

Response of juvenile progeny of seven forest tree species and their populations to simulated climate change-related stressors, heat, elevated humidity and drought

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The study aimed to evaluate response and phenotypic plasticity of juvenile progeny of seven forest tree species *Pinus sylvestris*, *Picea abies*, *Quercus robur*, *Fraxinus excelsior*, *Alnus glutinosa*, *Betula pendula* and *Populus tremula* and their populations to climate change-related stressors, simulated in a phytotron – heat and elevated humidity and heat and drought – in comparison to performance in ambient (control) conditions. Treatment effect on sapling morphometric, physiological and biochemical traits was significant except for health condition, transpiration and photosynthetic rates and water use efficiency (WUE). Species effect and species-by-treatment interaction were strongly significant in most traits studied, indicating a great inter-specific variability of responses to the applied treatments. Compared to control, stem diameter increment was lower for most species following both hot-wet and hot-dry treatments, while treatment impact on height increment was less pronounced and sometimes even positive. Drought caused significant defoliation in *P. tremula*, *A. glutinosa* and *B. pendula*, while under hot-wet treatment the defoliation in most species was lower than in control. Following hot dry treatment, WUE in *P. abies*, *P. sylvestris* and *B. pendula* was lower than following both hot-wet treatment and control, while in *P. tremula*, *A. glutinosa* and *Q. robur* WUE was higher. This suggests that the latter species are able to maintain a balance between photosynthesis and transpiration. Photosynthetic rate was highest in *P. tremula*, *B. pendula* and *A. glutinosa*, however it was much more negatively affected by water deficit in these three species than in other tested species. In most cases, drought had a negative effect on production of pigments in deciduous tree species, which, together with increased amounts of malondialdehyde and hydrogen peroxide, indicated a presence of an oxidative stress. Significant population effect and population-by-treatment interactions found for most traits showed different plasticity and response of tree populations to the treatments. Although, only 19% of the populations showed significant ecovalencies. Some of the observed reactions may not be considered as adaptive acclimation as decreasing growth of some species and populations indicates deteriorating performance which may lead to changes in their competitiveness, thus compromising regeneration, persistence of natural successions and sustainability of forest ecosystems.

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Introduction

Over the few last millennia forest tree species were subjected to conspicuous climatic and environmental changes and were forced to react to these changes by migration and genetic adaptation to new growing conditions (Huntley & Birks 1983). Current environmental changes are significantly faster compared to those that took place during post-glacial climatic shifts. Therefore, there are widespread concerns that trees, as long-lived organisms, may not be able to cope with rapid climate changes. Increasing frequency and amplitude of abiotic and biotic disturbances driven by recent rapid climate change may compromise the sustainability of forest ecosystems, forest regeneration, biodiver-

sity and persistence of natural ecological successions in European boreal and northern temperate forests. Global climate changes are leading to increase in temperature as well as alterations in rainfall patterns (Bhargava & Sawant 2013, Shah 2015).

With prolonged periods of drought and temperature extremes becoming more frequent (Kattenberg et al. 1996), the environmental stress on trees will increase. For example, in Lithuania, there were several local and country-wide periods of prolonged drought and heat waves/temperature extremes (>+30°C) during the last two decades (1995-2015). These climatic extremes occurred about three times more often than during the preceding two de-

cares (1975-1995): during 1995-2015 there were 102 days with day temperatures exceeding +30°C, during 1975-1995 there were only 35 such days (Anonymous 2017). Changes in physical climate will subsequently induce changes in plant morphology, physiology and chemistry that may increase plant susceptibility to diseases (Chakraborty et al. 2000). The risk of drought increases in some regions because of reduced soil moisture caused by enhanced evapotranspiration under a rising temperature (Mitchell et al. 1990). Such changes have the potential to directly affect the physiological processes of plants such as photosynthesis and transpiration (Norby et al. 1999). Water deficit is regarded to be a major factor limiting plant growth (Hsiao 1973, Rhodenbaugh & Pallardy 1993). Drought is one of the most important abiotic stress factors that influence the natural distribution of woody plants and limit ecosystem production (Yang & Miao 2010). Recent studies demonstrated that drought affects a wide range of plant responses, from changes in plant metabolism to the alteration in leaf and wood anatomy (Topić et al. 2013, Stojnić et al. 2016). High temperature and drought stresses alter the physiological, biochemical and molecular responses of plants (Wahid et al. 2007, Song et al. 2014). These stresses increase tree mortality world-wide revealing climate change risk for forests (Allen et al. 2010).

Photosynthetic rate is considered to be a sensitive physiological parameter of plant metabolism and development which can be measured and used to reflect plant response to changes in environmental conditions and to evaluate the resistance of tree populations to drought and high temperatures (Liu et al. 2013, Ma et al. 2014). Induced inhibition of photosynthesis and oxidative stress well reveal the effect of lack of water in the cell, reducing plant dry biomass accumulation and productivity (Griffin et al. 2004, Wahid et al. 2007, Zhao et al. 2014). It has been demonstrated that the inhibition of photosynthesis and magnitude of oxidative stress strength largely depends on stomatal conductivity (Bacelar et al. 2007, Medrano et al. 2015). Initial response to the reduced amount of water in soil is stomatal closure, which decreases evaporation, gas exchange intensity and intercellular CO₂ concentration, thus reducing photosynthetic productivity (Bacelar et al. 2007, Song et al. 2014). Severe drought during one growing season may induce physiological responses that are carried over into the following years (Irvine et al. 1998).

Generation of reactive oxygen species (ROS) is one of the earliest responses of plant cells to heat and drought stresses. Moreover, free radicals damage plant cellular macromolecules, biomembranes and increase lipid peroxidation (Bacelar et al. 2007, Cheeseman 2007). On the other hand, they also play a signaling role, and changes in production of ROS can act as a

signal that changes the transcription of genes, thereby favoring the adaptation of plants to stresses (Jajic et al. 2015). Among ROS species, the hydrogen peroxide (H₂O₂) is an important compound involved in plant response to different environmental stressors (Belkadi et al. 2014, Jajic et al. 2015). Moreover, H₂O₂ protects photosynthetic membranes during light stress (Laloi et al. 2007). Lipid peroxidation is well-established mechanism of controlling cellular injures in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues (Dalle-Donne et al. 2003). Malondialdehyde (MDA) is usually used to evaluate status of redox and osmotic adjustment, which is important in the adaptation of plants to environmental stresses. A lower MDA gives indication of a higher anti-oxidative ability (high oxidative stress tolerance), which reflects a higher drought resistance (Apel & Hirt 2004).

Plant pigments are of key importance for photosynthetic processes and for preventing photo-oxidation and photo-inhibition (García-Plazaola & Becerril 2000). The decrease of chlorophyll content is a typical symptom of oxidative stress (Bacelar et al. 2007, Morais et al. 2007). Photosynthetic pigments are important to plants mainly for harvesting light and for the production of reducing molecules such as ATP and NADPH. Plants respond to changing environmental conditions by changes in composition of pigments in the chloroplasts (Morais et al. 2007). For proper functioning of the photosynthetic mechanism, the chlorophyll *a/b* ratio may decrease in leaves because chlorophyll *a* has a tendency to degrade faster in stressful conditions than chlorophyll *b* (Morais et al. 2007). Carotenoids are also present in plant cellular membranes and protect the membranes from light-dependent oxidative damage (Morais et al. 2007, Zhang et al. 2008). Comparison of drought-tolerant and sensitive cultivars of herbaceous species suggested that tolerance positively correlates with concentration of carotenoids in plant tissues (Davison et al. 2002, Zhang et al. 2008).

Such a high pressure by climate change-related stressors may not only affect plant physiology, but also through an intensified natural selection may result in changes in plants' genetic properties, reduction of genetic diversity and, subsequently, decline in adaptability potential of plant populations (Koskela & Lefèvre 2013). The wide distribution of temperate and boreal forest tree species throughout Europe exposed them to a variety of environmental conditions. Such a capacity for the dynamic colonisation has been attributed to a large species' adaptability (Eriksson 1998). Previous studies have shown a large variation in forest trees' responses to changing environmental conditions or impact by various climate change-related stressors (Dormling et al. 1968, Skre 1991, Weiser 1995, Kleinschmit et al. 1996, Clapham et al. 1998, Baliuckas

2002, Pliura & Eriksson 2002, Pliura & Kundrotas 2002, Baliuckas & Pliura 2003, Lauteri et al. 2004, Eriksson et al. 2005, Pliura & Baliuckas 2007, Pliura et al. 2008, 2016, Petrokas & Pliura 2014). Ecophysiological studies highlighted differential mechanisms of response to water stress depending on site conditions at the origins (Lauteri et al. 1997a, 1997b, 2004). A higher capacity of photosynthesis and transpiration in seedlings originating from arid sites than in those from mesic sites was reported (Lauteri et al. 1999). Studies on genetic variation in these traits in material from the whole distribution area of a certain tree species indicated a presence of among- and within-population genetic variation in drought tolerance (Pliura & Eriksson 2002, Lauteri et al. 2004). It has been found that different populations and genotypes show different plasticity and patterns of response to stressors. This builds a basis for a natural selection to act upon and loss of less adaptive genotypes may change a genotypic structure and reduce genetic diversity of populations. These may have a negative effect on evolution and survival of separate populations or even of the entire species. Under climate change temperate deciduous forests are predicted to shift northwards, where they are likely to replace mixed forests (Bachelet et al. 2001). Lithuania is located in the transition between the continental and boreal biogeographical regions of Europe – continental and boreal (EEA 2002) – thus northwards shifting of climatic envelope may have more pronounced impact on those forest ecosystems than in centres of biogeographical regions.

Despite extensive research efforts there is still a certain lack of knowledge on how different tree species and populations may respond to rapidly changing environmental conditions and extreme climate change-related stressors, what are the mechanisms of species' adaptation, which species have developed best strategies to cope with the stress-induced damages, and how adaptive response and phenotypic plasticity can contribute to adaptation to stressful environmental conditions. The aim of the present study was to evaluate response and plasticity of juvenile progeny of seven forest tree species, Scots pine, Norway spruce, pedunculate oak, common ash, black alder, silver birch and common aspen, and their different populations to climate change-related stressors – heat in combination with drought or elevated humidity – simulated in a controlled environment. A complex of morphometric (tree height and diameter), health condition (survival, sanitary condition and defoliation), physiological (rate of photosynthesis as net CO₂ assimilation, transpiration, stomatal conductance and intercellular CO₂ concentration, rate of photosynthetic gas exchange) and biochemical parameters (amount of photosynthetic pigments, stress-indicating biochemical compounds

like hydrogen peroxide and malondialdehyde) were used as indicators of response variability among species and populations.

Materials and methods

Tree species studied

Saplings of seven forest tree species (Scots pine, *Pinus sylvestris* L.; Norway spruce, *Picea abies* [L.] Karst.; pedunculate oak, *Quercus robur* L.; European ash, *Fraxinus excelsior* L.; black alder, *Alnus glutinosa* [L.] Gaertn.; silver birch, *Betula pendula* Roth.; and common aspen, *Populus tremula* L.) were used in the present study. Each species was represented by open-pollinated progenies from three populations representing three different ecoclimatic sub-regions of Lithuania and two biogeographical regions of Europe – continental and boreal (EEA 2002 – Tab. 1).

Treatment experiments

Prior to the treatment experiments, the saplings of all tree species except common aspen were grown in an open-air nursery, and in autumn 2015 they were transplanted into plastic squared pots (15×15×17 cm) filled with 3.5 l of peat substratum (Klasmann KTS-1, pH 5.5–6.0, with N 140 mg l⁻¹, P₂O₅ 160 mg l⁻¹, K₂O 18 mg l⁻¹, Mg 85 mg l⁻¹). Each treatment was performed using a randomized complete block design with 27 single-tree plots of each population. Two such blocks were established. In total, over 5000 saplings of all the tested tree species were used in this experiment.

The experiments were carried out in two closed walk-in chambers of greenhouse type with semi-controlled environment in a phytotron of the Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry (Central Lithuania, 55° 52' N 24° 07' E). The area of each chamber covered 60 m² and the interior volume was 240 m³. Two treatments were set to mimic forecasted stressors of climate change (namely, heat and drought, and heat and elevated humidity), and the third was control (ambient conditions). Specifically, the treatments were:

(1) Hot-wet (HW) treatment. Air temperature ranged between 25 and 40 °C during day and between 15 and 20 °C during night, and air humidity during day ranged between 60 and 80%. Plants were watered as necessary throughout the experiment. The total duration of the hot-wet treatment was two months (July and August).

(2) Hot-dry (HD) treatment. In mid-July 2016, artificial drought conditions have been created by reducing watering of tree saplings for three weeks and keeping substrate moisture at 20–30% of the total water holding capacity. To estimate soil water content, evapotranspiration and plant water status, in each treatment, 15 pots with substrate and saplings of each tree species (5 per population) were weighted every second day, allowing the estimation of water loss in the pots at a given moment. The

Tab. 1 - Data on origin of populations of seven forest tree species used in the experiment. (a): <http://www.geoportal.lt> (2016); (b): Chromov (1968); (c): EEA (2002).

Tree species	Population	Long Lat	Altitude (m a.s.l.)	Climatic sub-region ^(a)	Climate continentality index ^(b)	Temperature sum >10 °C (days) ^(c)	Annual precipitation (mm) ^(c)	Biogeographic region ^(c)
<i>Pinus sylvestris</i>	Jonava	55°01' N 24°28' E	40	6	30	2200	620	Contin.
	Varena	54°04' N 24°30' E	125	9	31	2170	650	Contin.
	Anykščiai	55°38' N 25°06' E	120	8	30	2150	700	Boreal
<i>Picea abies</i>	Rietavas	55°32' N 22°24' E	140	5	26	2000	850	Boreal
	Kaunas	54°59' N 23°55' E	74	7	30	2220	640	Contin.
	Anykščiai	55°32' N 24°59' E	125	8	30	2150	700	Boreal
<i>Quercus robur</i>	Tytuvėnai	55°39' N 23°01' E	122	5	27	2100	700	Boreal
	Jonava	55°05' N 24°15' E	90	6	30	2200	620	Contin.
	Anykščiai	55°33' N 24°55' E	103	8	30	2150	700	Boreal
<i>Fraxinus excelsior</i>	Bartoszyce	54°13' N 20°46' E	72	9	31	2400	700	Contin.
	Lelkovo	54°15' N 20°11' E	111	9	31	2400	700	Contin.
	Kozlovo	53°31' N 20°22' E	339	9	31	2300	700	Contin.
<i>Betula pendula</i>	Raseiniai	55°20' N 22°57' E	110	7	28	2070	700	Contin.
	Ukmerge	55°22' N 24°32' E	126	6	30	2200	630	Boreal
	Anykščiai	55°40' N 25°10' E	113	8	30	2150	700	Boreal
<i>Alnus glutinosa</i>	Tytuvėnai	55°34' N 23°18' E	131	5	27	2100	700	Boreal
	Jonava	55°00' N 24°15' E	61	6	30	2200	620	Contin.
	Valkininkai	54°24' N 24°34' E	159	9	31	2150	670	Boreal
<i>Populus tremula</i>	Tytuvėnai	55°37' N 23°21' E	110	5	27	2100	700	Boreal
	Dubrava	54°51' N 24°04' E	80	7	30	2210	650	Contin.
	Anykščiai	55°34' N 24°50' E	115	8	30	2150	700	Boreal

water loss was calculated by comparing weight of these pots with that of the same pots with fully saturated substrate (weighted prior to treatment). When soil moisture for certain tree species dropped below 20% of the total water capacity, a limited irrigation (flooding) from below was applied. During the period of artificial drought, air humidity in the climatic chamber during day ranged between 40 and 60%. After this treatment the plants were watered to restore full water holding capacity of peat substrate.

(3) Control (ambient conditions, Amb). Saplings were grown outside the phytotron in an open air. During the experiment, outside air temperature ranged between 20 and 28 °C during day and between 12 and 17 °C during night, and air humidity ranged between 60 and 80%. Plants were watered when necessary throughout

the experiment to keep substrate moisture in pots between 70 and 90% of full water holding capacity. The total duration of the control treatment was four months (May–August). In the year the present study was performed (2016), no natural droughts or heat waves have occurred at the experimental site, thus control treatment corresponded to ambient climatic conditions almost unaffected by climate change.

Temperature regimes were equal both in HW and HD treatments. In growth chambers, light intensity (PAR 400–700 nm) at the top of the sapling crowns in clear days ranged between 1220–1370 μmol m⁻² s⁻¹ while in open air between 1670–2090 μmol m⁻² s⁻¹.

Assessment of sapling morphometric and physiological parameters

Total height (above the ground) and stem

diameter at the root collar were measured for each sapling before and by the end of vegetation season of 2016. Height and diameter increments were calculated for each sapling based on their corresponding heights and diameters. Sapling health condition was scored in autumn 2016 using a categorical scale ranging from 5 (excellent condition, no visible damages) to 1 (very bad condition, dying). Survival was assessed in autumn 2016 as the ratio between a number of survived trees and the total number of alive trees at the beginning of vegetation season. Sapling defoliation (leaf/needle loss) was estimated as a fraction of shed leaves/needles at the end of each treatment of the initial amount of leaves/needles on a certain sapling (recorded at the beginning of the experiment).

Rates of photosynthesis as net CO₂ assimilation (A, μmol m⁻² s⁻¹), transpiration (E, mmol m⁻² s⁻¹), stomatal conductance (g_s, mol m⁻² s⁻¹) and intercellular CO₂ concentration (C_i, μmol mol⁻¹) were recorded using a LCpro-SD[®] portable photosynthesis system (ADC BioScientific Ltd, UK). Light conditions inside a leaf chamber were set using the LCpro-SD light unit, which emitted photosynthetically active radiation (PAR) at 1000 μmol m⁻² s⁻¹. The air supply unit provided a flow of ambient air to the leaf chamber at a constant rate of 200 μmol s⁻¹. Air humidity was set at 20 mBar of partial water pressure; temperature and CO₂ concentration were at ambient levels. Readings were taken under steady-state conditions (after 1 min). In each treatment, photosynthetic gas exchange was assessed for nine randomly selected trees of each of the seven species from each population (Tab. 1). For the gas exchange measurements we selected: (i) for deciduous trees, a fully expanded and exposed fifth leaf from the top of the leader shoot of each sapling, and (ii) for conifers, all needles on the first (from tree top) lateral annual shoot of each sapling. The measurements were carried out at the end of experiment (beginning of August) from 10:00 to 14:30 a.m.

Instantaneous water use efficiency (WUE, μmol mmol⁻¹) was computed as the ratio between net photosynthesis and transpiration (A/E), and intrinsic water use efficiency (WUE_i, μmol mol⁻¹) was computed as the ratio between net photosynthesis and stomatal conductance (A/g_s – Farquhar et al. 1989).

To estimate sapling response to different stressors, the amounts of produced malondialdehyde (MDA), hydrogen peroxide (H₂O₂), and photosynthetic pigments (chlorophylls *a* and *b* and carotenoids) were assessed. For analysis of MDA and H₂O₂, fresh leaf material (0.5 g) was homogenized using 5% (w/v) trichloroacetic acid (TCA). Lipid peroxidation was estimated as the amount of MDA produced by 2-thiobarbituric acid (TBA – Kramer et al. 1991). The homogenates were centrifuged at 12,130 g for 17 min, and 2.0 ml of supernatant was added

to 3.0 ml of 20% (w/v) TCA containing 0.50% (w/v) TBA. The homogenates were incubated at 95 °C for 30 min and subsequently cooled on ice. After cooling, the homogenates were centrifuged at 12,130 g for 17 min, and MDA content was determined by subtracting absorbance value recorded at 600 nm from the absorbance value recorded at 532 nm and using an absorbance coefficient of 155 mM⁻¹ cm⁻¹. H₂O₂ content in leaves was determined according to Velikova et al. (2000). A 0.5 ml of supernatant was added to 0.5 ml of potassium phosphate buffer (10 mM, pH 7.0) mixed with 1.0 ml of potassium iodide (1 M). The reaction solution was incubated for 30 min at 25 °C in the dark. The absorbance of the supernatant was measured at 390 nm wavelength. The amount of H₂O₂ was calculated using a standard curve based on known concentrations.

The photosynthetic pigments were extracted from 0.05 g of fresh leaf/needle material in 3 ml of N,N'-dimethylformamide (DMF). The light absorption was measured at 480, 664, 647 nm wavelengths. Chlorophyll *a/b* ratios and amounts of chlorophylls *a* and *b* and carotenoids were calculated according to Wellburn (1994).

All spectrophotometrical measurements were made using the spectrophotometer SPECORD[®] 210 Plus (Analytik Jena AG, Germany). All data are expressed on a leaf fresh weight basis.

Statistical analysis

The variance analysis of data was performed using the MIXED procedure in SAS (SAS[®] Analytics Pro 12.1, 9.3, SAS Institute Inc. 2016) which is based on Mixed model equations (MME) and restricted maximum likelihood (REML) method. The significance of fixed effects was tested using an *F*-test. The following linear models were used for joint analyses (eqn. 1) of all treatments together and for separate analyses (eqn. 2) of individual treatments:

$$Y_{ijklm} = \mu + T_j + S_k + P_{i(k)} + S_{i(k)} \cdot T_j + P_k \cdot T_j + B_m + e_{ijklm} \quad (1)$$

$$Y_{iklm} = \mu + S_k + P_{i(k)} + B_m + e_{iklm} \quad (2)$$

where Y_{ijklm} is an observation on l^{th} tree from i^{th} population in k^{th} tree species in m^{th} block in j^{th} treatment, Y_{iklm} is an observation on l^{th} tree from i^{th} population in k^{th} species in m^{th} block, μ is the overall mean, T_j is the effect of j^{th} treatment, B_m is the effect due to the m^{th} block, S_k is the effect due to the k^{th} tree species, $P_{i(k)}$ is the effect of i^{th} population in k^{th} species, $P_{i(k)} \cdot T_j$ is the effect of interaction between i^{th} population in k^{th} species and j^{th} treatment, $S_k \cdot T_j$ is the effect of interaction between k^{th} species and j^{th} treatment, ε_{ijklm} and ε_{iklm} are the random residuals. The models assume that all effects are fixed.

Assumptions of normal distribution of residuals and variance homogeneity for each trait were tested by using the Shapiro-Wilk tests (Sabin & Stafford 1990)

with the GLM and UNIVARIATE procedures in SAS. Least-square means estimates were obtained for species and populations at each treatment and statistical significance (at $P < 0.05$) of differences between least-squares means was tested using a *t*-test. Pearson's correlation between growth, tree condition and physiological parameters were calculated for each tree species, SAS CORR procedure was used.

To evaluate the stability of individual populations across treatments (including control) and the contribution of each of the populations to the population × treatment interaction variances, the Wricke ecovalence values (Wricke 1962) were calculated using variances and least-square means obtained within each treatment with SAS MIXED procedure and expressed as a percent of the total population × treatment interaction variance. The Shukla stability variances were computed and the statistical significance of the ecovalences was tested using the *F*-test developed by Shukla (1972). In calculating ecovalences, to better fulfill the assumptions behind the linear model and thus to reduce scale effects of different treatments in joint analysis, the data were transformed to equal population variance. Phenotypic plasticity of each population was estimated as difference between maximum and minimum least-squares means obtained across treatments.

In order to evaluate multidimensional differences among populations and among species based on the complex of tree growth, health condition, physiological and biochemical traits, a Rotated Principal Factor Analysis was performed on all data across all treatments using the FACTOR procedure in SAS software (SAS[®] Analytics Pro 12.1, 9.3, SAS Institute Inc 2016). SAS procedure SCORE was used to score population means based on output from FACTOR procedure. Its outputs were used to plot populations' means in coordinates of main factors applying the SAS procedure PLOT.

Results

Treatment effect

The results of multifactor analysis of variance using a statistical model (1) showed that the impact of treatment on sapling morphometric, physiological and biochemical traits was highly significant ($P < 0.001$) or significant ($P < 0.01$), except tree health condition, transpiration, photosynthetic rates and WUE (Tab. 2). Compared to control, stem diameter increment was lower in most tested tree species following both hot-wet and hot-dry treatments, while differences in height growth were less pronounced (Fig. 1). For most species this resulted in an increase of height to diameter ratio under both hot-wet and hot-dry conditions compared to control (Fig. 1c). In general, an increase in tree defoliation was observed in hot-dry conditions, and among

Tab. 2 - Results from joint mixed linear model (eqn. 1) analysis of variance of different morphometric, health condition, physiological and biochemical traits in twenty one tested populations of seven forest tree species following hot-wet and hot-dry treatments and in ambient conditions (control): *F*-criteria and probabilities (*P*) of the fixed effects. For the definition of traits, see Materials and Methods. (ne): Not estimated.

Trait	Treatment		Block		Species		Populations		Species × treatment		Population × treatment	
	F	P	F	P	F	P	F	P	F	P	F	P
Height increment (ZH)	8.8	<0.001	0.3	0.559	3471.1	<0.001	71.6	<0.001	5.0	<0.001	2.1	0.001
Diameter increment (ZD)	37.8	<0.001	0.9	0.333	1891.4	<0.001	11.5	<0.001	3.9	<0.001	2.1	0.001
H/D ratio	49.9	<0.001	0.1	0.766	2249.2	<0.001	18.4	<0.001	11.2	<0.001	1.8	0.005
Defoliation	108.6	<0.001	43.5	<0.001	1018.1	<0.001	22.4	<0.001	29.9	<0.001	3.0	<0.001
Tree condition	0.6	0.540	2.7	0.099	47.2	<0.001	14.1	<0.001	1.7	0.065	1.1	0.327
Survival	7.0	0.001	0.1	0.708	18.1	<0.001	7.1	<0.001	2.3	0.007	0.5	0.994
Transpiration rate	3.8	0.051	ne	ne	161.4	<0.001	1.7	0.050	42.6	<0.001	3.5	<0.001
Stomatal conductance	15.3	<0.001	ne	ne	103.0	<0.001	1.7	0.058	14.0	<0.001	3.4	<0.001
Photosynthetic rate	1.4	0.232	ne	ne	92.0	<0.001	4.6	<0.001	10.7	<0.001	3.8	<0.001
WUE	0.6	0.440	ne	ne	8.5	<0.001	2.0	0.014	8.7	<0.001	2.1	0.001
WUEi	6.5	0.011	ne	ne	4.0	0.001	2.4	0.003	3.3	<0.001	2.4	<0.001
Chlorophyll <i>a</i>	3469.4	<0.001	ne	ne	20794.3	<0.001	846.9	<0.001	445.8	<0.001	292.7	<0.001
Chlorophyll <i>b</i>	2528.9	<0.001	ne	ne	6001.3	<0.001	330.8	<0.001	140.1	<0.001	89.5	<0.001
Chlorophyll <i>a/b</i> ratio	2251.8	<0.001	ne	ne	441.2	<0.001	109.0	<0.001	41.3	<0.001	29.4	<0.001
Carotenoids	550.7	<0.001	ne	ne	11439.4	<0.001	459.4	<0.001	132.7	<0.001	141.4	<0.001
MDA	1170.5	<0.001	ne	ne	12166.8	<0.001	279.3	<0.001	693.7	<0.001	228.5	<0.001
H ₂ O ₂	7761.9	<0.001	ne	ne	32792.7	<0.001	1813.5	<0.001	955.4	<0.001	704.7	<0.001

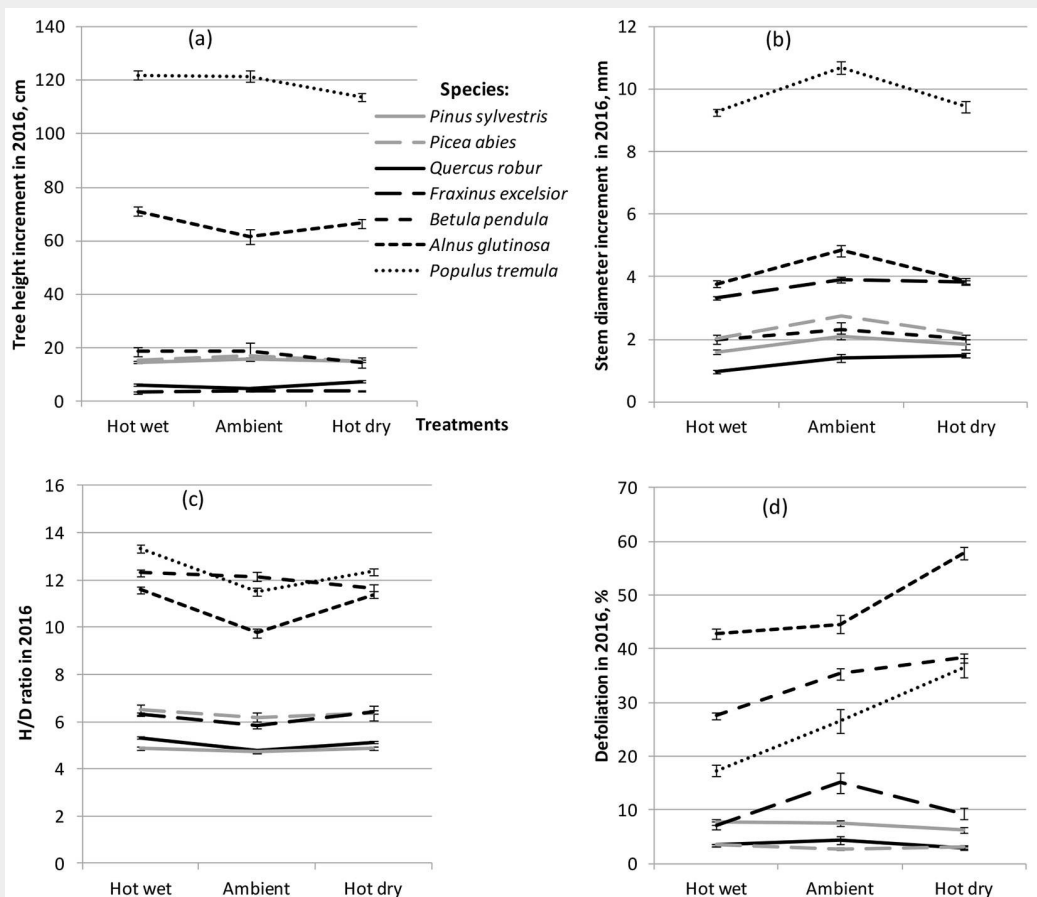
the most severely defoliated were three pioneer tree species *P. tremula*, *A. glutinosa* and *B. pendula* (Fig. 1d). Meanwhile, defoliation rates were lower under the hot-wet treatment for most of the tested tree species.

Absence of significant treatment effect in variation of transpiration rate, photosyn-

thetic rate and WUE (Tab. 2) may be explained by highly differing reaction of different tree species and populations to the treatments applied (Fig. 2a, Fig. 2b, Fig. 2c) which is indicated by highly significant species and species-by-treatment interaction effects (Tab. 2). For most tested species transpiration and photosynthetic rates

were higher following hot-wet treatment than under the hot-dry treatment (Fig. 2a, Fig. 2b). Highly different reactions of the tested species were observed in the hot-wet treatment, while under the hot-dry treatment all species showed lower transpiration and photosynthetic rates as compared to control (Fig. 2a, Fig. 2b). For WUE,

Fig. 1 - Performance of seven forest tree species following hot-wet, hot-dry and control (Ambient) treatments. (a) Tree height increment; (b) stem diameter increment; (c) tree height to stem diameter ratio (H/D); (d) tree defoliation.



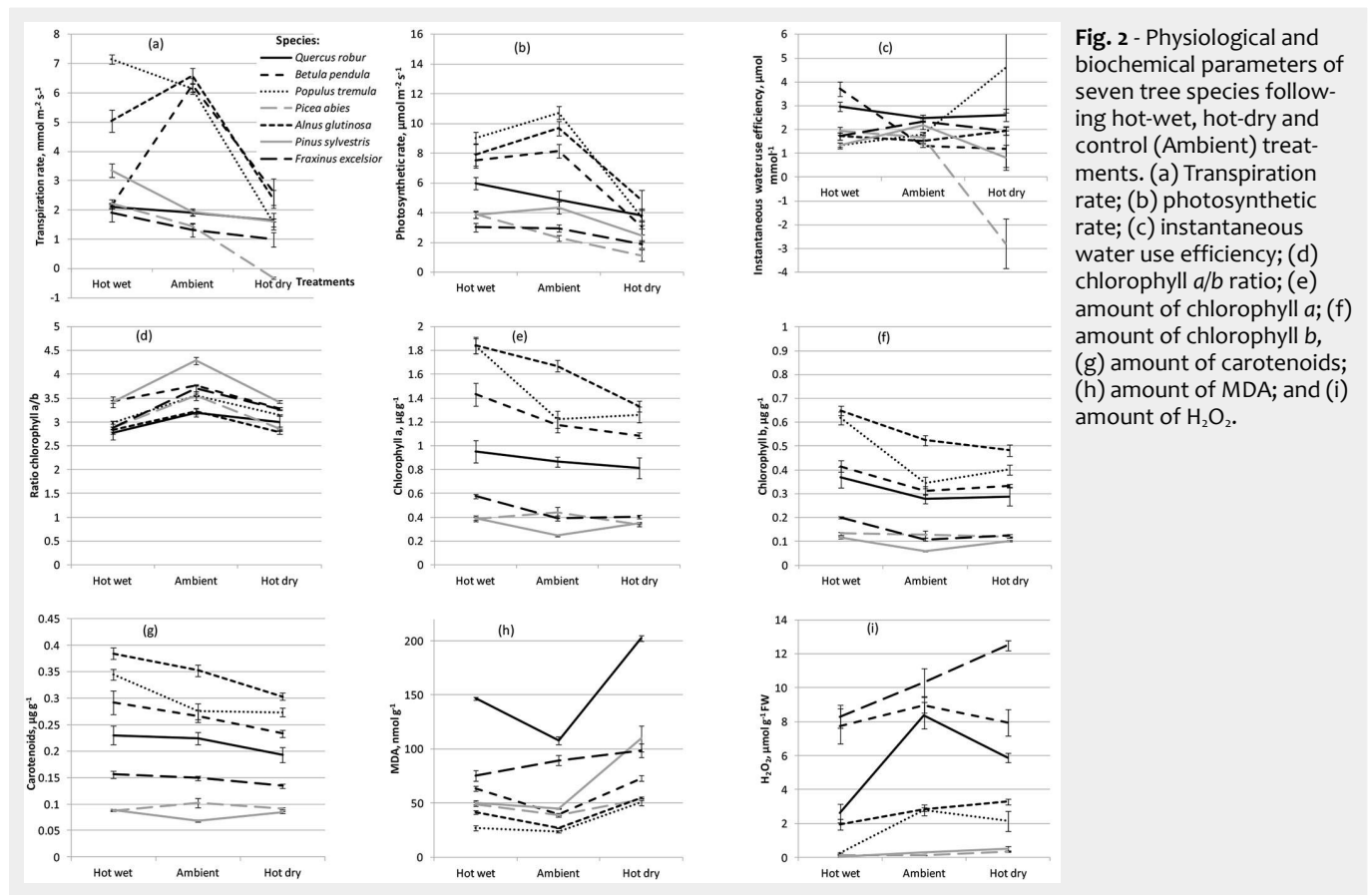


Fig. 2 - Physiological and biochemical parameters of seven tree species following hot-wet, hot-dry and control (Ambient) treatments. (a) Transpiration rate; (b) photosynthetic rate; (c) instantaneous water use efficiency; (d) chlorophyll *a/b* ratio; (e) amount of chlorophyll *a*; (f) amount of chlorophyll *b*; (g) amount of carotenoids; (h) amount of MDA; and (i) amount of H_2O_2 .

the reactions varied a lot among treatments and tree species (Fig. 2c). Compared to control, a clear reduction in chlorophyll *a/b* ratio was observed following both hot-wet and hot-dry treatments (Fig. 2d). Amounts of chlorophylls *a* and *b* as well as amount of carotenoids in leaves and needles in most tree species (except *P. abies*) under hot-wet conditions were greater than under ambient and hot-dry conditions (Fig. 2e-2g).

Compared to control, the amount of MDA was greater under both hot-wet and hot-dry treatments in all tree species except *F. excelsior*; for *F. excelsior* MDA production was higher under hot-dry but lower under hot-wet conditions (Fig. 2h). Amount of H_2O_2 was lower following hot-wet treatment (compared to control), while under hot-dry treatment it largely varied among different species. For example, *Q. robur* showed a clear reduction in production of H_2O_2 following both heat treatments, while both conifer species showed almost no reaction (Fig. 2i).

Species effect

ANOVA showed that species effect was highly significant ($P < 0.001$) for all the studied traits (Tab. 2). The largest height and diameter increments in all treatments were observed in fast growing deciduous tree species *P. tremula* and *A. glutinosa* (Fig. 1a, Fig. 1b). Height increment in these tree species varied from 113.6 ± 1.4 (mean \pm standard deviation) to 122.0 ± 1.7 cm and from 66.5 ± 1.7 to 71.1 ± 1.8 cm, respec-

tively. In noble hardwoods *Q. robur* and *F. excelsior*, height increment was much smaller and ranged between 4.6 ± 0.4 and 7.5 ± 0.5 cm in *Q. robur* and between 3.3 ± 0.3 and 3.9 ± 0.4 cm in *F. excelsior* (Fig. 1a). Largest height to diameter ratio was observed for both these species and also for *B. pendula* (Fig. 1c). Nevertheless, *A. glutinosa*, *B. pendula* and *P. tremula* showed the highest degree of defoliation among all treatments (Fig. 1d). Rather intense defoliation of these species was observed even at ambient (control) conditions, which most likely was a result of the limited light availability because of close plant spacing.

Transpiration and photosynthetic rates were also the highest in *P. tremula*, *B. pendula* and *A. glutinosa*, although for all three species these parameters were much more affected by water deficit than for other investigated tree species (Fig. 2a, Fig. 2b).

The smallest, though significant, differences among the tested tree species were observed in chlorophyll *a/b* ratio (Fig. 2d). Compared to control, very large differences among species were found in the amount of chlorophyll *a*, while for chlorophyll *b* the differences were smaller (Fig. 2e, Fig. 2f). As regards species ranking, very similar pattern in the content of all pigments (chlorophylls *a* and *b* and carotenoids) were observed. For example, in *A. glutinosa* and *P. tremula* amounts of pigment were always the highest, while in *P. sylvestris* always the lowest, regardless the treatment. The highest amount of MDA was found in both noble hardwoods, *Q.*

robur and *F. excelsior*, thus indicating the highest levels of lipid peroxidation as a response to oxidative stress, while the smallest MDA amount was in *A. glutinosa* and *P. tremula* (Fig. 2h). The highest amount of H_2O_2 was found in *F. excelsior* and *B. pendula* followed by *Q. robur*, while the lowest amount was observed in both conifer species *P. sylvestris* and *P. abies*, regardless the treatment (Fig. 2i).

Species-by-treatment interaction

The species-by-treatment interaction was found to be significant ($P < 0.05$) for all studied traits except tree condition (Tab. 2). This interaction is manifested by differing patterns of reaction and species rank changes across treatments (Fig. 1, Fig. 2). The most variable species' performances across treatments were observed for transpiration rate, concentration of chlorophyll *a*, and amount of MDA and H_2O_2 (Tab. 2, Fig. 2a, Fig. 2e, Fig. 2h, Fig. 2i). Under the hot-dry treatment, most species showed slightly reduced height and stem diameter increments (compared to control); however, some species such as *Q. robur* for both height and stem diameter increment and *A. glutinosa* for height increment, showed opposite reactions (Fig. 1a, Fig. 1b). All tested tree species except *Q. robur* showed somehow lower stem diameter increment in both stressful environments as compared to ambient conditions (Fig. 1b). *P. tremula* and *B. pendula* displayed the lowest level of drought tolerance, i.e., for these species a noticeable reduction in

height growth following hot-dry treatment was observed (Fig. 1a). Compared to control, height/diameter ratio was clearly higher in *P. tremula* and *A. glutinosa* under both heat treatments, while in other tree species the treatment effect was rather small (Fig. 1c). *Q. robur*, however, showed the highest diameter increment following hot-dry treatment (Fig. 1b). In the present study, most severe negative effect of drought was manifested by the dramatic loss of foliage in fast growing tree species *P. tremula*, *A. glutinosa* and *B. pendula* (Fig. 1d). Meanwhile, for *F. excelsior*, *Q. robur* and conifers the defoliation rate was at the same level or even lower compared to control (Fig. 1d).

Transpiration, stomatal conductance and photosynthetic rates in fast growing deciduous species *P. tremula*, *B. pendula* and *A. glutinosa* were much more affected by water deficit than in other investigated tree species (Fig. 2a, Fig. 2b). These three species showed the highest transpiration rate in ambient conditions and, compared to control, have substantially reduced this rate under hot-dry treatment, while following hot-wet treatment the reduction in transpiration was observed only for *A. glutinosa* and *B. pendula*. In addition to *P. tremula*, noble hardwoods *Q. robur* and *F. excelsior* as well as coniferous species *P. sylvestris* and *P. abies* showed the highest transpiration rates in hot-wet treatment compared to both ambient and hot-dry conditions (Fig. 2a). All investigated tree species except *Q. robur* has decreased CO₂ stomatal conductance following hot-dry treatment, while following hot-wet treatment it increased in all tree species except *A. glutinosa* and *P. tremula* (data not shown). All species showed a decrease in photosynthetic rate under the hot-dry treatment, with sharpest reduction in *P. tremula*, *A. glutinosa* and *B. pendula* (Fig. 2b). All species except *Q. robur* and *P. abies* showed lower (compared to control) photosynthetic rate in hot-wet treatment (Fig. 2b). As regards WUE, differences among the tested tree species were not large in ambient conditions, although after the hot-dry treatment large differences in response among species were observed: *P. tremula*, *A. glutinosa* and *Q. robur* showed an increase, while *P. abies*, *P. sylvestris* and *B. pendula* showed a clear decrease compared to both control and hot-wet treatments (Fig. 2c). For most species (except *P. abies*), higher pigment concentrations were observed following hot-wet treatment compared to both ambient and hot dry conditions (Fig. 2e-Fig. 2g).

Amounts of MDA and H₂O₂ showed significant fluctuations across treatments for different tree species (Fig. 2h, Fig. 2i). Compared to control, the amount of MDA under hot-dry conditions was higher for all tested tree species, and under hot-wet conditions the reactions of most tested tree species were similar: only *F. excelsior* showed lower MDA amount compared to

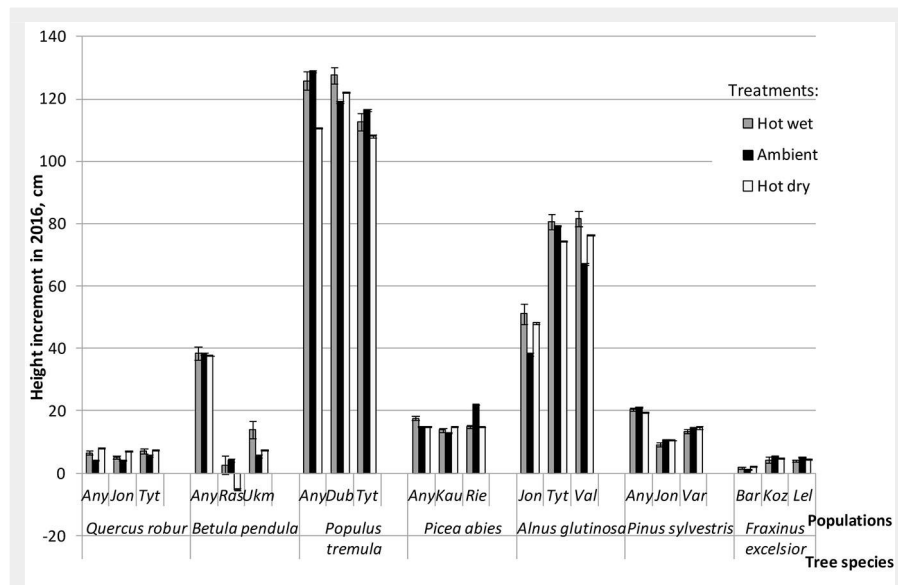


Fig. 3 - Mean tree height increment of populations of seven forest tree species following hot-wet (grey bars), hot-dry (white bars) and control (Ambient – black bars) treatments. Error bars represent the standard error.

ambient conditions (Fig. 2h). For *Q. robur*, the amount of MDA was significantly higher and the amount of H₂O₂ was significantly lower (compared to control) following both heat treatments. For *P. sylvestris*, a clear (statistically significant) increase in MDA amount (compared to control) was observed following hot-dry treatment, while under hot-wet treatment the increase was negligible. Four tree species (*A. glutinosa*, *F. excelsior*, *P. sylvestris* and *P. abies*) under hot dry conditions showed higher H₂O₂ levels than under both ambient and hot wet conditions (Fig. 2i). *Q. robur*, *B. pendula* and *B. tremula* showed the highest amount of H₂O₂ in ambient conditions com-

pared to both hot-wet and hot-dry treatments (Fig. 2i).

Population effect

ANOVA showed that population effect (within tree species) was highly significant ($P < 0.001$) for most traits except for stomatal conductance, transpiration rate, WUE and WUE_i, where significance of the population effect was lower (Tab. 2). Within tree species, significant inter-population differences were observed following each of the three treatments: in height increment for *B. pendula* and *A. glutinosa* (Fig. 3), in defoliation rate for *B. pendula* and *P. tremula* (Fig. 4), while for photosynthetic rate the

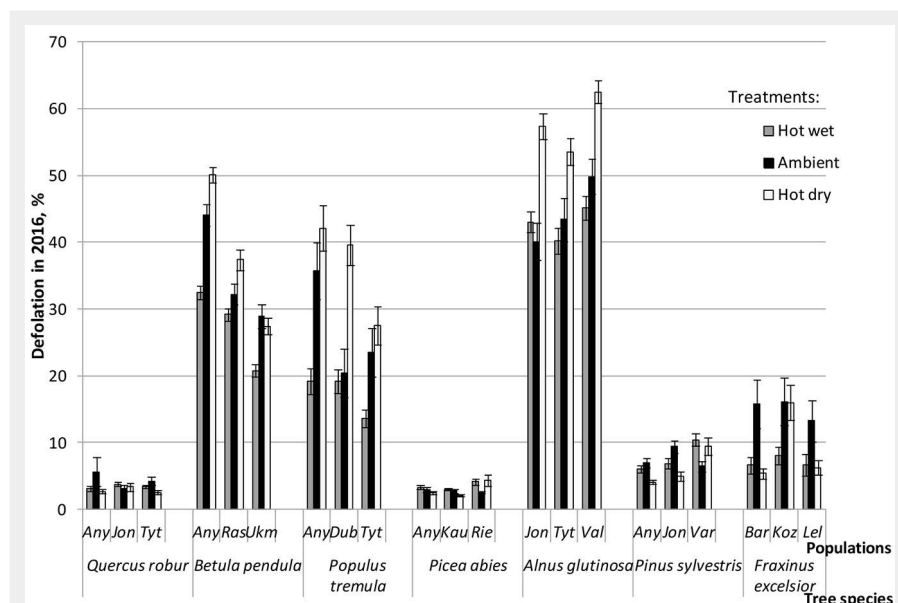


Fig. 4 - Mean defoliation of populations of seven forest tree species following hot-wet (grey bars), hot-dry (white bars) and control (Ambient – black bars) treatments. Error bars represent the standard error.

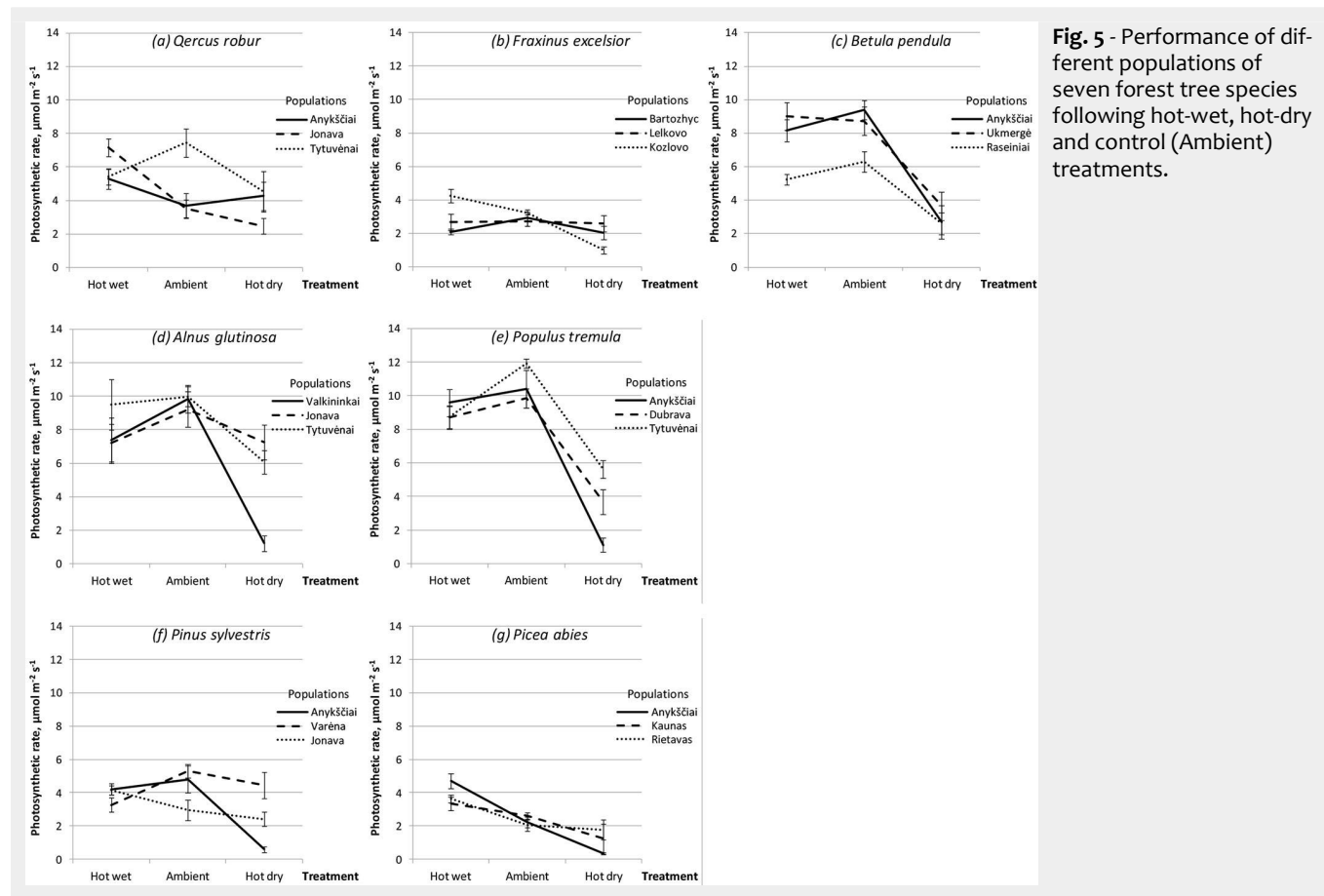


Fig. 5 - Performance of different populations of seven forest tree species following hot-wet, hot-dry and control (Ambient) treatments.

magnitude of these differences varied a lot among populations depending on treatment (Fig. 5). Very high inter-population genetic variation (CV_p) in height increment following all treatments was found for *B. pendula* and *F. excelsior*, reaching up to 166.2 and 59.5%, respectively (Tab. 3). High variation was also found for these two species also in diameter increment, reaching up to 51.2 and 39.2%, respectively. Inter-population variation in defoliation rate ranged from low to medium for most tree

Tab. 3 - Inter-population genetic variation (CV_p , %) of morphometric, health condition, physiological and biochemical traits of seven tree species following hot-wet (HW) and hot-dry (HD) treatments and in ambient conditions (control, Amb). For the definition of traits, see Materials and Methods.

Species	Treatment	Height increment	Diameter increment	Defoliation	Tree condition	Survival	Height/diameter ratio	Transpiration rate	Stomal conductance rate	Photosynthetic rate	WUE	Chlorophyll a/b ratio	Amount				
													Chlorophyll a	Chlorophyll b	Carotenoids	MDA	H ₂ O ₂
<i>Pinus sylvestris</i>	HW	39.9	10.9	30.0	7.0	7.3	8.1	12.4	19.4	13.4	7.0	15.0	19.1	20.2	10.6	18.6	149.2
	Amb	34.0	11.1	20.5	3.0	3.5	3.0	16.6	12.9	28.6	14.2	6.9	13.9	18.7	16.3	5.1	35.9
	HD	30.3	27.3	46.2	6.3	6.6	6.3	51.4	58.7	77.1	174.2	3.2	11.5	14.2	8.8	50.2	119.9
<i>Picea abies</i>	HW	12.8	22.6	16.8	0.5	0.9	4.3	13.5	39.0	18.2	19.6	5.4	22.9	17.3	3.2	19.6	133.4
	Amb	28.4	8.0	7.3	0.6	0.0	5.3	20.5	37.0	12.5	20.0	11.6	44.7	54.3	39.4	20.6	20.5
	HD	0.4	23.8	41.6	1.3	1.4	4.1	37.0	96.2	63.8	81.4	7.4	18.9	13.2	9.7	10.1	28.1
<i>Quercus robur</i>	HW	16.8	23.0	9.5	2.7	1.2	18.3	20.3	16.6	17.4	20.9	21.8	45.2	54.1	33.7	4.6	93.4
	Amb	15.9	36.4	28.7	1.3	0.0	18.3	26.2	44.2	45.5	15.3	11.6	21.9	33.4	21.7	15.5	42.7
	HD	5.7	14.0	15.3	0.4	1.1	15.5	4.9	3.3	30.0	34.6	22.5	48.7	59.4	33.0	6.1	21.3
<i>Fraxinus excelsior</i>	HW	42.6	15.7	11.2	8.4	2.4	3.7	32.5	37.8	36.8	13.0	5.0	13.4	9.5	20.5	28.2	36.9
	Amb	59.5	39.2	10.4	2.5	0.0	9.9	9.9	6.9	8.6	16.3	8.1	22.5	26.4	11.7	23.7	37.0
	HD	37.8	34.8	63.9	5.7	0.7	1.6	18.4	35.8	43.0	13.8	3.6	17.6	21.0	13.8	29.2	11.0
<i>Betula pendula</i>	HW	99.8	41.6	22.0	8.5	4.7	10.0	9.9	10.6	26.4	34.5	4.4	30.7	26.6	34.2	15.8	60.3
	Amb	121.2	29.2	22.6	5.5	4.8	4.2	16.8	19.0	20.0	13.8	1.4	24.8	25.8	19.1	10.7	23.8
	HD	166.2	51.2	29.6	10.7	6.5	7.5	17.4	19.4	19.2	154.5	2.9	9.6	9.0	14.4	16.3	45.5
<i>Alnus glutinosa</i>	HW	24.4	12.6	5.8	2.2	0.0	3.6	14.7	8.7	15.8	8.4	5.0	17.6	13.2	13.1	19.0	77.1
	Amb	34.3	6.7	11.1	5.5	1.2	1.6	10.5	19.3	4.1	8.4	1.8	12.8	14.6	12.9	3.8	17.3
	HD	23.7	13.7	7.8	4.7	2.3	3.6	53.6	74.3	65.7	31.2	6.1	16.2	22.6	10.3	2.5	24.9
<i>Populus tremula</i>	HW	6.7	4.7	18.4	0.6	0.0	1.6	12.5	9.3	5.6	19.0	4.8	15.8	20.0	13.0	34.9	92.0
	Amb	5.4	7.9	30.4	1.1	0.0	3.1	12.3	4.9	9.9	18.9	4.5	27.2	32.3	24.4	17.7	50.8
	HD	6.5	2.1	21.4	0.8	0.0	4.1	60.7	99.0	65.0	83.6	1.2	23.8	23.2	13.5	29.2	124.3

Tab. 4 - Phenotypic plasticity and ecovalence in height increment over treatments of different populations of seven tree species following hot-wet (HW), hot-dry (HD) and control (Amb) treatments. For the definition of variables, see Materials and Methods.

Species	Population	Mean deviation estimate	Estimates of deviation from treatment means			Phenotypic plasticity	Wricke ecovalence	Shukla variance	F	P
			HW	Amb	HD					
<i>Pinus sylvestris</i>	Anykščiai	-14	-14.6	-13.1	-14.2	1.4	0.2	-0.1	-0.01	.
	Jonava	-24.2	-25.5	-23.5	-23.6	2	0.5	0.8	0.08	0.921
	Varena	-20.2	-21.4	-19.7	-19.4	2.1	0.5	0.7	0.08	0.928
<i>Picea abies</i>	Anykščiai	-18.6	-17.3	-19.5	-19	2.3	0.6	0.9	0.14	0.868
	Kaunas	-20.4	-21	-21.3	-19	2.3	0.6	1.1	0.14	0.872
	Rietavas	-17.1	-19.8	-12.4	-19.1	7.4	6.7	17.7	2.73	0.065
<i>Quercus robur</i>	Anykščiai	-28.1	-28.1	-30.1	-26.2	3.8	1.5	3.4	0.39	0.677
	Jonava	-28.9	-29.5	-30.1	-27.1	3	1	2.1	0.24	0.783
	Tytuvenai	-27.7	-27.6	-28.8	-26.8	2	0.4	0.5	0.06	0.944
<i>Fraxinus excelsior</i>	Bartoszyc	-32.7	-32.8	-33	-32.2	0.8	0.1	-0.5	-0.05	.
	Kozlowo	-29.6	-30.3	-28.9	-29.5	1.4	0.2	-0.1	-0.01	.
	Lelkowo	-29.8	-30.5	-29.2	-29.8	1.3	0.2	-0.2	-0.02	.
<i>Betula pendula</i>	Anykščiai	3.8	3	3.8	4.7	1.7	0.3	0.1	0.02	0.981
	Raseiniai	-34	-31.8	-30.1	-39.9	9.8	11.1	29.8	3.37	0.034
	Ukmerge	-25.5	-20.9	-28.9	-26.8	8	6.9	18.3	2.33	0.098
<i>Alnus glutinosa</i>	Jonava	11.5	15.2	3.7	15.5	11.9	18.3	49.6	5.68	0.003
	Tytuvenai	43.7	43.9	44.5	42.8	1.6	0.3	0.1	0.01	0.986
	Valkininkai	40.7	44.9	32.3	44.9	12.7	21.4	58.1	6.77	0.001
<i>Populus tremula</i>	Anykščiai	87.4	87.7	93.8	80.6	13.3	17.7	48	5.6	0.004
	Dubrava	88.6	89.4	84	92.5	8.5	7.4	19.8	2.32	0.099
	Tytuvenai	78.1	74.9	81.4	77.9	6.5	4.3	11	1.29	0.277

species, although following hot dry treatment the variation was rather substantial for *F. excelsior*, *P. sylvestris* and *P. abies*, reaching up to 63.9, 46.2 and 41.6%, respectively (Tab. 3).

Inter-population genetic variation in transpiration, stomatal conduction and photosynthetic rates varied a lot depending on treatment (Tab. 3). Clearly the highest inter-population variation in these traits was observed for *P. sylvestris*, *P. abies*, *A. glutinosa* and *P. tremula* under the hot-dry treatment, for *F. excelsior* in the hot-wet treatment (in transpiration and stomatal conduction rates only), and for *Q. robur* at ambient conditions (Tab. 3). In the most stressful hot-dry treatment, inter-population genetic variation in WUE was significantly larger compared to both hot-wet treatment and control for all tree species except *F. excelsior* (Tab. 3). Extremely large inter-population variation in WUE was found for *P. sylvestris*, *B. pendula*, *P. tremula* and *P. abies*. The highest inter-population variation in amount of chlorophylls *a* and *b* and their ratio was observed for *Q. robur* and *A. glutinosa* following hot-dry treatment, for *P. sylvestris* and *B. pendula* following hot-wet treatment, and for *P. abies*, *F. excelsior* and *P. tremula* at ambient conditions. Similar variation patterns among the tested tree species were observed in the concentration of carotenoids (Tab. 3).

As regards the amount of MDA, the highest inter-population variation was observed for *P. sylvestris*, *F. excelsior* and *B. pendula* under the hot-dry treatment, for *A. glutinosa* and *P. tremula* following hot-wet treatment, and for *P. abies* and *Q. robur* in ambient conditions (Tab. 3). Very

high variation in the amount of H₂O₂ was found for *P. sylvestris* and *P. tremula* following the hot-dry treatment, meanwhile in the hot-wet treatment the variation was very high for *P. sylvestris*, *P. abies*, *Q. robur* and *P. tremula*. In ambient conditions (control), the variation in the amount of H₂O₂ was the lowest across treatments for most of the tested species (Tab. 3).

Population-by-treatment interaction

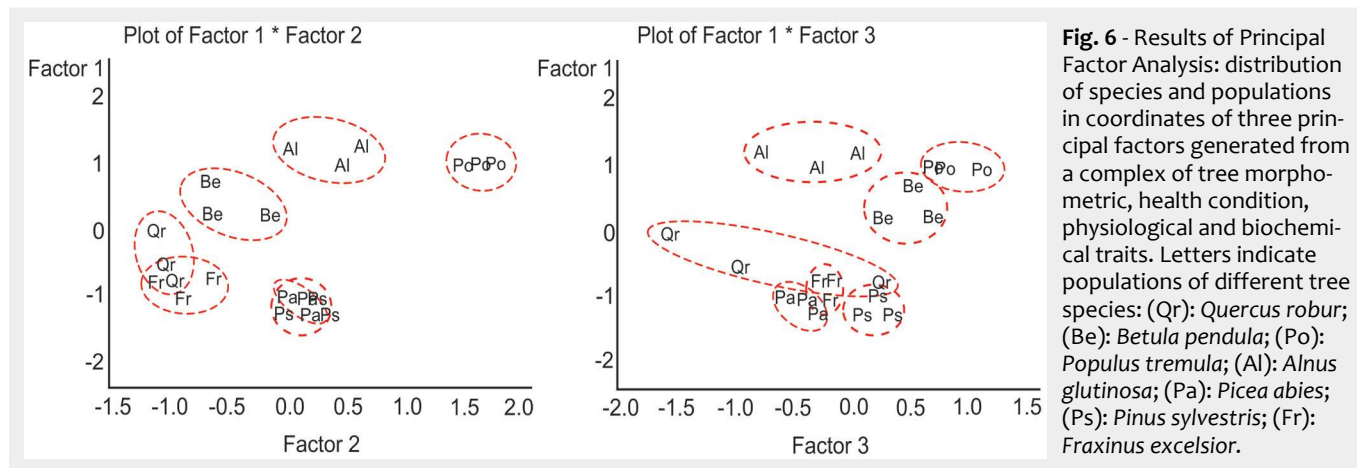
Significant ($P < 0.05$) population-by-treatment interaction (hereafter referred to as $G_p \times E$ interaction) was found for most morphometric, health condition, physiological and biochemical traits except tree condition and survival rates (Tab. 2).

In general, the contribution of individual

populations to the $G_p \times E$ interaction in height growth was not high, and only four out of 21 studied populations showed high plasticity and have contributed significantly to the interaction with ecovalences ranging between 11.1 and 21.4% (Tab. 4). This interaction was due to both reduction and increase of height increment in most sensitive/reactive populations of *B. pendula*, *P. tremula* and *A. glutinosa* across treatments (Fig. 3, Tab. 4). These species also showed the largest variation in defoliation rates among the three investigated populations across all treatments compared to other tested species (Fig. 4). Largest rank changes among populations in defoliation following different treatments were observed for *P. tremula* and *A. glutinosa* (Fig.

Tab. 5 - Results of Principal Factor Analysis of a complex of morphometric, health condition, physiological and biochemical traits in seven investigated tree species and their populations: factor structure - semipartial correlations. (a): For trait definitions see Materials and Methods. The strongest correlations are indicated (b).

Trait (a)	Semipartial Correlations		
	Factor1	Factor2	Factor3
Height increment	0.308	0.538 ^b	0.050
Diameter increment	0.191	0.521 ^b	0.123
Height/diameter ratio	0.602 ^b	0.150	0.091
Defoliation	0.588 ^b	-0.023	-0.084
Health condition	-0.118	0.180	-0.023
Transpiration rate	0.483 ^b	0.074	0.290
Photosynthetic rate	0.482 ^b	0.015	0.427 ^b
Carotenoids	0.916 ^b	-0.167	-0.062
MDA	-0.121	-0.469 ^b	-0.009
H ₂ O ₂	0.158	-0.618 ^b	0.346 ^b
Chlorophyll <i>b</i>	0.879 ^b	-0.049	-0.109
Chlorophyll <i>a</i>	0.845 ^b	-0.001	-0.260
Chlorophyll <i>a/b</i> ratio	-0.211	-0.124	0.648 ^b



). Defoliation rate in three populations of *B. pendula* was very different, the Anykščiai population had highest defoliation rate in all treatments, however no rank changes were observed following different treatments (Fig. 4).

The most significant $G_p \times E$ interaction was found for concentrations of chlorophylls *a* and *b* and carotenoids, and for the amounts of MDA and H_2O_2 (Tab. 2). All populations of all investigated tree species showed different response to treatments in photosynthetic rate (Fig. 5). For example, in ambient conditions *Q. robur* from the Tytuvėnai population showed a photosynthetic rate higher by 51% as compared to that recorded for the other two populations (Fig. 5a). Compared to control, the photosynthetic rate of oak from Tytuvėnai population was by 40% lower in the hot-wet and by 30% lower in the hot-dry treatments. Saplings from Anykščiai and Jonava populations showed the highest photosynthetic rates following hot-wet treatment. Under the hot-dry treatment, the photosynthetic rate of Jonava population has decreased by 30%, while in Anykščiai population it increased by 16% and reached the same rate as the Tytuvėnai population (Fig. 5a).

Principal Factor Analysis of the multiple characteristics of species and populations across treatments has revealed three main factors (Tab. 5). The factor structure indicates that Factor 1 with the highest positive semipartial coefficients of correlation accounted for the variation in concentration of carotenoids, chlorophylls *a* and *b*, H/D ratio, defoliation, transpiration and photosynthetic rates (Tab. 5). Factor 2 showed a strong positive correlation with tree height and diameter increments, while negatively correlated with the amounts of MDA and H_2O_2 . Factor 3 strongly positively correlated with chlorophyll *a/b* ratio, photosynthetic rate and amount of H_2O_2 . Fig. 6 shows that populations of three deciduous tree species *P. tremula*, *A. glutinosa* and *B. pendula* were clearly separated from each other and from the rest of the species studied. These three species had the highest values of Factor 1. Populations of *P. tremula* had the highest values of all three

factors. Populations of *P. abies* and *P. sylvestris* had the lowest values of Factor 1 and overlapped in coordinates of Factors 1 and 2 with a small separation in Factor 3. Populations of *Q. robur* and *F. excelsior* had the lowest values of Factors 1 and 2 and partially overlapped (Fig. 6).

Discussion

In this study significant inter-species effects and species-by-treatment interaction for almost all tree morphometric, health condition, physiological and biochemical traits show that tree responses to the applied treatments are species-specific. The investigated tree species showed different sensitivity to stressful conditions, exhibiting different physiological reactions and growth performance which can be considered as specific acclimation strategies to cope with stress. This suggests that effects of different treatments should not be generalized and have to be analyzed for each species separately. At the same time, tree reaction to treatments is expressed differently in different tree traits as they do not have the same acclimative/adaptive value. Tree height increment is regarded to be the most important trait in competition for light at juvenile age. The present study showed that elevated temperatures neither at wet nor at simulated drought conditions had significant positive effect on sapling height increment, except *A. glutinosa*, and *Q. robur* (Fig. 1a). Stress-induced differences (as compared to control) were more pronounced in stem diameter increment (mostly having a negative effect, in particular for *A. glutinosa* and *P. tremula*) than in height increment. This suggests that increment losses in stem diameter due to drought are compensated *via* internal relocation of biomass to height growth to sustain the competitive ability of trees affected by stress. The unequal treatment effect observed in this study is in agreement with earlier findings by Matyssek et al. (1992), who showed that the reduction of increment under stress condition is more pronounced for diameter than for height. The increase of height to diameter ratio under both hot-wet and hot-dry conditions compared to control might also be related

to stress-induced acceleration of growth as reaction to lower light intensity in phytotron than in ambient conditions.

Limited water availability during vegetation season can cause premature tree leaf shedding and lead to growth reduction. Tree species that had the highest transpiration rate in ambient conditions (*A. glutinosa*, *B. pendula* and *P. tremula*) demonstrated a specific combined strategy to cope with drought: in the hot-dry treatment they have closed stomata to reduce stomatal conductance and transpiration, which has negatively affected photosynthesis rate, and also have shed a portion of leaves. Interestingly, *B. pendula* and *A. glutinosa*, compared to control showed lower transpiration rate also in the hot-wet treatment, while *P. tremula* showed the opposite reaction (Fig. 2a). It should be noted that in control treatment, *F. excelsior* showed the highest defoliation among all treatments most likely because of damages by an ash dieback pathogen *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya, whose infection rate was higher outdoors than in the phytotron.

Larger WUE values observed in *P. tremula*, *Q. robur* and *A. glutinosa* under the hot-dry treatment (as compared to control) gives an indication that these species are more efficient responders to heat and drought stress as they are able to maintain a balance between photosynthesis and transpiration. Our observations are consistent with the results of previous studies on reactions of various plant species tested under hot and dry growth conditions (Gratani et al. 2000, Guo et al. 2006, Wikberg & Ogren 2007, Medrano et al. 2015). It has been demonstrated that drought causes a significant increase, while high temperature causes a significant decrease, in the intrinsic water use efficiency (WUE_i , A/g_s) for several forest tree species, e.g., *Populus* spp. (Zhang et al. 2004, Yin et al. 2005, Monclus et al. 2006), *Quercus ilex* L. (Gratani et al. 2000) and *B. pendula* (Kellomäki & Wang 2001). The increase in WUE_i is mainly due to a decrease in the stomatal conductance caused by drought (Zhang et al. 2005), while the decrease in WUE results from an increase in the transpiration

rate due to high temperature (Kellomäki & Wang 2001). Significantly lower WUE values (compared to control) found during the present study for *P. abies*, *P. sylvestris* and *B. pendula* in the hot-dry treatment are in agreement with the results obtained in studies on poplars, almonds and Mediterranean shrubs, where a clear decrease of instantaneous WUE under water deficit has been demonstrated (Klein et al. 2001, Llorens et al. 2003, Liang et al. 2006). Interestingly, in the present study a poplar species *P. tremula* showed the opposite reaction: higher WUE values were found following hot-dry treatment. It has been demonstrated that even closely related poplar species react to drought differently (Pallardy & Kozlowski 1981, Strong & Hansen 1991, Tschaplinski et al. 1994). Pattern of WUE changes might be related to environments of species' or populations' origin. For example, studies on *Castanea sativa* Mill. populations from contrasting xeric and mesic habitats showed that drought-adapted Mediterranean ecotype exhibited lower WUE values than the eastern wet-adapted one (Lauteri et al. 1997b, 2004).

Our study confirmed the results obtained by Griffin et al. (2004) who showed that elevated temperature (up to 40 °C) increased the carbon fixation in two ecotypes of redbud (*Cercis canadensis* L.), and that watered plants had significantly greater carbon fixation rates than plants grown under water deficit stress. In our study, photosynthetic rate and pigment concentrations were in most cases much greater in hot-wet than in hot-dry treatment, indicating that water deficit inhibits photosynthetic processes and reduces positive effect of the temperature increase. Similarly, reduction of net photosynthesis rate caused by drought was found in numerous studies (Li et al. 2004, Reddy et al. 2004, Yin et al. 2005, Ma et al. 2014). Stomata closure and photoinhibitory damage are considered as the major factors responsible for the depression of assimilation rate (Muraoka et al. 2000). Xu et al. (2008) demonstrated that drought treatment of *Populus cathayana* Rehd. juveniles (cuttings) had significant negative effect on their growth and net photosynthesis rate, and increased WUE_i and malondialdehyde (MDA) content. In contrast, elevated temperatures and good water supply significantly accelerated *P. cathayana* growth and net photosynthesis rate, but decreased WUE_i and MDA content.

Stomatal limitations largely account for a reduction of CO₂ assimilation under mild to moderate drought stress, whereas non-stomatal limitations may become dominant under severe drought (Tezara & Lawlor 1995, Flexas et al. 2004, Flexas et al. 2006, Ma et al. 2014). Although in field studies trees of genus *Quercus* often display high photosynthetic capacity and stomatal conductance, the stomatal control of gas exchange in response to water availability is more common than in other co-

curing tree species (Bahari et al. 1985, Ni & Pallardy 1991). In our study, *Q. robur* has increased CO₂ stomatal conductance following hot-dry treatment (while in all other tree species it decreased) and this may help to avoid such severe loss of photosynthetic rate observed in other tree species. It has been shown that stomatal control of gas exchange may act as an important physiological mechanism influencing distribution of *Quercus rubra* L., *Q. pirinus* L. and *Acer rubrum* L. (Turnbull et al. 2002). In a study by Ni & Pallardy (1991), more xeric species showed higher photosynthetic rate and increased leaf water vapor conductance under both well-watered and water-deficient conditions. However, a trend towards increased WUE in more xeric species has not been observed either in the above-mentioned (Ni & Pallardy 1991) nor in our study for *P. sylvestris*.

Results of the present study suggest that the production of most important plant pigments is enhanced under hot and wet conditions, while arid conditions have a more negative impact on pigment production. This, however, does not hold true for all tested species. For example, for *P. sylvestris* both treatments had positive effect (compared to control) on production of all the analysed pigments, while for *P. abies* the effect was always negative or neutral. Decline in carotenoid concentration following drought stress and high CO₂ concentrations was observed by Schwanz & Polle (2001) in *Q. robur* and *Pinus pinaster* Ait. Higher H₂O₂ levels (compared to control) recorded following drought stress in *A. glutinosa*, *F. excelsior*, *P. sylvestris* and *P. abies* suggest that these four tree species at young age are sensitive to water deficit. The greater increase of MDA concentration following the hot-dry treatment also indicates that oxidative damage is taking place in membranes of all tested tree species. Maintenance of membrane stability is critical for plant survival under environmental stress (Wahid et al. 2007). In the present study, combined stressful conditions, drought and heat, caused significantly stronger oxidative stress in tree leaves compared to that recorded in the hot-wet treatment. These results are consistent with the findings of similar studies on responses of olive trees (Sofa et al. 2004, Bacelar et al. 2007) and cowpea (Souza et al. 2004). H₂O₂ plays dual role in plants; as a signaling molecule it is involved in the regulation of various environmental stresses at optimal level between 0.1 and 5 μmol g⁻¹ FW, while high concentrations are capable to induce cellular damage (Veljovic-Jovanovic et al. 2002, Cheeseman 2007). In the present study, *F. excelsior*, *B. pendula* and *Q. robur* had much higher H₂O₂ levels than optimal, thus indicating tree response to stress and causing cell oxidative damage, which is partially responsible for plant growth inhibition.

It is argued that certain external stressors may reduce plant tolerance to secondary

stressors, i.e., when internal reserves of an organism are being depleted for coping with the primary stressors it gets more vulnerable (Pianka 1978, Duchovskis et al. 2002). On the other hand, if plant adaptive mechanisms are built to cope with different kinds of stress, such a plant, being adapted to one stressor, may be more tolerant to another one (Cox & Hutchinson 1980, Larcher 1995, Pliura et al. 2008). The observed significant treatment effects indicate generally high phenotypic plasticity of all tree species tested in the present study. Our results suggest that in a short term trees (at least at juvenile age) are able to respond to stressors, although plastic response and possibilities for acclimation and adaptation of different tree species may vary considerably. Large phenotypic plasticity in adaptive traits may be useful under changing conditions in a short-time perspective, but may be detrimental in a long term since it may not allow natural selection to act in full power (Eriksson 2001).

The highly-significant population effect found in our study for most traits – except stomatal conductance, transpiration rate, WUE and WUE_i – shows that populations of certain species differ in their adaptive response. The absence or presence of a weak population effect in these four traits may be explained by differing reaction of different populations to treatments, which is indicated by a highly significant population-by-treatment interaction effect. Very high inter-population genetic variation in height and diameter increments found for *B. pendula* and *F. excelsior* shows that populations of these species are the most different among all populations tested, which can be due to existing adaptations to specific site conditions in the place of their origin. Jensen & Hansen (2010) also found a significant population effect in tree height, stem diameter, shoot and root biomass in their study of 22 *Q. robur* populations from Central and Northern Europe in a greenhouse irrigation experiment.

Survival of plant populations and even of separate species will depend on abilities to acclimate and to adapt to relatively rapidly changing environmental conditions both physiologically and genetically. Genetic adaptability is determined by genetic variation of adaptive traits within and among populations, by ecogenetic plasticity, genotype by environment interaction, inter- and intra-population structure, population size and other factors (Eriksson 1998, 2001). Tree species differ from each other by a complex of biological and ecological traits such as successional stage in the ecosystem, character of self- and cross-pollination and seed dispersal, tree social status, distribution patterns in the natural range, size and structure of populations, particularities of post-glacial migration, etc. (Hamrick & Godt 1989). All these traits affect genetic variation also at intra-population levels, which in turn determines the possibility of natural selection and adaptation. Large dis-

tribution ranges of most deciduous tree species and high diversity of environmental conditions within these ranges precondition the climatic and edaphic differentiation of populations in the course of species' evolution. Wide distribution range in connection with occurrence of small isolated populations of a certain species determines large intra-specific genetic variation (Weisgerber 1974). Widely distributed social tree species are characterized by high inter-population variability, whereas their intra-population variability is smaller. Species-specific combinations of bioecological traits affect species' genetic inter-population diversity in different directions. Logically, such tree species as *P. sylvestris*, *P. abies*, *B. pendula* and *P. tremula* should be characterized by high inter-population variability, while *Q. robur* and *F. excelsior* by lower variability. No such trend, however, was observed in the present study, the inter-population variability varied a lot among species depending on treatments and traits studied (Tab. 3).

Results of Principal Factor Analysis (PFA) on the complex of studied traits allowed to discriminate and/or to group tree species and populations according to their ecological reaction across treatments. Clear separation of three deciduous species *P. tremula*, *A. glutinosa* and *B. pendula* from each other and from the rest of the tested species in plots of principal factors of PFA indicates largely different ecological behavior of those three species. In Europe, *A. glutinosa* is considered an asocial tree species with scattered distribution. In Lithuania, this species has a wide distribution and demonstrates a social character by forming forest stands. Our study confirms these ecological features of *A. glutinosa*; indeed, in the factor diagram populations of this species are located closer to *P. tremula* than to *Q. robur* and *F. excelsior*. Populations of *P. abies* and *P. sylvestris* may be considered as exhibiting a generally similar reaction to treatments, as suggested by their partial overlap in PFA plots (Factor 1 × Factor 2 – Fig. 6). Populations of *Q. robur* and *F. excelsior* formed a distinct group (Fig. 6), thus confirming their similar ecological behavior.

Significant population-by-treatment interaction found in the present study for most morphometric, health condition (except tree condition and survival), physiological and biochemical traits shows different response and plasticity of tree populations to the applied treatments and indicates a presence of specific site-related or climate-related adaptations. The absence of interaction between tree conditions and survival may be explained by a delay in the occurrence of visible tree damages and/or mortality, when treatment impact on morphometric, photosynthesis rate and other physiological and biochemical traits was already evident and scorable. Responses of the tested populations varied depending on different combinations of temperature

and water regimes. Skre (1991) investigated the response of progenies from geographically distant populations of *B. pendula* and *B. pubescens* under different temperature (and photoperiod) treatments in climatic chambers. Some of the investigated populations showed good growth at extreme temperatures, while other populations at moderate temperatures; differences among populations in net assimilation rate were found to be smaller at lower temperatures (Skre 1991), which is in agreement with our findings. In a study of 22 *Q. robur* populations from Central and Northern Europe in a greenhouse irrigation experiment, population × watering interaction effect was significant and more pronounced than population effect for stem diameter, similarly pronounced for tree height and number of lamma shoots, and less pronounced for shoot biomass and shoot/root ratio, while no interaction was found for root biomass and growth cessation (Jensen & Hansen 2010). In Turkey, two physiological types of *Castanea sativa* Mill. populations, drought-adapted and wet-adapted, were identified (Lauteri et al. 1999, Villani et al. 1999). WUE has been found to be a reliable indicator of complex adaptive mechanisms that differentiate wet- and drought-adapted populations (Lauteri et al. 1997b, 1999, 2004). At juvenile stage, a slower increment of above-ground biomass was found for drought-adapted Mediterranean *C. sativa* genotypes compared to eastern wet-adapted genotypes, while at maturity growth patterns have changed in the opposite way (Villani et al. 1992, Lauteri et al. 1997a).

In our previous study (Pliura et al. 2016), a significant genotype by environment (G × E) interaction for tree survival was found in *F. excelsior* juveniles tested under simulated summer drought and spring frost conditions, indicating that condition of different genotypes unequally depends on environmental conditions. Ecophysiological studies highlighted different mechanisms of response to water stress depending on site conditions at the origin location (Lauteri et al. 1997a, 1997b, 2004). Lauteri et al. (1999) reported a higher capacity of photosynthesis and transpiration in *C. sativa* seedlings from populations from arid sites than in those from mesic sites. Studies of genetic variation in these traits in material from the whole distribution range of *C. sativa* indicated inter- and intra-population genetic variation in drought tolerance (Pliura & Eriksson 2002, Lauteri et al. 2004). High phenotypic plasticity, which is an outcome of poor performance of certain genotypes, can aid natural selection by eliminating the least adaptive genotypes or populations thus enhancing general population/species adaptiveness. This somehow contradicts a conclusion by Ghalambor et al. (2007), who claimed that only plasticity that enhances fitness can facilitate adaptive evolution of a species. Genetically depleted populations are less likely to cope

with environmental stress under changing environmental conditions and may be more prone to inbreeding depression, with loss of productive performance (Ellstrand 1992).

Deciduous forest-forming tree species have immigrated to the current territory of Lithuania after the last glacial period (6-9 thousand years ago) from refugee populations in Balkans, Apennines and other areas of southern Europe (Csaikl et al. 2002). The offspring from these southern populations had too short time to adapt to rapidly changing environmental conditions in north-temperate regions because of long tree ontogenesis and prolonged period of generational change. Adaptation of forest trees has also been encumbered by frequent shifts and fluctuations of climatic and local environmental conditions during tree ontogenesis and through the ages, by high phenogenetic plasticity of trees and other factors. It can be speculated that ancient Lithuanian populations of deciduous forest-forming tree species have conserved part of their former environment-specific adaptation to southern drought-prone refugia, as well as the plasticity and specific reactions to environmental fluctuations; however, their current adaptive significance is unclear. If growth, morphological and physiological reactions of species and populations observed in the present study are not acclimative/adaptive, the negative consequences of this may get expressed in the next future via reduced growth, declining health conditions and decreasing survival rate. Therefore, further studies on how the induced physiological responses will be carried over into the following years and how they will affect growth and development of the treated trees are needed.

Conclusions

Our results showed a great response variability (as expressed by different morphometric, health condition, physiological and biochemical traits) among tested tree species and their populations exposed to climate change-related stressors at juvenile age. Under the hot-dry treatment, most deciduous species showed smaller height and diameter increment, lower transpiration and photosynthetic rates and higher defoliation rates compared to ambient conditions, meanwhile different reactions of different tree species, both negative and positive (compared to control), were observed under the hot-wet treatment. However, for conifers and *Q. robur*, the effect of treatment on these traits was negligible. Photosynthetic and transpiration rates were the highest in the fast-growing species *P. tremula*, *B. pendula* and *A. glutinosa*, however these rates were much more negatively affected by water deficit than in other tree species. These three species demonstrated a specific combined acclimation strategy to cope with drought stress, including stomata closure to reduce stomal conductance and transpiration rate, and (partial) leaf

shedding.

Lower photosynthetic rate observed under hot-wet treatment in all tested species (except *Q. robur* and *P. abies*) indicates that heat waves can negatively affect the physiology of certain tree species, even at good availability of water. Moreover, the large amount of MDA observed for most species under the hot-dry treatment suggest a stronger oxidative stress compared to hot-wet and ambient conditions. Production of chlorophylls *a* and *b* and carotenoids is likely positively affected by elevated temperatures and water availability, while hot-dry conditions are likely to cause some negative effect on pigment production, though quite a few exceptions were observed.

Significant population effect and population-by-treatment interaction for most traits indicate different plasticity and response of each population to stressful conditions. However, only a small fraction of populations showed high plasticity/ecovariance and significantly contributed to the population-by-treatment interaction. Specific reactions of such populations may be related to a presence of specific site- or climate-related pre-adaptations.

Some of the observed responses of species and populations to hot-dry treatment resulted in lower performances and a decrease in tree growth, and therefore cannot be considered as adaptive acclimation. Different physiological responses of populations/species at juvenile stages could unbalance the competition among species, particularly following heat waves and drought. This may compromise forest regeneration, cause atypical species successions and reduce the sustainability of future forest ecosystems. Specific short-term reactions/acclimation performances observed in this study represent a solid incentive for further investigations on the adaptive potential of forest trees.

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