Significance to the Horticulture Industry

Prescribed use of local tree populations not only for silviculture but also for plantings in the landscape will complicate the submission process and increase the cost of production due to a separated cultivation of different populations within each nursery. This can lead to problems concerning the supply of an adequate quantity of trees and shrubs. Our results show that all investigated populations concerning the supply of an adequate quantity of trees and shrubs. Our results show that all investigated populations of Quercus robur and Tilia cordata can cope with different drought conditions. Previous results showed that this is also the case for early and late frost stress (Selig and Bohne 2016a, 2016b). Hence prescribed use of local populations is an important one that involves several physiological and biochemical mechanisms.

Specific drought responses, matching ecological conditions at the site of origin, may enhance survival and performance of a local population. However, for regions with small differences of geographical and/or climatic conditions, local adaptation is not consistently demonstrated (Peuke et al. 2002). Using locally adapted populations for plantations requires the certification of suitable seed source areas and a separated cultivation within nurseries. This will increase the costs for production and, for some purposes, this can lead to problems concerning the supply of an adequate quantity of trees and shrubs.

For silvicultural purposes, Germany is divided into different provenances, depending on the species. The use of stock plants from these provenances has been recommended for many years and is regulated by law (FoVG 2002). For landscaping purposes in Germany, the Federal Nature Conservation Act (BNatschG 2010) was amended in 2010 and aims to preserve populations with suggested local adaptations. Six “defined areas of origin” independent of species were assigned according to a map by Schmidt and Krause (1997) based on ecological fundamental units (BMU 2012). After a transition period until March 1, 2020, it is forbidden to plant tree populations outside their defined area of origin in free nature without permission. Free nature in the BNatschG is a juristic term and is defined as all non-built-up areas with exception of forestry and vegetation in the immediate vicinity of streets (e.g. alley trees) (BMU 2012). Quercus robur and Tilia cordata are two important species for landscaping purposes in free nature and are affected by BNatschG.

This greenhouse study investigated if populations of Quercus robur and Tilia cordata from three of the German defined areas of origin differ in their reaction to drought. To cover a bigger geographic range, one Hungarian population of Quercus robur was included. In this experiment a slow and a fast developing drought stress

Species used in this study: pedunculate oak (Quercus robur L.); small-leaved linden (Tilia cordata Mill.).

Abstract

Locally adapted trees and shrubs are suggested to show higher fitness at their site of origin than non-locally adapted ones. The production in tree nurseries could take this into account to improve outplanting performance. However, separate production of specific ecotypes in nurseries increases the costs of production and may reduce the availability of a specific species. In this study, we investigated drought stress reactions of 2 year-old container-grown seedlings of Quercus robur L. and Tilia cordata Mill. from four and three different populations, respectively, each from a different site of origin. Slow- and fast-developing drought stress treatments were applied in a greenhouse study to determine any difference in drought tolerance. Control plants were well irrigated. Depending on the species and stress development, only marginal interpopulation differences in particular physiological (pre-dawn water potential, stomatal conductance, relative water content) and/or biochemical (glucose, fructose, sucrose, starch, proline) reactions to drought existed. These differences did not consistently reflect precipitation conditions at the population sites of origin, indicating only low level of local adaptation for populations of the target species.

Index words: adaptation, carbohydrates, pedunculate oak, provenances, proline, small-leaved lime.

Species used in this study: pedunculate oak (Quercus robur L.); small-leaved linden (Tilia cordata Mill.).
were imposed to cover different adaptation challenges which can occur in the landscape. We addressed the following questions: (1) do populations of Quercus robur and Tilia cordata differ in their drought response; (2) can possible interpopulation differences be related to local ecological conditions at the site of origin?

Material and Methods

Plants and cultivation. In April 2011, 2 year-old seedlings from three German populations of Tilia cordata and Quercus robur and one Hungarian population of Quercus robur were bought from nurseries (Table 1). Referring to § 40 BNatschG (BMU 2012), three defined areas of origin were included in the investigations. A certificate (prescription of FoVG) was provided to verify the provenance. The populations of Quercus robur originated from the west (Nordrhein-Westfalen (NW)), east (Brandenburg (BB)) and south (Bayern (BY)) of Germany and from northwest part of Hungary (near Tiszadob (HU)). Tilia cordata populations originated from west (Nordrhein-Westfalen (NW)), east (Sachsen (SN)) and south (Baden-Württemberg (BW)) of Germany. Regarding the precipitation range, three populations originated from relative dry habitats (Quercus robur: BB and HU; Tilia cordata: SN) and four populations from relative wet habitats (Quercus robur: NW, BY; Tilia cordata: NW, BW).

The seedlings were potted into 3 L (3.17 qt) containers with peat as the growing medium (Klasmann Deilmann GmbH, Georg-Klasmann-Strasse 2-10, 49744 Geeste, Germany) and were fertilized with Osmocote® Exact Standard 8-9M (15% N, 9% P2O5, 11% K2O, 2% MgO + trace elements; Everris NA Inc., 4190 CA Geldermalsen, Netherlands) to result in 0.8 g N L−1 (0.1 oz gal−1) substrate. Until the drought experiment started, the plants were arranged, separated by populations (each divided into two blocks), on the container area at the experimental site in Tiszadob (52°14’N, 9°49’E). In sum, 54 seedlings per population (27 per block) with a distance of 45 cm (17.7 in) between containers were cultivated. The irrigation took place automatically by a drip irrigation system.

Experimental drought conditions. Drought experiments were carried out from July 1 to 11, 2012 (Tilia cordata) and July 27 to August 14, 2012 (Quercus robur) in a greenhouse. At the beginning of the experiment, all root balls were saturated with water by submerging them for five minutes. After saturation and drainage for 5 minutes, the mass of each individual plant, including the container and the root ball, was weighed as the starting point for evapotranspiration. A total of 54 plants from each species and population were arranged completely randomized on four tables. Containers of the control plants were sited directly on the table and were irrigated twice a day for 20 minutes by an ebb and flow system. Containers of plants to be drought stressed were put on 7 cm (2.7 in) high spacers to prevent contact with the irrigation water of the control plants.

Two different drought treatments were applied: a slow developing and a fast developing drought stress. To achieve this, the plants to be stressed were irrigated manually and individually every other day with only 50% (slow developing drought, SDD) or 25% (fast developing drought, FDD) of the lost mass (evapotranspiration) since the last irrigation. To calculate the amount of water needed per stress treatment and plant, the pots were reweighed every other day.

For every treatment, 18 plants from each population were used. These 18 plants were again divided into 9 plants for measurements/analyses and 9 plants were used as “regeneration plants”. The regeneration plants were re-watered at the end of experiment, placed back on the container area, and further irrigated according to the gardener’s code of best practice to determine their regeneration ability. One month after re-watering, survival of the regeneration plants was determined.

Sampling. For Tilia cordata, the experiment ended when severe wilting symptoms (droopy leaves with initiating chlorosis) of more than half of all plants of one treatment were visible. For the FDD treatment, this was reached after 6 days (July 6, 2012) and for SDD after 11 days (July 11, 2012). The control treatment ended after 11 days as well.

Because of the inflexible leaves of Quercus robur, wilting symptoms were not as clear as for Tilia cordata. To achieve similar stress status like Tilia cordata plants, the Quercus robur experiment ended when half of all plants of one treatment showed pre dawn leaf water potential of −2.5 MPa. FDD treatment reached this status after 13 days (August 8, 2012). SDD treatment was harvested after 19 days (August 14, 2012), however, pre-dawn water potential already was more negative than the target value. The control treatment was sampled after 19 days as well.

For analyses of biochemical parameters at the end of the experiment (proline, sugars, starch, N, P, K), about 10 g of
the youngest fully expanded leaves were taken (biochemical samples). To stop enzymatic activity, the leaves were cut into small pieces and treated in a microwave for 2.5 minutes at 700 Watt immediately after sampling. Afterwards the leaves were dried for nutrient and biochemical analyses at 70 °C (158 °F) and 60 °C (140 °F), respectively and milled by a planetary ball mill (PM 100, Retsch GmbH, Retsch-Allee 1-5, 42781 Haan, Germany).

**Measurements during experiment.** During the experiments, physiological parameters of the plants were measured to determine any different responses among the populations to drought stress. The measurements were carried out every other day before irrigation with three plants per population and treatment, which were randomly chosen. For all measurements, the second fully expanded leaf of the longest shoot was taken. Pre-dawn leaf water potential (Ψpd) was measured between 4 and 5 a.m. with a Scholander type pressure chamber (Model 600, PMS Instrument Company, 1725 Geary Street SE, Albany, USA). Leaf relative water content (RWCleaf) was calculated based on fresh weight (FW), turgid weight (TW), and dry weight (DW). Leaf FW was determined on a mass balance immediately after being excised from the plants. TWs were determined after soaking the leaves in deionized water for 24 h in a covered glass vessel; they were weighed immediately after being blotted. Leaves were then dried at 80 °C (176 °F) for at least 72 h prior to being weighed for DW. RWCleaf [%] was calculated using the formula: (FW–DW)/(TW–DW) x 100 (Barrs and Weathery 1962). Stomatal conductance (gs) was measured with a porometer (AP4, Delta-T Devices Ltd, 130 Low Road, Cambridge, UK) and expressed as [mmol H2O m–2 s–1]. To determine any damage to the photosynthetic apparatus, chlorophyll fluorescence (Chl fluorescence) was measured with a chlorophyll fluorimeter (Handy PEA, Hansatech Instruments Ltd., Narborough Road, King’s Lynn, UK) at the sampling dates. Parameter Fv/Fm was used to describe the maximum quantum efficiency of photosystem II photochemistry (Baker 2008). At the end of the experiment, samples for biochemical analyses were taken as described above (Sampling section) and all plants of the respective treatment were chosen for physiological measurements.

**Proline analyses.** Free proline was analyzed spectrophotometrically using a modified ninhydrin method of Bates et al. (1973). Material of biochemical samples (50 mg) was extracted with 1.8 ml sulfosalicylic acid (3%). After centrifugation (14,800 rpm) for 15 minutes, 150 μl of the supernatant, 90 μl glacial acetic acid and 90 μl of acid ninhydrin were heated for 45 minutes at 100 °C (212 °F) and extracted with 1.5 ml toluene by mixing and shaking and 200 μl of toluene phase was pipetted in a quartz-microplate and absorbance was read at 520 nm with a VersaMax Microplate reader (VersaMax ELISA, Molecular Devices, 1311 Orleans Drive, Sunnyvale CA 94089, USA). Proline concentration was determined from a standard curve and calculated on a dry weight (DW) basis (mg g–1 DW).

**Carbohydrate analysis.** Material of biochemical samples (30 mg) was extracted three times with 1.5 ml of hot (80 °C) (176 °F) ethanol (80%). After centrifugation, the supernatants were combined and analyzed enzymatically for glucose, fructose and sucrose in a microplate as detailed by Zhao et al. (2010). Results were calculated on dry weight basis [mg g–1 DW] and combined as soluble sugars (GFS).

After extraction of sugars, the remaining pellet was used for starch analysis (Boehringer 1984). The pellet was solved in NaOH (0.5 M) and incubated at 60 °C for 30 minutes. To adjust the pH approximately to 4.7, glacial acetic acid was added. After centrifugation at 5,000 rpm for 5 minutes, the supernatant (10 μl) was placed to a microplate. Starch was hydrolyzed to glucose by adding 20 μl of amyloglucosidase and incubated for 60 minutes at 30 °C (86 °F). Starch concentration was determined by glucose based standard curve (see glucose assay above) and calculated on a dry weight basis [mg g–1 DW].

**Statistical analysis.** Statistical analyses were performed with statistic software R 3.1.3 (R Core Team 2014) with an additional statistical package ‘multcomp’ (Hothorn et al. 2008). All physiological parameters (RWCleaf, Ψpd, gs, chlorophyll fluorescence) and biochemical parameters (GFS, starch and proline) were analyzed by a two-way ANOVA and Tukey all-pair comparison with population and treatment as factors. Data were checked for heterogeneity of variance and normal distribution and if necessary logarithmic transformed to satisfy requirements of ANOVA.

**Results and Discussion**

Populations of *Quercus robur* and *Tilia cordata* did not differ significantly in the measured physiological parameters during the drought progress (data not shown). Hence, only the results from the sampling day at the end of the experiment are shown.

Closing stomata to reduce water loss under water deficit conditions is a typical physiological response of trees to drought stress (Peuke et al. 2002, Steiner et al 2014). Both target species showed decreasing stomatal conductance (gs) at both drought treatments compared to the control (Fig. 1). However, drought development rate (FDD vs. SDD) did not affect this response. Water loss was not entirely limited as shown by decreasing Ψpd and RWCleaf at drought treatments for both species compared to the control treatment (Figs. 2 and 3). However, while Ψpd decreased more at SDD compared to FDD for both target species, a greater reduction of RWCleaf at SDD was only true for *Quercus robur*. While for *Quercus robur* Fv/Fm was not affected by FDD, it decreased at SDD. For *Tilia cordata*, neither FDD nor SDD affected Fv/Fm (Fig. 4). Greater reduction of Ψpd at SDD for both species and RWCleaf for *Quercus robur* indicates more severe drought stress at this treatment. This is in accordance with results of Epron et al. (1993) for different *Quercus* species, who found rapid reduction of PSII photochemistry (Fv/Fm) only at very severe drought stress (Ψpd < –4 MPa). As shown by Epron and Dreyer (1992) for *Quercus petraea* (a species similar to pedunculate oak), Fv/Fm also declined rapidly after a critical RWCleaf (approximately 25%) value is reached. It
seems likely that critical $RWC_{\text{leaf}}$ values, affecting PS II photochemistry, were not reached at FDD for *Quercus robur* and *Tilia cordata*. In the Climate-Species-Matrix by Roloff et al. (2009), *Quercus robur* and *Tilia cordata* are categorized as "suitable" concerning dry climates. A high drought tolerance is also found in our results. Differences in stomatal closure among *Quercus robur* and *Tilia cordata* populations did not exist (Fig. 1). This is contrary to findings of Aspelmeier and Leuschner (2004) for *Betula pendula* as they recorded higher $g_s$ for genotypes of low-rainfall origin than genotypes of high-rainfall origin under drought conditions. Peuke et al. (2002) observed the highest $g_s$ from populations of wet and lowest from dry regions under drought conditions for *Fagus sylvatica* seedlings. These contrary results indicate a species-specific response of stomatal closure to drought on a population level. While no interpopulation differences occurred for *Quercus robur* populations, *Tilia cordata* populations did differ in $RWC_{\text{leaf}}$ and $W_{pd}$ at SDD (Figs. 2 and 3). Population SN from a relative dry habitat showed lower $W_{pd}$ compared to population NW and the lowest $RWC_{\text{leaf}}$ of all populations, indicating a more severe drought response for this population. This is in contrast to results of Ying et al. (2015) of *Camptotheca acuminata* Decne.
Table 2. Mean concentrations of GFS (glucose, fructose and sucrose), starch and proline (± standard error) in the newest leaves of four populations of Quercus robur and three populations of Tilia cordata, all container-grown, after different drought treatments (FDD [fast-developing drought], SDD [slow-developing drought]) in July/August 2012. Different lower case letters indicate significant differences between populations within a drought treatment. Populations code: NW = Nordrhein-Westfalen (Germany), BB = Brandenburg (Germany), BW = Baden-Württemberg (Germany), SN = Sachsen-Anhalt (Germany), HY = Hunsrück (Germany).

| Drought treatment | Control | FDD | SDD |
|-------------------|---------|-----|-----|
| Quercus robur     |         |     |     |
| NW                | 4.5 ± 0.4 | 6.0 ± 0.6 | 5.4 ± 0.3 |
| BB                | 3.3 ± 0.2 | 6.2 ± 0.6 | 5.8 ± 0.5 |
| BY                | 3.1 ± 0.2 | 5.6 ± 0.5 | 6.6 ± 0.6 |
| HU                | 4.7 ± 0.4 | 6.4 ± 0.7 | 5.9 ± 0.4 |
| Starch (mg g⁻¹ DW) |         |     |     |
| NW                | 0.18 ± 0.03 | 0.16 ± 0.01 | 0.11 ± 0.01 |
| BB                | 0.09 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.01 |
| BY                | 0.14 ± 0.01 | 0.10 ± 0.01 | 0.12 ± 0.01 |
| HU                | 0.27 ± 0.03 | 0.12 ± 0.03 | 0.12 ± 0.04 |
| Proline (mg g⁻¹ DW) |       |     |     |
| NW                | 177 ± 10 | 616 ± 169 | 1296 ± 380 |
| BB                | 208 ± 21 | 616 ± 159 | 2963 ± 548 |
| BY                | 249 ± 22 | 402 ± 193 | 2366 ± 358 |
| HU                | 271 ± 10 | 551 ± 89  | 1729 ± 413 |
| Tilia cordata     |         |     |     |
| NW                | 5.9 ± 0.5 | 5.9 ± 0.3 | 6.4 ± 0.3 |
| SN                | 5.6 ± 0.5 | 6.0 ± 0.4 | 6.8 ± 0.3 |
| BW                | 6.6 ± 0.2 | 5.3 ± 0.2 | 5.5 ± 0.3 |
| Starch (mg g⁻¹ DW) |         |     |     |
| NW                | 0.40 ± 0.10 | 0.04 ± 0.01 | 0.02 ± 0.01 |
| SN                | 0.42 ± 0.10 | 0.06 ± 0.02 | 0.04 ± 0.01 |
| BW                | 1.13 ± 0.13 | 0.07 ± 0.02 | 0.05 ± 0.01 |
| Proline (mg g⁻¹ DW) |       |     |     |
| NW                | 217 ± 23 | 310 ± 116 | 561 ± 148 |
| SN                | 162 ± 6  | 607 ± 140 | 1038 ± 192 |
| BW                | 204 ± 14 | 435 ± 101 | 499 ± 79  |

Seedlings with higher RWCleaf of a population from a drier habitat compared to a wet one. However, SN did not differ in survival of regeneration plants (data not shown) compared with the other populations, indicating that SN can survive such low RWCleaf and decrease in the reaction of PSI Si photochemistry (Fv / Fm) to drought stress among the populations. With the exception of the population HU, an increase of GFS concentration in response to drought stress was observed for Quercus robur as also reported by Spieß et al. (2012) (Table 2). Accumulation of soluble sugars due to alterations in expression of genes encoding for enzymes involved in the sugar conversion pathway is a response to a water deficit (e.g. Kempa et al. 2008). None of the populations of Tilia cordata showed an increase in GFS concentration as a response to drought stress. Callister et al. (2008) reported an absence or only little osmotic adjustment for Eucalyptus hybrids with a high ability to increase cell wall elasticity under drought. Thomas and Gausling (2000) reported that Quercus robur has a low ability to increase cell wall elasticity under drought. Literature regarding drought stress reactions of Tilia cordata is rare, so we only can speculate an increase of cell wall elasticity under drought conditions of this species and absence of GFS accumulation. Degradation of starch to supply energy and carbohydrate skeletons is a typical response under osmotic stress (Kempa et al. 2008). This behavior was also observed for the populations of both species in the current study with the exception of BW (Quercus robur) (Table 2). Depletion of starch associated with an increase in the GFS concentration of Quercus robur was also reported by Eppron and Dryer (1996). This did not occur in any of the Tilia cordata populations. Maybe energy and carbon skeletons supplied by depletion of starch were utilized for other compatible solutes, such as other sugars (e.g. raffinose) or sugar alcohols (e.g. mannitol) involved in osmotic adjustment under drought conditions. Accumulation of proline under water and osmotic stress is reported for many species (Kempa et al. 2008, Watanabe et al. 2000). This indicates the important role of proline for osmotic adjustment and stabilizing cell membranes and proteins (Hare et al. 1998, Szabados and Savouré 2010). However, compared to the control treatment, proline concentration was only increased in the SDD treatment for both target species (Table 2). This is in accordance with results from other authors measuring higher osmoregulation if drought develops slowly (Arndt et al. 2001, Aspelmeier and Leuschner 2004).

Differences in sugar concentrations among Quercus robur populations only occurred in the control treatment and disappeared in both FDD and SDD treatments. However, the differences among populations for the controls were not related to total rainfall (Pannual) or summer rainfall (Psummer) (Table 1), because both the population with the lowest precipitation (BB) and the highest precipitation (BY) did not differ significantly. In contrast to Quercus robur, the sugar concentration of Tilia cordata population BW decreased under drought conditions and resulted in significantly lower GFS concentrations in BW at SDD compared to population SN. Sanchez-Rodriguez et al. (1999) also observed a decrease of soluble sugars under drought for Casuarina equisetifolia L. Differences among populations in starch concentration only existed at control treatment for both target species. For Quercus robur, only population HU showed a higher starch concentration, while German populations did not differ from each other. The Tilia cordata population BW showed the highest starch concentration under well irrigated conditions (control treatment). However, these differences disappeared under drought conditions, indicating stronger degradation of starch for population BW. Differences among populations in proline concentration occurred in the control treatment for Quercus robur and at SDD treatment for both target species. Well watered plants of population BY and HU showed higher proline concentrations compared to NW. At SDD, BW also had the numerically lowest concentration of proline. Tilia cordata population SN had a higher proline concentration compared to population BW at SDD. These interpopulation differences at SDD could be carefully related to precipi-
Populus cathayana seem to accumulate highest amounts of proline under water deficit as also reported for Populus cathayana Rehder populations (Xiao et al. 2008). Nevertheless, also the Quercus robur population BY with the highest $P_{\text{summer}}$ showed high accumulation of proline under drought conditions comparable to population BB and HU. Among protective functions for cell membranes and proteins, proline is also known to be important as an energy and substrate supply for resumed growth after stress events (Szabados and Savouré 2010, Taylor 1996). Therefore, the high accumulation of proline of Tilia cordata population SN could be the reason for survival of regeneration plants in drought reactions. GFS only increased for Tilia cordata (German populations) at drought stress treatments compared to the control treatment and not for Quercus robur, Betula pendula and Rhamnus cathartica, as reported by Arend et al. (2011) compared to our studies, provenance differences could not be related to climatic conditions at their site of origin. We can only speculate on the reasons. However, compared to Quercus robur with glacial refugia in south-west and south-east Europe, those of Tilia cordata were concentrated in south-east Europe (Willis 1996), suggesting less interaction of different genotypes during post-glacial immigration.

On the biochemical level, species differed in their drought reactions. GFS only increased for Quercus robur (German populations) at drought stress treatments compared to the control treatment and not for Tilia cordata (Tab. 3). Osmoregulation seems to be a high plastic trait with large interspecific variations (Aspelmeier and Leuschner 2004). In contrast to GFS, proline accumulation could be observed for both species at SDD and interpopulation differences occurred (Table 2). The manifold features of proline under several environmental stresses (e.g. Szabados and Savouré 2010; Taylor 1996) could be a reason for the species-unspecific accumulation of proline. In both species, proline was significantly synthesized only with slowly developing drought (SDD) (Table 2), suggesting a higher vulnerability of short and intense drought events.

Regarding drought stress reactions, the current study shows only minor hints of local adaptation of Quercus robur and Tilia cordata populations. This is in accordance with previous investigations regarding early (Selig and Bohne 2016a) and late frost reactions (Selig and Bohne 2016b). Regulations within § 40 of BNAtschG do not differ between species, because of assumed local adaptation of populations of woody species per se. However, these assumptions could not be confirmed by the current study. This should be considered by the generalized restrictions of BNAtschG § 40 and the assignment of the defined areas of origin. Otherwise, the costs for nursery production will be unnecessarily increased and the availability of a specific species for landscaping purposes may be reduced.

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