisks were removed by the model. The parameters assimilation rate, length of the leafy period and total leaf area were not included in the multiple regression analysis, because they were previously used for calculating carbon gain.

	P. tremula	*P. tremuloides*
	Carbon gain	Carbon gain
Date of peak leaf number	0.57*	0.04
Relative leaf area increment rate	0.40*	0.00
Leaf production rate	0.18	0.87*
Leaf loss rate	0.13	0.02
Ratio of leaves lost to leaves produced	0.00	0.00

3.3 Leaf gas exchange and leaf water status

The response of net photosynthesis rate to variable photon flux densities (0-1500 μmol m^{-2} s^{-1}) and leaf-internal CO_2 concentrations (50-2000 μmol CO_2 mol^{-1} air) was remarkably similar for sun leaves of the two species (Fig. 3a, b). None of the parameters characterizing photosynthetic efficiency (light-saturated and CO_2-saturated photosynthesis, apparent quantum efficiency and carboxylation efficiency, maximum rates of electron transport and of carboxylation) differed significantly between *P. tremula* and *P. tremuloides* (Table 4). The only exception was the saturating photon flux density for A_{max} that was significantly lower in *P. tremuloides*; however, this had no consequences for the efficiency of photosynthetic light and CO_2 use and resulted in very similar means of A_{max} (16.3 μmol CO_2 m^{-2} s^{-1}) in both species. The two species were more different with respect to leaf conductance and leaf water potential. Leaf conductance (G_s), measured at standard conditions and averaged over the whole growing season, showed only a non-significant tendency to lower values in *P. tremuloides*, while the monthly means were significantly smaller in June in the American species (Table 4, Fig. 4a).

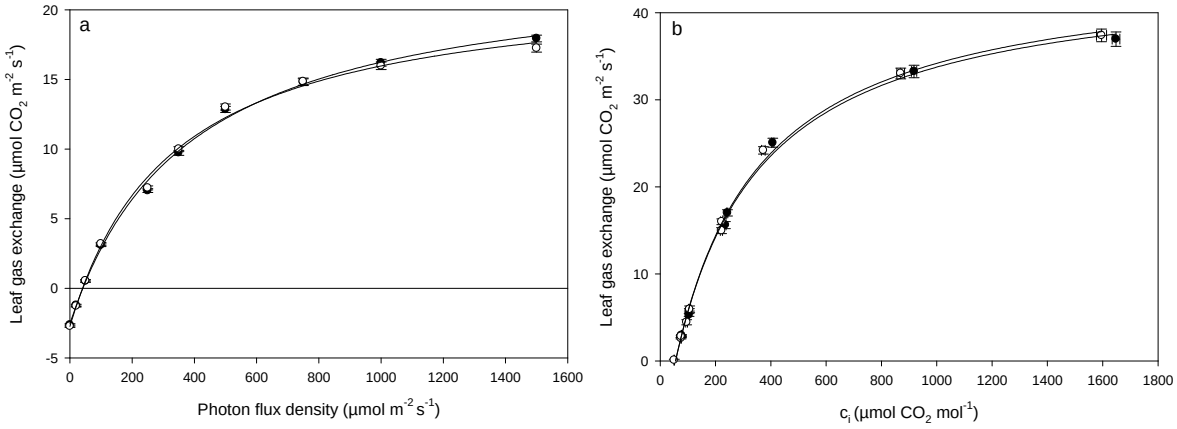

Figure 3 Dependence on photon flux density (a) and on intercellular CO_2 concentration of the photosynthesis of *P. tremula* (filled circles) and *P. tremuloides* (open circles) (means and standard error of 30 trees per species). The values were fitted with a non-rectangular hyperbolic function of the form: $y = \frac{a \cdot bx}{(a + bx) - y0}$.

Lower leaf conductance, but similar photosynthetic rates, in *P. tremuloides* led to significantly higher water-use-efficiencies (WUE) at the leaf-level in this species during the growing season (Table 4, Fig. 4b). However, we found no correlation between WUE and canopy carbon gain. Daily minima of leaf water potential were significantly lower in *P. tremula* than in *P. tremuloides*, probably caused by the lower leaf conductance and thus reduced transpiration of the American species. The more pronounced reduction in leaf water potential over midday had no effect on the predawn values of Ψ that were similar for the two species (Table 4).

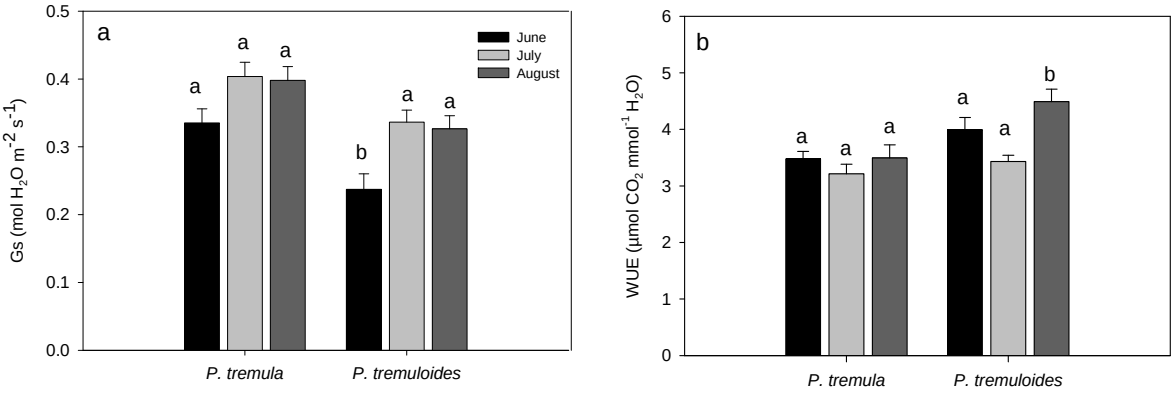

Figure 4 Stomatal conductance (G_s) (a) and photosynthetic water-use-efficiency (WUE) (b) of *P. tremula* and *P. tremuloides* at 1500 µmol photons m^{-2} s^{-1}, VPD = 1kPa and 25°C in June, July and August. Different letters indicate significant differences between the species for each month at $P<0.05$ (means and standard error of 10 leaves per species).

Table 4 Means and standard error (30 measurements per species conducted on different individuals between June and August 2008) of 15 physiological traits related to photosynthesis and plant water status of *P. tremula* and *P. tremuloides*. Light-saturated photosynthesis rate (A_{max}: µmol m^{-2} s^{-1} of CO_2 at 25°C and 1kPa VPD), leaf dark respiration (DR: µmol m^{-2} s^{-1} of CO_2 at 25°C and zero quantum flux), light saturation point (LSP: µmol m^{-2} s^{-1} of photons), light compensation point (LCP: µmol m^{-2} s^{-1} of photons), apparent quantum efficiency (Q_e: µmol µmol^{-1}of CO_2), CO_2 compensation point (CO_{2comp}: µmol mol^{-1} of CO_2), CO_2 saturation point (CO_{2sat}: µmol mol^{-1} of CO_2), net photosynthesis rate at CO_2 and light saturation (A_{CO2}: µmol m^{-2} s^{-1} of CO_2), carboxylation efficiency (CE: mol m^{-2} s^{-1} of air), maximum rate of carboxylation (V_{cmax}: µmol m^{-2} s^{-1} of CO_2), maximum rate of electron transport (J_{max}: µmol m^{-2} s^{-1} of electrons), stomatal conductance (G_s: mol m^{-2} s^{-1} of H_2O at 1500 µmol PAR and 1kPa VPD), photosynthetic water-use-efficiency (WUE: µmol $mmol^{-1}$ of CO_2) and leaf water potential recorded before dawn (ψ_{max}: MPa) and around midday (ψ_{min}: MPa). For every trait the associated coefficients of variation within a species (CV_{intra}) and between the species (CV_{inter}, %) are also given. Different letters indicate significant differences in trait means between *P. tremula* and *P. tremuloides* (P<0.05).

	P. tremula		*P. tremuloides*		
	Mean ± SE	CV_{intra}	Mean ± SE	CV_{intra}	CV_{inter}
A_{max}	16.37 ± 0.28 a	5.75	16.35 ± 0.26 a	4.67	0.0
DR	- 2.83 ± 0.09 a	10.83	-2.81 ± 0.13 a	13.57	0.0
LSP	970.93 ± 6.98 a	2.39	947.51 ± 9.20 b	2.75	2.7
LCP	25.50 ± 0.46 a	6.02	25.08 ± 0.40 a	4.62	0.0
Q_e	0.080 ± 0.001 a	7.49	0.086 ± 0.002 a	8.10	7.2
CO_{2comp}	54.96 ± 0.97 a	5.91	54.98 ± 1.35 a	6.99	0.0
CO_{2sat}	977.24 ± 14.80 a	5.02	980.21 ± 20.75 a	5.99	0.0
A_{CO2}	33.41 ± 0.68 a	6.79	33.73 ± 0.87 a	7.31	0.0
CE	0.24 ± 0.018 a	25.84	0.22 ± 0.014 a	17.68	0.0
V_{cmax}	41.13 ± 1.26 a	16.82	41.57 ± 1.24 a	16.68	0.0
J_{max}	145.63 ± 4.99 a	18.75	146.44 ± 4.82 a	18.33	0.0
G_s	0.38 ± 0.02 a	14.91	0.33 ± 0.01 a	11.55	15.3
WUE	3.47 ±0.13 a	12.18	3.99 ± 0.21 b	16.95	33.5
ψ_{max}	-0.14 ± 0.02 a	63.08	-0.14 ± 0.01 a	33.65	0.0
ψ_{min}	-1.02 ± 0.06 a	23.15	-0.76 ± 0.04 b	16.47	37.7

4. Discussion

P. tremula and *P. tremuloides* are typical pioneer tree species with rapid growth rate, high light demand and short longevity that occupy equivalent ecological niches in the natural forest vegetation of Western Eurasia and North America (Dickmann and Kuzovkina 2008). Both species belong to the phylogenetically oldest of the six poplar sections, which contains the genus *Populus*. It is assumed that both species derived from a common ancestor (Eckenwalder 1996), but followed separate lines of evolution on the two continents for quite a long time. Most likely, the two species split in the Miocene about 23.5 Ma ago with the break-up of Laurasia into Eurasia and North America (Parrish 1987). There exists some controversy about the degree of genetic similarity of the two sister species. According to AFLP analyses in members of the genus *Populus*, Cervera et al. (2005) emphasized the remarkable genetic similarity between *P. tremula* and *P. tremuloides* (similarity coefficient of 80%) and interpreted their results as an argument to merge the two systematic groups in one species, similar to an earlier proposal by Eckenwalder (1996) which based on morphological similarity. Our study provides controversial evidence on the relatedness of *P. tremula* and *P. tremuloides*: the nuclear microsatellite data (Nei´s genetic distance: 77%) and the leaf morphological and phenological data rather emphasize the distance and independence of the two systematic groups, while the physiological data seem to indicate a close relatedness.

The high similarity of the two species in terms of physiological traits related to photosynthesis and leaf water status is indeed astonishing: from the 15 physiological traits investigated, only three (light saturation point of photosynthesis, WUE and daily minimum of leaf water potential) were found to differ significantly between the species. A remarkable similarity was found for the variables characterizing the capacity and resource use efficiency of the photosynthetic apparatus, measured in 30 trees per species under controlled conditions in the field. A_{max}, V_{cmax}, J_{max}, CE, quantum yield, and the light and CO_2 compensation points of standard leaves in the sun canopy were nearly identical in their means, as was leaf dark respiration rate at standard temperature. Moreover, the variation in physiological performance within and among the investigated 30 individuals of a species was remarkably small for the photosynthetic traits (coefficient of within-species variation mostly <15%, except for V_{cmax}, J_{max} and CE) which documents a uniform physiological performance across a collective of individuals with a limited genetic variability. One reason for the uniformity is the origin of all test plants from the same mother-tree, while different fathers are possible.

The low variation in physiological properties between the two species may have two possible reasons, (1) a particularly conservative inheritance of physiological traits, or (2) similar

selection forces having been active in the environment of *P. tremula* and *P. tremuloides*. While both *Populus* species may indeed be exposed to similar microclimate and soil conditions in the early phases of forest succession today, and probably were so in their evolutionary history, it is unrealistic to assume that a uniform environmental setting has resulted in such a high degree of similarity in the physiological constitution. More likely is the first explanation, because a uniforming effect of selection should also have acted on the morphological traits, which is not the case. Moreover, several other studies in *Populus* and other tree genera provided indeed evidence of a conservative inheritance of traits related to photosynthesis (Rowland 2001), which weakens their indicative value with respect to systematic relatedness and speciation.

The within-population variability was markedly larger in the traits related to foliage structure and leaf dynamics (within-population CV values typically >25%) and 7 of the 14 variables investigated were significantly different between the two *Populus* species. This is in accordance with the findings of Müller et al. (subm.a, subm.b) that intraspecific genetic variation in *P. tremula* is particularly large in foliage-related morphological and phenological traits. More important, canopy carbon gain in the vegetation period was remarkably different between the species (about 20% larger in *P. tremuloides*) despite very similar photosynthetic characteristics and mean daily assimilation rates. The two species showed striking differences in their carbon allocation patterns to leaves, branches and stems and with respect to leaf phenology, which matches with the relatively large genetic distance between the species visible in the microsatellite data, thus contradicting the one-species hypothesis.

Plants of *P. tremuloides* had relatively few side branches and only about half the number of longer twigs (>5cm) in comparison to *P. tremula*, and these twigs grew more slowly. However, the total axes length increment did not differ significantly between the two species because American aspen had a greater mean twig length than its European relative. These morphological differences developed in the first months of live, when the saplings of both species were reared under identical environmental conditions in the botanical garden. Due to the larger twig length, relative axes increment rate was smaller in *P. tremuloides*, even though absolute twig and stem length growth were similar for the two species, or tended to be even larger in *P. tremuloides.*

P. tremuloides reached an about 20% greater total leaf area than *P. tremula* (difference not significant) with a relatively small number of large leaves, which offers one explanation for the smaller number of long side branches in American aspen. Plants of *P. tremula* were able to compensate for the later start of leaf flushing through a higher leaf production rate,

resulting in a high number of small leaves. However, a larger mean (and maximum) plant leaf area was the decisive plant trait responsible for the more than 20% higher canopy carbon gain of *P. tremuloides*, and not a high leaf number or a higher mean assimilation rate nor a longer lasting leafy period, which both were only 3-4% higher in American than in European aspen. Two processes contributed to the trend toward a higher mean and maximum leaf area in *P. tremuloides*, the more rapid leaf expansion growth between the start of leaf enfolding and the date of the plant's peak leaf number, and the greater leaf longevity in August, when net leaf losses started to increase in *P. tremula*, while American aspen lost relatively few leaves with no net leaf loss occurring until the end of August. The longer-lasting plateau of peak leaf number in *P. tremuloides* in combination with a continued leaf enlargement until early autumn were the causes for the progressive leaf area increase in American aspen and its higher C gain in the late August. The results of the multiple regression analysis confirmed the prominent role played by traits related to leaf area development (relative leaf area increment rate and the date of peak leaf number) for the carbon gain of *P. tremula*, while leaf production rate was the key trait in *P. tremuloides*.

The phenomenon of delayed leaf abscission was also investigated in *Salix* species and identified as an important trait resulting in increased leaf area duration (i.e. leaf area x time), a variable closely related to productivity (Weih 2009). We observed that both species reached the peak of total leaf number in the middle of August (circa August 14) but the phase of maximum leaf number lasted for more than 20 days in *P. tremuloides* as compared to 10 days in *P. tremula*. While other studies identified extended growing season length as a key factor increasing productivity in *P. tremula* (Yu et al. 2001) and in other species used in short-rotation forestry such as *Salix* spp. (Weih 2009), we found an only insignificant influence of the length of the leafy period for carbon gain, but recognized the timing of the first phase of leaf abscission in mid-summer and early autumn to be decisive. The importance of other foliage-related traits for the productivity of *Populus* species was emphasized by e.g. Dillen et al. (2009: leaf production rate) and Bunn et al. (2004: total leaf area).

The superior growth performance of *P. tremuloides* in comparison to *P. tremula* was shown in other experiments as well which studied the suitability of these species for short-rotation forestry in Central Europe (Liesebach et al. 1999) focussing on wood mass production. Liesebach et al. (1999) reported a yield of 2.6 – 4.8 Mg of aboveground biomass ha^{-1} $year^{-1}$ for *P. tremula*, but about 3.4 - 8.7 Mg ha^{-1} for *P. tremuloides*. Other studies found aboveground dry mass production rates of aspen grown in Central Europe in the range of 1.4-11.2 Mg ha^{-1} $year^{-1}$, depending on soil fertility and management regime (Kauter et al. 2001,

Bemmann et al. 2007, Kollas et al. 2009). Our rough estimate of 1.2 (*P. tremula*) and 1.4 Mg dry mass ha^{-1} $year^{-1}$ (*P. tremuloides*) represents the lower limit of production values, which reflects the fact that the Solling Mountains are a rather unfavourable growing site and the trees are only 1.5 years old. On very poor sites, annual yield of aspen was found to be < 1.3 Mg dry mass ha^{-1} $year^{-1}$ in the first years (Landgraf et al. 2007). High biomass yields are typically reached in aspen plantations not before the sixth year (Liesebach et al. 1999) which promises for the Solling plantation increasing yields in the coming years.

The higher cumulative canopy carbon gain of *P. tremuloides* was linked to a higher mean water-use-efficiency. We recorded leaf-level WUE means for *P. tremuloides* between 3.5 and 4 µmol CO_2 $mmol^{-1}$ H_2O, which is higher than comparable values reported for this species in other studies (<3 µmol CO_2 $mmol^{-1}$ H_2O by Liu et al. 2006, or 2-4 µmol CO_2 $mmol^{-1}$ H_2O by Thomas et al. 1997). The 15% higher water-use-efficiency of American aspen was solely caused by a lower leaf conductance under standard conditions, while photosynthetic capacity was not different between the species. A likely consequence of the higher leaf conductance and transpirative water loss in *P. tremula* is the significantly lower daily minimum of leaf water potential observed in this species (-1.02 MPa in *P. tremuloides* vs. -0.76 MPa in *P. tremula*), which may indicate that American aspen is better adapted at tolerating summer droughts than its European relative. Other studies found evidence that WUE can indeed be used as an indicator of differences in drought susceptibility of *Populus* species and hybrids (e.g. Bassman and Zwier 1991). However, we found no significant correlation between leaf-level WUE and canopy carbon gain in the 10 individuals per species investigated, which is probably a consequence of the moist and cool climate at the study site where water shortage is no relevant growth-influencing factor. The larger leaves and larger total leaf area of *P. tremuloides*, on the other hand, may expose American aspen to higher water deficits. Therefore, the lower leaf conductance could perhaps represent an adaptive response of this species to its larger transpiring surface.

Growth trials with controlled soil drought and the monitoring of additional traits related to plant water status have to show whether *P. tremuloides* indeed maintains a lower leaf conductance under optimal and reduced soil water availabilities than *P. tremula* and thus offers more favourable adaptations for cultivation at drought-affected sites. The necessity to select drought-tolerant species for short-rotation forestry will increase in future because climate change scenarios predict more frequent droughts for parts of Europe (Saxe et al. 2001).

Conclusions

Our comparative growth trial with 1-year-old saplings of the native *P. tremula* and the introduced *P. tremuloides* revealed an about 20% higher canopy carbon gain in the vegetation period of the American species, indicating a higher growth potential at our study site. Canopy C gain as a measure of productivity has the advantage over more traditional growth indices (such as aboveground biomass production or height growth), that it covers the carbon assimilated and being available for whole-plant growth, thus including belowground production as well, which is most often ignored in tree growth trials. When the focus is on harvestable biomass, however, the direct measurement of stem and twig biomass or length increment is indispensable. The canopy carbon gain data of our study suggest to prefer the non-native species over the native one for maximising yield at this low-fertility site. However, it must be kept in mind that this study covered only the first one-and-a half years of cultivation and not the full 2 to 10-year-long rotation period used in short-rotation forestry. Moreover, our results are only valid for the montane sub-oceanic climate with relatively poor soil at the study site. The growth potential of the two species at more fertile and more drought-affected sites requires separate study. Further, other genotypes of *P. tremula* and *P. tremuloides* can differ in their growth potential which could result in different species rankings with respect to productivity. The selection of the most advantageous species or genotype of poplar for a given site is facilitated by our finding that the timing of the onset of leaf abscission in late summer and early autumn seems to be a key determinant of carbon gain and productivity.

If the choice is *P. tremuloides* (or another non-native species or hybrid), additional research should also address the susceptibility of this species to herbivore attack and pathogens, and must investigate the potential of the species to become invasive in Europe. Our study provided important evidence through microsatellite and leaf morphological investigations that *P. tremula* and *P. tremuloides* should be treated as two distinct species, despite astonishing similarities in physiological traits and high genomic similarity according to AFLP analyses. An important argument is that poplar productivity depends mostly on leaf morphological and phenological properties, that differ with species, and not on the physiological traits that seem to be inheritated conservatively. Additional studies on stress tolerance might provide further evidence for more profound functional differences between *P. tremula* and *P. tremuloides*.

Acknowledgements

We thank Lars Köhler and Frauke Kleemann for help with the installation of the field experiment. This work was funded by the Niedersächsisches Ministerium für Wissenschaft und Kultur and the "Niedersächsisches Vorab". The authors declare that they have no conflict of interest.

References

Bassman, J.H., Zwier, J.C., 1991. Gas exchange characteristics of *Populus trichocarpa*, *Populus deltoides* and *Populus trichocarpa* x *P. deltoides* clones. Tree Physiol. 8, 145-159.

Bemmann, A., Feger, K.H., Gerold, D., Große, W., Hartmann, K.U., Petzold, R., Röhle, H., Schweile, J., Steinke, C., 2007. Kurzumtriebsplantagen auf landwirtschaftlichen Flächen in der Region Großenhain im Freistaat Sachsen. Forstarchiv 78, 95-101.

Bernacchi, C.J., Pimentel, C., Long, S.P., 2003. In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. Plant Cell Environ. 26**,** 1419-1430.

Bunn, S.M., Rae, A.M., Herbert, C.S., Taylor, G., 2004. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. Forestry 77, 307-323.

Cervera, M.T., Storme, V., Soto, A., Ivens, B., Van Montagu, M., Rajora, O.P., Boerjan, W., 2005. Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. Theor. Appl. Genet. 111, 1440-1456.

Dayanandan, S., Rajora, O.P., Bawa, K.S., 1998. Isolation and characterization of microsatellites in trembling aspen (*Populus tremuloides*). Theor. Appl. Genet. 96, 950-6.

Dickmann, D.I., Kuzovkina, J., 2008. Poplars and willows of the world, with emphasis on silviculturally important species. in*:* Poplars and willows in the world. International poplar commission thematic papers. Food and Agriculture Organisation of the United Nations. Rome, Italy.

Dillen, S.Y., Marron, N., Bastien, C., Ricciotti, L., Salani, F., Sabatti, M., Pinel, M.P.C., Rae, A.M., Taylor, G., Ceulemans, R., 2007. Effects of environment and progeny on biomass estimations of five hybrid poplar families grown at three contrasting sites across Europe. Forest Ecol. Manag. 252, 12-23.

Dillen, S.Y., Marron, N., Sabatti, M., Ceulemans, R., Bastien, C., 2009. Relationships among productivity determinants in two hybrid poplar families grown during three years at two contrasting sites. Tree Physiol. 29, 975-987.

Eckenwalder, J.E., 1996. Systematics and evolution of *Populus*, in: Stettler, R.F., Bradshaw, H.D., Heilman, P.E., Hinckley, T.M. (Eds), Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Ontario, Canada, pp.7-32.

Edwards, N.T, Shugart JR, H.H., Mclaughlin, S.B., Harris, W.F., Reichle, D.E., 1980. Carbon metabolism in terrestrial ecosystems, in: Reichle, D.E. (Ed), Dynamic properties of forest ecosystems. Cambridge University Press, London, pp. 499-536.

Heilman, P.E., 1999. Planted forests: poplars. New Forests 17, 89-93.

Karp, A., Shield, I., 2008. Bioenergy from plants and the sustainable yield challenge. New Phytol. 179, 15-32.

Kauter, D., Lewandowski, I., Claupein, W., 2001. Pappeln in Kurzumtriebswirtschaft: Eigenschaften und Qualitätsmanagement bei der Festbrennstoffbereitstellung – Ein Überblick. Pflanzenbauwissenschaften 5(2), 64-74.

Kollas, C., Lasch, P., Rock, J., Suckow, F., 2009. Bioenergy potential in Germany – assessing spatial patterns of biomass production with aspen short-rotation coppice. Int. Agrophys. 23, 343-352.

Landgraf, D., Ertle, C., Böcker, L., 2007. Stockausschlagpotenzial von Aspe und Robinie. AFZ - der Wald 62(2), 80-83.

Lenz, K.E., Host, G.E., Roskoski, K., Noormets, A., Sober, A., Karnosky, D.F., 2010. Analysis of a Farquhar-von Caemmerer-Berry leaf-level photosynthetic rate model for *Populus tremuloides* in the context of modeling and measurement limitations. Environ. Pollut. 158**,** 1015-1022.

Liesebach, M., von Wuehlisch, G., Muhs, H.J., 1999. Aspen for short-rotation coppice plantations on agricultural sites in Germany: Effects of spacing and rotation time on growth and biomass production of aspen progenies. Forest Ecol. Manag. 121, 25-39.

Liu, N., Dang, Q.L., Parker, W.H., 2006. Genetic variation of *Populus tremuloides* in ecophysiological responses to CO_2 elevation. Can. J. Botany 84, 294-302.

Müller A., Horna, V., Kleemann, F., Vornam, B., Leuschner, C., a. Physiological vs. morphological traits controlling the productivity of six aspen full-sib families. Unpublished results.

Müller A., Horna, V., Zhang, C., Leuschner, C., b. Different growth strategies determine the carbon gain and productivity of aspen collectives to be used in short-rotation plantations. Unpublished results.

Meir, P., Levy, P.E., Grace, J., Jarvis, P.G., 2007. Photosynthetic parameters from two contrasting woody vegetation types in West Africa. Plant Ecol. 192, 277-287.

Nei, M., 1972. Genetic distance between populations. Am. Nat. 106, 283-292.

Parrish, J.T., 1987. Global paleogeography and paleoclimatic of the late cretaceous and early tertiary, in: Friis, E.M., Chaloner, W.G., Crane, P.R. (Eds), The origins of angiosperms and their biological consequences. Cambridge University Press, Cambridge, UK, pp. 51-73.

Peakall R., Smouse, P.E., 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.

Pellis, A., Laureysens, I., Ceulemans, R., 2004. Genetic variation of the bud and leaf phenology of seventeen poplar clones in a short rotation coppice culture. Plant Biol. 6**,** 38-46.

Rahman M.H., Rajora, O.P., 2002. Microsatellite DNA fingerprinting, differentiation, and genetic relationships of clones, cultivars, and varieties of six poplar species from three sections of the genus *Populus*. Genome 45, 1083–1094.

Rowland, D.L., 2001. Diversity in physiological and morphological characteristics of four cottonwood (*Populus deltoides* var. *wislizenii*) populations in New Mexico: evidence for a genetic component of variation. Can. J. Forest Res. 31, 845-853.

Saxe, H., Cannell, M.G.R., Johnsen, B., Ryan, M.G., Vourlitis, G., 2001. Tree and forest functioning in response to global warming. New Phytol. 149, 369-399.

Smulders, M.J.M., van der Schoot, J., Arens, P., Vosman, B., 2001. Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). Mol. Ecol. Notes 1, 188–190.

Stanturf, J.A., van Oosten, C., Netzer, D.A., Coleman, M.D., Portwood, C.J., 2001. Ecology and silviculture of poplar plantations, in: Dickmann, D.I., Isebrands, J.G., Eckenwalder, J.E., Richardson, J. (Eds), Poplar Culture in North America. NRC Research Press, Ottawa, Ontario, Canada.

Suvanto, L.I., Latva-Karjanmaa, T.B., 2005. Clone identification and clonal structure of the European aspen (*Populus tremula*). Mol. Ecol. 14, 2851–2860.

Thomas, B.R., MacDonald, S.E., Dancik, B.P., 1997. Variance components, heritabilities and gain estimates for growth chamber and field performance of *Populus tremuloides*: Gas exchange parameters. Silvae Genet. 46, 309-317.

Tuskan G., DiFazio, S., Hellsten, U., Jansson, S., Rombauts, S., Putnam, N., Sterck, L., Bohlmann, J., Schein, J., Bhalerao, R.R., Bhalerao, R.P., Blaudez, D., Boerjan, W., Brun, A., Brunner, A., Busov, V., Campbell, M., Carlson, J., Chalot, M., Chapman, J., Chen, G., Cooper, D., Coutinho, P.M. , Couturier, J., Covert, S., Cunningham, R., Davis, J., Degroeve, S., dePamphilis, C., Detter, J., Dirks, B., Dubchak, I., Duplessis, S., Ehlting, J., Ellis, B., Gendler, K., Goodstein, D., Gribskov, M., Grigoriev, I., Groover, A., Gunter, L., Hamberger, B., Heinze, B., Helariutta, Y., Henrissat, B., Holligan, D., Islam-Faridi, N., Jones-Rhoades, M., Jorgensen, R., Joshi, C., Kangasjärvi, J., Karlsson, J., Kelleher, C., Kirkpatrick, R., Kirst, M., Kohler, A., Kalluri, U., Larimer, F., Leebens-Mack, J., Leplé, J.C., Déjardin, A., Pilate, G., Locascio, P., Lucas, S., Martin, F., Montanini, B., Napoli, C., Nelson, D.R., Nelson, C.D., Nieminen, K.M., Nilsson, O., Peter, G., Philippe, R., Poliakov, A., Ralph, S., Richardson, P., Rinaldi, C., Ritland, K., Rouzé, P., Ryaboy, D., Salamov, A., Schrader, J., Segerman, B., Sterky, F., Souza, C., Tsai, C., Unneberg, P., Wall, K., 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313, 1596–1604.

UPOV: International Union for the Protection of New Varieties of Plants, 1981. Guidelines for the conduct of tests for distinctness, homogeneity and stability, Test guidelines 21/7. Geneva, Switzerland.

Weih, M., 2009. Genetic and environmental variation in spring and autumn phenology of biomass willows (*Salix* spp.): effects on shoot growth and nitrogen economy. Tree Physiol. 29, 1479-1490.

Yu, Q.B., Tigerstedt, P.M.A., Haapanen, M., 2001. Growth and phenology of hybrid aspen clones (*Populus tremula* L. x *Populus tremuloides* Michx.). Silva Fenn. 35, 15-25.

Zhang, X.L., Zang, R.G., Li, C.Y., 2004. Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. Plant Sci. 166, 791-797.

Chapter 7

Synopsis

Why is aspen research necessary?

Due to the increasing demand for renewable energy sources and the associated interest in short-rotation forestry, growth experiments with highly productive *Populus* species as a model tree have been undertaken for decades (Rose and DeBell 1978, Pellis et al. 2004, Marron and Ceulemans 2006, Monclus et al. 2006, Dillen et al. 2009, Rae et al. 2009). Most research has been focused on the productivity of *P. nigra*, *P. trichocarpa* or *P. deltoides* and the associated molecular or phenotypic traits controlling it. The main reason why the focus was on the mentioned species is because of their superior biomass gain in comparison to other *Populus* species. However, the popularity of aspen (*P. tremula* and *P. tremuloides*) for short-rotation forestry will probably increase in the near future, because they cope better with drought in poor soils and have a lower demand on habitat conditions than other poplar species. These features make aspen a promising alternative in comparison to more drought-sensitive species, especially in the face of future climate scenarios which include increasing temperatures and decreasing summer precipitation (Kollas et al. 2009).

Furthermore, aspen are an ecologically important species, because they act as pioneers, colonizing disturbed habitats and sequestering high amounts of carbon (Chen et al. 1999, David et al. 2001). Additionally, aspen-dominated forests provide habitat for endangered species (e.g. breeding birds and small mammals) (Turner et al. 2003, Kouki et al. 2004). An "extended" phenotypic effect of aspen forests could be shown for soil processes, as the variation in genetic diversity of aspen forests has been related to patchiness of belowground activity, due to differences in chemical composition of the leaf litter (Madritch et al. 2007, 2009). Even non-dominated aspen forests seem to benefit from the presence of aspen trees, which enhance forest productivity, increase the amount of cyanolichens due to an altered nutrient cycle, and increase moisture and light conditions (Legare et al. 2005, Campbell et al. 2010). Therefore, aspen can be said to act as keystone species and contribute to ecosystem services and functioning. Considering all the mentioned aspects, there is no doubt about the importance of investigating plant morphological and physiological traits of aspen in relation to their genetic constitution and places of origin. This research can be either economically motivated and aimed at giving advice for plant breeding and forest-plantation management or non-economically prompted in order to support the understanding of the functioning of aspen communities.

In this thesis I quantified the variability of productivity along a gradient of genetic relatedness including closely and distantly related *P. tremula* assemblages as well as two different aspen species (*P. tremula* and *P. tremuloides*). We selected the plant material in order to simulate a

genetic constellation which could be the result of natural pair-crossing by an aspen founder population and investigated more than 30 phenotypical traits (morphological, physiological, phenological traits and aspen associated organisms) related to growth performance.

Variability of productivity along a gradient of genetic relatedness

We analysed three aspen assemblages which differed in their genetic distance (Nei 1972, 1978): six full-sib families with German origin (2-30% genetic distance), four *P. tremula* collectives originating from Central Europe (Germany, Switzerland and Austria: 20-40% genetic distance) and two aspen species (*P. tremula* with German origin and *P. tremuloides* with American background differing 77% in their genetic distance). The growth analysis for every assemblage revealed that the level of variation in terms of productivity (either carbon gain or relative biomass gain) differed within every sub-group (either in each full-sib family, collective or species) between 25 and 40%, whereas the variation between the sub-groups reached values between 10 and 40% (Table 1). The highest variation in productivity was reached within the closely related full-sib families and between the analysed Central European collectives (each 40%), whereas the variation among the studied full-sib families and the two aspen species was characterized by the lowest variation with 10 and 20%, respectively. The total variation in productivity in each aspen assemblage ranged between 50-60%.

Therefore, we conclude 1) that the markedly higher values in the genetic distance between *P. tremula* and *P. tremuloides* in comparison to the relatedness to the German or Central European aspen assemblages is not reflected in biomass yield in the first year of growth (results of the between group variation). We conclude further 2) that the increase in genetic diversity in the single collectives or species caused by more than one pollen donor in comparison to the considered full-sib families, with only one father, is not mirrored in a greater variation in productivity within each studied assemblage (results of the within group variation). The plant material used in this study was selected in order to simulate an assemblage which could be naturally arise by pair-crossing of a founder population of aspen trees. We simulated different numbers of mother and father trees. Hence, the results of the total variation in productivity (50-60%) indicate 3) that the variation in productivity of natural aspen populations is not decisively increased by a parallel increase in pollen donors, and that also an aspen assemblage based on a small founder population is able to obtain high variation levels in terms of productivity. The high variation in productivity was closely related to high variation in plant functional traits (e.g. leaf-level traits), which will be explained in detail in

the following section. A high level of variation in functional traits has been related to ecosystem stability in other studies (Lhomme and Winkel 2002, Boege and Dirzo 2004, Lecerf and Chauvet 2008). Therefore we believe 4) that also these small founder populations could provide high ecosystem stability and ecosystem services, due to their high levels of intraspecific variability in functional traits. This further enables a successful occupation of different sites and under different environmental conditions (Joshi et al. 2001), which is a key feature of the widespread aspen species.

Aspen productivity and its associated traits

Tree growth and related traits are fundamental components of planted forests but also of survival and productivity in natural undomesticated forests (Grattapaglia et al. 2009). With the help of my results we were able to reveal the parameters which were most decisive for the variation in productivity within the studied aspen assemblages. For every study assemblage, despite genetic distance or geographical background, we demonstrated that leaf phenology and leaf area related traits were the prime parameters controlling productivity. In the assemblage of closely related aspen full-sib families (Chapter 4), the different families differed significantly in their leaf number and total leaf area, which were positively associated with yield gain and negatively with the time of leaf flushing. The variation in leaf number and the time of leaf flush could be significantly attributed to the genetic constitution (results of the quantitative genetic analysis) and a multiple regression analysis confirmed the prominent role of leaf number for the productivity of the full-sib families. Next to the variation in leaf-level traits, we found that the amount of phenolic compounds was negatively related with the level of aspen herbivory. But, neither increasing levels of phenolic compounds nor the suppression of endophytic fungal infection had negative impacts on biomass gain. The trial to relate the genetic variance and phenotypic characteristics based on molecular markers failed, because only five neutral markers were investigated (Chapter 3). Nevertheless, the strong genetic control for bud phenology, leaf number and the concentration of phenolics in *Populus* has been emphasized by other authors such as Wu et al. (1998), Frewen et al. (2000) or Donaldson and Lindroth (2007).

Traditional biomass indicators like plant height or twig growth could also be used as responsive traits, but this was not true for the Central European aspen assemblage (Chapter 5). The collectives differed significantly from each other in their growth performance and especially plant height and twig increment as biomass indicators could lead to misleading results in terms of final productivity. A large plant height in combination with a low number

of leaves and leaf area resulted in low values of carbon gain. More importantly, leaf phenology and leaf area associated traits were again detected as being the most relevant triggers for productivity and key factors for carbon gain, which was also marked for other *Populus* species (Bradshaw et al. 2000). This was applicable for *P. tremula* and *P. tremuloides* as well (Chapter 5 & 6).

The calculated canopy carbon gain was based on plant leaf area, the mean assimilation rate per unit leaf area, and the species specific length of the leafy period, whereas the latter played a less important role for the variation in carbon gain. This seems to be in contrast with other studies which associated a longer growing season with better growth for poplar families and other tree species (Rae et al. 2004, Picard et al. 2005, Dillen et al. 2009). However, the variation in growing season length between the collectives and species differed by merely 2% and 4.5% respectively, and hence could not be the decisive trait in order to explain the 20-40% variation in productivity (Table 1). As already mentioned, the variation in productivity was mainly associated with differences in total leaf area and related traits (variation in total leaf area among collectives and species: 15 and 40%, respectively, Table 1). From our selected plant material, a late onset of net leaf loss in mid-summer and hence low values in the ratio of leaves lost to leaves produced were determined to be the major impact factors for leaf area in the late summer and hence for carbon gain (Chapter 5 & 6). Therefore, we believe that next to canopy duration, the critical point of the time when leaf loss rate exceeds the leaf production rate should also be captured in leaf phenological monitoring. We conclude that a great leaf longevity and the ability to produce new leaves and leaf area at a stage in late summer (leaf production rate > leaf loss rate) is of major importance for successful biomass gain. With the help of a quantitative genetics analysis (Chapter 5), we confirm that leaf phenology associated traits are highly heritable and a better guide for plant breeding programmes than classical traits like plant height. Our results show firstly that associated traits are stable and have a great impact on yield irrespective of the variation in the plant material and secondly, the phenotypic variation of these traits is moderately high with a great proportion of genetic variation and heritability indices.

Next to morphological traits we monitored the ecophysiological traits in the three aspen assemblages. For most of the analysed physiological traits (except plant water-balance related traits) we found no significant differences between the full-sib families, between the collectives or between the aspen species (Chapters 4-6). More important than the homogenous pattern of the physiological traits were the results that photosynthesis related traits (e.g. assimilation rate, apparent quantum yield or A_{max}) could not be directly related with

productivity in any of the studied aspen assemblages, which is in accordance with Bunn et al. (2004). Nevertheless, assimilation rate as driving force for carbon uptake was mathematically involved in carbon gain, but with a variation between 2-5% among collectives and species, it could not be the explanatory variable for the productivity variation of 20-40%. As already mentioned, the variation in productivity was mainly associated with differences in total leaf area and related traits. This is in line with the results of Poorter and Remkes (1990) who found a much weaker relationship between net assimilation rate and relative growth rate than with leaf area ratio across 24 species. Furthermore, Henderson and Jose (2005) demonstrated that an increase in leaf area index alone is able to increase canopy assimilation and lead to higher values in productivity in fast-growing tree species. This is linked with the fact that an efficient investment of photosynthetic assimilate could be more relevant for successful growth than net photosynthesis rates. Photosynthetic assimilate may not be directly invested in plant growth but may accumulate in leaves in order to maximise leaf area relative to total plant weight, which is in most species more related to successful growth than net photosynthesis rates (Stitt and Schulze 1994). Hence, productivity can be increased by a trade-off between maximum photosynthetic surface and minimum energy investment in branches (Wu 1998).

None of our studied ecophysiological parameters could be proven to be a reliable biomass predictor, and had a low phenotypic variance with a low genetic impact on trait variation. On the one hand, the low genetic variation makes the physiological traits almost unsuitable for breeding approaches and selection, due to the fact that fitness associated traits usually have a high genetic variability (Houle 1992). But on the other hand we cannot exclude the fact that genetic variation in photosynthesis or related traits exists in *P. tremula* or *P. tremuloides*, even if it was neither expressed within the closely related full-sib families, nor within the assemblage of distantly related aspen collectives or different aspen species during the vegetation period and under uniform environmental conditions. It has been shown for *Populus* and other species that if a significant genetic variation in net photosynthesis exists, this may only be expressed under specific environmental conditions like drought. This genotype x environment interaction would be expressed in an association between photosynthesis rate and productivity in a water-limited environment, which is mainly reduced due to stomatal limitation, but it would not be expressed under well watered conditions (Ullah et al. 2008, Silim et al. 2009). Hence, we conclude that physiological associated traits are less suitable as plant biomass predictors than morphological ones, according to their low phenotypic and genetic variance, but should not be neglected, especially in terms of stress related conditions. Especially plant water status related traits should be considered as important traits for growth

analyses, because species with a good adaptation to drought will be in demand for areas with low precipitation. The intraspecific comparison of *P. tremula* collectives did not reveal significant differences in water-use-efficiency, but the interspecific comparison did, with a better drought adaptation for *P. tremuloides* linked with higher values of carbon gain. The mean values of WUE for *P. tremuloides* were recorded at 4 µmol CO_2 mmol H_2O^{-1} (Chapter 6), which is about 40% higher than in other experiments that studied the water-use efficiency of *P. deltoides* x *P. nigra* hybrids (Guo et al. 2010).

Are aspen suitable for short-rotation forestry?

In this study, through the field experiment performed in 2009 and our selected plant material from four Central European *P. tremula* collectives and one *P. tremuloides* collective from the US, we could reveal a gradient according to successful productivity (cumulative carbon gain), which was associated with the above mentioned leaf phenological and leaf area associated traits.

The plants of the American collective were the most productive, followed by the Austrian (AU) and German collectives (Gm_1) and lastly the German and the Swiss collectives Gm_2 and CH. Due to this productivity gradient, we extrapolated a rough estimate of harvestable woody biomass of 0.9 -1.3 (*P. tremula*) – 1.4 (*P. tremuloides*) Mg dry mass ha^{-1} yr^{-1} for the studied aspen in our experiment in the Solling Mountains on poor soil and under poor climate conditions. The calculation is based on a moderate stem density (8500 plants ha^{-1}) and the assumption that about 50% of the assimilated carbon is invested in aboveground dry weight with a carbon content of 50% (Edwards et al. 1980). Liesebach et al. (1999) reported a yield of 2.6 – 4.8 Mg of aboveground biomass ha^{-1} yr^{-1} for *P. tremula*, but about 3.4 - 8.7 Mg absolute dry biomass ha^{-1} for *P. tremuloides*. Other studies found aboveground dry mass production rates of aspen grown in Central Europe in the range of 1.4 and 11.2 Mg dry mass ha^{-1} yr^{-1} depending on soil fertility and management regime (Kauter et al. 2001, Bemmann et al. 2007, Kollas et al. 2009). Our rough estimate of 0.9 - 1.3 (*P. tremula*) and 1.4 Mg dry mass ha^{-1} yr^{-1} (*P. tremuloides*) represents the lower limit of production values, which reflects the fact that the Solling Mountains are a rather unfavourable growing site and the trees are only 1.5 years old. On very poor sites, annual yield of aspen was found to be < 1.3 Mg dry weight ha^{-1} yr^{-1} in the first years (Landgraf et al. 2007). High biomass yields are not typically reached in aspen plantations before the sixth year (Liesebach et al. 1999) which promises increasing production values for the coming years for the Solling plantations. Maximum yearly increment could be expected to occur at the age of ten and onward, which leads to the

conclusion that short-rotation cultures with aspen and rotation times under 15 years are not worthwhile (Liesebach et al. 1999).

Previous studies pointed out that *P. tremula* and *P. tremuloides* are capable of producing suitable amounts of biomass, even on unfavourable soil conditions and longer rotation times (Hofmann-Schielle 1999, Liesebach et al. 1999, Lasch et al. 2010). The strength of *P. tremuloides* as emphasized by Liesebach et al. (1999) was also found in our results as well (Chapter 6). We believe that the differences between the two species are related to higher values in leaf area and a later onset of leaf abscission in mid-summer for *P. tremuloides.* This will lead to greater differences in carbon gain in the upcoming years of growth than currently exist, making *P. tremuloides* the more favourable seed stock. The higher carbon gain of *P. tremuloides* was linked to a higher mean water-use-efficiency, which is in accordance with Silim et al. (2009) who stated the importance of poplar clones combine high productivity with drought tolerance traits for biomass plantations. Other studies found evidence that WUE can indeed be used as an indicator of differences in drought susceptibility of *Populus* species and hybrids (Bassman and Zwier 1991).

Nevertheless, the question of seed stock is also related to the question of the introduction of non-native species. The introduction of the exotic North American *P. tremuloides* in Europe also bears the risk that the species will become invasive with unpredictable effects on native ecosystems. Our results revealed several significant differences in morphology between the two aspen species (Chapter 6), and even if the final yield production and the associated leaf area exhibited a relatively low variation among the two species, the leaf dynamics (leaf area increment and leaf size) and branching pattern were decisively different and exceeded the variation among the *P. tremula* collectives which were native to Europe (Table 1). Such differences are mainly caused by the long evolutionary separation on two continents. *P. tremula* and *P. tremuloides* should be treated as two different species, and not as proposed by some authors as one single species (Eckenwalder 1996, Cervera et al. 2005). This point has to be considered for making a decision for or against non-native seed stock in terms of short-rotation forestry.

Therefore, we suggest preferring *P. tremuloides* for short-rotation forestry due to its higher growth potential. However, the risk of introducing exotic species should be considered, when deciding for or against non-native seed stock. Our results show further, that the choice of the plant material for maximum yield gain in short-rotation forestry is clearly a question which should be addressed not only regarding different species, but also different collectives, demes or genotypes of the same species, which is indicated by the higher variation in productivity

among the four European collectives than the variation among *P. tremula* and *P. tremuloides* (38 vs. 17%, Table 1).

Table 1 Coefficients of variation (%) of selected traits for the analysed full-sib families, collectives and species. The mean variation within each sub-group (either in each full-sib family or collective or species), the variation between the sub-groups and the total variation are presented. The detailed calculations for each are given in Chapters 4 to 6, respectively. Productivity is either defined as the relative biomass gain (mg dry weight g^{-1} d^{-1}) or the canopy carbon gain (g C $plant^{-1}$ vegetation $period^{-1}$).

		Full-sib families (Chapter 4)	Collectives (Chapter 5)	Species (Chapter 6)
within group variation	Productivity	39.4	25.2	32.4
	Assimilation rate	-	7.9	7.7
	Net photosynthesis	19.6	7.7	5.2
	Leaf conductance	34.3	20.4	13.2
	Leaf number	43.9	30.1	27.8
	Leaf size	35.7	32.2	34.6
	Leaf area	65.5	29.2	31.0
	Specific leaf area	16.7	15.0	10.8
	Leaf area increment	-	19.9	41.9
	Number of long branches > 5cm	-	28.6	29.3
	Length of leafy period	-	3.1	3.0
between group variation	Productivity	9.8	38.2	17.7
	Assimilation rate	-	5.4	1.7
	Net photosynthesis	0.0	6.1	0.0
	Leaf conductance	0.0	7.4	15.3
	Leaf number	25.1	24.7	35.4
	Leaf size	14.9	14.6	47.1
	Leaf area	23.0	39.0	14.9
	Specific leaf area	3.9	10.9	0.0
	Leaf area increment	-	0.0	118.1
	Number of long branches > 5cm	-	23.8	79.5
	Length of leafy period	-	2.0	4.3
total variation	Productivity	49.2	63.4	50.1
	Assimilation rate	-	13.3	9.4
	Net photosynthesis	19.6	13.8	5.2
	Leaf conductance	34.3	27.8	28.5
	Leaf number	69.0	54.8	63.2
	Leaf size	50.6	46.8	81.7
	Leaf area	88.5	68.2	45.9
	Specific leaf area	20.6	25.9	10.8
	Leaf area increment	-	19.9	160.0
	Number of long branches > 5cm	-	52.4	108.8
	Length of leafy period	-	5.1	7.3

References

Bassman, J. H., and J. C Zwier. 1991. Gas exchange characteristics of *Populus trichocarpa*, *Populus deltoides* and *Populus trichocarpa* x *P. deltoides* clones. Tree Physiology **8**:145-159.

Bemmann, A., K. H. Feger, D. Gerold, W. Große, K. U. Hartmann, R. Petzold, H. Röhle, J. Schweile, and C. Steinke. 2007. Kurzumtriebsplantagen auf landwirtschaftlichen Flächen in der Region Großenhain im Freistaat Sachsen. Forstarchiv **78**:95-101.

Boege, K., and R. Dirzo. 2004. Intraspecific variation in growth, defense and herbivory in *Dialium guianense* (Caesalpiniacea) mediated by edaphic heterogeneity. Plant Ecology **175**:59-69.

Bradshaw, H. D., R. Ceulemans, J. Davis, and R. Stettler. 2000. Emerging model systems in plant Biology: Poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulation **19**:306-313.

Bunn, S. M., A. M. Rae, C. S. Herbert, and G. Taylor. 2004. Leaf-level productivity traits in *Populus* grown in short rotation coppice for biomass energy. Forestry **77**:307-323.

Campbell, J., G. E. Bradfield, C. E. Prescott, and A. L. Fredeen. 2010. The influence of overstorey *Populus* on epiphytic lichens in subboreal spruce forests of British Columbia. Canadian Journal of Forest Research **40**:143-154.

Cervera, M. T., V. Storme, A. Soto, B. Ivens, M. Van Montagu, O. P. Rajora, and W. Boerjan. 2005. Intraspecific and interspecific genetic and phylogenetic relationships in the genus *Populus* based on AFLP markers. Theoretical and Applied Genetics **111**:1440-1456.

Chen, W. J., T. A. Black, P. C. Yang, A. G. Barr, H. H. Neumann, Z. Nesic, P. D. Blanken, M. D. Novak, J. Eley, R. J. Ketler and R. Cuenca. 1999. Effects of climatic variability on the annual carbon sequestration by a boreal aspen forest. Global Change Biology **5**:41-53.

David, A. J., J. C. Zasada, D. W. Gilmore, and S. M. Landhäusser. 2001. Current trends in the management of aspen and mixed aspen forests for sustainable production. Forestry Chronicle **77**:525-532.

Dillen, S. Y., N. Marron, M. Sabatti, R. Ceulemans, and C. Bastien. 2009. Relationships among productivity determinants in two hybrid poplar families grown during three years at two contrasting sites. Tree Physiology **29**:975-987.

Donaldson, J. R., and R. L. Lindroth. 2007. Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. Ecology **88**:729-739.

Eckenwalder, J. E. 1996. Systematics and evolution of *Populus*. *in*: R. F. Stettler, H. D. Bradshaw, P. E. Heilman, and T. M. Hinckley, editors. Biology of *Populus* and its implications for management and conservation. NRC Research Press, Ottawa, Ontario, Canada, pp. 7-32.

Edwards, N. T, H. H. Shugart JR, S. B. Mclaughlin, W. F. Harris and D. E. Reichle. 1980. Carbon metabolism in terrestrial ecosystems. *in:* D. E. Reichle editor. Dynamic properties of forest ecosystems. Cambridge University Press, London, pp. 499-536.

Frewen, B. E., T. H. H. Chen, G. T. Howe, J. Davis, A. Rohde, W. Boerjan, and H. D. Bradshaw. 2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics **154**:837-845.

Grattapaglia, D., C. Plomion, M. Kirst, and R. R. Sederoff. 2009. Genomics of growth traits in forest trees. Current Opinion in Plant Biology **12**:148-156.

Guo, X., X. Zhang, and Z. Huang. 2010. Drought tolerance in three hybrid poplar clones submitted to different watering regimes. Journal of Plant Ecology **3**:79-87.

Henderson, D. E., and S. B. Jose. 2005. Production physiology of three fast-growing hardwood species along a soil resource gradient. Tree Physiology **25**:1487-1494.

Hofmann-Schielle, C., A. Jug, F. Makeschin, K. E. Rehfuess. 1999. Short rotation plantations of balsam poplars, aspen and willows on former arable land in the Federal Republic of Germany. I. Site-growth relationships. Forest Ecology and Management **121**:41-55.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics **130**:195-204.

Kauter, D., I. Lewandowski, and W. Claupein. 2001. Pappeln in Kurzumtriebswirtschaft: Eigenschaften und Qualitätsmanagement bei der Festbrennstoffbereitstellung – Ein Überblick. Pflanzenbauwissenschaften **5(2)**:64-74.

Kollas, C., P. Lasch, J. Rock, and F. Suckow. 2009. Bioenergy potential in Germany - assessing spatial patterns of biomass production with aspen short-rotation coppice. International Agrophysics **23**:343-352.

Kouki, J., K. Arnold, and P. Martikainen. 2004. Long-term persistence of aspen - a key host for many threatened species - is endangered in old-growth conservation areas in Finland. Journal for Nature Conservation **12**:41-52.

Joshi, J., B. Schmid, M. C. Caldeira, P. G. Dimitrakopoulos, J. Good, R. Harris, A. Hector, K. Huss-Danell, A. Jumpponen, A. Minns, C. P. H. Mulder, J. S. Pereira, A. Prinz, M. Scherer-Lorenzen, A.-S. D. Siamantziouas, A. C. Terry, A. C. Terry, A. Y. Troumbis, and J. H. Lawton. 2001. Local adaptation enhances performance of common plant species. Ecology Letters **4**:536-544.

Lasch, P., C. Kollas, J. Rock, and F. Suckow. 2010. Potentials and impacts of short-rotation coppice plantation with aspen in Easterm Germany under conditions of climate change. Regional Environmental Change **10**:83-94.

Landgraf, D., C. Ertle, and L. Böcker. 2007. Stockausschlagpotenzial von Aspe und Robinie. AFZ - der Wald **62(2)**:80-83.

Lecerf, A., and E. Chauvet. 2008. Intraspecific variability in leaf traits strongly affects alder leaf decomposition in stream. Basic and Applied Ecology **9**:598-605.

Legare, S., D. Pare, and Y. Bergeron. 2005. Influence of aspen on forest floor properties in black spruce-dominated stands. Plant and Soil **275**:207-220.

Lhomme, J.-P., and T. Winkel. 2002. Diversity-Stability relationships in community ecology: Re-examination of the portfolio effect. Theoretical Population Biology **62**:271-279.

Liesebach, M., G. von Wuehlisch, and H. J. Muhs. 1999. Aspen for short-rotation coppice plantations on agricultural sites in Germany: Effects of spacing and rotation time on growth and biomass production of aspen progenies. Forest Ecology and Management **121**:25-39.

Marron, N., and R. Ceulemans. 2006. Genetic variation of leaf traits related to productivity in a *Populus deltoides* x *Populus nigra* family. Canadian Journal of Forest Research **36**:390-400.

Madritch, M. D., J. R. Donaldson, and R. L. Lindroth. 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. Soil Biology & Biochemistry **39**:1192-1201.

Madritch, M. D., S. L. Greene, and R. L. Lindroth. 2009. Genetic mosaics of ecosystem functioning across aspen-dominated landscapes. Oecologia **160**:119-127.

Monclus, R., E. Dreyer, M. Villar, F. M. Delmotte, D. Delay, J. M. Petit, C. Barbaroux, D. Le Thiec, C. Brechet, and F. Brignolas. 2006. Impacts of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* x *Populus nigra*. New Phytologist **169**:765-777.

Nei, M. 1972. Genetic distance between populations. American Naturalist **106**:283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583-590.

Pellis, A., I. Laureysens, and R. Ceulemans. 2004. Genetic variation of the bud and leaf phenology of seventeen poplar clones in a short rotation coppice culture. Plant Biology **6**:38-46.

Picard, G., S. Quegan, N. Delbart, M. R. Lomas, T. Le Toan, and F. I. Woodward. 2005. Bud-burst modelling in Siberia and its impact on quantifying the carbon budget. Global Change Biology **11**:2164-2176.

Poorter, H., and C. Remkes. 1990. Leaf-area ratio and net assimilation rate of 24 wild-species differing in relative growth-rate. Oecologia **83**:553-559.

Rae, A. M., K. M. Robinson, N. R. Street, and G. Taylor. 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. Canadian Journal of Forest Research **34**:1488-1498.

Rae, A. M., N. R. Street, K. M. Robinson, N. Harris, and G. Taylor. 2009. Five QTL hotspots for yield in short rotation coppice bioenergy poplar: The poplar biomass loci. BMC Plant Biology **9**:23-35.

Rose, D. W., and D. S. DeBell. 1978. Economic assessment of intensive culture of short-rotation hardwood crops. Journal of Forestry **76**:706-711.

Silim, S., R. Nash, D. Reynard, B. White, and W. Schroeder. 2009. Leaf gas exchange and water potential responses to drought in nine poplar (*Populus* spp.) clones with contrasting drought tolerance. Trees-Structure and Function **23**:959-969.

Stitt, M., and D. Schulze. 1994. Does Rubisco control the rate of photosynthesis and plant-growth - An exercise in molecular ecophysiology. Plant Cell and Environment **17**:465-487.

Turner, M. G., W. H. Romme, R. A. Reed, and G. A. Tuskan. 2003. Post-fire aspen seedling recruitment across the Yellowstone (USA) landscape. Lanscape Ecology **18**:127-140.

Ullah, I., R. Mehboob, M. Ashraf, and Y. Zafar. 2008. Genotypic variation for drought tolerance in cotton (*Gossypium hirsutum* L.): Leaf gas exchange and productivity. Flora **203**:105-115.

Wu, R. L. 1998. Genetic mapping of QTLs affecting tree growth and architecture in *Populus*: implication for ideotype breeding. Theoretical and Applied Genetics **96**:447-457.

Wu, R., H. D. Bradshaw, and R. F. Stettler. 1998. Developmental quantitative genetics of growth in *Populus*. Theoretical and Applied Genetics **97**:1110-1119.

Acknowledgements

Without the big support of many people, finishing this PhD-thesis would have been impossible….

First of all, I would like to thank my supervisor Prof. Christoph Leuschner for providing me an interesting research topic and for the constant support during my work and for the never ending proof reading of my manuscripts.
Special thanks go to Dr. Viviana Horna for her help, support and discussions and enthusiasm in my work.
I thank Prof. Christoph Kleinn and Dr. Dirk Gansert for being part in my PhD committee and supporting me when ever necessary.
I am grateful to Prof. Andrea Polle for the good organisation of the Göttingen poplar diversity experiment and our poplar meetings.
I also thank Laura Sutcliff for proof reading my thesis and to all my colleagues of the plant ecology group: thank you for the very nice working atmosphere and the coffee breaks.
Special thanks go to Bernd Raufeisen for providing us with spareribs and "Mettbrötchen".
I also thank Dr. Heinz Coners for providing technical support, Dr. Karsten Wesche for his help with statistics, and Dr. Lars Köhler, Dr. Frauke Kleemann and Maximilian von Fragstein und Niemsdorf for helping in the field.
Special thanks go to my roommates Dominik Seidel and Benjamin Krause for being there during my ups and downs, for all the useful and also useless discussions, for their constant support during my work, for their growing interest in poplars and for their good jokes. I will miss them.
I would like to thank Friderike Beyer for sharing the office with me in the first two years and for standing the disastrous conditions of my desk.
Thank you Ute Schlonsog and Uta Nüsse-Hahne for helping with the never ending harvest of my plants, which should be finished after a week, but actually took us eight.
I am grateful to Astrid Rodriguez for her support with all the paperwork.
Finally, I would like to thank my family for their support and believe in me and my work.

Curriculum Vitae

Name:	Annika Müller
Date of birth:	12-09-1982
Place of birth:	Bad Oeynhausen
Study:	Since March 2008 PhD at the Albrecht-von-Haller Institute for Plant Sciences at the University of Göttingen (Department of Ecology and Ecosystem Research) October 2002 - June 2007 Study of Biology at the University of Bielefeld September 2006 – June 2007 Diploma-thesis at the Department of Experimental and Systems Ecology Topic of the Diploma-thesis: Seedling establishment of the invasive *Acacia longifolia* along a North,- South and microclimatic gradient in Portuguese dune ecosystems March 2006 - September 2006 Stay at the University of Lisboa at the Department of Applied Sciences and Technology (ICAT Instituto de Ciencia Aplicada e Tecnologia)
Education:	August 1993 - May 2002 Secondary school: Städtisches Gymnasium Löhne Qualification: A-levels
Language skills:	English