

# **Leaky taps in trees: Exploring leaf minimum water conductance in temperate European trees**

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## **General introduction**

Forests create diverse habitats for life, drive water, carbon, and nutrient cycles, and provide significant socioeconomic benefits to human civilization (Bonan, 2008; Ilstedt et al., 2016; Millar & Stephenson, 2015; Pan et al., 2011). With ongoing climate change, however, summer droughts accompanied by hot temperatures (hereafter referred as hot droughts) are emerging as one of the most critical challenges to maintaining forest health (Adams et al., 2017; Allen et al., 2010; McDowell et al., 2022; Teuling, 2018). In 2018 – 2019, for example, two consecutive hot drought events caused a series of detrimental consequences for forest communities in Central Europe, including premature leaf shedding, severe stem dehydration, and extensive canopy dieback (Arend et al., 2021; Brun et al., 2020; Salomón et al., 2022; Schuldt et al., 2020). Globally, tree mortality induced by hot droughts will significantly affect forest composition, structure, productivity, and climate interactions (Ciais et al., 2005; Dyderski et al., 2018; Reichstein et al., 2013; Senf et al., 2021). Therefore, a nuanced understanding of the underlying physiological mechanisms behind the critical responses of trees to hot droughts is fundamental for accurate prediction of tree mortality and better forest management.

As root water uptake becomes limited under severe drought, trees gradually close leaf stomata to reduce transpiration and keep their tissues hydrated (Körner, 2019; Martin-StPaul et al., 2017; Peters et al., 2023). It has been estimated that when leaf stomata are entirely closed, the transpiration demand of water flux within the tree can be reduced to 1 – 2 % of that under well-watered conditions (Körner, 2019). Although stomatal closure can effectively control most of the water loss from trees, the water content of trees continues to decline due to this residual transpiration, including leaf cuticular transpiration, water leakage from incompletely closed or broken stomata, and bark transpiration (Blackman et al., 2019; Duursma et al., 2019; Körner, 2019; Schuster et al., 2017; Wolfe, 2020). Thus, the surviving time of a tree after full stomatal closure under severe drought stress will mainly depend on the magnitudes of canopy leaf and bark residual transpiration, tree water capacitance, and the dehydration tolerance of

critical tissues or organs (Blackman et al., 2016, 2019; Hammond & Adams, 2019; Körner, 2019; Mantova et al., 2021; Martinez-Vilalta et al., 2019).

Canopy leaf residual transpiration consists of water transpired from the leaf cuticle and water leaked from the stomata (Duursma et al., 2019). Although canopy leaf residual transpiration plays a crucial role in the drought tolerance of trees, it is difficult to directly obtain a trustworthy amount of residual water loss in situ after full stomatal closure (Körner, 2019). Theoretically, the canopy leaf residual transpiration mainly depends on leaf minimum water conductance after full stomatal closure (hereafter referred as  $g_{min}$ ), a tree's total leaf surface area, and the vapor pressure deficit (VPD) that the canopy leaves experience. However, to date, different lines of evidence have demonstrated that  $g_{min}$  varies considerably between and within plant species and that  $g_{min}$  is highly responsive to the environmental drivers, including soil water availability and temperature, and also varies with leaf age (see literature compilation by Duursma et al., 2019). Therefore, determining the variability of  $g_{min}$  is essential and instructive for estimating canopy leaf residual transpiration of different tree species in the forest.

According to a data review by Schuster et al. (2017),  $g_{min}$  ranged from 0.01 (*Zamioculcas zamiifolia*) to 11.67 mmol m<sup>-2</sup> s<sup>-1</sup> (*Pinus pumila*) in 160 species from 11 life form groups, indicating a large amount of variation in  $g_{min}$  among plant species. This considerable interspecies variation can be partly or even mainly attributed to variation in cuticular traits, as in some plant species there was no significant difference between  $g_{min}$  and cuticular conductance (Burghardt & Riederer, 2003; Schuster et al., 2017). One of the most controversial cuticular traits associated with  $g_{min}$  is cuticular thickness. In some plant species, such as *Arabidopsis thaliana*, an increase in cuticular thickness is often accompanied by decreased  $g_{min}$  values (Kosma et al., 2009; Patwari et al., 2019). However, the relationship between cuticular thickness and  $g_{min}$  in tree species is generally weak. For example, there was no significant relationship between cuticular thickness and  $g_{min}$  for 30 tropical savanna tree species (Machado et al., 2021). In addition, Grünhofer et al. (2022) showed that leaf residual transpiration in *Populus × canescens* detached leaves remained unchanged after a 12.5-fold increase in leaf cuticular wax thickness. Nevertheless, the effect of cuticular thickness on  $g_{min}$  for

temperate tree species remains unclear.

In recent years, it has been increasingly recognized that stomatal water leakage plays an important role in  $g_{min}$ . The average value of  $g_{min}$  was estimated to be  $4.9 \text{ mmol m}^{-2} \text{ s}^{-1}$  for 221 plant species, which was 10-fold higher than cuticular conductance ( $0.45 \text{ mmol m}^{-2} \text{ s}^{-1}$  for 139 species), suggesting that stomatal water leakage has a significant impact on  $g_{min}$  (Duursma et al., 2019). A more precise quantitative study on  $g_{min}$  showed that stomatal water leakage accounted for 6.6 to 43.6 % of  $g_{min}$  in 30 tropical savanna tree species, supporting the leaky stomata hypothesis (Machado et al., 2021). Therefore, it is highly plausible that the interspecies variation of  $g_{min}$  is closely related to leaf stomatal traits such as stomatal size and density (Šantrůček et al., 2004). In the same study, Machado et al. (2021) found that leaves with smaller stomatal size and higher stomatal density generally had higher  $g_{min}$  values in 30 tropical savanna tree species. In addition,  $g_{min}$  was also positively correlated with stomatal density in 10 *Sorghum* genotypes (Muchow & Sinclair, 1989). Therefore, it is reasonable to assume that a similar relationship between stomatal traits and  $g_{min}$  exists in temperate tree species, but this requires conclusive research evidence.

In addition to remarkable interspecies variation,  $g_{min}$  may exhibit significant intraspecific variation, as leaf stomata and cuticle are highly responsive to environmental factors, especially soil water availability (Duursma et al., 2019). Intraspecific variation for a given plant functional trait is mainly depended on the genotypic and phenotypic plasticity (Aitken et al., 2008; Alberto et al., 2013; Nicotra et al., 2010; Westerband et al., 2021). With the increasing severity of drought stress, genotypic plasticity may provide more opportunities for tree populations (i.e., genotypes) to form higher drought tolerance and thus maintain the species' fitness. A large body of crop studies have reported genotypic plasticity in  $g_{min}$ , as  $g_{min}$  has long been recognized as a critical drought tolerance trait in agricultural breeding work (Sinclair & Ludlow, 1986). For instance, Muchow and Sinclair (1989) showed that  $g_{min}$  varied from  $6.3$  to  $17.6 \text{ mmol m}^{-2} \text{ s}^{-1}$  among 10 *Sorghum* genotypes and that  $g_{min}$  was also positively correlated with stomatal density. In addition,  $g_{min}$  in the flag leaves ranged from  $16.0$  to  $28.6 \text{ mmol m}^{-2} \text{ s}^{-1}$  in 11 durum wheat genotypes grown under well-



watered conditions (Araus et al., 1991). However, the genotypic plasticity of  $g_{min}$  is poorly understood in tree species. When functional traits show limited genotypic plasticity, phenotypic plasticity can ensure that functional traits can be quickly adjusted in response to the changes in local environments to facilitate tree acclimation. Some studies have demonstrated that  $g_{min}$  can quickly adapt to local soil water conditions, suggesting a strong phenotypic plasticity in  $g_{min}$  (Cavender-Bares et al., 2007; Duursma et al., 2019; Villar-Salvador et al., 1999; Warren et al., 2011). For example, after two months of drought treatment,  $g_{min}$  in one-year-old *Pinus halepensis* seedlings decreased by an average of 28 % (Villar-Salvador et al., 1999). In addition, for 11 *Hekea* species,  $g_{min}$  decreased 31 % to 63 % when plants experienced chronic drought stress (Duursma et al., 2019). However, for trees from the same *Corymbia calophylla* population, no significant differences were found between trees grown in wet and dry environments (Challis et al., 2021). Therefore, a more explicit panorama on the degree of genotypic and phenotypic plasticity of  $g_{min}$  is imperative to understand the differential drought-induced tree mortality within tree species.

Recent studies have reported that  $g_{min}$  can respond to instantaneous changes in air temperatures, showing higher values under hotter temperatures. For instance, Billon et al. (2020) demonstrated that  $g_{min}$  increased two- to four-fold in five temperate tree species along an ascending temperature gradient from 30 to 55 °C. For two desert plants,  $g_{min}$  of *Citrullus colocynthis* increased three-fold when the temperature increased from 25 to 50 °C, whereas  $g_{min}$  remained stable in *Phoenix dactylifera* (Bueno et al., 2019). Similar results were also found in tropical broadleaf tree species (Slot et al., 2021) and cool-temperate evergreen angiosperms (Hartill et al., 2023), suggesting that the thermal sensitivity of  $g_{min}$  is highly species-specific. Some studies have linked the temperature response of  $g_{min}$  to increased leaf cuticular permeability, as the structure of the polymer chains of cutin matrix or the crystalline wax barrier may change at high temperatures, leading to an increase in cuticular permeability and ultimately to an increase in  $g_{min}$  (Schuster et al., 2016). In particular, phase transition temperatures ( $T_p$ ) have been found in some plant species, indicating the onset of the substantial increase in  $g_{min}$  at high temperatures due to significant changes in the structure of leaf cuticular wax. Different

lines of evidence have demonstrated that  $T_p$  is also highly species-specific, ranging from 30 to 50 °C in different tree species. For example, Hartill et al. (2023) found that of the 14 eucalypt species in Tasmania, *Eucalyptus vernicosa* had the lowest  $T_p$  at about 29.8 °C, while *Eucalyptus dalrympleana* had the highest  $T_p$  at about 40.9 °C. With the increasing temperatures during summer drought periods, drought vulnerability of trees may become more elusive, as temperature responses of  $g_{min}$  and  $T_p$  are highly species-specific.

Interestingly,  $g_{min}$  may vary as the leaf matures or ages. Burghardt and Riederer (2006) showed that cuticular conductance can increase two to three orders of magnitude after removing the outermost wax layer of the cuticle, suggesting the paramount importance of cuticular integrity for controlling leaf water loss. At the same time, leaf cuticular wax is typically synthesized at the early stage of leaf ontogeny, and in some species, it maintains stable water permeability throughout the growing season (Gamarra & Kahmen, 2016; Hauke & Schreiber, 1998; Kahmen et al., 2011; Sachse et al., 2010). However, wind, rain, and particle abrasion, as well as radiation and bacterial damage, can undermine cuticular integrity, leading to seasonal variation of  $g_{min}$ , especially in evergreen plant species (Baker & Hunt, 1986; Elliott-Kingston et al., 2014; Hoad et al., 1992). For example, in the central Rocky Mountains of the United States,  $g_{min}$  of *Picea engelmannii* increased by 40 % in the current-year needles after the first winter (Hadley & Smith, 1994). In another study,  $g_{min}$  in evergreen shrub *Agastachys odorata* also increased progressively with leaf age, with  $g_{min}$  in 10-year-old leaves nearly seven-fold higher than in 1-year-old leaves (Jordan & Brodribb, 2007). Notably, some studies have demonstrated that leaf aging is also accompanied by a significant loss of stomatal control, showing higher stomatal water loss, especially in deciduous species (Reich, 1984; Reich & Borchert, 1988). For the sake of simplicity, however, most of the literature usually considered  $g_{min}$  to be a seasonally stable leaf trait and ignored the potential effects of cuticle and stomatal aging, which leaves some uncertainty in the estimation of canopy residual water loss.

Bark transpiration mainly depends on bark conductance (hereafter referred as  $g_{bark}$ ), total stem and branch bark area, and VPD that the bark experiences. However,

there is comparatively little research on  $g_{bark}$ , and most focus on tropical tree species. It has been reported that  $g_{bark}$  ranged from 0.86 to 12.98 mmol m<sup>-2</sup> s<sup>-1</sup> in eight tropical tree species (Wolfe, 2020). In another study, Loram-Lourenço et al. (2022) found similar  $g_{bark}$  in ten tropical savanna tree species, ranging from 2.97 to 11.0 mmol m<sup>-2</sup> s<sup>-1</sup>. These studies have suggested that  $g_{bark}$  values are close to  $g_{min}$  values in woody plants and that  $g_{bark}$  also varies considerably among tree species. High  $g_{bark}$  can assist stems in acquiring resources and promote stem growth, but at the same time, it can also lead to a high risk of stem dehydration during drought. Wolfe (2020) showed a significant positive relationship between  $g_{bark}$  and stem water deficit for eight tropical tree species under drought stress. Similarly, in a semi-arid environment, water loss through bark transpiration accounted for 64 – 78% of the total water loss from *Pinus halepensis* trees after stomatal closure (Lintunen et al., 2021). Although several studies have demonstrated that  $g_{bark}$  is intimately linked to the outmost layer of bark (i.e., the phellem, Leite & Pereira, 2017; Lenzian, 2006), and in particular to hydrophobic waxes (e.g., suberin, Rains et al., 2018) in the phellem cell wall, the temperature response of  $g_{bark}$  remains untested.

Despite the widespread recognition that  $g_{min}$  and  $g_{bark}$  play a critical role in determining trees' hydraulic safe time under drought after stomatal closure, the methodology has hindered quantifying these two physiological traits in a wide range of tree species. The most widely used method for determining  $g_{min}$  and  $g_{bark}$  is repeated measurements of mass loss in detached leaves or stems (i.e., MLD, Duursma et al., 2019). Theoretically, MLD simulates the trajectories of leaf dehydration during a prolonged drought event, while full stomatal closure becomes much faster under MLD (ca. 0.5 – 2 h) than under natural drought conditions. Notably, the progression of leaf dehydration needs to be carried out in a known stable environment, especially constant air temperature and relative humidity, as changes in the environment can lead to changes in leaf dehydration rate. However, when studying the temperature response of  $g_{min}$  and  $g_{bark}$ , extremely high temperatures (e.g., 40 – 50 ° C) are often employed, making fine control of the environment challenging. In addition, depending on plant species and environmental conditions, repeated measurements of water loss throughout

the plant dehydration can last for tens of hours, which is labor-intensive and may also destabilize the environment. Therefore, improving the traditional MLD is integral to the in-depth study of  $g_{min}$  and  $g_{bark}$ .

In summary, under severe hot droughts, the minimal water use of a tree after full stomatal closure will directly affect the surviving time of the tree. Yet, this minimal water use has not been quantified, mainly because  $g_{min}$  and  $g_{bark}$  vary considerably between and within species and exhibit great plasticity potential. Therefore, a systematic study on the variability of  $g_{min}$  and  $g_{bark}$  is necessary to deepen our understanding of tree minimum water consumption and to improve plant drought response models.

### ***Thesis outline***

The aim of this thesis was to quantify the minimal water use of trees under hot droughts by comprehensively exploring the variation of  $g_{min}$  and  $g_{bark}$  in temperate European tree species. Specifically, we asked: (1) if the Drought Box is a reliable tool for determining  $g_{min}$  and  $g_{bark}$  and how  $g_{bark}$  responds to increasing temperatures for eight temperate tree species, (2) the degree of genotypic and phenotypic plasticity of  $g_{min}$  in four temperate tree species, and (3) how much is the minimal water use of trees in nine temperate tree species during a severe hot drought? Based on these research questions, this thesis is divided into the following three chapters:

Since the commonly used method (i.e., MLD) for measuring  $g_{min}$  and  $g_{bark}$  is labor-intensive and difficult to maintain a stable environment, in **Chapter 1**, we constructed and tested the "Drought Box", a novel research tool that allows semi-automated measurements of  $g_{min}$  and  $g_{bark}$  in a highly controlled environment. Using the Drought Box, we determined  $g_{min}$  and the temperature response of  $g_{bark}$  for eight temperate European tree species at our forest research site, Swiss Canopy Crane II (SCCII). Specifically, we asked the following questions: (1) whether the Drought Box can maintain stable temperature (T) and relative humidity (RH) over time while accurately recording plant water loss, (2) whether the  $g_{min}$  values measured with twigs in the Drought Box are not flawed by  $g_{bark}$ , and (3) whether  $g_{bark}$  can respond to

increasing air temperatures.

Interspecies variation of  $g_{min}$  is widely reported among woody species from contrasting climates, but little is known about intraspecific variation resulting from genotypic and phenotypic plasticity. Therefore, in **Chapter 2**, we quantified the genotypic and phenotypic plasticity of  $g_{min}$  and its thermal sensitivity for four temperate tree species grown in three common gardens in Switzerland with different hydroclimates. For each species, we tested different provenances originating from different populations and climatic regions across Europe. At the same time, we investigated the leaf cuticular and stomatal traits to understand differences in  $g_{min}$  between provenances or common garden trials. Specifically, we ask: (1) if  $g_{min}$  and its temperature response differ between tree provenances originating from wet and dry regions when they are grown in the same common garden, (2) for a given provenance, if  $g_{min}$  and its temperature response differ between different common garden sites, (3) if there are genotype-by-phenotype interaction effects on  $g_{min}$  and its temperature response, and (4) if the variation in  $g_{min}$  is correlated with leaf cuticular and stomatal traits.

Finally, to quantify the canopy residual water loss for adult trees during severe hot droughts, in **Chapter 3**, we conducted a comprehensive investigation of  $g_{min}$  variability in nine European tree species at the SCCII research site. Specifically, we explored the seasonal variation of  $g_{min}$  among these nine tree species and the relationship between leaf cuticular and stomatal traits and interspecific variation in  $g_{min}$ . Given the increasing temperatures during drought, we determined the species-specific temperature response of  $g_{min}$  for all studied species. With these newly obtained insights, we calculated daily canopy residual transpiration for the nine species using climate data from the hot drought that occurred at our research site in 2022.



## **Chapter I**

### **Determining leaf minimum conductance and bark conductance in 8 temperate tree species using the “Drought Box”**

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## Abstract

Trees typically close their stomata under hot and dry conditions to prevent excessive water loss. However, after full stomatal closure, uncontrolled minimum water loss from leaves ( $g_{min}$ ) and bark ( $g_{bark}$ ) can lead to slow dehydration and even death of the tree. The Drought Box is a novel tool that allows semi-automated measurements of minimum water loss of branches in a finely controlled environment. Yet, it is unclear whether  $g_{bark}$  could confound  $g_{min}$  measurements in the Drought Box. In this study, we constructed the Drought Box and tested it under various conditions. Using the Drought Box, we determined  $g_{min}$  and  $g_{bark}$  for eight temperate European tree species. Test results show that the Drought Box enables precise control of target temperature and relative humidity and maintains stable environmental conditions for a long time. At the same time, the Drought Box can record the water loss of plants under different environmental conditions with high resolution. Our results show that  $g_{min}$  varied considerably among the eight studied tree species, ranging from 0.7 to 3.0 mmol m<sup>-2</sup> s<sup>-1</sup>. In addition, the range of  $g_{bark}$  values was very close to  $g_{min}$ , ranging from 1.7 to 2.8 mmol m<sup>-2</sup> s<sup>-1</sup>. At the same time, most species kept stable  $g_{bark}$  when the temperature increased from 25 to 45 °C. Therefore, our study suggests that the Drought Box is a reliable and efficient tool for studying the variability of  $g_{min}$  and  $g_{bark}$  among tree species, especially their responses to high temperatures. Given the small surface area of bark compared to leaves in twigs (or shoots) and the limited weighing precision of the Drought Box, measuring  $g_{min}$  on twigs in the Drought Box is recommended, and the resulting values are not flawed by  $g_{bark}$ .

## Introduction

The co-occurrence of summer heatwaves and droughts (i.e., hot droughts) have led to profound changes in composition, structure, and function of global forests by affecting tree eco-physiological processes (e.g., transpiration and photosynthesis, (Adams et al., 2009; Allen et al., 2015; Anderegg et al., 2019; Bauman et al., 2022; Dyderski et al., 2018; Reichstein et al., 2013). Furthermore, the intensity and frequency of hot droughts are expected to increase (Adams et al., 2017; Breshears et al., 2021; Reyer et al., 2013), making the fate of global forests even more elusive. Trees will close leaf stomata to avoid fatal dehydration during severe hot droughts, thereby reducing their transpirational water demands to 1 – 2% of that under ample soil water conditions (Körner, 2019; Martinez-Vilalta et al., 2019; Wang et al., 2024). This residual transpiration is mainly caused by two processes: one is water loss through leaf cuticle and leaky stomata, the other is bark transpiration from stems and branches (Duursma et al., 2019; Loram-Lourenço et al., 2022; Machado et al., 2021). Although residual transpiration only represents a small fraction of the total water loss of a tree, if the tree's water reservoirs cannot be replenished promptly under prolonged hot droughts, it may lead to the ultimate death of the tree when the dehydration in the key tissues or organs reaches lethal thresholds (Blackman et al., 2016, 2019; Körner, 2019; Machado et al., 2021; Mantova et al., 2021).

The rate of leaf residual transpiration depends on the leaf minimum water conductance ( $g_{min}$ ) after full stomatal closure. Based on a compilation of earlier data, the average  $g_{min}$  for temperate forest tree leaves was about  $5.8 \pm 0.8 \text{ mmol m}^{-2} \text{ s}^{-1}$  ( $n = 16$  species), while the average leaf maximum stomatal conductance was about  $218 \pm 24 \text{ mmol m}^{-2} \text{ s}^{-1}$  (Körner, 2005). Although absolute values of  $g_{min}$  are typically low, different lines of evidence have demonstrated that  $g_{min}$  varies considerably among tree species (Schuster et al., 2017). Duursma et al. (2019) found that  $g_{min}$  ranged from 0.1 to  $25.0 \text{ mmol m}^{-2} \text{ s}^{-1}$  for 127 woody species. Several studies have suggested that the interspecific variation in leaf cuticular and (or) stomatal traits may partly explain the wide range of  $g_{min}$  values between tree species. For example, Machado et al. (2021)



found that among 30 tropical savanna tree species, those with small and numerous stomata had higher  $g_{min}$  values. Furthermore, increasing air temperatures can lead to structural changes in leaf cuticular wax, resulting in increased cuticular permeability and, ultimately, higher  $g_{min}$  (Riederer & Schreiber, 2001; Schuster et al., 2016). Based on Billon et al. (2020),  $g_{min}$  increased two- to four-fold in five temperate tree species when temperatures increased from 30 to 55 °C. At the same time, modeling of plant drought responses has also shown a strong negative relationship between  $g_{min}$  and tree's surviving time under prolonged drought conditions (Blackman et al., 2016, 2019, 2023; Challis et al., 2021; Cochard, 2021; Martin-StPaul et al., 2017)

Although  $g_{min}$  plays a critical role in the drought tolerance of trees, the methodology for determining  $g_{min}$  has largely limited research on a wide range of tree species. The most widely used approach to determining  $g_{min}$  is quantifying the mass loss of detached leaves (MLD), which relies on repeated measurements of leaf water loss in a stable environment (Burghardt & Riederer, 2003; Duursma et al., 2019). MLD is time-consuming and laborious, especially when studying tree species with low  $g_{min}$  values (e.g., evergreen conifers), where repeated measurements can take dozens of hours. In addition, as leaf water loss rates will be subjected to the vapor pressure deficit (VPD) during dehydration, MLD requires a stable environment (i.e., air temperature and relative humidity), which may become challenging when exploring the temperature response of  $g_{min}$  at high temperatures (e.g., 40 – 50 °C). Some studies have determined the temperature response of  $g_{min}$  by using modified drying ovens (Hartill et al., 2023) or plant incubators (Schuster et al., 2016). Nevertheless, these research methods still require repeated measurements of plant water loss, during which VPD may change occasionally.

Recently, Billon et al. (2020) proposed a novel research tool, the Drought Box, for semi-automatically determining  $g_{min}$  in a highly controlled environment. Compared to other research tools, the main innovation of the Drought Box is the use of micro load cells to continuously measure the weight of the plant samples during dehydration, thereby obtaining the amount of leaf minimal water loss at a given period. At the same time, the Drought Box enables accurate regulation of temperature and humidity over a

wide range. In their study, Billon et al. (2020) determined the temperature response of  $g_{min}$  for five tree species using the Drought Box and found that  $g_{min}$  increased two- to four-fold when the temperature increased from 30 to 55 °C. However, it is worth noting that these authors used shoots (ca. 40 cm) for their measurements and reported the residual shoot conductance, including  $g_{min}$  and bark conductance ( $g_{bark}$ ). Considering the light leaf mass of many tree species and the precision of load cells in the Drought Box (precision:  $\pm 50$  mg), the use of branches or twigs to measure  $g_{min}$  may be necessary, especially when measuring at high temperatures, where plant samples lose most of their water in a short period. It is, however, unclear whether  $g_{bark}$  could confound  $g_{min}$  measurements in the Drought Box, especially when exploring the temperature response of  $g_{min}$ .

In contrast to  $g_{min}$ , there are only a few studies on  $g_{bark}$ , and most of the available  $g_{bark}$  data are from tropical species. For example, Loram-Lourenço et al. (2022) found that  $g_{bark}$  ranged from 2.97 to 11.0 mmol m<sup>-2</sup> s<sup>-1</sup> among ten tropical tree species, significantly higher than  $g_{min}$  for these species. Wolfe (2020) also reported similar  $g_{bark}$  values for eight tropical tree species, ranging from 0.86 to 12.98 mmol m<sup>-2</sup> s<sup>-1</sup>. Some studies have demonstrated that the effective barrier of bark against water loss is provided by the outer bark, especially the phellem in the outer bark (Leite & Pereira, 2017; Lenzian, 2006; Wittmann & Pfanz, 2008). At the same time, lenticels pierce phellem and provide efficient tunnels for water and gas exchange between the atmosphere and internal stem tissues, directly affecting  $g_{bark}$  (Leite & Pereira, 2017; Loram-Lourenço et al., 2022). Thus, these studies suggest that  $g_{bark}$  also exhibits significant interspecific variation among tree species, which may be related to the phellem and lenticel traits. Although high  $g_{bark}$  is likely to help trees access resources and promote stem and branch growth, it may simultaneously lead to stem excessive water loss under prolonged drought conditions (Lintunen et al., 2021; Loram-Lourenço et al., 2022; Wolfe, 2020). For instance, Wolfe (2020) has shown a strong positive correlation between  $g_{bark}$  and stem water deficit. Interestingly, the physiologically important role of  $g_{bark}$  in the whole-tree water loss can proportionally increase with tree size in some species (Lintunen et al., 2021), as the ratio of total bark surface to total

leaf area will typically increase as the tree matures (Hölttä et al., 2013). Given the increasing severity of hot droughts, one of the critical questions is whether  $g_{bark}$  can respond to instantaneous changes in air temperatures. However, the temperature response of  $g_{bark}$  has never been tested.

In this study, we built the Drought Box following the instruction of Billon et al. (2020) and tested its reliability under a range of environmental conditions. We then explored  $g_{min}$  and the temperature response of  $g_{bark}$  for eight temperate European tree species at our forest research site, Swiss Canopy Crane II (SCCII). Specifically, we asked: (1) whether the Drought Box can maintain stable temperature (T) and relative humidity (RH) over time while accurately recording plant water loss, (2) whether the  $g_{min}$  values measured with twigs are not flawed by  $g_{bark}$ , and (3) whether  $g_{bark}$  can respond to increasing air temperatures

## **Materials and methods**

### *Description of the Drought Box*

The Drought Box is a rectangular wooden structure, 1.65 m long, 0.72 m wide, and 0.97 m high, with a total volume of about 1.15 m<sup>3</sup> (Figure S1). It consists of three main compartments and a front door: compartment A is the weighing chamber, compartment B is the drying chamber, and compartment C is the control chamber. Compartment A contains 12 single-point load cells (capacity: 100g, precision: ± 50 mg, SEN-14727, SparkFun Electronics, USA) fixed in two columns on an aluminum frame. Every four load cells are connected to a load cell interface (PhidgetBridge 4-Input, Phidgets Inc. Canada), where pressure variations in the load cells are amplified and transmitted as voltage changes and ultimately converted into the corresponding mass changes. The interior of compartment B is lined with 50 mm thick polystyrene foam board, and heating resistor wires (HK-5.0-12, Arnold Rak Wärmetechnik GmbH, Germany) are installed at the bottom to control temperature in the Drought Box up to 50 °C. The relative humidity is controlled by a humidifier (SKU399824, Warmtoo, China), and the consumed water is automatically replenished by a 2 L water tank in compartment C. An air temperature and humidity sensor (AM2315, Aosong Electronics, China) is placed in the middle of compartment B to monitor the environment in real time and transmit the data to the control panel. According to the target environmental conditions, dry ambient air will be pumped into compartment B to regulate the temperature and humidity inside the chamber. The plant materials will be fixed to each of the 12 hooks connected to the load cells through small holes at the top of compartment B. In order to promote air circulation inside the Drought Box and reduce the boundary layer resistance at the plant material's surface, fans (HA40201V4-1000U-A99, SUNON, China) are installed in both compartments A and B and are switched on throughout the experiment. The Drought Box is controlled by a nano-computer, Raspberry-Pi 3 (Raspberry Pi Foundation, United Kingdom) in compartment C, where the researchers can set the target temperature and relative humidity. At the same time, the weight change from each load cell is also displayed on the monitor. Once the measurements

are complete, the Drought Box provides a download of the data file, which includes the time series, temperature, relative humidity, and sample mass.

### ***Drought Box test***

To test whether the Drought Box can maintain stable temperature (T) and relative humidity (RH) for an extended period, we set T and RH at 35 °C and 40 %, respectively, for 80 h. In addition, to test the Drought Box's ability to record the weight of the samples in different environments accurately, different weights of aluminum foil leaves (27.8, 36.8, 30.2, 25.2, 20.0, 24.3, 50.7, 23.4, 9.8, 17.4, 7.6, 14.6 g) were fixed to each of the 12 weighing hooks. The temperatures were set to 25, 30, 40, and 50 °C, and the relative humidity was 40, 35, 30, and 25 %, respectively. These tests were carried out continuously, with each environmental condition tested for 10 to 12 hours.

### ***g<sub>min</sub> and g<sub>bark</sub> measurements***

Plant samples were collected at the Swiss Canopy Crane II (SCCII) research site (47°26'17"N, 7°46'37"E) close to Hölstein, Basel-Landschaft, Switzerland. The research site is at the top of a Swiss Jura hill at 550 m a.s.l., where a mature temperate mixed forest grows on high clay soils. The site covers an area of approximately 1.68 ha and comprises 458 trees from 14 common European tree species, with a diameter at tree breast height > 10 cm (Kahmen et al., 2022). At the center of the site, there is a 50 m high canopy crane with a radius of 62.5 m, allowing access to 333 trees. According to the mean data (1991 – 2020) from the closest MeteoSwiss climate station, Rünenberg, the mean annual temperature and precipitation in this region are 9.6 °C and 972 mm, respectively. In the study, we investigated  $g_{min}$  and the temperature response of  $g_{bark}$  for eight tree species, including *Acer pseudoplatanus* L., *Carpinus betulus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Quercus* sp. (hybrid forms by *Q. petraea* Liebl. and *Q. robur* L.), *Abies alba* Mill., *Picea abies* Karst., *Pinus sylvestris* L. For simplicity, all tree species are referred by their genus names in the following.

The measurements of  $g_{min}$  of detached leaves had been described by Wang et al. (2024). Therefore, we focused on determining  $g_{bark}$  and compared these two values for

each tree species in this study. Specifically, the investigation of  $g_{bark}$  was conducted at the SCCII research site from December 2022 to February 2023. Each species consisted of four different individual replicates. It was worth noting that *Acer* and *Fraxinus* samples were taken from young understory trees (ca. 3 m high). Samples for the other six species were collected from adult trees using a canopy crane or long pruning shear. On the morning of each sampling day, four different south-exposed shoots (ca. 1.5 cm in diameter and 70 cm long) were cut from different trees. The cut ends were immediately wrapped in wet towels, packed in black bags and brought to the laboratory in Basel. For five deciduous species (*Acer*, *Carpinus*, *Fagus*, *Fraxinus*, and *Quercus*), we assumed all leafless shoots had been fully rehydrated in the field during the wet winter of 2022. In contrast, shoots of the three evergreen conifers (*Abies*, *Picea*, and *Pinus*) were rehydrated entirely in the lab. Specifically, conifer shoots were recut underwater to release xylem tension (ca. 90 s) and rehydrated in degassed water overnight in the dark. After rehydration, all shoots were trimmed off the bottom, leaving only the healthy leafless upper or middle segment (ca. 15 – 30 cm long) for the measurements, and the cut ends were sealed by high-melting paraffin wax (melting point 68 °C). For simplicity, we considered the segment as a cylinder and measured the diameter and length using a vernier caliper (Burg-Wächter, Germany) to estimate the surface area ( $A$ ). Segments were then measured on balance (precision:  $\pm 1.0$  mg, PL303, METTLER TOLEDO, Switzerland) to obtain the saturated weight ( $W_{sat}$ ). Subsequently, segments were suspended in the Drought Box to progressively dehydrate for 15 – 70 h, depending on the environmental conditions in the Drought Box. In particular,  $g_{bark}$  was measured with different samples at 25, 30, 35, 40, and 45 °C, and the corresponding relative humidity was 35, 38, 40, 40, and 40 %. Meanwhile, the fresh weight ( $W_{fre}$ ) was automatically measured every minute. After the measurements in the Drought Box, all segments were dried down in the oven for 72 h at 80 °C to obtain the dry mass ( $W_{dry}$ ). Therefore, the relative water content ( $RWC$ ) of segments was calculated as:

$$\text{Eq. 1} \quad RWC = \frac{W_{fre} - W_{dry}}{W_{sat} - W_{dry}}$$

Subsequently, based on the curve of bark water loss against dehydration time,  $g_{bark}$  was calculated as the slope of the linear portion of the curve, normalized by the total bark area ( $A$ ) and the driving force VPD, following:

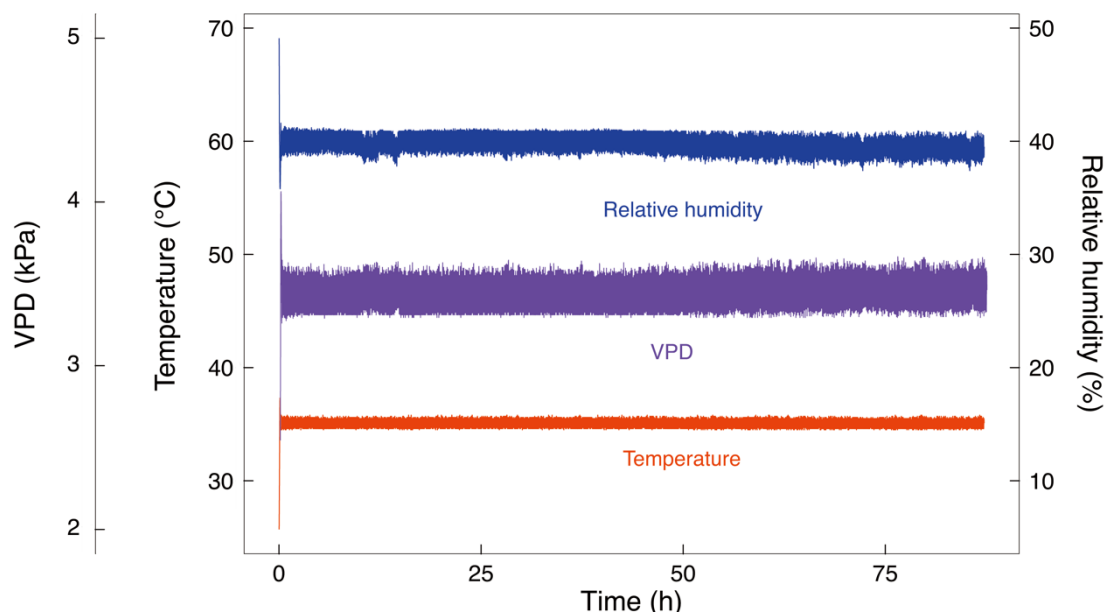
$$\text{Eq. 2} \quad g_{bark} = \frac{\text{Slope} * 98.0}{A * VPD}$$

It is worth noting that we only used the linear portion of the curve to determine  $g_{bark}$  when the corresponding  $RWC$  was still above 50 %, as the bark may not be functionally intact at a more serious water deficit and bark surface may crack due to shrinkage. The atmospheric pressure in the Drought Box was assumed to be 98.0 kPa. Additionally, we assumed the bark internal air to be fully saturated and in equilibrium with air temperature.

### ***Statistics***

The difference in  $g_{min}$  and  $g_{bark}$  between the two functional groups (deciduous vs evergreen species) was determined with the Mann–Whitney  $U$  test. Significant differences in  $g_{bark}$  values measured at different temperatures were determined with ANOVA tests followed by a Student’s T post-hoc test when hypothesis tests were met. Otherwise, a Kruskal Wallis test was used and followed by the Durbin-Conover post hoc test when homoscedasticity of variances was violated. The significance threshold was 0.05 throughout all analyses. All the statistical analyses were performed in R v.4.1.2 (R Core Team, 2021).

## Results

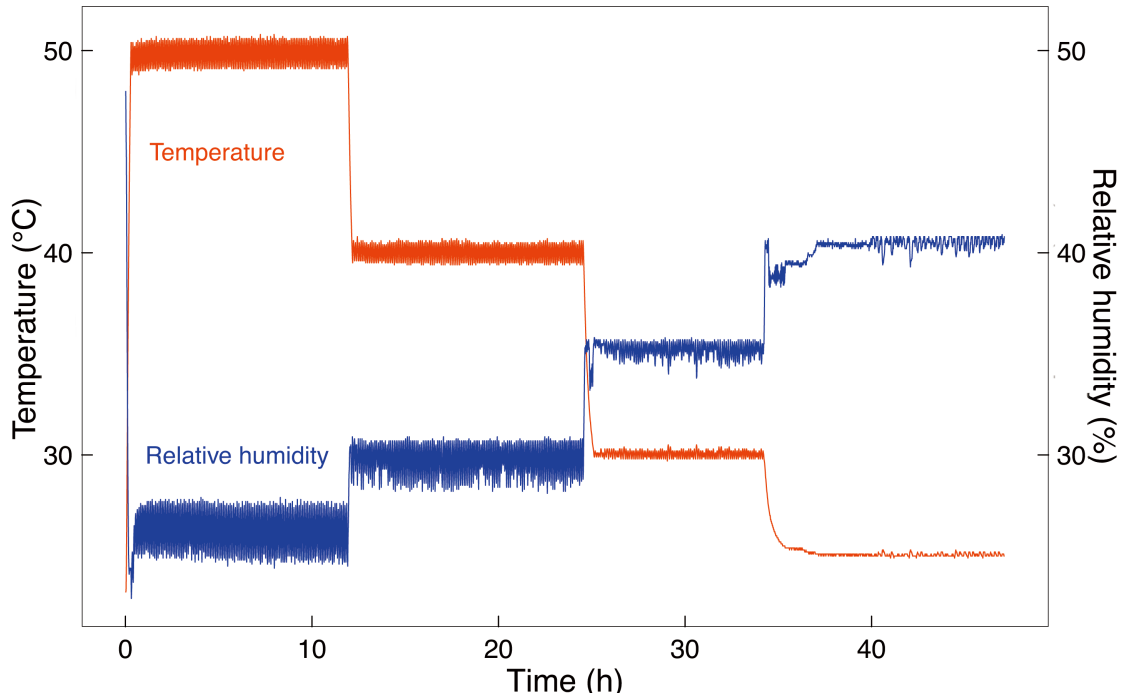


**Figure 1.** Time course of temperature (red line), relative humidity (blue line), and vapor pressure deficit (VPD, purple line) in the Drought Box at target 35 °C with a relative humidity of 40 %.

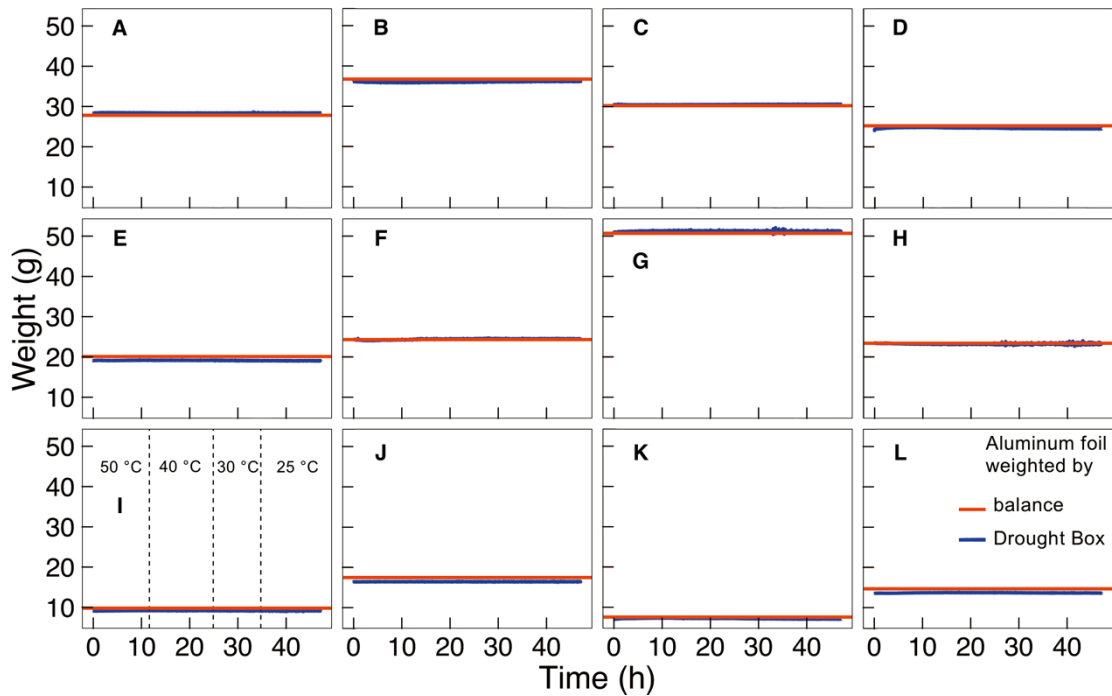
### *The Drought Box Performance*

When the target T and RH were 35 °C and 40 %, respectively, our results show that the Drought Box maintained a stable T ( $35.1 \pm 0.4$  °C, mean  $\pm$  standard deviation) and RH ( $39.7 \pm 0.9$  %) during the 80-hour-long test (Figure 1). The corresponding VPD was about  $3.4 \pm 0.1$  kPa. During the consecutive tests, the Drought Box took 17 min to heat from 23.2 to 49.5 °C, and RH decreased from 48.0 to 25.1 % in 10 min (Figure 2). After each adjustment, the Drought Box maintained all target T (Figure 2, Table S1) at  $25.1 \pm 0.1$  °C,  $30.1 \pm 0.2$  °C,  $40.0 \pm 0.4$  °C, and  $49.9 \pm 0.6$  °C, respectively. The corresponding RH values were at  $40.3 \pm 0.4$  %,  $35.2 \pm 0.4$  %,  $29.8 \pm 0.8$  %, and  $26.2 \pm 1.0$  %. Meanwhile, the Drought Box measured the weights of the material accurately and consistently (Figure 3). There was no evident measurement drift in the Drought Box under various environmental conditions (Figure 3, Table S2).





**Figure 2.** Time course of environmental conditions in the Drought Box at target temperatures of 50, 40, 30 and 25 °C and corresponding relative humidity of 25, 30, 35 and 40 %.



**Figure 3.** Time course of the weights of aluminum foil under different environmental conditions (25 – 50 °C) in the Drought Box. Red and blue lines indicate the weights measured by the lab balance and the 12 weighing hooks (A to L) in the Drought Box, respectively.

### *g<sub>min</sub>* and *g<sub>bark</sub>*

According to the high-resolution measurements of plant water loss in the Drought Box, leaf and bark dehydration trajectories were divided into three distinct stages (Figure S1). First, the water content of leaves and bark declined rapidly over 1 – 2 h until the full stomatal closure or the diminishment of condensation in the bark, showing a high slope of sample weight versus dehydration time (Stage A). Subsequently, leaves and bark lost water at relatively constant rates (*g<sub>min</sub>* and *g<sub>bark</sub>*) with a stable and shallower slope (Stage B). Finally, leaf and bark water content was depleted, and the curve became flat (Stage C).

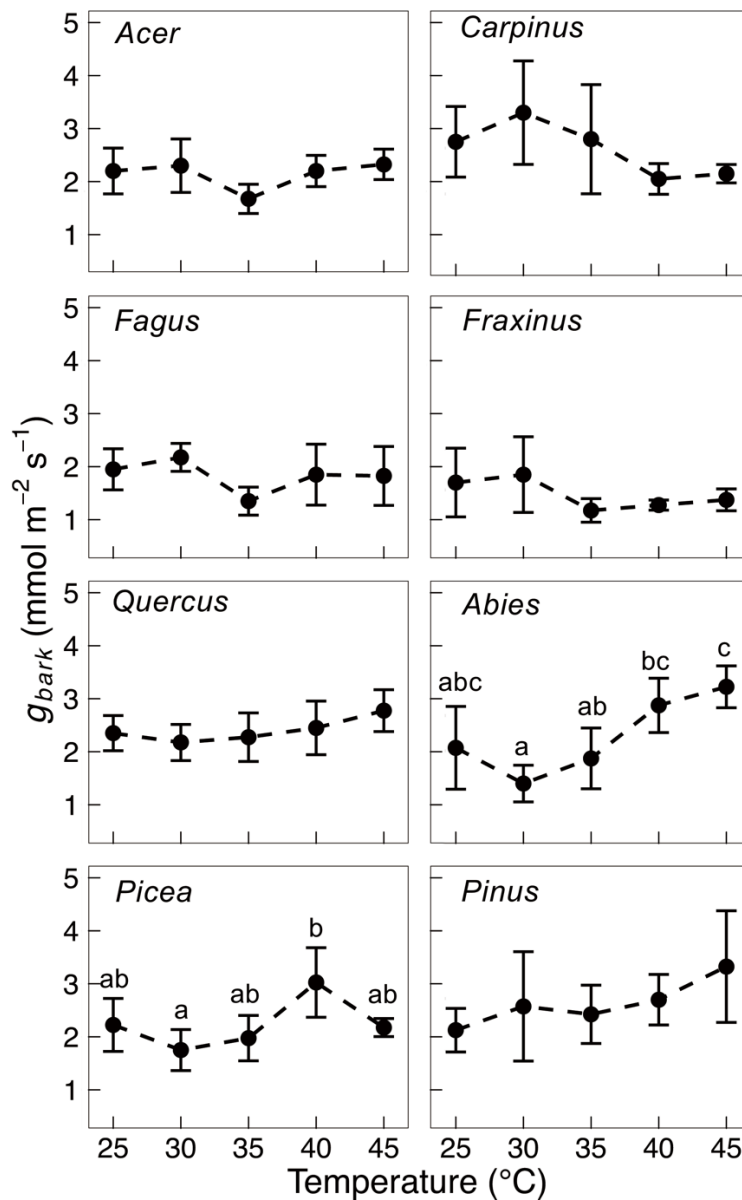
**Table 1.** *g<sub>min</sub>* (mmol m<sup>-2</sup> s<sup>-1</sup>) and *g<sub>bark</sub>* (mmol m<sup>-2</sup> s<sup>-1</sup>) ± standard deviation measured at 25 °C for eight studied species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus sp.*, *Abies alba*, *Picea abies*, *Pinus sylvestris*).

Species	<i>g<sub>min</sub></i> * mmol m <sup>-2</sup> s <sup>-1</sup>	Replicates (tree)	<i>g<sub>bark</sub></i> mmol m <sup>-2</sup> s <sup>-1</sup>	Replicates (tree)
<i>Acer</i>	2.8 ± 0.6	4	2.2 ± 0.4	4
<i>Carpinus</i>	1.1 ± 0.2	4	2.8 ± 0.7	4
<i>Fagus</i>	1.1 ± 0.6	4	2.0 ± 0.4	4
<i>Fraxinus</i>	1.9 ± 0.2	4	1.7 ± 0.6	4
<i>Quercus</i>	3.0 ± 0.6	4	2.4 ± 0.3	4
<i>Abies</i>	0.8 ± 0.2	4	2.1 ± 0.8	4
<i>Picea</i>	0.7 ± 0.2	4	2.2 ± 0.5	4
<i>Pinus</i>	0.7 ± 0.2	4	2.1 ± 0.4	4
Deciduous	2.0 ± 0.4 a	-	2.2 ± 0.4	-
Evergreen	0.7 ± 0.1 b	-	2.1 ± 0.1	-

\**g<sub>min</sub>* values were from Wang et al. (2024). Specifically, *g<sub>min</sub>* was determined with detached leaves for deciduous species and with small twigs for evergreen conifer species. Significant differences (*p* < 0.05) across functional groups are indicated by lowercase letters.

At a temperature of 25 °C, *g<sub>min</sub>* of *Quercus* was the highest of the eight studied tree species, about 3.0 mmol m<sup>-2</sup> s<sup>-1</sup>, which was four times higher than that of the lowest, *Picea* and *Pinus* (0.7 mmol m<sup>-2</sup> s<sup>-1</sup>, Table 1). Overall, deciduous tree species had

significantly higher  $g_{min}$  ( $2.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ ,  $p < 0.001$ ) than evergreen species ( $0.7 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). At a temperature of  $25 \text{ }^{\circ}\text{C}$  and  $40 \%$ ,  $g_{bark}$  ranged from  $1.7$  to  $2.8 \text{ mmol m}^{-2} \text{ s}^{-1}$  among eight studied tree species (Table 1). Deciduous broadleaf and evergreen conifer species showed similar  $g_{bark}$  values, about  $2.2$  and  $2.1 \text{ mmol m}^{-2} \text{ s}^{-1}$ , respectively.  $g_{min}$  and  $g_{bark}$  values for deciduous broadleaf trees showed a similar range, but  $g_{bark}$  values for evergreen conifers were about three times as high as  $g_{min}$  (Table 1).



**Figure 4.** Temperature response of  $g_{bark}$  for eight tree species. Each point is the mean value ( $n = 4$ ), and the error bar represents the standard deviation. Significant differences ( $p < 0.05$ ) across temperatures within a single species are indicated by lowercase letters.

Across the 25 – 45 °C temperature range, only *Abies* and *Picea* exhibited temperature response of  $g_{bark}$  with a significant increase at high temperatures (Figure 4;  $F = 7.5, p < 0.01$ ;  $F = 4.1, p < 0.01$ ), but the absolute differences were very small. Specifically, when the temperature increased from 30 to 45 °C,  $g_{bark}$  of *Abies* increased from 1.4 to 3.2 mmol m<sup>-2</sup> s<sup>-1</sup> (Table S3). For *Picea*,  $g_{bark}$  increased from 1.8 to 3.0 mmol m<sup>-2</sup> s<sup>-1</sup> as the temperature increased from 30 °C to 40 °C, but  $g_{bark}$  decreased at 45 °C (Figure 4, Table S3). Other six studied tree species kept relatively stable  $g_{bark}$  when temperature increased.

## Discussion

In the present study, we tested a novel tool, the Drought Box, for semi-automated measurements of  $g_{min}$  and  $g_{bark}$  in a finely controlled environment. Our results showed that the Drought Box can create and maintain stable environments across various set points. At the same time, it can also accurately record plant water loss at high resolution. At a given temperature of 25 °C,  $g_{min}$  varied considerably among the eight studied tree species, ranging from 0.7 to 3.0 mmol m<sup>-2</sup> s<sup>-1</sup>. At the same time, the range of  $g_{bark}$  values was very close to  $g_{min}$ , ranging from 1.7 to 2.8 mmol m<sup>-2</sup> s<sup>-1</sup>. Notably,  $g_{bark}$  exhibited weak thermal sensitivity in most species along an ascending temperature gradient from 25 to 45 °C.

An essential requirement for determining  $g_{min}$  and  $g_{bark}$  is a stable environment (i.e., air temperature and relative humidity) during leaf and bark dehydration, as environmental changes can lead to changes in plant transpiration rates over time. Although some studies used bench laboratory dehydration to determine  $g_{min}$  (Burghardt & Riederer, 2003; Machado et al., 2021), this method can only provide some ambient temperatures (ca. 25 °C) and is difficult to achieve fine control of the environment, thereby significantly limiting the study of the temperature response of  $g_{min}$  and  $g_{bark}$ , which typically requires high temperatures above 40 °C. For example, Bueno et al. (2019) reported that  $g_{min}$  of *Citrullus colocynthis* measured at 50 °C was 3.2 times higher than at 25 °C. Previous studies have used modified drying ovens (Hartill et al., 2023; Slot et al., 2021) or plant incubators (Schuster et al., 2016) to explore the temperature response of  $g_{min}$ . However, repeated measurements are still needed and thus may lead to uncertain changes in leaf temperatures and (or) environmental conditions. Our results showed that the Drought Box maintains a stable environment for a long time, and accurately measures plant water loss under different environmental conditions (e.g., 25 – 50 °C). Therefore, the Drought Box can greatly promote the study of the temperature response of  $g_{min}$  and (or)  $g_{bark}$  in a wide range of tree species.

Determining  $g_{min}$  and  $g_{bark}$  requires repeated measurements of plant water loss because the leaf and bark dehydration process can be divided into several stages

through which  $g_{min}$  and  $g_{bark}$  need to be correctly confirmed. It is well known that some of the stomata remain open after leaf rehydration in the dark. According to a recent data review, for 221 species from 57 taxonomic families, the average of  $g_{min}$  was about 4.9  $\text{mmol m}^{-2} \text{s}^{-1}$ , eight-fold lower than leaf conductance (mean value = 41.1  $\text{mmol m}^{-2} \text{s}^{-1}$ ) during the night or after dark adaptation (Duursma et al., 2019), suggesting that there is a substantial stomatal leakage in the latter. In our study, we found that the leaf dehydration process displayed three distinct stages, ranging from the initially rapid water loss due to incompletely closed stomata (stage A) to a stable rate of leaf water loss after full stomatal closure (phase B,  $g_{min}$ ), and finally to the depletion of leaf water (stage C). This result is consistent with that reported by Billon et al. (2020). For bark samples, stage A may not be as pronounced as in leaf samples due to the lack of active stomatal regulation. However, it is worth noting that in some studies where bark samples were immersed in water for rehydration, oversaturated bark samples may also result in high rates of bark water loss within the first few hours of dehydration (Loram-Lourenço et al., 2022). Therefore, our study demonstrated that the Drought Box could provide a complete picture of the physiological trajectory of leaf and bark dehydration through automated high-resolution measurements of plant water loss, which will significantly improve the efficiency in the study of  $g_{min}$  and  $g_{bark}$ .

In our study,  $g_{min}$  varied considerably among the eight studied tree species at an ambient temperature of 25°C, ranging from 0.7 to 3.0  $\text{mmol m}^{-2} \text{s}^{-1}$ . These results are within a range of previously published  $g_{min}$  values for 127 woody species (0.1 – 25.0  $\text{mmol m}^{-2} \text{s}^{-1}$ , Duursma et al., 2019). Interestingly, our study shows that the range of  $g_{bark}$  values (1.7 – 2.8  $\text{mmol m}^{-2} \text{s}^{-1}$  at 25 °C) was very close to that of  $g_{min}$ . Few studies have explored  $g_{bark}$  in woody species, and most of the available data are from tropical tree species. Our findings keep in line with previous  $g_{bark}$  studies on *Pinus halepensis* (1.46 – 1.96  $\text{mmol m}^{-2} \text{s}^{-1}$ , Lintunen et al., 2021), ten tropical savanna tree species (2.97 – 11.0  $\text{mmol m}^{-2} \text{s}^{-1}$ , Loram-Lourenço et al., 2022), five temperate broadleaf tree species (5.01 – 27.3  $\text{mmol m}^{-2} \text{s}^{-1}$ , Wittmann & Pfanz, 2008), suggesting that  $g_{bark}$  also varies among tree species. In addition, we found that  $g_{bark}$  was about three times higher than  $g_{min}$  among the three studied evergreen conifers. Similarly, for ten tropical savanna

tree species, Loram-Lourenço et al. (2022) showed that  $g_{bark}$  was significantly higher than  $g_{min}$  for both deciduous and evergreen species. It is worth noting that we used conifer small twigs to determine  $g_{min}$  without calculating bark area in our study. However, our results indicate that  $g_{bark}$  had little impact on  $g_{min}$ , as the  $g_{min}$  values we measured were very close to other  $g_{min}$  values measured using only needles (see details in Anfodillo et al., 2002; Schuster et al., 2017). Therefore, we conclude that  $g_{min}$  values measured with small twigs are not flawed by  $g_{bark}$ . Given the weighing precision of the Drought Box and the small amount of plant water loss after full stomatal closure, it is reasonable and feasible to use twigs (or shoots) rather than individual leaves to determine  $g_{min}$  in the Drought Box.

Different lines of evidence have suggested that one of the most critical mechanisms by which hot temperatures increase the mortality risk of drought-stressed trees is the increase in canopy residual transpiration, which is induced by higher  $g_{min}$  and VPD (Billon et al., 2020; Cochard, 2020). Given the fact that the temperature response of  $g_{min}$  has been reported across different forest types (Billon et al., 2020; Bueno et al., 2019; Hartill et al., 2023; Slot et al., 2021), it is highly plausible that trees display the temperature response of  $g_{bark}$ . Unexpectedly, we found that most of the studied tree species showed relatively stable  $g_{bark}$  under hot temperatures, indicating that the bark of these species is highly heat resistant. Considering that the water permeability of bark is mainly related to suberin and lignin in phellem cell walls (Rains et al., 2016), the weak thermal sensitivity of  $g_{bark}$  may depend on the excellent heat resistance of these hydrophobic compounds. According to Şen et al. (2012), for *Quercus cerris* and *Quercus suber*, suberin and lignin in the phellem cell walls could tolerate 200 °C without significant degradation. Furthermore, many studies have shown that outer bark, especially the phellem, plays a crucial role in protecting tree stems from fire, which also suggests that the phellem can withstand extreme heat (Hoffmann et al., 2012; Lawes et al., 2013; Pausas, 2015; Schafer et al., 2015). To our knowledge, the current study is the first to examine the temperature response of  $g_{bark}$  among temperate tree species, and our results suggest that most of the studied tree species can maintain stable  $g_{bark}$  values under hot droughts. At the same time, our results indicate that  $g_{bark}$  is

unlikely to significantly affect the temperature response of  $g_{min}$  determined with small twigs or shoots in the Drought Box

## **Conclusion**

With ongoing global warming, trees will encounter higher transpirational demands under drought, and their capacity to retain water will largely depend on the residual transpiration rate after stomatal closure, including  $g_{min}$  and  $g_{bark}$  (Blackman et al., 2016, 2019; Machado et al., 2021; Mantova et al., 2023; Wolfe, 2020). Increasing knowledge of  $g_{min}$  and  $g_{bark}$  will greatly contribute to modelling tree drought response and predicting tree mortality. Our study demonstrates that the Drought Box is a reliable research tool capable of accurately measuring leaf and bark water loss in a finely controlled environment, thus significantly contributing to determining  $g_{min}$  and  $g_{bark}$ . Consistent with the previous studies, we showed that  $g_{min}$  and  $g_{bark}$  varied considerably across tree species, and at the same time, the ranges of  $g_{min}$  and  $g_{bark}$  were very close. In particular, most of the studied tree species showed stable  $g_{bark}$  with increasing air temperatures. Therefore, given the small surface area of bark compared to leaves in twigs, measuring  $g_{min}$  on twigs in the Drought Box is recommended, and the resulting values are not flawed by  $g_{bark}$ . Finally, our present study suggests a compelling demand to explore the variability of  $g_{min}$  and  $g_{bark}$  in more tree species and that the Drought Box is a promising tool that could greatly facilitate this research.

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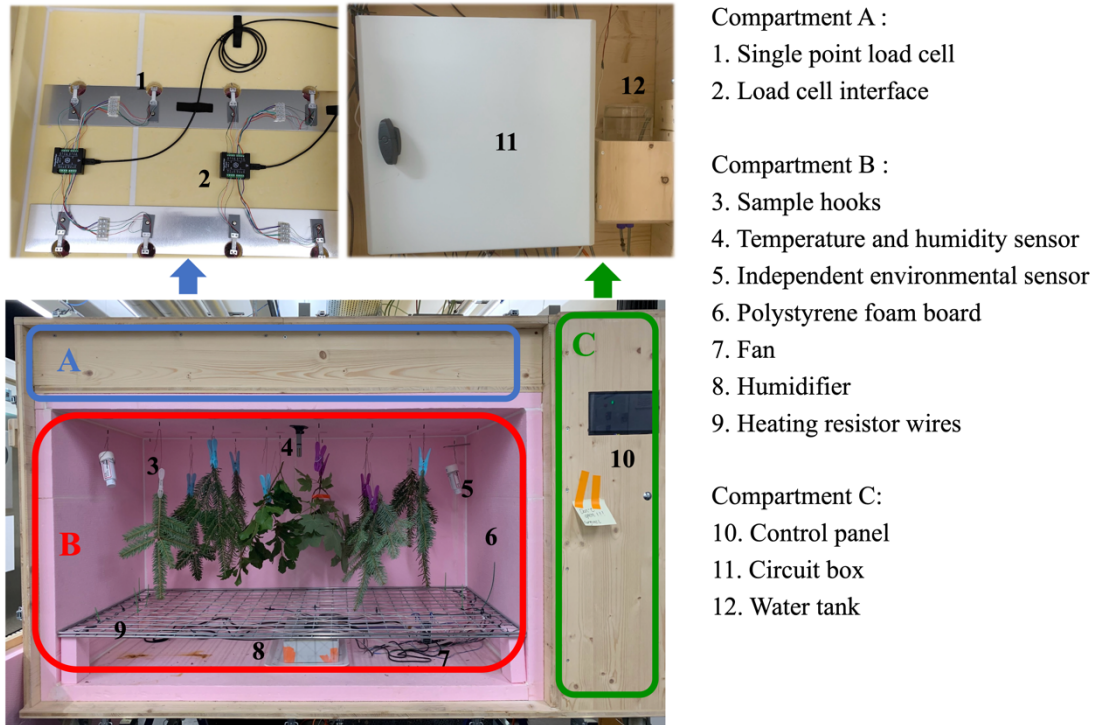
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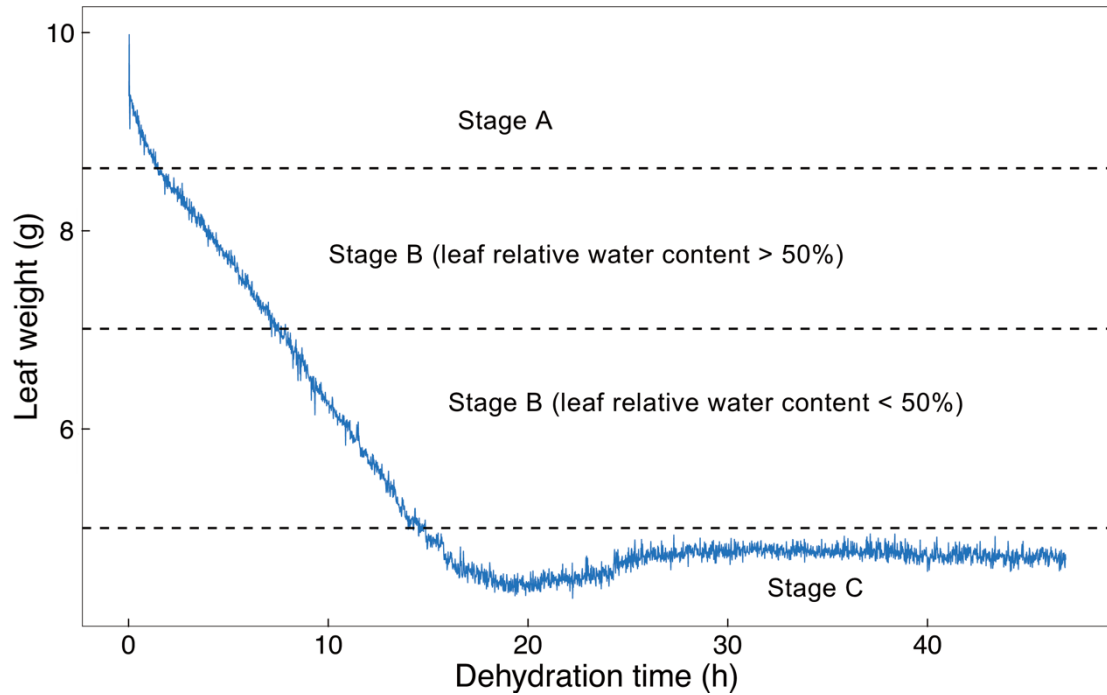
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## Chapter I – Supporting Information



**Figure S1.** Photos of the Drought Box with twig samples. It mainly consists of three compartments and a front door.



**Figure S2.** Changes in the fresh weight of *Acer* leaves with dehydration time at 25 °C. Stage A represents the rapid leaf water loss before complete stomatal closure. Stage B represents the relatively constant rates of leaf water loss ( $g_{min}$ ) after complete stomatal closure. Stage C indicates the depletion of leaf water content. Since stomata may open due to epidermal shrinkage or cuticle cracks at severe water deficits, we only used the stable slope to calculate  $g_{min}$  when the leaf relative water content is still above 50 %.

**Table S1.** Temperature and relative humidity (mean  $\pm$  SD) in the Drought Box during four consecutive tests. Data logged every minute.

	Drought Box condition		Duration (h)
	Temperature (°C)	Relative humidity (%)	
Test 1: 50 °C and 25 %	49.9 $\pm$ 0.6	26.2 $\pm$ 1.0	12.0
Test 2: 40 °C and 30 %	40.0 $\pm$ 0.4	29.8 $\pm$ 0.8	12.6
Test 3: 30 °C and 35 %	30.1 $\pm$ 0.2	35.2 $\pm$ 0.4	9.6
Test 4: 25 °C and 40 %	25.1 $\pm$ 0.1	40.3 $\pm$ 0.4	12.9

**Table S2.** the weights of aluminum foil measured by 12 weighing hooks (A to L) in the Drought Box under different environmental conditions. Values were shown in mean values and standard deviation.

	Measurements in the Drought Box (g)											
	A	B	C	D	E	F	J	H	I	J	K	L
Aluminum foil (g)	27.8	36.8	30.2	25.2	20.0	24.3	50.7	23.4	9.8	17.4	7.6	14.6
Test 1: 50 °C and 25 %	28.4 (0.03)	36.0 (0.08)	30.4 (0.03)	24.7 (0.13)	19.1 (0.03)	24.2 (0.08)	51.2 (0.12)	23.3 (0.08)	9.2 (0.04)	16.4 (0.03)	7.3 (0.07)	13.6 (0.05)
Test 2: 40 °C and 30 %	28.4 (0.02)	36.0 (0.05)	30.5 (0.02)	24.7 (0.04)	19.1 (0.02)	24.4 (0.06)	51.3 (0.08)	23.2 (0.06)	9.2 (0.03)	16.4 (0.03)	7.3 (0.03)	13.7 (0.02)
Test 3: 30 °C and 35 %	28.4 (0.03)	36.1 (0.06)	30.5 (0.02)	24.5 (0.05)	19.1 (0.04)	24.5 (0.06)	51.2 (0.12)	23.2 (0.13)	9.2 (0.04)	16.4 (0.04)	7.2 (0.04)	13.6 (0.03)
Test 4: 25 °C and 40 %	28.4 (0.03)	36.2 (0.05)	30.5 (0.02)	24.5 (0.03)	19.1 (0.03)	24.5 (0.05)	51.2 (0.13)	23.3 (0.15)	9.2 (0.05)	16.4 (0.04)	7.1 (0.03)	13.6 (0.03)



**Table S3.**  $g_{bark}$  ( $\text{mmol m}^{-2} \text{s}^{-1}$ )  $\pm$  standard deviation for eight studied species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus sp.*, *Abies alba*, *Picea abies*, *Pinus sylvestris*) at the different temperature levels.

Temperature (°C)	$g_{bark}$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )							
	<i>Acer</i>	<i>Carpinus</i>	<i>Fagus</i>	<i>Fraxinus</i>	<i>Quercus</i>	<i>Abies</i>	<i>Picea</i>	<i>Pinus</i>
25	2.2 $\pm$ 0.4	2.8 $\pm$ 0.7	2.0 $\pm$ 0.4	1.7 $\pm$ 0.6	2.4 $\pm$ 0.3	2.1 $\pm$ 0.8	2.2 $\pm$ 0.5	2.1 $\pm$ 0.4
30	2.3 $\pm$ 0.5	3.3 $\pm$ 1.0	2.2 $\pm$ 0.3	1.9 $\pm$ 0.7	2.2 $\pm$ 0.3	1.4 $\pm$ 0.4	1.8 $\pm$ 0.4	2.6 $\pm$ 1.0
35	1.7 $\pm$ 0.3	2.8 $\pm$ 1.0	1.4 $\pm$ 0.3	1.2 $\pm$ 0.2	2.3 $\pm$ 0.5	1.9 $\pm$ 0.6	2.0 $\pm$ 0.4	2.4 $\pm$ 0.6
40	2.2 $\pm$ 0.3	2.1 $\pm$ 0.3	1.9 $\pm$ 0.6	1.3 $\pm$ 0.1	2.5 $\pm$ 0.5	2.9 $\pm$ 0.5	3.0 $\pm$ 0.7	2.7 $\pm$ 0.5
45	2.3 $\pm$ 0.3	2.2 $\pm$ 0.2	1.8 $\pm$ 0.6	1.4 $\pm$ 0.2	2.8 $\pm$ 0.4	3.2 $\pm$ 0.4	2.2 $\pm$ 0.2	3.3 $\pm$ 1.1



## **Chapter II**

### **Genotypic variation and phenotypic plasticity of leaf minimum conductance in 4 temperate tree species**

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## Abstract

The survival time of trees under drought is intimately linked to leaf minimum water conductance ( $g_{min}$ ), which determines the residual water loss of trees after full stomatal closure. Particularly,  $g_{min}$  exhibits strong and highly species-specific thermal sensitivity ( $T$ ) with rising temperatures. Considerable interspecies variation in  $g_{min}$  is known among tree species, but intraspecific variation resulting from genotypic variation ( $G$ ) and phenotypic plasticity ( $E$ ) remains unclear. We measured  $T$  of four temperate tree species in three common garden trials to assess  $G$ ,  $E$ , and  $G \times E$  of  $g_{min}$  and  $T$ . Additionally, we explored how leaf cuticular and stomatal traits are related to the intraspecific variation of  $g_{min}$ . Our results showed strong  $T$  in all studied tree species. However, little evidence of  $G$  for  $g_{min}$  was found in these four species. All species displayed significant  $E$  effects, while being more pronounced in deciduous tree species than in evergreen species. Surprisingly, some tree species showed a significant interaction between  $E$  and  $T$ . Intraspecific variation of  $g_{min}$ , however, could not be explained by leaf stomatal and cuticular traits. Our study provides novel insights into the long-term evolution and short-term adaptation of  $g_{min}$ , suggesting that deciduous species may benefit from  $E$  of  $g_{min}$  under future hotter and drier environments.

## Introduction

Forests worldwide offer a range of essential ecological goods and services, including carbon stocks, hydrological and nutrient cycles (Bonan, 2008; Ilstedt et al., 2016; Pan et al., 2011). In many instances, climate change is expected to significantly impact global forests' structure, composition, and distribution, thereby affecting the goods and services provided by forests (Ehbrecht et al., 2021; Khaine & Woo, 2015). Particularly, frequent summer droughts accompanied by heatwaves (i.e., hot droughts) are emerging as one of the major challenges to global forest ecosystems (Allen et al., 2010, 2015; Breshears et al., 2021; Teuling, 2018). A range of traits and trait combinations have been identified to determine the drought responses of different tree species (Martínez-Vilalta et al., 2023; Mencuccini et al., 2015; Sanchez-Martinez et al., 2020). However, the drought response of a tree species also depends on the genotypic variation ( $G$ ), phenotypic plasticity ( $E$ ), and (or) genotype-by-phenotype interaction ( $G \times E$ ), which determine the expression of drought-related traits or trait combinations (Aitken et al., 2008; Alberto et al., 2013; Nicotra et al., 2010).  $G$  can influence the drought performance of a species at the population (i.e., different genotypes) and ecosystem level.  $E$  can ensure that drought-related traits can be quickly adjusted in response to the changes in local environments, thus facilitating tree acclimation. To obtain a more nuanced understanding of the long-term responses of tree species to drought, understanding the  $G$ ,  $E$ , and (or)  $G \times E$  of drought-related traits is highly relevant.

A critical trait associated with the drought resistance of trees is leaf minimum water conductance ( $g_{min}$ ) (Duursma et al., 2019; Machado et al., 2021; Márquez et al., 2022).  $g_{min}$  plays an important role in determining trees' surviving time under drought (Blackman et al., 2016, 2019; Körner, 2019; Wang et al., 2024). Although leaf transpirational water loss is significantly reduced after stomatal closure (Körner, 2019), the leaf water content will continue to decline due to  $g_{min}$  once a tree has lost most access to soil water (Machado et al., 2021; Márquez et al., 2022). Therefore, trees with higher  $g_{min}$  values may encounter more significant risks of canopy desiccation under severe drought conditions (Billon et al., 2020; Blackman et al., 2019; Machado et al.,

2021). According to Schuster et al. (2017), the median of  $g_{min}$  for 39 deciduous woody plants (trees and shrubs) was about  $1.93 \text{ mmol m}^{-2} \text{ s}^{-1}$ , and the upper and lower limits of the interquartile range were from 0.8 to  $3.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ , suggesting that  $g_{min}$  varies considerably among tree species. Some studies have linked the variation of  $g_{min}$  to leaf cuticular and stomatal traits. For instance, the increment of cuticular thickness was thought to reduce  $g_{min}$  and thus enhance drought resistance in *Arabidopsis thaliana* (Kosma et al., 2009; Patwari et al., 2019). However, a significant effect of cuticle thickness on  $g_{min}$  was rarely found in tree species. Additionally, for 30 tropical savanna tree species, Machado et al. (2021) found that smaller and more numerous stomata were associated with higher  $g_{min}$  values.

Interestingly,  $g_{min}$  can respond to instantaneous changes in air temperatures, showing higher values under hotter conditions (Billon et al., 2020; Eamus et al., 2008; Hartill et al., 2023; Schuster et al., 2016; Slot et al., 2021; Wang et al., 2024). For example, Wang et al. (2024) found that  $g_{min}$  typically increased by a factor of two to four along an ascending temperature gradient from 25 to 50 °C for nine temperate tree species. Initially, the temperature response of  $g_{min}$  may be moderate, but once a particular threshold temperature ( $T_p$ ) is exceeded,  $g_{min}$  increases rapidly, eventually displaying non-linear responses. (Billon et al., 2020; Bueno et al., 2019a; Slot et al., 2021; Wang et al., 2024). Different lines of evidence have demonstrated that  $T_p$  is also highly species-specific, ranging from 30 to 50 °C (Billon et al., 2020; Hartill et al., 2023; Slot et al., 2021; Wang et al., 2023). Some studies have attributed these non-linear responses to changes in the structure of cuticular waxes, leading to higher water permeability (Schreiber & Schönherr, 1989; Schuster et al., 2016). Given the anticipated increasing hot temperatures during summer droughts, the strong thermal sensitivity of  $g_{min}$  may lead to excessive residual canopy water loss after stomatal closure, increasing the drought vulnerability of trees (Billon et al., 2020; Cochard, 2021; Wang et al., 2023).

Many studies have demonstrated that  $g_{min}$  can exhibit significant  $G$  in crop plants (Duursma et al., 2019). For example, Saito and Futakuchi (2010) found that  $g_{min}$  ranged from 11.6 to  $17.2 \text{ mmol m}^{-2} \text{ s}^{-1}$  among ten rice cultivars (*Oryza spp.*). Similarly,

$g_{min}$  varied more than two-fold among 58 soybean (*Glycine max*) genotypes under well-watered conditions (James et al., 2008). For woody plants, Challis et al. (2021) found that trees from dry provenances had lower  $g_{min}$  values than trees from wet provenances for evergreen *Corymbia calophylla* when grown in the same environment. On the other hand, soil water availability seems to affect  $g_{min}$  in woody plants of the same genotype, suggesting the strong  $E$  of  $g_{min}$  (Bengtson et al., 1978; Duursma et al., 2019; Warren et al., 2011). For instance, for 4 *Eucalyptus* and 2 *Acacia* tree species, Warren et al. (2011) showed that seedlings subjected to eight weeks of drought stress had a remarkable decrease in  $g_{min}$  (-40% to -70%) compared to controls. Nevertheless, the potential effect of soil water availability on the thermal sensitivity of  $g_{min}$  remains untested. Here, Slot et al. (2021) found no significant relationship between the thermal sensitivity of  $g_{min}$  and soil moisture conditions for 24 tree species from humid, intermediate, and dry tropical forests. However, as this research was not conducted in common gardens, it did not allow researchers to accurately unravel the intraspecies variation in the thermal sensitivity of  $g_{min}$ . To date, systematic studies on the degree of  $G$  and  $E$  of  $g_{min}$ , particularly its thermal sensitivity, are virtually nonexistent, making it challenging to predict trees' acclimation and adaptation potential to the anticipated increases in summer hot drought.

In this study, we determined the  $G$  and  $E$  of  $g_{min}$  and its temperature sensitivity along a gradient from 30 to 50 °C for four temperate tree species. For each species, we tested different provenances originating from different populations and climatic regions across Europe. The trees were grown in three common gardens in Switzerland that differed in their hydroclimates. To explain the variability of  $g_{min}$  based on  $G$  or  $E$ , leaf cuticular and stomatal traits for two deciduous broadleaf tree species were also assessed. Specifically, we tested: (i) if  $g_{min}$  and its temperature response ( $T$ ) differ between tree provenances originating from a wet to dry climatic gradient when they are grown in the same climate (i.e., the evidence of  $G$  and  $G \times T$ ); (ii) if  $g_{min}$  and  $T$  of a given provenance differ when trees are grown in different climates (i.e., the evidence of  $E$  and  $E \times T$ ); (iii) if there are  $G \times E$  effects on  $g_{min}$  and (or)  $T$  (i.e., the evidence of  $G \times E \times T$ ); and (iv) if  $G$  and  $E$  of  $g_{min}$  is correlated with leaf cuticular and stomatal traits.

## Materials and methods

### *Common garden design and studied species*

The common garden trials were established in northwestern and central Switzerland. The sites differed slightly in mean annual temperature but mainly in mean annual precipitation and soil water availability. The Hünenberg Garden (hereafter called “wet trial”), located in Zug in central Switzerland, is the wettest site, with a mean annual precipitation (MAP) of 1212 mm and a mean annual temperature (MAT) of 9.7 °C (Table 1). In contrast, the Hard Garden in Basel-land of northwestern Switzerland is the driest site (hereafter called “dry trial”), with MAP and MAT of 864 mm and 10.6 °C, respectively. The last site, Sulzchopf Garden (hereafter called “moderate trial”), is also in Basel-land, with MAP of 995 mm and MAT of 9.7 °C.

Eight different temperate tree species are grown in these three common gardens, four of which we focused on in our survey. Seeds for the trees in the common garden trials were collected in 2010 from 32 European natural populations. Some seeds originated from arid regions in Bulgaria, Poland, Italy, Spain, and Switzerland. Other seeds came from wet regions in central Switzerland or northern Europe (Table 1). The seeds were germinated in 2012 and grown in a nursery in Muri, Switzerland. At the end of March 2014, a total of 1233 saplings were transplanted to the three common garden trials and planted at a distance of 1.5 X 1.5 m. In March 2015, transplantations were also completed for the remaining six conifer populations totaling 270 trees, which were too small in the spring of 2014 and, therefore, remained in the nursery for another year.

The four tree species we examined in this study were *Acer pseudoplatanus*, *Fagus sylvatica*, *Picea abies*, and *Pseudotsuga menziesii*. Hereafter, tree species will be referred to by their genus names. Except for *Fagus*, for which we tested five provenances, four provenances were studied for each of the other three species (Table 1). In each trial, there are four replicated blocks for each species. In each block, four (*Acer*, *Picea*, and *Pseudotsuga*) or five (*Fagus*) replicates were grown per provenance, resulting in 16 or 25 trees per block. It is worth noting that *Picea* and *Pseudotsuga* had only one block in the dry trial. *Fagus* and *Acer* were not planted in the moderate trial.

**Table 1.** Characteristics of three common gardens and of the different provenances evaluated in this study. MAP and MAT are mean annual precipitation and mean annual temperature from 1999 to 2015, respectively. MAWB is mean annual water balance (precipitation minus potential evapotranspiration).

Species	Country	Provenances	Longitude (°)	Latitude (°)	Altitude (m)	MAP (mm)	MAT (°C)	MAWB (mm)
<i>Acer</i>	Switzerland	Muri (Mur)	8°21'20"E	47°16'23"N	420	1074.7	10.0	-51
	Switzerland	Pfynwald (Pfy)	7°33'33"E	46°17'37"N	576	683.8	10.2	-514.5
	Italy	Toskana (Tos)	11°59'13"E	43°41'52"N	862	1197.2	13.3	-714.2
	Bulgaria	Sliven (Sli)	26°19'13"E	42°41'56"N	405	645.7	13.1	-800.3
<i>Fagus</i>	Switzerland	Auw (Auw)	8°22'56"E	47°12'33"N	516	1072.3	10.0	-56.4
	Switzerland	Wilchingen (Wil)	8°29'39"E	47°39'19"N	586	1046.1	9.2	-70.3
	Switzerland	Champagne (Cha)	6°38'36"E	46°50'26"N	615	929.2	9.3	-126.3
	Bulgaria	Petrohan (Pet)	23°14'20"E	43°15'18"N	463	808.5	9.1	-267.7
	Italy	Cimini (Cim)	12°12'7"E	42°24'28"N	1036	963.1	11.3	-467.1
<i>Picea</i>	Switzerland	Bünzen (Bün)	8°19'30"E	47°19'36"N	468	1065.5	9.9	-62.0
	Poland	Smolarz (Smo)	15°57'E	52°54'N	35	572.5	9.3	-345.1
	Switzerland	Gampel (Gam)	7°43'33"E	46°18'9"N	700	628.8	9.7	-620.7
	Italy	Trentino (Tre)	11°20'24"E	46°29'42"N	1292	758.9	13.0	-832.6
<i>Pseudotsuga</i>	Switzerland	Biel (Bie)	7°15'58"E	47°9'53"N	697	1098.1	8.7	-60.3
	Poland	Barlinek (Bar)	15°8'E	52°56'N	89	599.1	9.4	-342.7
	Poland	Rzepin (Rze)	14°41'E	52°21'N	69	581.9	10.0	-403.0
	Italy	Toskana (Tos)	11°35'42"E	43°46'47"N	1017	1329.2	13.1	-607.5
Common garden	Country	Trial	Longitude (°)	Latitude (°)	Altitude (m)	MAP (mm)	MAT (°C)	MAWB (mm)
Hünenberg	Switzerland	Wet	8°25'7"E	47°11'50"N	412	1212	9.7	-20
Sulzchopf	Switzerland	Moderate	7°39'12"E	47°29'48"N	585	995	9.7	-60
Hard	Switzerland	Dry	7°38'53"E	47°32'28"N	276	864	10.6	-150



### ***g<sub>min</sub> measurement***

*g<sub>min</sub>* and its temperature response was measured using the Drought Box (Billon et al., 2020). Derived from the commonly used method for the determination of *g<sub>min</sub>* (i.e., the mass loss of detached leaves, Duursma et al., 2019), the Drought Box can not only create and maintain stable environmental conditions at a range of set points, but also can provide an accurate, high-resolution record of plant water loss during dehydration.

*Acer* and *Fagus* samples were collected from the common gardens in July and August 2023 after full leaf expansion. For *Picea* and *Pseudotsuga*, samples were collected in winter 2022 and early spring 2023 before the new bud burst. As each block consisted of 16 or 25 individual trees of the same species belonging to four or five different provenances, and there were typically four replicated blocks per common garden, we thus selected only one tree per provenance in each block and visited all four replicated blocks. However, we sampled all trees for *Picea* and *Pseudotsuga* in the dry trial because only one block was available for these two species in the dry trial.

On the morning of each sampling day, a healthy and sun-exposed shoot (ca. 30 – 40 cm) was cut from the upper canopy of each tree. For evergreen conifers, each sampled shoot consisted of the current-year and 1-year-old needles. The cut ends were immediately immersed in the degassed water and transported to the laboratory in cool boxes. In the lab, shoots were re-cut under water to allow the relaxation of xylem tension for 90 s. Then, the shoots (ca. 20 cm) were covered in black plastic bags to ensure complete rehydration in degassed water overnight. The following day, the wet cut ends were cleaned with dry tissues and sealed with high-melting paraffin wax (melting point at 68 °C). The saturated fresh weight of each shoot ( $W_{sat}$ ) was immediately measured on balance (precision:  $\pm 1.0$  mg, PL303, METTLER TOLEDO, Switzerland). The shoots were then fixed on each of the 12 strain gauge hooks (precision:  $\pm 50$  mg) and progressively dried down in the Drought Box for 15 – 70 h, depending on the environmental conditions in the box. The fresh weight of each shoot ( $W_{fre}$ ) was automatically measured at 1 min intervals in the Drought Box. *g<sub>min</sub>* measurements for each tree were done in the Drought Box with independent samples at 30, 35, 40, 45, and 50 °C, and the corresponding relative humidity was at 40, 40, 40,

35, and 35 %. A previous study has demonstrated that only high relative humidity (> 70 %) significantly increased cuticular permeability. At the same time, the authors noted that the effect of humidity on  $g_{min}$  was several orders of magnitude lower than the temperature effect (Schreiber et al., 2001). Therefore, it is unlikely that the slight changes in relative humidity in our study had a significant impact on the  $g_{min}$  values determined at different temperatures.

After the measurements, shoots were removed from the Drought Box and thoroughly dried in the oven for 72 h at 80 °C to obtain the dry mass ( $W_{dry}$ ). The shoot relative water content ( $RWC$ ) was then calculated with equation (1):

$$\text{Eq. 1} \quad RWC = \frac{W_{fre} - W_{dry}}{W_{sat} - W_{dry}}$$

The determination of  $g_{min}$  was based on the trajectories of shoot dehydration progression (i.e.,  $RWC$  decline over time, Figure S1).  $RWC$  decreased rapidly during the early drying stage and before the stomata closed completely. This usually took 1 – 2 h depending on the species and the environmental conditions in the Drought Box (Figure S1, Phase 1). After full stomatal closure,  $RWC$  continued to decrease at a relatively constant rate, showing a stable slope of water loss versus time (Phase 2). Then, this slope gradually became flat, indicating that most of the water in the shoot had been depleted (Phase 3). Taking into account the risk of artifacts at severe water deficits, as stomata may open due to epidermal shrinkage or cuticle cracks, we used the slope of the linear region of actual shoot mass versus drying time to determine  $g_{min}$  when  $RWC$  was > 50 % (Figure S1).  $g_{min}$  was then calculated following equation (2):

$$\text{Eq. 2} \quad g_{min} = \frac{\text{Slope} * 98.0}{A * VPD}$$

Where the atmospheric pressure in the Drought Box was assumed to be 98.0 kPa.  $A$  is the two-sided leaf area ( $m^2$ ), which was determined by specific leaf area (SLA) and leaf dry mass after  $g_{min}$  measurements. SLA was calculated as the ratio of fresh leaf area to

leaf dry mass. For this, leaves from the upper canopy of each labeled tree were collected and scanned. The fresh leaf area was measured by our self-developed leaf image analysis tool (<https://github.com/dabasler/LeafAreaExtraction>). Leaves were then dried in the oven at 80 °C for 72 h to obtain dry mass. It is worth noting that the bark area of the shoot was not considered in the calculation of  $g_{min}$  because it only represented a small fraction of the total shoot area. However, we acknowledge that this may have led to a slight overestimation of  $g_{min}$  in our study.

### ***Stomatal traits***

The epidermal impression technique was used to obtain *Fagus* leaf stomatal traits. *Acer* was not examined here because of its' sunk stomata in the epidermal layer. For each sampled tree, three healthy twigs were cut from the upper canopy in the morning and transported to the laboratory in a cool box. Then, one fully expanded leaf from each twig was used to observe the leaf stomata. Specifically, a small amount of clear nail polish was applied to the leaf adaxial surface and allowed to dry for several minutes. According to our preliminary experiment, there were no stomata on the leaf abaxial surface. Subsequently, transparent sticky tape was pressed on the nail polish and lifted. The tape with the leaf epidermis was trimmed and fixed onto microscopy slides. All slides were observed with the aid of a light microscope (model CX43; Olympus, Tokyo, Japan). For each slide, five pictures were taken at  $\times 200$  magnification to determine the stomatal density (SD), and ten pictures were taken at  $\times 1000$  magnification (i.e., ten clear stomata) to measure guard cell length (L), guard cell pair width (W), using ImageJ software. Stomatal size (SS) was calculated as L multiplied by W (Franks et al., 2009). Therefore, 15 pictures and 30 stomata per tree were used to measure SD and SS, respectively. Finally, the results were reported as averages per provenance based on the individual sampled tree.

### ***Cuticular traits***

For *Acer* and *Fagus*, leaf cuticle was obtained by hand-sectioning fresh leaves. Specifically, after the measurements of stomatal traits, two remaining leaves from each

tree were used to determine cuticular thickness. Leaves were sectioned at the upper, middle, and lower positions, respectively. These thin leaf slices were immediately stained with Sudan III solution and observed at  $\times 400$  magnification with the microscope described above. For each slide, we took three clear pictures of the cuticle at the leaf adaxial and abaxial surfaces. Cuticular thickness was measured at three clear points for each image to calculate the mean values for the leaf adaxial ( $CT_{adaxial}$ ) and abaxial surface ( $CT_{abaxial}$ ). The total cuticular thickness ( $CT_{total}$ ) was calculated as the average mean values of  $CT_{adaxial}$  and  $CT_{abaxial}$ . Finally, the result was reported as averages per provenance based on the individual sampled tree.

### **Relative distance plasticity index**

To quantitatively compare variation in phenotypic plasticity ( $E$ ) of  $g_{min}$  between provenances of a given species, the relative distance plasticity index (RDPI) was calculated following the methodology of Valladares et al. (2006) using equation (3). Specifically,  $j$  and  $j'$  are two individual trees of the same population grown in different trials  $i$  and  $i'$ , respectively. In the calculation, we only considered the wet and dry trials, not the moderate trial. Then for a given temperature, the relative distance of  $g_{min}(x)$  can be calculated as the absolute distance between  $j$  and  $j'$  ( $x_{ij'} - x_{ij}$ ) divided by the sum of  $j$  and  $j'$  ( $x_{ij'} + x_{ij}$ ). Finally, RDPI for all pairs of individuals of the same provenance grown in different trials was calculated as:

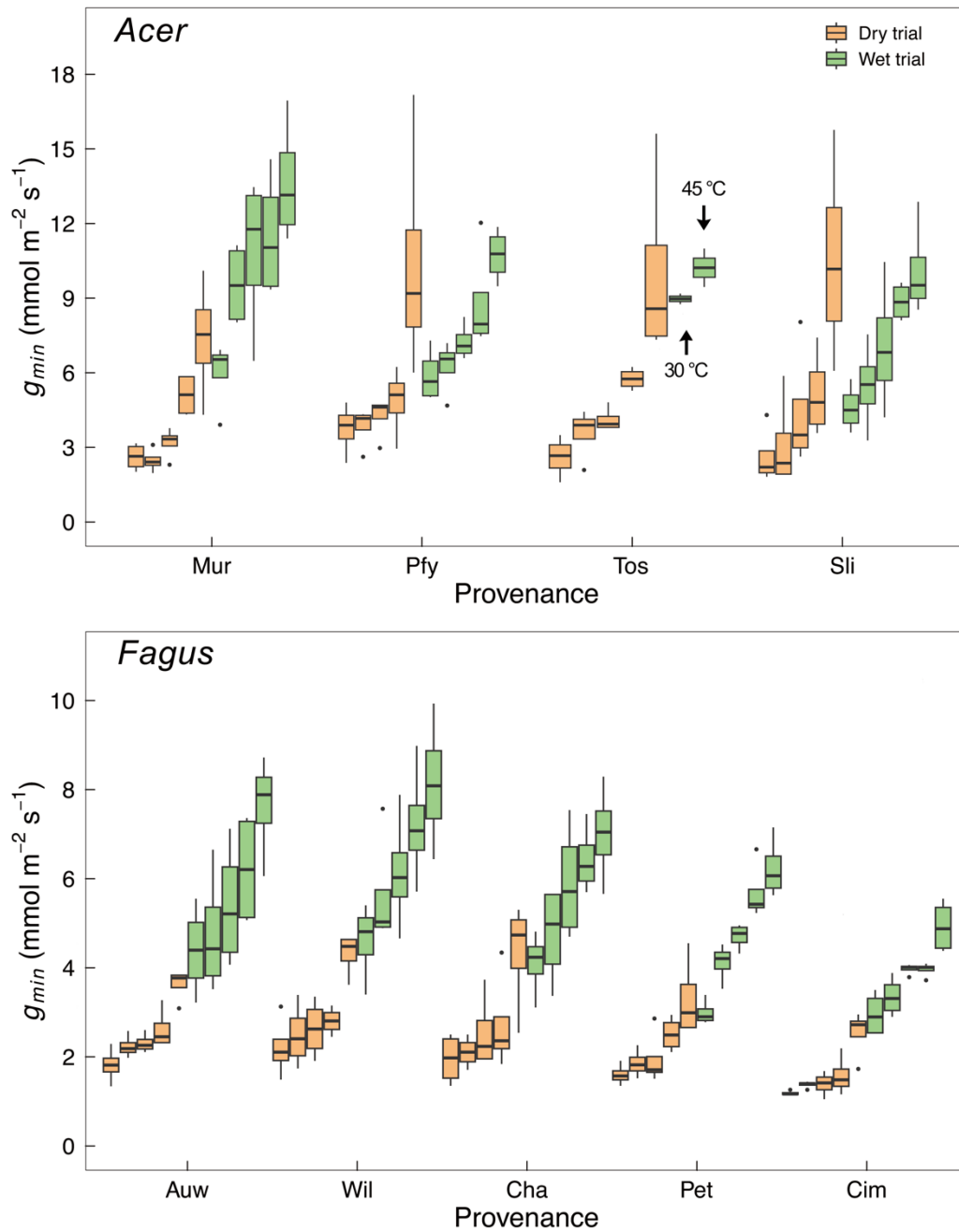
$$\text{Eq. 3} \quad \text{RDPI} = \frac{\sum \frac{d_{ij \rightarrow i'j'}}{x_{i'j'} + x_{ij}}}{n}$$

where  $n$  is the number of relative distances (or pairs). In the study, for instance,  $n = 16$  for 4 individual trees from the same provenance grown in two different trials. The final result was reported as the mean values of RDPI at five different temperatures (30 to 50 °C) for each provenance, ranging from 0 (no plasticity) to 1 (maximal plasticity).

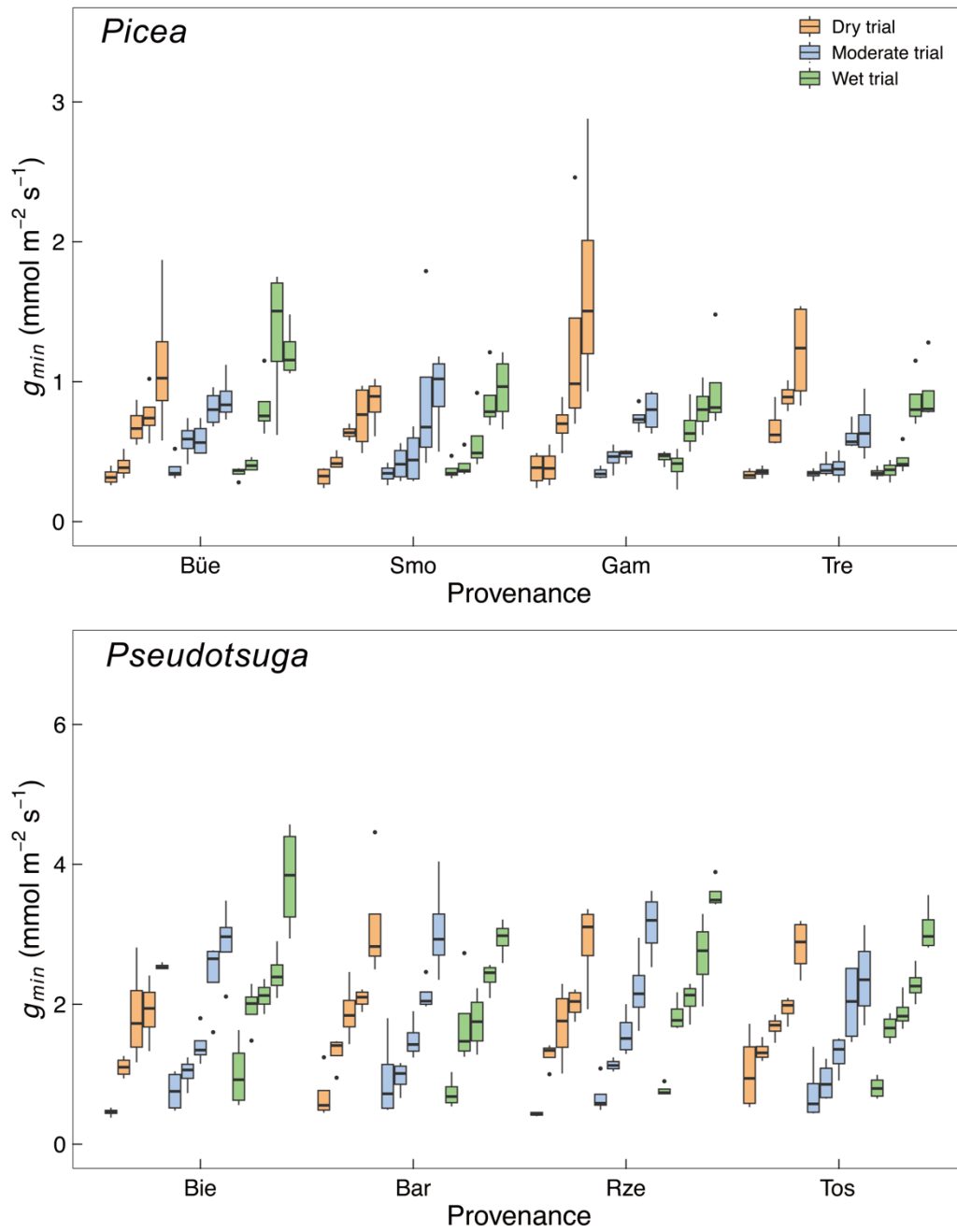
### ***Statistics***

We used linear mixed models (`lme()`; *lmerTest* package; Kuznetsova et al., 2017) to explore the questions (i), (ii), and (iii). Specifically, trials, provenances, and temperatures were fixed effects, while individual trees nested within blocks were included as random factors to account for unmeasured error terms. For *Acer*, provenance Tos was excluded in the model analysis, as only two trees were available for this population in the wet trial.  $g_{min}$  data were first log<sub>10</sub>-transformed to improve the normality and homoscedasticity. Model selections were based on Akaike's information criterion corrected for small sample size (AICc), and were conducted from the full model with a function "step ()" (*lmerTest* package; Kuznetsova et al., 2017). The marginal and conditional pseudo- $r^2$  values in the mixed models were calculated with *performance* package (Nakagawa et al., 2016). Furthermore, the significant differences in RDPI among populations for each species were determined by One-way ANOVA. Specially, when the normal distribution (or homogeneity of variance) was violated, the Kruskal–Wallis test (or Welch's ANOVA) was applied. All tests were followed by corresponding post hoc tests and adjustments were made to  $p$  values. Finally, the relationships between  $g_{min}$  variation and leaf stomatal and cuticular traits were determined with Pearson's linear correlation analyses. Before analysis, data were first log<sub>10</sub>- transformed to improve the normality and homoscedasticity. The significance threshold was 0.05 throughout all analyses above. All the statistical analyses were performed in R v.4.1.2 (R Core Team, 2021).

## Results



**Figure 1.** The temperature responses of  $g_{min}$  for different provenances of *Acer* and *Fagus* in two trials. Different colors indicate the different trials. Provenances are listed from left to right in order from humid to arid regions. For a given provenance at a specific trial, the temperature gradients are 30, 35, 40, 45 and 50 °C from left to right. Note that  $g_{min}$  of provenance Tos for *Acer* in the wet trial was measured only at 30 and 45 °C.



**Figure 2.** The temperature responses of  $g_{min}$  for different provenances of *Picea* and *Pseudotsuga* in three trials. Different colors indicate the different trials. Provenances are listed from left to right in order from humid to arid regions. For a given provenance in a specific trial, the temperature gradients are 30, 35, 40, 45 and 50 °C from left to right.

**Table 2.** Results of the optimal linear mixed-effect models for the effects of genotype ( $G$ ), phenotype ( $E$ ), temperatures ( $T$ ) on  $\log_{10}(g_{min})$ . Nested random factor Block/Tree\_id was used in all models. Significant  $p$  values ( $< 0.05$ ) were shown in bold.  $R_m^2$  and  $R_c^2$  are marginal and conditional  $R^2$ , respectively.

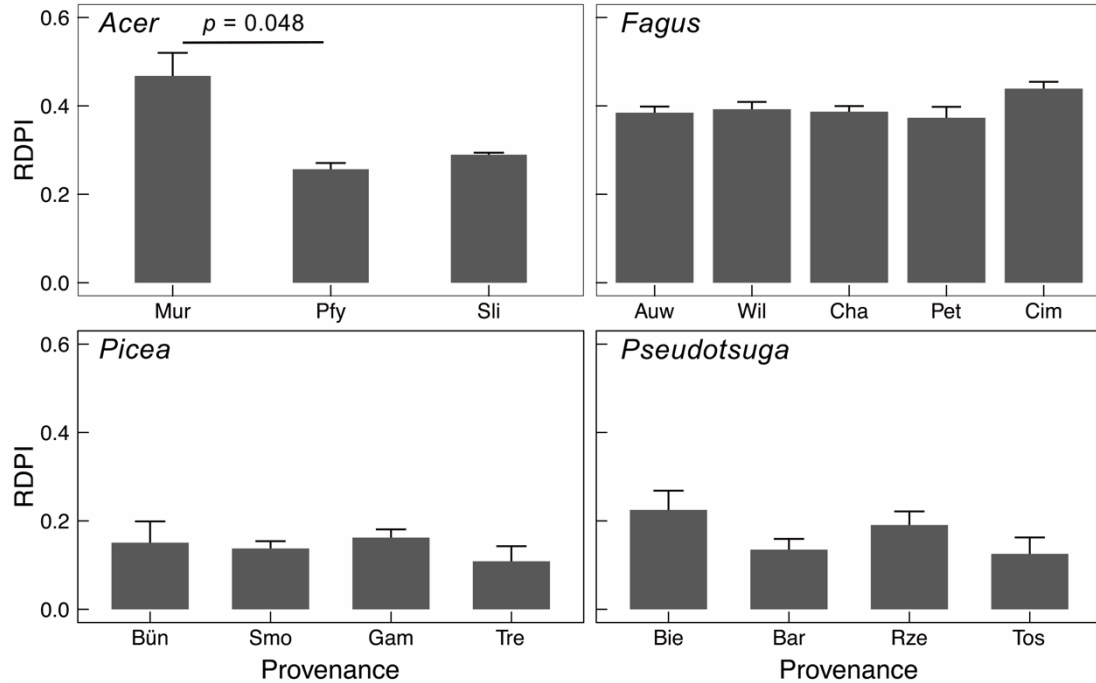
Best model	Fixed effects	NumDf	DenDF	F value	P-value
<i>Acer</i> : $\log_{10}(g_{min}) = E + T + E : T$ + random (Block/Tree_id) $R_m^2 = 0.67, R_c^2 = 0.84$	E	1	115.66	29.36	<b>&lt;0.001</b>
	T	1	94	258.23	<b>&lt;0.001</b>
	E : T	1	94	10.25	<b>0.002</b>
<i>Fagus</i> : $\log_{10}(g_{min}) = G + E + T$ + random (Block/Tree_id) $R_m^2 = 0.86, R_c^2 = 0.93$	G	4	34	15.74	<b>&lt;0.001</b>
	E	1	34	293.11	<b>&lt;0.001</b>
	T	1	159	440.84	<b>&lt;0.001</b>
<i>Picea</i> : $\log_{10}(g_{min}) = G + E + T + G : E$ + $G : T + E : T + G : E : T$ + random (Block/Tree_id) $R_m^2 = 0.69, R_c^2 = 0.79$	G	3	209.43	0.10	0.961
	E	2	209.43	6.27	<b>0.002</b>
	T	1	180	683.76	<b>&lt;0.001</b>
	G : E	6	209.43	2.32	<b>0.035</b>
	G : T	3	180	0.48	0.696
<i>Pseudotsuga</i> : $\log_{10}(g_{min}) = E + T$ + random (Block/Tree_id) $R_m^2 = 0.76, R_c^2 = 0.81$	E : T	2	180	9.92	<b>&lt;0.001</b>
	G : E : T	6	180	3.34	<b>0.004</b>
	E	2	45	12.00	<b>&lt;0.001</b>
	T	1	191	888.26	<b>&lt;0.001</b>

### **Temperature response of $g_{min}$**

With increasing temperatures from 30 to 50 °C, all tested tree species showed significant temperature responses of  $g_{min}$  across provenances and trials (Figure 1, 2; Table 2,  $p < 0.001$ ). The strongest temperature response was found in the provenance Rze of *Pseudotsuga* in the dry trial, where  $g_{min}$  increased about 6.7-fold from 0.43 to 2.88  $\text{mmol m}^{-2} \text{s}^{-1}$  (Table S1). In contrast, the provenance Cim of *Fagus* in the wet trial exhibited the least temperature response, with a 1.7-fold increase in  $g_{min}$  from 1.19 to 2.53  $\text{mmol m}^{-2} \text{s}^{-1}$  (Table S1). Additionally, the provenance Sli of *Acer* in the dry trial showed the largest absolute increase in  $g_{min}$  with an increase of 7.92  $\text{mmol m}^{-2} \text{s}^{-1}$  (Table S1). Despite the evident temperature responses of  $g_{min}$ , only seven significant  $T_p$  were



found for all studied tree species, ranging from 36.8 °C (Table S2, Gam of *Picea* in the dry trial,  $p < 0.05$ ) to 43.8 °C (Tos of *Acer* in the dry trial,  $p < 0.01$ ).



**Figure 3.** Relative distance plasticity index (RDPI) of  $g_{min}$  between provenances of four tree species. Significant differences ( $p < 0.05$ ) between provenances were shown. RDPI is from 0 (no plasticity) to 1 (maximal plasticity).

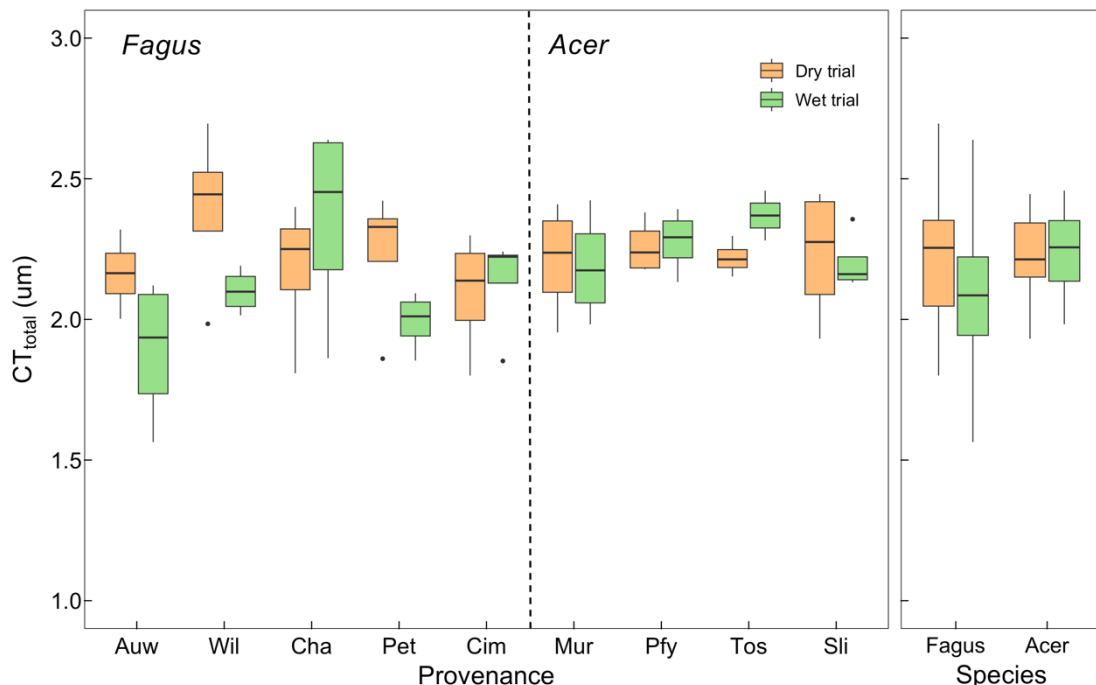
### ***Genotypic (G) and phenotypic (E) plasticity of $g_{min}$***

Significant  $G$  of  $g_{min}$  was only found in *Fagus* (Table 2,  $F = 15.74$ ,  $p < 0.001$ ). Although the effect was not particularly large, there was a statistically significant tendency for *Fagus* provenances from wet regions to have higher  $g_{min}$  values than provenances from dry regions (Figure 1). In contrast, there were no significant  $G$  effects for the other three species. Statistically, there was significant  $E$  in  $g_{min}$  for all studied tree species. For *Acer* and *Fagus*,  $g_{min}$  values were dramatically higher in the wet trial than in the dry trial (Figure 1; Table 2,  $F = 29.36$ ,  $p < 0.001$ ;  $F = 293.11$ ,  $p < 0.001$ ). Although *Picea* (Table 2,  $F = 6.27$ ,  $p < 0.01$ ) and *Pseudotsuga* ( $F = 12.00$ ,  $p < 0.001$ ) also showed significantly higher  $g_{min}$  values in the wet trial than in the moderate and dry trials, the absolute differences were rather small (Figure 2). Provenances of *Picea* and *Pseudotsuga* also

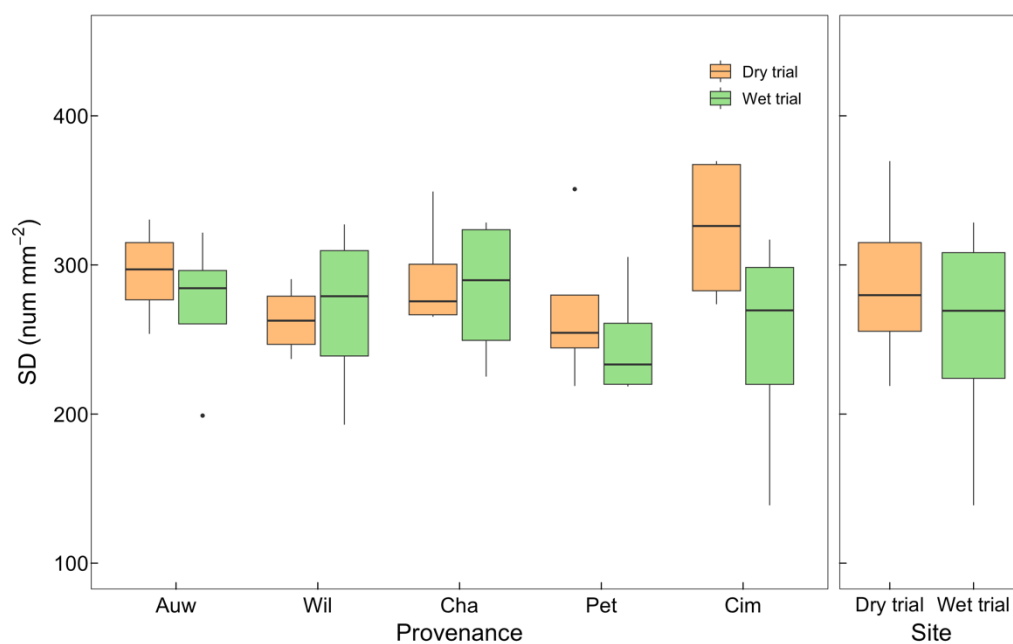
showed typically low RDPI values, with most provenances having  $RDPI < 0.2$ . A marginally significant difference ( $p = 0.048$ ) in RDPI was found between the two provenances of *Acer* (Figure 3, Mur vs Pfy). The RDPI values of the different *Fagus* provenances were relatively high, about 0.4.

### *Interactions of G and E with T*

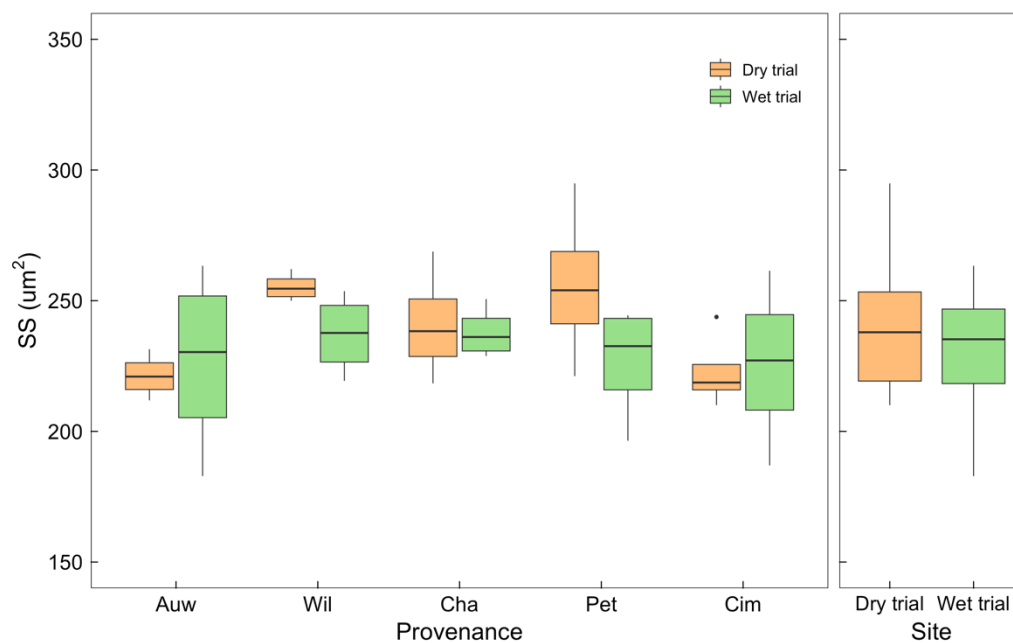
We found no interaction between *G* and *T*, suggesting that temperature responses of  $g_{min}$  were similar across the proveniences of a given species. A significant interaction between *E* and *T* was found in *Acer*, with trees of a given provenance in the dry trial showing more potent *T* effects than trees in the wet trial (Table 2, Figure S2,  $F = 10.25$ ,  $p < 0.01$ ). Nevertheless, at a given temperature (30 – 45 °C),  $g_{min}$  of *Acer* was still typically higher in the wet trial than in the dry trial. In addition, there was significant interaction between *G*, *E*, and *T* in *Picea* (Table 2, Figure S3,  $F = 3.33$ ,  $p < 0.01$ ), but the interaction did not show a consistent pattern across either trials or provenances.



**Figure 4.** The variation of cuticle thickness ( $CT_{total}$ ) of *Fagus* and *Acer* across provenances and trials. Different colors indicate different trials. Provenances are listed from left to right in order from humid to arid regions.



**Figure 5.** The variation of stomatal density (SD) of *Fagus* across provenances and trials. Different colors indicate different trials. Provenances are listed from left to right in order from humid to arid regions.



**Figure 6.** The variation of stomatal size (SS) of *Fagus* across provenances and trials. Different colors indicate different trials. Provenances are listed from left to right in order from humid to arid regions.

### ***Cuticular and stomatal traits***

$CT_{total}$  was similar across provenances and trials for *Fagus* and *Acer* (Figure 4). Additionally, there were non-significant differences in SD and SS across provenances of *Fagus* and trials (Figure 5, 6). Finally, leaf cuticular and stomatal traits were not significantly correlated with variation of  $g_{min}$ .

## Discussion

Exploring  $G$  and  $E$  of key hydraulic traits is paramount for understanding forest ecosystem responses to compounded hot drought. To our knowledge, this is the first comprehensive study to quantify  $G$  and  $E$  of  $g_{min}$  and its thermal sensitivity for tree species. Our study shows that  $g_{min}$  strongly increased by a factor of two to seven along an ascending temperature gradient from 30 to 50 °C for all studied tree species. However, only *Fagus* displayed significant  $G$  in  $g_{min}$ , with  $g_{min}$  values slightly higher in tree provenances from wet regions than in provenances from dry regions. In contrast, there was little  $G$  effect in  $g_{min}$  for the other three tree species. In addition, significant  $E$  of  $g_{min}$  was found in all studied tree species, with generally higher  $g_{min}$  values in the wet trial than in the moderate and dry trials. While these  $E$  effects were pronounced in the two angiosperm species, they were rather low across provenances in the two conifer species, suggesting the limited absolute differences in  $g_{min}$  across trials for these two conifer species. At the same time, there was a significant interaction of  $E \times T$  in *Acer*, showing stronger thermal sensitivity of  $g_{min}$  in the dry trial than in the wet trial. Interestingly, the variation of  $g_{min}$  across provenances and trials could not be explained by the variation in leaf stomatal and cuticular traits.

### *Strong temperature response of $g_{min}$*

One of the most striking patterns we observed in this study was the strong temperature response of  $g_{min}$  across either provenances or trials in all studied tree species. Similarly, several studies have also demonstrated that  $g_{min}$  increased non-linearly with rising temperatures for tree species from different climates (Billon et al., 2020; Bueno et al., 2019a; Hartill et al., 2023; Slot et al., 2021; Wang et al., 2024), and that higher  $g_{min}$  values are likely to cause larger canopy residual transpiration and thus faster xylem hydraulic failure (Billon et al., 2020; Cochard, 2021; Wang et al., 2024). For instance, Billon et al. (2020) showed that  $g_{min}$  dramatically increased two- to four-fold in five temperate tree species when the temperature increased from 30 to 55 °C, and that hot temperatures led to a rapid decline in shoot relative water content and xylem

conductivity. However, in 24 tropical tree species, Slot et al. (2021) found that most studied species did not show significant temperature responses of  $g_{min}$  between 25 and 50 °C, suggesting that the thermal sensitivity of  $g_{min}$  varies considerably among tree species. Particularly, some studies have reported a phase transition temperature ( $T_p$ ) in the temperature response of  $g_{min}$ , ranging from 30 to 50 °C, which also indicates the significant differences in the thermal sensitivities of  $g_{min}$  (Billon et al., 2020; Bueno et al., 2019a; Hartill et al., 2023; Wang et al., 2023). Consistent with previous research, our results not only showed a minimum two-fold and a maximum seven-fold increase in  $g_{min}$  for four studied tree species when the temperature increased from 30 to 50 °C, but also revealed some significant  $T_p$ , ranging from 36.8 to 43.8 °C. According to Schuster et al. (2016), the structure of leaf cuticular waxes will change under hot temperatures, leading to higher cuticular water permeability and, thus, higher  $g_{min}$ . This may explain the considerable variation of thermal sensitivity of  $g_{min}$ , as leaf cuticular structure and composition varies greatly between plant species.

### ***Genotypic variation of $g_{min}$***

One of the promising strategies for forest management to cope with future climate scenarios is to plant trees with genotypes that show better adaptation to hot and dry stress. Previous studies have mainly investigated the genetic diversity of xylem hydraulic efficiency and safety in different tree species, including Huber value, turgor loss point, P50 and P88 (i.e., water potential at 50 or 88 % loss of hydraulic conductivity, Blackman et al., 2017; Lamy et al., 2014; Paudel et al., 2021; Pritzkow et al., 2020; Wortemann et al., 2011). Although there is a wealth of research on the genetic variation of  $g_{min}$  for crops (Duursma et al., 2019; James et al., 2008; Saito & Futakuchi, 2010), few studies have explored this important issue for tree species. In the present study, we found that only *Fagus* displayed significant  $G$  of  $g_{min}$ , but the absolute differences in  $g_{min}$  between provenances resulting from this effect were slight. Our results show that *Fagus* trees originating from wet regions had significantly higher  $g_{min}$  values than those from dry regions, suggesting that  $g_{min}$  was under strong genetic control in this tree species. Concurring with our results, Challis et al. (2021) reported that *Corymbia*

*calophylla* trees from dry habitats had lower  $g_{min}$  values than trees from wet habitats. However, as we did not determine neutral genetic differentiation across and within populations, the significant  $G$  of  $g_{min}$  that we observed between *Fagus* provenances might be caused by other processes unrelated to selection (e.g., genetic drift, Lamy et al., 2011; Spitze, 1993). On the other hand, according to our results,  $g_{min}$  and its temperature responses were very similar among different provenances of *Acer*, *Picea*, and *Pseudotsuga*, although the hydroclimatic contrasts of the studied provenances were more pronounced in these three species than in *Fagus* (Table 1). It is worth noting that we also measured  $g_{min}$  at 30 °C for five different provenances of *Abies alba* in the dry trial (Figure S4). Again, we found little  $G$  of  $g_{min}$  in this species. Therefore, our data points to  $G$  of  $g_{min}$  as small or non-existent in the four studied species. This result is surprising, given that the investigated tree provenances spanned a large part of these species' natural distribution in Europe. However, the causal mechanisms leading to the absence of genotypic plasticity in functional traits remain unclear. According to Lamy et al. (2011), some processes, such as weak molecular variation, genetic constraints, and canalized traits, may be responsible for this phenomenon.

Our findings provide valuable knowledge for foresters to improve European forest management in the context of global-change-type drought. Specifically, replanting seeds from dry provenances for the four studied tree species during reforestation will be neither effective in reducing the  $g_{min}$  values of trees nor in affecting the thermal sensitivity of  $g_{min}$ . Despite this fact, we cannot rule out the possibility that tree populations growing at the warm/dry or cold/wet trailing edges of species' distribution may have evolved more strongly contrasting ecotypes, such as “low- $g_{min}$ -adapted” genotypes, which were not considered in this study. Therefore, it is still worth comparing  $G$  of  $g_{min}$  across a broader range of populations.

### ***Phenotypic plasticity of $g_{min}$***

A series of experiments have demonstrated that  $g_{min}$  can be adjusted quickly in response to changes in soil moisture conditions and thus exhibits high  $E$  (Cavender-Bares et al., 2007; Duursma et al., 2019; Villar-Salvador et al., 1999; Warren et al., 2011). For

instance, Duursma et al. (2019) showed that after planting 11 *Hakea* species in contrasting water environments, there was a substantial decrease in  $g_{min}$  in individuals of all species under water-stressed conditions compared to well-watered conditions. Consistent with previous findings, we found that all studied tree species displayed significant  $E$  in  $g_{min}$ , and  $g_{min}$  was generally lower in the dry trial than in the moderate and wet trials for a given provenance and temperature. However, it is noteworthy that the degree of the  $E$  effect on  $g_{min}$  contrasted strongly between the two functional plant groups. The absolute differences in  $g_{min}$  between trials were rather limited in evergreen conifers, which was also supported by their typically low RDPI values across provenances ( $< 0.2$ ). Therefore, it is unlikely that  $E$  effects will lead to physiologically and ecologically significant changes in  $g_{min}$  and (or) canopy residual water loss for these two conifers. The considerable variation in  $E$  effects between deciduous broadleaf and evergreen conifer species may be attributed to different resource strategies (i.e., leaf economics spectrum, Stotz et al., 2022; Zhang et al., 2020). In a meta-analysis of 168 plant species, including herbs, woody deciduous and woody evergreens, Stotz et al. (2022) found a generally positive relationship between SLA and phenotypic plasticity in leaf morphological and physiological traits, implying that resource-conservative species (e.g., *Picea* and *Pseudotsuga* in our study) tend to maximize survival rather than growth and become more stress-tolerant, but may be less able to respond to resource fluctuations due to their generally lower phenotypic plasticity. According to Challis et al. (2021),  $g_{min}$  of the evergreen species *Corymbia calophylla* also did not show a significant  $E$  effect. Thus, our study suggests that  $g_{min}$  is capable of adapting to future drier environments, but this occurs mainly in deciduous broadleaf tree species.

Surprisingly, our work also showed that in some species (i.e., *Acer*), the thermal sensitivity of  $g_{min}$  can also respond to changes in local pedo-climatic conditions, becoming less resistant to heat when trees grow in a water-stressed environment. In contrast, for 24 tropical tree species, Slot et al. (2021) found that the temperature responses of  $g_{min}$  differed considerably among species but were not related to rainfall conditions. In addition, for 14 *Eucalyptus* tree species widely distributed in Tasmania (Australia), Hartill et al. (2023) found that climate of origin had no evident effect on



the thermal sensitivity of  $g_{min}$ . These studies, however, were not conducted in common garden trials and thus may be insufficient to delineate the intricate interaction between  $E$  and  $T$ . According to our study, the more potent  $T$  effects in the dry trial may diminish the benefits of  $g_{min}$  adaptation to the local dry environment (i.e.,  $E$  effects). Nevertheless, the effect of  $E \times T$  should not be over-interpreted, especially on tree residual water loss. Our results showed that although  $T$  was stronger in the dry trial,  $g_{min}$  was still much higher in the wet trial for a given temperature (Figure 1, 30 - 45 °C). Based on Wang et al. (2024), there is a substantial amount of canopy residual water loss during the hot drought period after stomatal closure, and these minimal water consumptions are predicted to rapidly increase with rising temperatures. Therefore, our study suggests that as the climate continues to warm and dry, forest trees, depending on the species, are likely to reduce their canopy residual transpiration by adapting to drier environments, but this may become counteracted by a concurrent increase in the thermal sensitivity of  $g_{min}$ .

#### ***$g_{min}$ variation and leaf morphology***

Given that  $g_{min}$  consists of cuticular transpiration and stomatal water leakage, some studies investigated the relationships between  $g_{min}$  and leaf cuticular and stomatal traits. Typically, increment of cuticular thickness is recognized as one of the features of plant drought adaptation, as thicker cuticle was found to be associated with lower  $g_{min}$  in some species, such as in *Arabidopsis thaliana* (Kosma et al., 2009; Patwari et al., 2019). However, few studies observed this relationship in tree species. For nine temperate tree species, Wang et al. (2024) found that thicker cuticle was correlated with higher  $g_{min}$  values, suggesting an opposite relationship. In the present study, we found that some *Fagus* provenances had slightly thicker cuticle in the dry trial than in the wet trial. However, there was no correlation between  $g_{min}$  and cuticular thickness across trials or provenances, which keeps in line with previous studies (Bueno et al., 2019b; Grünhofer et al., 2022; Machado et al., 2021). According to Jetter & Riederer (2016), cuticular transpiration resistance is mainly dependent on the intracuticular wax layer, in which some very-long-chain fatty acid derivatives play a critical role. Furthermore, some

studies showed that the integrity of the cuticle was associated with cuticular permeability, especially for long-lived leaves (Anfodillo et al., 2002; Hadley & Smith, 1994; Jordan & Brodribb, 2007).

The contribution of stomatal water leakage to  $g_{min}$  varies substantially among plant species (Brodribb et al., 2014; Márquez et al., 2022; Šantrůček et al., 2004; Schuster et al., 2017). For instance, in 30 tropical savanna tree species, stomatal water leakage accounted for 6.6 to 43.6 % of  $g_{min}$  (Machado et al., 2021). A positive correlation between SD and  $g_{min}$  was reported in some crops and tree species, suggesting numerous stomata may result in higher  $g_{min}$  (Muchow & Sinclair, 1989; Machado et al., 2021). For instance, Muchow and Sinclair (1989) showed that  $g_{min}$  ranged from 6.3 to 17.6 mmol m<sup>-2</sup> s<sup>-1</sup> among ten sorghum genotypes and that this variation was positively correlated with the variation of SD. However, our study found no significant difference in stomatal traits (i.e., SD, SS) between *Fagus* provenances or trials. Therefore, the variation of  $g_{min}$  in *Fagus* may be mainly driven by other factors (i.e., the chemical composition of the cuticle) and cannot be simply explained by the limited variation of stomatal traits. Current ambiguities on the role of cuticular and stomatal traits on  $g_{min}$  deserve closer scrutiny.

## Conclusion

Tree species that fail to adapt and acclimate to compounded hot droughts will likely experience surges in tree mortality events (Williams et al., 2013; Allen et al., 2015; Zhang et al., 2017; Yi et al., 2022). Consistent with previous research on  $g_{min}$ , our study shows that all tested tree species exhibited strong temperature responses of  $g_{min}$  along a temperature gradient from 30 to 50 °C, implying a greater risk of canopy residual water loss under hotter droughts. Surprisingly, most studied species showed little evidence of  $G$  effects for  $g_{min}$ . At the same time, we found that  $g_{min}$  was typically lower in the dry trial than in the moderate and wet trials for all studied tree species. Yet, the absolute differences in  $g_{min}$  between trials were rather limited for conifers. Interestingly, the thermal sensitivity of  $g_{min}$  also adapted to the local hydroclimate in some tree species

(i.e., *Acer*), being more potent in the dry trial. Leaf cuticular and stomatal traits were, however, insufficient to explain the variation of  $g_{min}$  across trials and provenances. As a whole, our study provides novel insights into the long-term evolution and short-term adaptation of  $g_{min}$  for four temperate European tree species: (1) strong  $E$  effects on  $g_{min}$  can promote the drought resistance of *Acer* and *Fagus*; (2) *Picea* and *Pseudotsuga* may be impracticable to rely on  $G$  and  $E$  of  $g_{min}$  to cope with increasing severity of hot drought.

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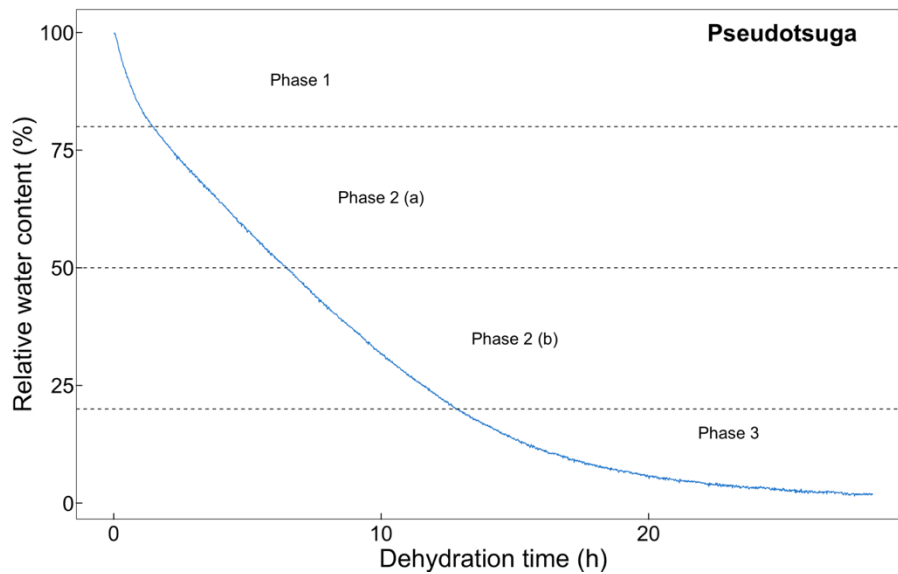
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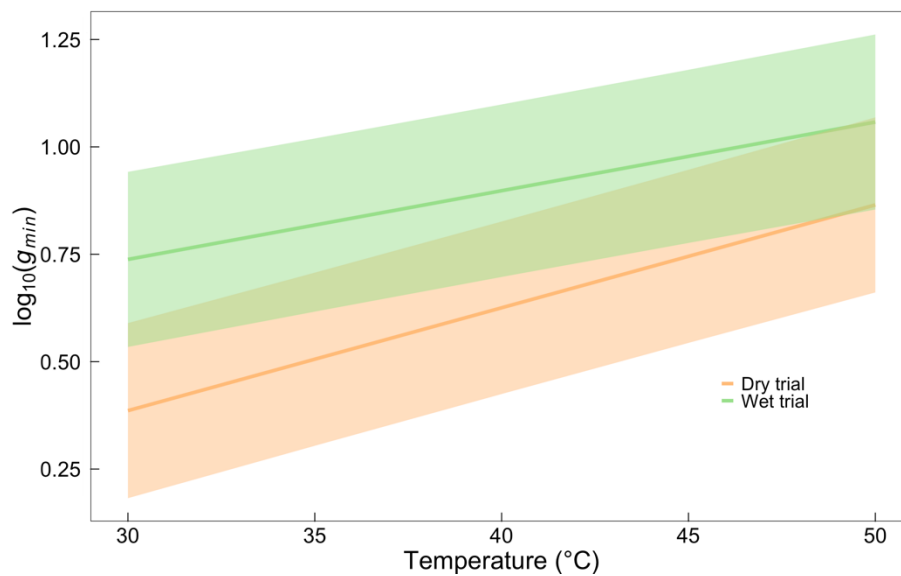
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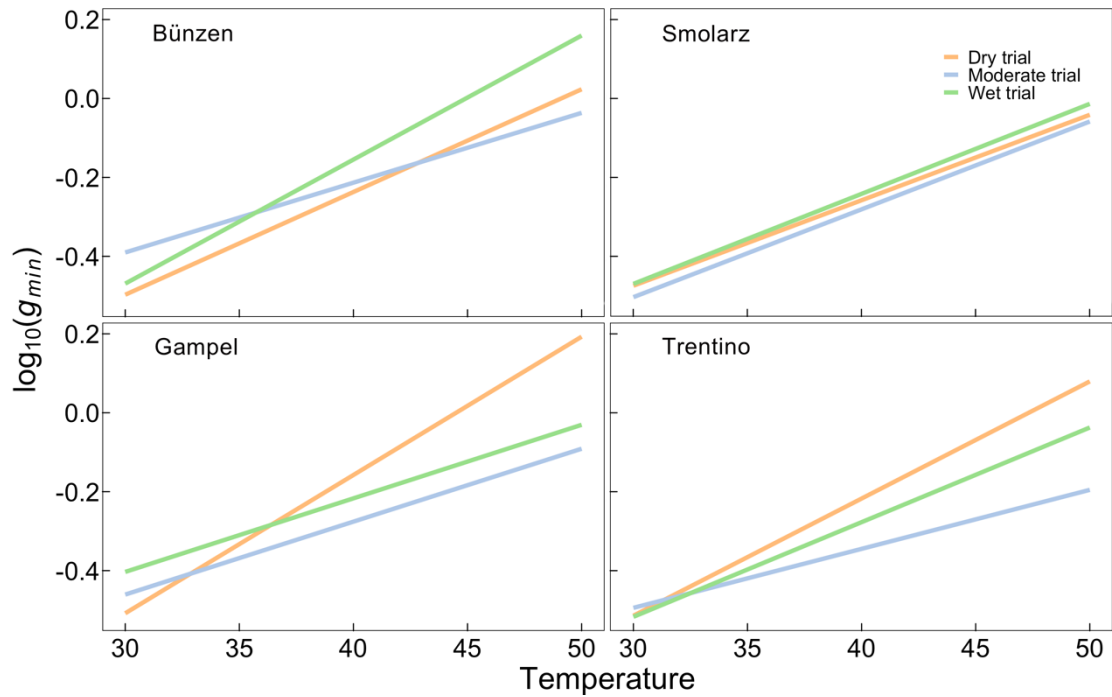
## Chapter II – Supporting Information



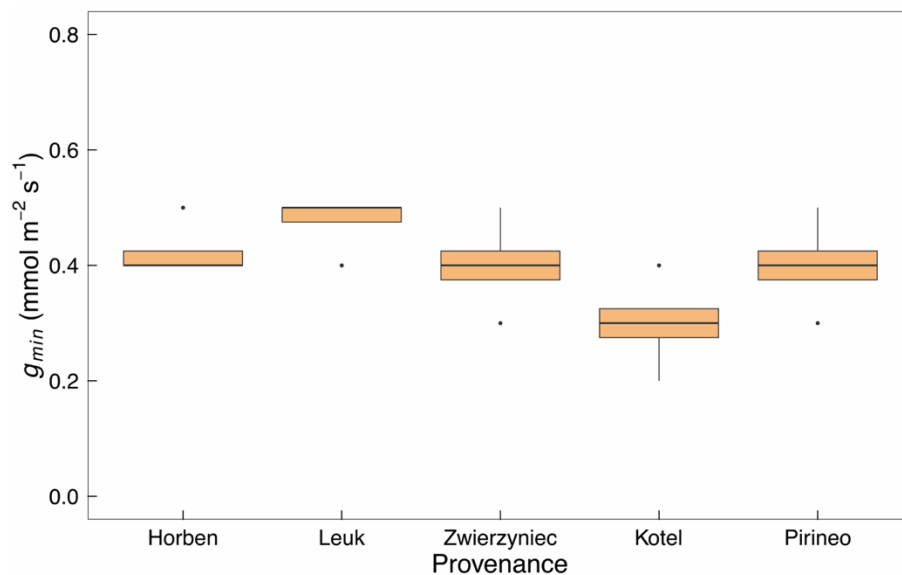
**Figure S1.** Schematic graph of changes in relative water content of *Pseudotsuga* shoots as a function of dehydration time at 40 °C. P



**Figure S2.** Effects of trials on the temperature responses of  $g_{min}$  for *Acer*. Y-axis means the predicted values from the corresponding linear mixed models. X-axis is temperature gradient from 30 to 50 °C. Different colors indicate different trials and 95% confidence interval of predicted values. Trees in the dry trial showing stronger thermal sensitivity of  $g_{min}$  than trees in the wet trial ( $F = 10.25$ ,  $p < 0.01$ ).



**Figure S3.** Effects of provenances and trials on the temperature responses of  $g_{min}$  for *Picea*. Y-axis means the predicted values from the corresponding linear mixed models. X-axis is temperature gradient from 30 to 50 °C. Different colors indicate different trials. Significant interaction between provenances, trials, and temperature responses of  $g_{min}$  was found in *Picea* ( $F = 3.34, p < 0.01$ ), but the interaction did not show a consistent pattern across either trials or provenances.



**Figure S4.**  $g_{min}$  at 30 °C for different provenances of *Abies alba* in the dry trial. Provenances are listed from left to right in order from humid to arid regions. There was no significant difference in  $g_{min}$  between provenances.

**Table S1.** Temperature response of  $g_{min}$  (mean  $\pm$  standard deviation) between different provenances of four studied tree species at different soil moisture trials. N.A. indicates the unmeasured data.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Acer	Wet	Muri	30	5.98 $\pm$ 1.39
Acer	Wet	Muri	35	9.54 $\pm$ 1.66
Acer	Wet	Muri	40	10.87 $\pm$ 3.20
Acer	Wet	Muri	45	11.50 $\pm$ 2.53
Acer	Wet	Muri	50	13.66 $\pm$ 2.48
Acer	Wet	Pfynwald	30	5.90 $\pm$ 1.07
Acer	Wet	Pfynwald	35	6.25 $\pm$ 1.09
Acer	Wet	Pfynwald	40	7.25 $\pm$ 0.73
Acer	Wet	Pfynwald	45	8.85 $\pm$ 2.15
Acer	Wet	Pfynwald	50	10.73 $\pm$ 1.07
Acer	Wet	Toskana	30	8.97
Acer	Wet	Toskana	35	N.A.
Acer	Wet	Toskana	40	N.A.
Acer	Wet	Toskana	45	10.22
Acer	Wet	Toskana	50	N.A.
Acer	Wet	Sliven	30	4.58 $\pm$ 0.94
Acer	Wet	Sliven	35	5.46 $\pm$ 1.76
Acer	Wet	Sliven	40	7.08 $\pm$ 2.62
Acer	Wet	Sliven	45	8.85 $\pm$ 0.76
Acer	Wet	Sliven	50	10.11 $\pm$ 1.92
Acer	Dry	Muri	30	2.62 $\pm$ 0.55
Acer	Dry	Muri	35	2.47 $\pm$ 0.47
Acer	Dry	Muri	40	3.19 $\pm$ 0.62
Acer	Dry	Muri	45	5.10 $\pm$ 0.86
Acer	Dry	Muri	50	7.37 $\pm$ 0.24

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Acer	Dry	Pfynwald	30	3.74 ± 1.02
Acer	Dry	Pfynwald	35	3.82 ± 0.81
Acer	Dry	Pfynwald	40	4.23 ± 0.84
Acer	Dry	Pfynwald	45	4.85 ± 1.39
Acer	Dry	Pfynwald	50	10.39 ± 4.80
Acer	Dry	Toscana	30	2.61 ± 0.81
Acer	Dry	Toscana	35	3.58 ± 1.03
Acer	Dry	Toscana	40	4.12 ± 0.48
Acer	Dry	Toscana	45	5.75 ± 0.43
Acer	Dry	Toscana	50	10.02 ± 3.87
Acer	Dry	Sliven	30	2.63 ± 1.14
Acer	Dry	Sliven	35	3.13 ± 1.88
Acer	Dry	Sliven	40	4.42 ± 2.47
Acer	Dry	Sliven	45	5.15 ± 1.73
Acer	Dry	Sliven	50	10.55 ± 4.14
Fagus	Wet	Auw	30	4.39 ± 1.02
Fagus	Wet	Auw	35	4.76 ± 1.40
Fagus	Wet	Auw	40	5.40 ± 1.41
Fagus	Wet	Auw	45	6.21 ± 1.27
Fagus	Wet	Auw	50	7.64 ± 1.14
Fagus	Wet	Wilchingen	30	4.61 ± 0.87
Fagus	Wet	Wilchingen	35	5.63 ± 1.30
Fagus	Wet	Wilchingen	40	6.15 ± 1.33
Fagus	Wet	Wilchingen	45	7.21 ± 1.35
Fagus	Wet	Wilchingen	50	8.14 ± 1.47

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Fagus	Wet	Champagne	30	4.10 ± 0.72
Fagus	Wet	Champagne	35	4.75 ± 1.11
Fagus	Wet	Champagne	40	5.92 ± 1.32
Fagus	Wet	Champagne	45	6.43 ± 0.76
Fagus	Wet	Champagne	50	7.01 ± 1.09
Fagus	Wet	Petrohan	30	2.99 ± 0.28
Fagus	Wet	Petrohan	35	4.12 ± 0.42
Fagus	Wet	Petrohan	40	4.70 ± 0.29
Fagus	Wet	Petrohan	45	5.69 ± 0.66
Fagus	Wet	Petrohan	50	6.23 ± 0.67
Fagus	Wet	Cimini	30	2.96 ± 0.50
Fagus	Wet	Cimini	35	3.35 ± 0.44
Fagus	Wet	Cimini	40	3.97 ± 0.12
Fagus	Wet	Cimini	45	3.96 ± 0.16
Fagus	Wet	Cimini	50	4.92 ± 0.59
Fagus	Dry	Auw	30	1.82 ± 0.39
Fagus	Dry	Auw	35	2.23 ± 0.25
Fagus	Dry	Auw	40	2.31 ± 0.21
Fagus	Dry	Auw	45	2.62 ± 0.45
Fagus	Dry	Auw	50	3.62 ± 0.36
Fagus	Dry	Wilchingen	30	2.21 ± 0.68
Fagus	Dry	Wilchingen	35	2.49 ± 0.72
Fagus	Dry	Wilchingen	40	2.63 ± 0.65
Fagus	Dry	Wilchingen	45	2.80 ± 0.31
Fagus	Dry	Wilchingen	50	4.31 ± 0.48

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Fagus	Dry	Champagne	30	1.95 ± 0.57
Fagus	Dry	Champagne	35	2.11 ± 0.35
Fagus	Dry	Champagne	40	2.54 ± 0.84
Fagus	Dry	Champagne	45	2.73 ± 1.11
Fagus	Dry	Champagne	50	4.33 ± 1.24
Fagus	Dry	Petrohan	30	1.60 ± 0.23
Fagus	Dry	Petrohan	35	1.86 ± 0.31
Fagus	Dry	Petrohan	40	1.95 ± 0.62
Fagus	Dry	Petrohan	45	2.51 ± 0.38
Fagus	Dry	Petrohan	50	3.30 ± 0.89
Fagus	Dry	Cimini	30	1.19 ± 0.05
Fagus	Dry	Cimini	35	1.38 ± 0.08
Fagus	Dry	Cimini	40	1.39 ± 0.27
Fagus	Dry	Cimini	45	1.58 ± 0.44
Fagus	Dry	Cimini	50	2.53 ± 0.54
Picea	Wet	Bünzen	30	0.35 ± 0.05
Picea	Wet	Bünzen	35	0.41 ± 0.05
Picea	Wet	Bünzen	40	0.82 ± 0.23
Picea	Wet	Bünzen	45	1.35 ± 0.52
Picea	Wet	Bünzen	50	1.21 ± 0.19
Picea	Wet	Smolarz	30	0.37 ± 0.07
Picea	Wet	Smolarz	35	0.41 ± 0.10
Picea	Wet	Smolarz	40	0.58 ± 0.23
Picea	Wet	Smolarz	45	0.87 ± 0.23
Picea	Wet	Smolarz	50	0.95 ± 0.25



Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Picea	Wet	Gampel	30	0.46 ± 0.05
Picea	Wet	Gampel	35	0.40 ± 0.12
Picea	Wet	Gampel	40	0.67 ± 0.17
Picea	Wet	Gampel	45	0.81 ± 0.17
Picea	Wet	Gampel	50	0.96 ± 0.35
Picea	Wet	Trentino	30	0.35 ± 0.04
Picea	Wet	Trentino	35	0.37 ± 0.07
Picea	Wet	Trentino	40	0.44 ± 0.10
Picea	Wet	Trentino	45	0.86 ± 0.20
Picea	Wet	Trentino	50	0.92 ± 0.24
Picea	Moderate	Bünzen	30	0.38 ± 0.09
Picea	Moderate	Bünzen	35	0.58 ± 0.14
Picea	Moderate	Bünzen	40	0.59 ± 0.12
Picea	Moderate	Bünzen	45	0.81 ± 0.13
Picea	Moderate	Bünzen	50	0.88 ± 0.17
Picea	Moderate	Smolarz	30	0.34 ± 0.07
Picea	Moderate	Smolarz	35	0.42 ± 0.13
Picea	Moderate	Smolarz	40	0.46 ± 0.19
Picea	Moderate	Smolarz	45	0.89 ± 0.62
Picea	Moderate	Smolarz	50	0.93 ± 0.31
Picea	Moderate	Gampel	30	0.35 ± 0.04
Picea	Moderate	Gampel	35	0.45 ± 0.09
Picea	Moderate	Gampel	40	0.48 ± 0.05
Picea	Moderate	Gampel	45	0.74 ± 0.09
Picea	Moderate	Gampel	50	0.79 ± 0.15

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Picea	Moderate	Trentino	30	0.34 ± 0.04
Picea	Moderate	Trentino	35	0.39 ± 0.08
Picea	Moderate	Trentino	40	0.39 ± 0.10
Picea	Moderate	Trentino	45	0.61 ± 0.10
Picea	Moderate	Trentino	50	0.67 ± 0.22
Picea	Dry	Bünzen	30	0.32 ± 0.06
Picea	Dry	Bünzen	35	0.40 ± 0.09
Picea	Dry	Bünzen	40	0.69 ± 0.14
Picea	Dry	Bünzen	45	0.77 ± 0.19
Picea	Dry	Bünzen	50	1.13 ± 0.54
Picea	Dry	Smolarz	30	0.32 ± 0.07
Picea	Dry	Smolarz	35	0.43 ± 0.06
Picea	Dry	Smolarz	40	0.64 ± 0.05
Picea	Dry	Smolarz	45	0.75 ± 0.24
Picea	Dry	Smolarz	50	0.86 ± 0.18
Picea	Dry	Gampel	30	0.38 ± 0.12
Picea	Dry	Gampel	35	0.39 ± 0.13
Picea	Dry	Gampel	40	0.70 ± 0.16
Picea	Dry	Gampel	45	1.28 ± 0.80
Picea	Dry	Gampel	50	1.71 ± 0.85
Picea	Dry	Trentino	30	0.34 ± 0.03
Picea	Dry	Trentino	35	0.36 ± 0.04
Picea	Dry	Trentino	40	0.67 ± 0.15
Picea	Dry	Trentino	45	0.90 ± 0.09
Picea	Dry	Trentino	50	1.21 ± 0.37

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Pseudotsuga	Wet	Biel	30	1.00 ± 0.50
Pseudotsuga	Wet	Biel	35	1.95 ± 0.34
Pseudotsuga	Wet	Biel	40	2.12 ± 0.21
Pseudotsuga	Wet	Biel	45	2.44 ± 0.34
Pseudotsuga	Wet	Biel	50	3.80 ± 0.78
Pseudotsuga	Wet	Barlinek	30	0.73 ± 0.22
Pseudotsuga	Wet	Barlinek	35	1.73 ± 0.68
Pseudotsuga	Wet	Barlinek	40	1.75 ± 0.42
Pseudotsuga	Wet	Barlinek	45	2.39 ± 0.21
Pseudotsuga	Wet	Barlinek	50	2.94 ± 0.26
Pseudotsuga	Wet	Rzepin	30	0.77 ± 0.09
Pseudotsuga	Wet	Rzepin	35	1.84 ± 0.23
Pseudotsuga	Wet	Rzepin	40	2.07 ± 0.25
Pseudotsuga	Wet	Rzepin	45	2.70 ± 0.57
Pseudotsuga	Wet	Rzepin	50	3.58 ± 0.21
Pseudotsuga	Wet	Toskana	30	0.81 ± 0.16
Pseudotsuga	Wet	Toskana	35	1.66 ± 0.19
Pseudotsuga	Wet	Toskana	40	1.89 ± 0.25
Pseudotsuga	Wet	Toskana	45	2.29 ± 0.26
Pseudotsuga	Wet	Toskana	50	3.08 ± 0.34
Pseudotsuga	Moderate	Biel	30	0.76 ± 0.29
Pseudotsuga	Moderate	Biel	35	1.02 ± 0.22
Pseudotsuga	Moderate	Biel	40	1.41 ± 0.28
Pseudotsuga	Moderate	Biel	45	2.42 ± 0.55
Pseudotsuga	Moderate	Biel	50	2.88 ± 0.57

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Pseudotsuga	Moderate	Barlinek	30	0.93 ± 0.61
Pseudotsuga	Moderate	Barlinek	35	0.96 ± 0.22
Pseudotsuga	Moderate	Barlinek	40	1.50 ± 0.29
Pseudotsuga	Moderate	Barlinek	45	2.13 ± 0.22
Pseudotsuga	Moderate	Barlinek	50	3.06 ± 0.71
Pseudotsuga	Moderate	Rzepin	30	0.69 ± 0.27
Pseudotsuga	Moderate	Rzepin	35	1.13 ± 0.09
Pseudotsuga	Moderate	Rzepin	40	1.58 ± 0.32
Pseudotsuga	Moderate	Rzepin	45	2.22 ± 0.55
Pseudotsuga	Moderate	Rzepin	50	3.14 ± 0.48
Pseudotsuga	Moderate	Toskana	30	0.75 ± 0.44
Pseudotsuga	Moderate	Toskana	35	0.90 ± 0.28
Pseudotsuga	Moderate	Toskana	40	1.28 ± 0.28
Pseudotsuga	Moderate	Toskana	45	2.02 ± 0.58
Pseudotsuga	Moderate	Toskana	50	2.38 ± 0.63
Pseudotsuga	Dry	Biel	30	0.46 ± 0.06
Pseudotsuga	Dry	Biel	35	1.10 ± 0.15
Pseudotsuga	Dry	Biel	40	1.86 ± 0.72
Pseudotsuga	Dry	Biel	45	1.91 ± 0.46
Pseudotsuga	Dry	Biel	50	2.54 ± 0.04
Pseudotsuga	Dry	Barlinek	30	0.70 ± 0.37
Pseudotsuga	Dry	Barlinek	35	1.31 ± 0.24
Pseudotsuga	Dry	Barlinek	40	1.89 ± 0.43
Pseudotsuga	Dry	Barlinek	45	2.08 ± 0.14
Pseudotsuga	Dry	Barlinek	50	3.15 ± 0.89

Table S1 continued from previous page.

Species	Trial	Provenance	Temperature(°C)	$g_{min}$ (mmol m <sup>-2</sup> s <sup>-1</sup> )
Pseudotsuga	Dry	Rzepin	30	0.43 ± 0.03
Pseudotsuga	Dry	Rzepin	35	1.27 ± 0.19
Pseudotsuga	Dry	Rzepin	40	1.71 ± 0.56
Pseudotsuga	Dry	Rzepin	45	2.01 ± 0.21
Pseudotsuga	Dry	Rzepin	50	2.88 ± 0.65
Pseudotsuga	Dry	Toskana	30	1.03 ± 0.57
Pseudotsuga	Dry	Toskana	35	1.33 ± 0.15
Pseudotsuga	Dry	Toskana	40	1.68 ± 0.17
Pseudotsuga	Dry	Toskana	45	1.94 ± 0.18
Pseudotsuga	Dry	Toskana	50	2.83 ± 0.40

Table S2. Phase transition temperatures ( $T_p$ ) in the temperature responses of  $g_{min}$ .

Species	Trial	Provenance	$T_p$ (°C)	$p$ values*
Acer	Wet	Pfynwald	37.8	0.013
Acer	Dry	Muri	38.2	0.011
Acer	Dry	Toskana	43.8	0.007
Fagus	Dry	Petrohan	42.1	0.017
Picea	Dry	Gampel	36.8	0.023
Pseudotsuga	Moderate	Barlinek	37.0	0.027
Pseudotsuga	Moderate	Rzepin	43.0	<0.001

\* Based on segmented regression analysis



## **Chapter III**

### **Water loss after stomatal closure: quantifying leaf minimum conductance and minimal water use in 9 temperate European tree species during a severe drought**

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## Abstract

Residual canopy transpiration ( $E_{min\_canop}$ ) is a key physiological trait that determines trees' survival time under drought after stomatal closure and after trees have limited access to soil water.  $E_{min\_canop}$  mainly depends on leaf minimum conductance ( $g_{min}$ ) and vapor pressure deficit (VPD). Here we determined the seasonal variation of  $g_{min}$  and how  $g_{min}$  is related to interspecies variation in leaf cuticular and stomatal traits for nine European tree species in a mature forest. In addition, we determined the species-specific temperature responses of  $g_{min}$ . With this newly obtained insight, we calculated  $E_{min\_canop}$  for the nine species for one day at our research site during the 2022 central European hot drought. Our results show that at ambient temperatures  $g_{min}$  ranged from 0.8 to 4.8  $\text{mmol m}^{-2} \text{s}^{-1}$  across the nine species and was stable in most species throughout the growing season. The interspecies variation of  $g_{min}$  was associated with leaf cuticular and stomatal traits. Additionally,  $g_{min}$  exhibited strong temperature responses and increased, depending on species, by a factor of two to four in the range of 25 to 50 °C. For the studied species at the site, during a single hot drought day,  $E_{min\_canop}$  standardized by tree size (stem basal area) ranged from 2.0 to 36.7  $\text{L m}^{-2}$ , and non-standardized  $E_{min\_canop}$  for adult trees ranged from 0.3 to 5.3  $\text{L}$ .  $E_{min\_canop}$  also exhibited species-specific rapid increases under hotter temperatures. Our results suggest that trees, depending on species, need reasonable amounts of water during a drought, even when stomates are fully closed. Species differences in  $g_{min}$  and ultimately  $E_{min\_canop}$  can affect, together with other traits, the ability of a tree to keep its tissue hydrated during a drought and is likely to contribute to species-specific differences in drought vulnerability.

## Introduction

The intensity and frequency of both extreme high temperatures and droughts are predicted to increase worldwide in the 21<sup>st</sup> century (Teuling 2018). Already now, increasing drought-induced tree mortality has been reported across the globe (Allen et al. 2010, 2015, Rigling et al. 2013, Hammond et al. 2019, Powers et al. 2020, Schuldt et al. 2020). When it becomes difficult for trees to take up water from the soil, trees will close their stomata to maintain turgor pressure in their tissues and to avoid critical levels of dehydration and ultimately death (Bartlett et al. 2016, Martin-StPaul et al. 2017, Peters et al. 2023). Full stomatal closure cannot, however, completely prevent water loss from the canopy, and the transpiration through leaky stomata and (or) cuticle can lead to progressive dehydration of a tree (Schuster et al. 2017, Duursma et al. 2019). The dehydration rate and thus the surviving time of a tree after root water uptake is becoming limited in a severe drought will thus depend on the magnitude of residual canopy transpiration, the availability of stored mobile water inside the tree as well as the dehydration tolerance of critical tissues or organs (Blackman et al. 2016, Körner 2019, Hammond and Adams 2019, Martinez-Vilalta et al. 2019, Mantova et al. 2022).

Residual whole tree canopy transpiration depends on leaf minimum water conductance (hereafter referred as  $g_{min}$ ), a tree's total leaf surface area, and the vapor pressure deficit (VPD) that the canopy experiences. However, the rates of residual whole tree canopy transpiration during severe droughts and how these rates vary across species are rarely quantified. This is partly because the variability of  $g_{min}$  is not well constrained.  $g_{min}$  will vary across species, throughout a growing season as a consequence of leaf aging and in response to environmental drivers (Schuster et al. 2017, Duursma et al. 2019).

According to Schuster et al. (2017), for a total of 39 deciduous woody plants (trees and shrubs),  $g_{min}$  ranges from 0.15 mmol m<sup>-2</sup> s<sup>-1</sup> to 8.0 mmol m<sup>-2</sup> s<sup>-1</sup>. Across-species variability in  $g_{min}$  likely depends on cuticular and stomatal traits (Duursma et al. 2019, Machado et al. 2021). For example, a thicker leaf cuticle of *Arabidopsis thaliana* has been suggested to be an effective way to improve the plant's drought



performance, because of the accompanied lower  $g_{min}$  (Kosma et al. 2009, Patwari et al. 2019). However, an effect of cuticular thickness on  $g_{min}$  was not found in a range of different tree species indicating that uncertainties remain regarding the morphological and anatomical leaf properties that determine  $g_{min}$  (Bueno et al. 2020, Machado et al. 2021, Grünhofer et al. 2022). Additionally, Machado et al. (2021) reported that higher  $g_{min}$  was associated with small and numerous stomata among 30 tropical savanna tree species and that most deciduous tree species had higher  $g_{min}$  than evergreen trees.

As leaves mature and age,  $g_{min}$  might vary not only among species but also within a growing season. Stable isotope studies have demonstrated that leaf waxes were synthesized mostly in the early stage of the leaf development (Kahmen et al. 2011, Gamarra and Kahmen 2016), and that cuticular water permeability was maintained throughout the lifespan of a leaf (Hauke and Schreiber 1998). However, physical damage on the leaf surface due to impacts of radiation, wind, and rain erosion throughout the lifespan of a leaf can cause  $g_{min}$  to vary seasonally with leaf aging, especially in evergreen conifer trees (Hadley and Smith 1994, Anfodillo et al. 2002, Jordan and Brodribb, 2007). For instance, in the northeast Italian Alps, Anfodillo et al. (2002) reported that  $g_{min}$  of *Picea abies* Karst. varied largely with the progression of the growing season in current-year needles, and increased fourfold from 5-month-old needles to 13-month-old needles. For the sake of simplicity, however, in most literature  $g_{min}$  is generally considered a seasonally stable leaf trait, and potential effects of cuticle aging are not accounted for (Duursma et al. 2019).

Recently, several studies have reported a strong temperature sensitivity of  $g_{min}$  suggesting that  $g_{min}$  will increase with rising leaf surface temperature (Eamus et al. 2008, Schuster et al. 2016, Bueno et al. 2019, Billon et al. 2020, Slot et al. 2021). For instance, Billon et al. (2020) compared the response of  $g_{min}$  to a temperature gradient of 30 to 55 °C in five temperate tree species and found that  $g_{min}$  increased nonlinearly under hotter temperatures. A species-specific phase transition temperature was also suggested, beyond which  $g_{min}$  showed a rapid increase for some species (Bueno et al. 2019, Billon et al. 2020, Slot et al. 2021, Hartill et al. 2023). Given anticipated more frequent hot temperatures in summer (Teuling 2018), the residual whole tree canopy transpiration at

full stomatal closure may thus be underestimated due to the temperature dependency of  $g_{min}$ , and the surviving time of trees may be largely overestimated during such events (Billon et al. 2020, Cochard 2021, Breshears et al. 2021).

In this study, we conducted a comprehensive investigation of the variability of  $g_{min}$  in nine ecologically and economically important temperate European tree species, and we used these data to calculate the residual whole tree canopy transpiration at full stomatal closure ( $E_{min\_canop}$ ) for these species using a 2022 hot drought event as a case study. Specifically, we studied for all species: (I) the intraspecific variability of  $g_{min}$  across the growing season; (II) the relationships between  $g_{min}$  and leaf cuticular and stomatal traits; (III) the temperature response of  $g_{min}$ ; and (IV) the  $E_{min\_canop}$  during a single day of the 2022 central European hot drought event.

## Methods and material

### *Research site and climate*

This study was conducted with leaves collected from tree canopies at the Swiss Canopy Crane II (SCCII) research site (47°26'17"N, 7°46'37"E) close to Hölstein, about 20 km southeast of the city of Basel, Switzerland. The site is located on a plateau of the Jura Mountains at an elevation of 550 m a.s.l., and has high-clay soil and deep groundwater. *Fagus sylvatica* L. and *Picea abies* Karst. are dominant species at the site, forming a mixed temperate forest with 12 other tree species. At the center of the site is a 50 m tall canopy crane with a radius of 62.5 m that allows access to 333 trees.

The mean annual temperature and the mean annual precipitation in the region is 9.6 °C and 972 mm, respectively (mean data 1991 – 2020 from SwissMeteo, station Rünenberg). In 2022, the mean annual temperature and the total annual precipitation was 11.4 °C and 662 mm at the site. July was the hottest month with average and maximum temperatures of 21.0 and 34.8 °C, respectively, and a total precipitation of only 20 mm.

The nine species we investigated were *Acer pseudoplatanus* L., *Carpinus betulus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Quercus* sp. (hybrid forms by *Q. petraea* Liebl. and *Q. robur* L.), *Sorbus torminalis* Crantz, *Abies alba* Mill., *Picea abies* Karst., *Pinus sylvestris* L. For simplicity, all tree species are referred by their genus names in the following.

### *$g_{min}$ measurements*

Since vapor loss after stomatal closure is too low to be reliably detected by conventional gas exchange measurement methods, the approach of mass loss of detached leaves was used to determine  $g_{min}$  (Duursma et al. 2019).

### *Species differences and seasonal variation of $g_{min}$*

Species differences and the seasonal variability of  $g_{min}$  were investigated monthly from June to September 2020. Depending on the availability of trees under the canopy crane,

two to five individual trees of each species were sampled (Table 1). Each month, two shoots (ca. 25 cm long) from two different branches were cut in the morning at the south-exposed side of the upper canopy of a tree using the canopy crane. The cut ends of the shoots were immediately submerged in water and transported in a cool box to the laboratory in Basel. In the lab, two healthy and fully expanded leaves from each shoot were used for  $g_{min}$  measurements. Thus, four leaves were measured to calculate an average  $g_{min}$  value for each tree and monthly averages of species were reported (i.e.,  $n = 2 - 5$ ). In *Fraxinus*, we used individual leaflets of the dissected leaf. For the three conifer species, the current-year and 1-year-old needles were measured separately using four fascicles in *Pinus* and four small twigs in *Abies* and *Picea*.

For the measurements, leaves were cut at the petioles underwater with sharp razor blades from the shoot. Leaves, fascicles, or twigs were then rehydrated in de-gassed water via the standing rehydration method for 20 h in the dark (Arndt et al. 2015). After rehydration, the cut ends were sealed with high-melting paraffin wax (melting point 68 °C), and the saturated fresh weight of the leaves was immediately measured ( $W_{sat}$ ). All leaves were then placed into climate-controlled growth chambers to dry down for 30 h in the dark, with air temperature and relative humidity being stable at 20 °C and 69 %, respectively. During the desiccation, leaves were taken out of the chambers at regular intervals ( $\Delta T$ , 2 – 3 h) and weighed with a high precision balance (precision:  $\pm 0.2$  mg, Balance XPR204S, METTLER TOLEDO, Switzerland). This process took approximately ten minutes. Leaf water loss ( $W_{loss}$ ) was determined based on the difference in leaf fresh weight ( $W_{fre}$ ) between two weighing intervals. After 30 h, leaves were removed from the growth chamber and weighed for the last time, and then completely dried in a drying oven for 72 h at 80 °C to obtain the leaf dry mass ( $W_{dry}$ ).

Leaf water conductance ( $J$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) was calculated as:

$$\text{Equation (1): } J = \frac{W_{loss}}{\Delta T * A * VPD} * 98.0$$

where  $A$  is the total two-sided leaf area ( $\text{m}^2$ ). Leaf area was calculated based on values for specific leaf area (SLA) and dry mass of leaf samples after  $g_{min}$  measurements. SLA was calculated as the ratio of fresh leaf area to leaf dry mass. For this, leaves from adjacent twigs were scanned and fresh leaf area was extracted by our self-developed leaf image analysis tool (<https://github.com/dabasler/LeafAreaExtraction>). Leaves were then dried in the oven for 72 h to obtain dry mass. The bark area in conifer twigs was not considered in the calculation because of its' rather small surface area compared to the attached total needle area. VPD in Eq. (1) is the vapor pressure deficit (kPa), and the atmospheric pressure in the growth chamber was assumed to be 98.0 kPa. Additionally, the effect of boundary layer conductance on  $g_{min}$  was assumed to be insignificant, since the fan system fully operated in the growth chamber during leaf desiccation.  $g_{min}$  was obtained by plotting leaf water conductance over the remaining leaf relative water content (RWC; see Figure S1 available as Supplementary Data at Tree Physiology Online). According to our preliminary experiment, full stomatal closure occurred at  $\text{RWC} < 80 \%$  (commonly after 2-3 hours of exposure) for the studied tree species, and we assume that the leaf is functionally intact at  $\text{RWC} > 50 \%$ . At greater (including fatal) water deficits, there is a risk of artifacts because stomata may be pulled open by shrinking epidermis or cracks in the cuticle. Therefore, we used the horizontal part (values) of the curve between 80 % and 50 % of RWC to determine  $g_{min}$  (Figure S1). Leaf relative water content was calculated as:

$$\text{Equation (2): } RWC = \frac{W_{fre} - W_{dry}}{W_{sat} - W_{dry}}$$

#### *Temperature sensitivity of $g_{min}$ across species*

To determine the temperature sensitivity of  $g_{min}$ , field sampling started in mid-July 2021 after the full expansion of new leaves in all nine species, and ended in late August 2021. For each species, two to four individual trees were sampled according to the cover range of the crane. At the south-exposed side of the upper canopy of each tree, a small shoot from each of two different branches was cut in the morning and the cut ends were kept

in water in a cool box before the transportation to Basel. In the lab, five to seven healthy leaves from each shoot were cut under water and rehydrated as described above. For the three conifers, three or four twigs (including the current-year and 1-year-old needles) from each shoot were rehydrated.

For the temperature response of  $g_{min}$ , we employed an alternative, less labor-intensive  $g_{min}$  detection method as described above using the "Drought Box", an efficient tool for semi-automated measurements of  $g_{min}$  under controlled environments (Billon et al. 2020). Fully rehydrated leaves and (or) twigs were hung in the Drought Box to dehydrate progressively while the weights of leaves were automatically measured at 1 min intervals (precision:  $\pm 50$  mg). Meanwhile, three fans were running to ensure good air circulation inside the box. The balance of the Drought Box is not sensitive enough to record the weight loss of individual leaves with high precision. For this reason, we jointly measured the weight loss of several leaves (ten to fourteen leaves for broadleaf trees and six to eight twigs in conifers, as described above).  $g_{min}$  measurements were performed for different leaves at temperatures of 25, 35, 45, and 50 °C, and the corresponding relative humidity was 37, 56, 63, and 66 %. Relative humidity was moderately higher at high temperatures to slow the rate of water loss and to increase the precision of the  $g_{min}$  measurements. Although a previous study showed that extremely high air relative humidity ( $> 70$  %) caused a significant increase in cuticular permeability, the absolute difference in permeability was small and the effect of increasing temperature on cuticular permeability was several orders of magnitude higher than that caused by the changes in humidity (Schreiber et al. 2001). We tested if  $g_{min}$  was dependent on relative humidity before our measurements but found no such effect (see Figure S2 available as Supplementary Data at Tree Physiology Online).

To obtain a more complete picture of the temperature responses of  $g_{min}$  in these nine species,  $g_{min}$  measurements were repeated for all tree species under different temperatures in the Drought Box from July to August 2022. Sampled shoots were directly rehydrated as described above and used for  $g_{min}$  measurements (instead of isolated leaves). For the sake of simplicity, the bark area of the shoots was not considered in the calculation. Temperatures inside the Drought Box were 30, 37, 43,

and 48 °C, and the corresponding relative humidity was 40, 36, 33, and 37 %. The average values of species based on individual trees were reported.

A nonlinear temperature response of  $g_{min}$  has been reported for some species and phase transition temperatures had been extracted from these nonlinear functions (Eamus et al. 2008, Schuster et al. 2016, Bueno et al. 2019, Billon et al. 2020, Slot et al. 2021). In addition, there was no significant effect of sampling approaches or VPD on the temperature responses of  $g_{min}$  in our data (see Table S2 and S3 available as Supplementary Data at Tree Physiology Online). For the sake of simplicity, the  $g_{min}$  response to temperature in the present study was thus visualized by fitting exponential functions to the pooled data across both years. Phase transition temperatures were determined by using segmented regression.

### ***Stomatal traits***

The epidermal impression technique was used to investigate leaf stomatal traits for deciduous species (Machado et al. 2021), except for *Acer* due to sunken stomata in the epidermal layer. During the field campaign 2022, two healthy twigs were collected in the morning from the same branches used in the  $g_{min}$  measurements and transported to the laboratory in a cool box. Then two fully expanded leaves were re-cut under water from each twig. On the detached leaves, a small amount of clear nail polish was applied to both adaxial and abaxial leaf surfaces and allowed to dry for several minutes. After drying, nail polish was covered by transparent sticky tape and lifted. The tape with the leaf epidermis was trimmed and mounted onto microscopy slides. All slides were then observed with the aid of a light microscope (model CX43; Olympus, Tokyo, Japan), and photographed at  $\times 400$  magnification to calculate stomatal density (SD), and at  $\times 1000$  magnification for the measurements of stomatal size (SS). Picture analyses were done in ImageJ software (US National Institutes of Health). At least 10 pictures / slide (100 – 160 pictures in total for each species) and 15 clear stomata / slide (120 – 160 stomata in total for each species) were used to calculate SD and SS, respectively. The SS was determined as the guard cell length (L) multiplied by the width (W) of the guard cell pair (Franks et al. 2009). Additionally, the average fraction of the leaf epidermis

that is allocated to stomata ( $f_{gc}$ ) was calculated by the average area of the guard cell pair and stomatal density following

$$\text{Equation (3) (Boer et al. 2016): } f_{gc} = \frac{\pi}{2} * W * L * SD$$

Furthermore, the maximum stomatal conductance ( $g_{max}$ ) was extracted from our *in-situ* records of the canopy gas exchange, which was monthly measured with a LiCOR 6800 (LICOR, Lincoln, Nebraska, USA) during the growing seasons from 2020 to 2022 (unpublished data). All stomatal and following cuticular traits in the present study were calculated on individual trees and reported as averages of species.

### ***Cuticular thickness***

The hand-sectioning of fresh leaves was used to determine leaf cuticular thickness for six deciduous species. After the measurements of stomatal traits, one remaining leaf was cut from each twig (thus two leaves per tree). Each leaf was sectioned at the upper, middle, and lower positions, respectively. All leaf sections were stained with Sudan III solution and then observed and photographed at  $\times 400$  magnification with the microscope described above. For each leaf section, five clear pictures were taken, and then the cuticular thickness was measured at different three clear points in each picture to obtain the mean values of different leaf cuticle positions. The measurements were conducted both on adaxial ( $CT_{adaxial}$ ) and abaxial cuticle ( $CT_{abaxial}$ ), subsequently, cuticular thickness ( $CT_{total}$ ) was calculated as the mean values of  $CT_{adaxial}$  and  $CT_{abaxial}$ .

### ***The estimation of residual whole tree canopy transpiration at full stomatal closure ( $E_{min\_canop}$ )***

We calculated  $E_{min\_canop}$  for 3 August 2022, when the average predawn leaf water potential was about -2.0 MPa among nine species, and the minimum midday leaf water potential was close to the xylem water pressures inducing 12 % loss of branch hydraulic conductivity ( $\Psi_{12}$ ) in most studied species (Arend et al. 2021, Kahmen et al. 2022,



Peters et al. 2023). The diurnal records (from 8:00 a.m. to 8:00 p.m., time interval: 5 min) of canopy climate showed that maximum air temperature and lowest relative humidity were respectively 32.2 °C and 29.4 %, and the highest VPD reached 3.4 kPa during that day (see Figure S3 available as Supplementary Data at Tree Physiology Online). Although the dynamic changes in relative humidity were considerable during the day, the absolute humidity was rather stable (Figure S3). So, this day represented a hot day during the 2022 summer drought that compares to the extreme days that trees had experienced in the 2015, 2018 and 2020 droughts in this area (Dietrich et al. 2018, Schuldt et al. 2020, Kahmen et al. 2022). We chose the diurnal patterns in air temperature and absolute humidity of this day to estimate  $E_{min\_canop}$  every 5 min ( $\Delta T$ ) from 8:00 am to 8:00 pm for the nine investigated tree species and calculated the daily  $E_{min\_canop}$ .

To further simulate how temperatures increasing beyond the measured 32.2 °C on 3 August would impact  $E_{min\_canop}$ , we gradually elevated the original diurnal air temperature curve from 3 August by steps of 0.1 °C until a maximum temperature of 50 °C was reached (see Figure S4 available as Supplementary Data at Tree Physiology Online). Meanwhile, we kept the corresponding daily absolute humidity curve unchanged so that VPD covaried with the raising temperature. Before our simulations, we noticed that leaf temperatures of the nine studied species were slightly higher than air temperatures during most of the daytime (unpublished data). However, the dynamic differences between leaf temperature and air temperature were highly species-dependent. For simplicity, we therefore assumed in our simulations that leaf temperatures were equal to air temperatures, but acknowledge that this assumption might lead to a slight underestimation of  $E_{min\_canop}$  for some species. Similarly, we also assumed in our calculation that leaf boundary layer conductance would not cause a significant impact on  $E_{min\_canop}$  for all species, which might also cause deviations in calculated results from  $E_{min\_canop}$  in nature.

Total canopy leaf area is an important variable for determining  $E_{min\_canop}$ . Total canopy leaf area is, however, highly variable among individual trees of a species and depends on tree size, stand density, growing conditions and forest management

practices (Leuschner and Ellenberg 2017). For our calculations, we used the species' measured projected leaf area for our research site (see Kahmen et al. 2022 for details). To standardize our data and make estimates of  $E_{min\_canop}$  of the given species comparable to other sites, we calculated the ratio of the average canopy projected leaf area to the average stem basal area for each species ( $R$ ). Data for basal area per species were previously reported (Kahmen et al. 2022). As such, we calculated  $E_{min\_canop}$  as the daily residual whole tree canopy transpiration at full stomatal closure per square meter of basal area following Eq. (4). In a second approach, we simply calculated  $E_{min\_canop}$  as a function of canopy leaf area ranging from 10 to 200 m<sup>2</sup> for each species to estimate the actual daily water loss from varying canopy sizes in nature.

$$\text{Equation (4): } E_{min\_canop} = (g_{min} * VPD) / 94.9 * \Delta t * R$$

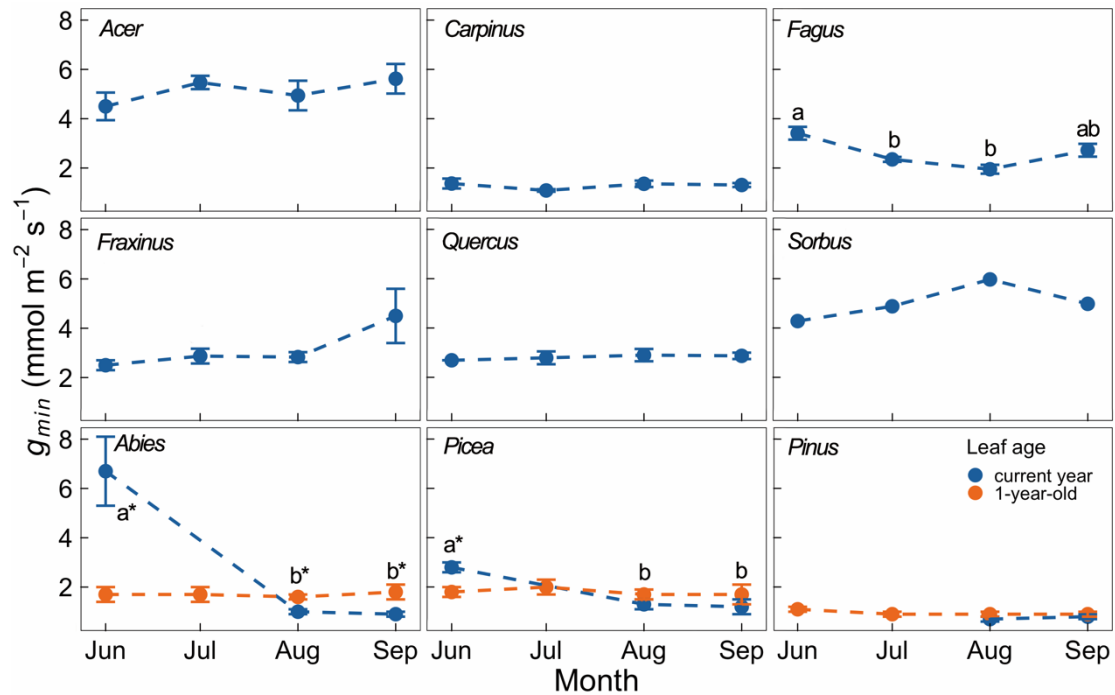
where 94.9 (kPa) is air pressure at our research site.  $\Delta T$  (s) is the time interval, and  $R$  is the ratio of the average canopy projected leaf area to the average stem basal area for each species (See Table 2).

### ***Statistics***

Seasonal significant differences in  $g_{min}$  values were determined with the within-subjects One-way ANOVA in each species. Specifically, when hypothesis tests were met, One-way repeated measures ANOVA were used and followed by Student's T post hoc test. Otherwise, Friedman rank sum test was adopted in the analysis followed by Durbin-Conover post hoc test. At the same time, the significant differences between the two age groups of needles in each month were determined with Mann-Whitney  $U$  test. The significant differences in  $g_{min}$  values across the nine species were determined based on the mean seasonal values of  $g_{min}$  in each species. Additionally, considering that the especially high  $g_{min}$  of the current-year needles in three conifer species only occurred at the early stage of the growing season, these special values were not taken into calculation. The results were analyzed with Kruskal-Wallis one-way ANOVA followed by Fisher's LSD test, because homoscedasticity of variances was not fulfilled and the

number of observations was relatively small among all species ( $N = 2 - 5$ ). Then the significant difference in  $g_{min}$  between the two functional groups was determined with Mann-Whitney  $U$  test because of the large difference in sample sizes between these two groups. Pearson's linear correlation analyses were used to explore the relationships between  $g_{min}$  and stomatal and cuticular traits among the species. Before analysis, species traits were log10-transformed, if necessary, to improve homoscedasticity and normality. It is worth noting that the values of  $g_{min}$  at 25 °C and SLA both measured in 2021 were used in these Pearson's linear correlation analyses. The significance threshold was 0.05 throughout all analyses. All the statistical analyses were performed in R v.4.1.2 (R Core Team, 2021).

## Results



**Figure 1.** Seasonal variation of  $g_{min}$  for nine species in 2020 measured at 20 °C. Significant differences ( $p < 0.05$ ) across months for current year leaves within a single species are indicated by lowercase letters. Significant differences between the current (blue) and 1-year-old needles (orange) in each month are indicated by asterisks (\*,  $p < 0.05$ ). Each point is a mean value ( $n = 2 - 5$ ), and the error bar represents the SE. Please note in *Sorbus*  $n = 2$ , only the mean values are shown without SE.

### *Seasonal course of $g_{min}$*

Of the six deciduous broadleaf species, only *Fagus* showed significant monthly variation in  $g_{min}$  in the 2020 growing season ( $p < 0.001$ , Figure 1). These variations were, however, small. The seasonal variations of  $g_{min}$  in the other five species were small and not significantly different among sampling dates. For the three evergreen conifer species, where different needle generations were measured,  $g_{min}$  was significantly higher at the first sampling date in June in current-year needles than in 1-year-old needles for *Abies* ( $p < 0.05$ ) and *Picea* ( $p < 0.05$ ), but this only occurred very early in the growing season (Figure 1). Then  $g_{min}$  substantially decreased over the next month in the current year needles and there was no statistical difference between the

two age groups of needles in *Picea* and *Pinus* after August. Additionally, we found current-year needles exhibited significantly lower  $g_{min}$  than 1-year-old needles for *Abies* in August and September ( $p < 0.05$ ), but absolute differences were small. The 1-year-old needles showed non-significant seasonal variation in  $g_{min}$  in all three conifer species.

**Table 1.**  $g_{min}$  and  $g_{max}$  ( $\text{mmol m}^{-2} \text{s}^{-1}$ )  $\pm$  standard errors (SE) for nine studied species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus sp.*, *Sorbus torminalis*, *Abies alba*, *Picea abies*, *Pinus sylvestris*).

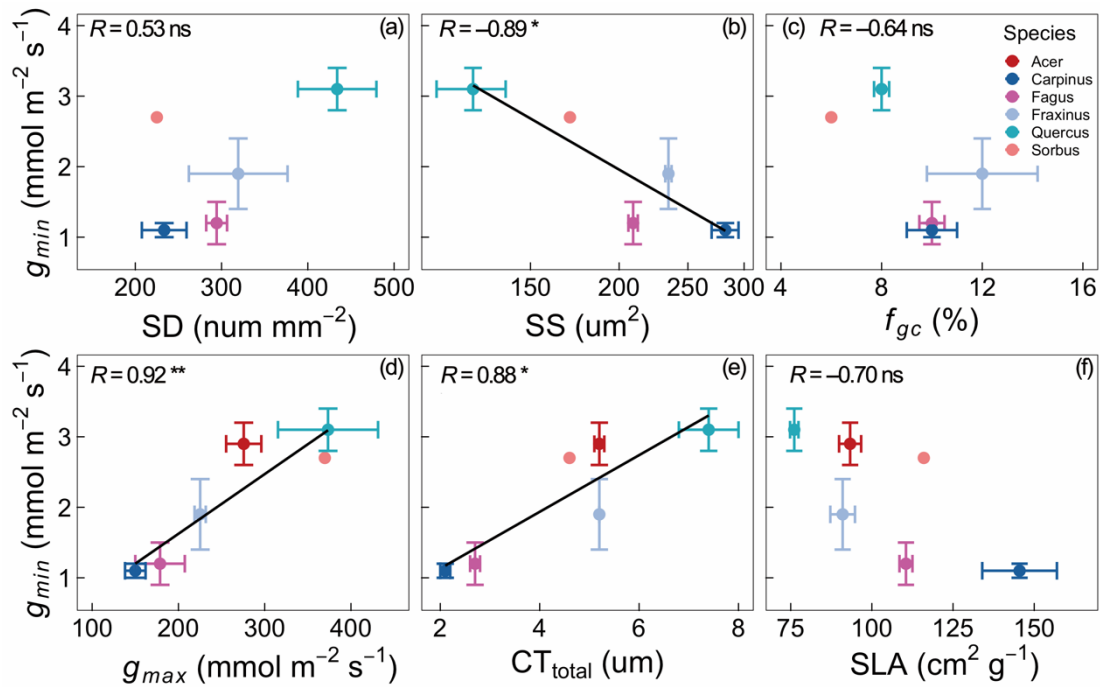
Species	$g_{min}$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	Replicates (tree)	$g_{max}$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	Replicates (tree)
<i>Acer</i>	4.8 $\pm$ 0.4 a	5	275.7 $\pm$ 20.4 ab	4
<i>Carpinus</i>	1.2 $\pm$ 0.1 bd	5	149.6 $\pm$ 11.9 bcd	4
<i>Fagus</i>	2.5 $\pm$ 0.2 c	5	178.7 $\pm$ 28.7 abcd	4
<i>Fraxinus</i>	2.9 $\pm$ 0.4 ac	3	225.2 $\pm$ 6.5 abc	3
<i>Quercus</i>	2.7 $\pm$ 0.1 c	5	373.4 $\pm$ 58.0 a	4
<i>Sorbus</i>	4.8	2	369.7	2
<i>Abies</i>	1.5 $\pm$ 0.2 b	4	114.6 $\pm$ 7.2 d	4
<i>Picea</i>	1.5 $\pm$ 0.1 b	5	146.7 $\pm$ 30.3 cd	4
<i>Pinus</i>	0.8 $\pm$ 0.1 d	5	158.1 $\pm$ 18.4 bcd	4
Angiosperm	3.2 $\pm$ 0.6 A	-	262.1 $\pm$ 38.8 A	-
Gymnosperm	1.3 $\pm$ 0.2 A	-	139.8 $\pm$ 13.0 A	-

$g_{min}$  was measured at 20 °C during 2020 growing season. And  $g_{max}$  was referred to *in-situ* records of the canopy gas exchange during the growing seasons from 2020 to 2022 (unpublished data). Significant differences ( $p < 0.05$ ) across species (functional groups) are indicated by lowercase (uppercase) letters.

### ***Species variation in $g_{min}$***

When averaged across the growing season of 2020,  $g_{min}$  differed significantly among the nine species ( $p < 0.001$ , Table 1). *Acer* (4.8  $\pm$  0.4  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and *Sorbus* (4.8

mmol m<sup>-2</sup> s<sup>-1</sup>) had similar  $g_{min}$  values and were the highest of the nine studied species. In deciduous broadleaf species, *Carpinus* ( $1.2 \pm 0.1$  mmol m<sup>-2</sup> s<sup>-1</sup>) had the lowest  $g_{min}$ . The lowest  $g_{min}$  among all nine studied species was found in *Pinus* with only  $0.8 \pm 0.1$  mmol m<sup>-2</sup> s<sup>-1</sup>, which was also significantly lower than that in *Abies* ( $1.5 \pm 0.2$  mmol m<sup>-2</sup> s<sup>-1</sup>) and *Picea* ( $1.5 \pm 0.1$  mmol m<sup>-2</sup> s<sup>-1</sup>). While there was a tendency for  $g_{min}$  being higher in angiosperms than in gymnosperm trees, this difference was not significant ( $p = 0.09$ ). Compared to the  $g_{max}$  of the nine studied species,  $g_{min}$  was approximately 1 to 2 % of  $g_{max}$  in all species (Table 1).

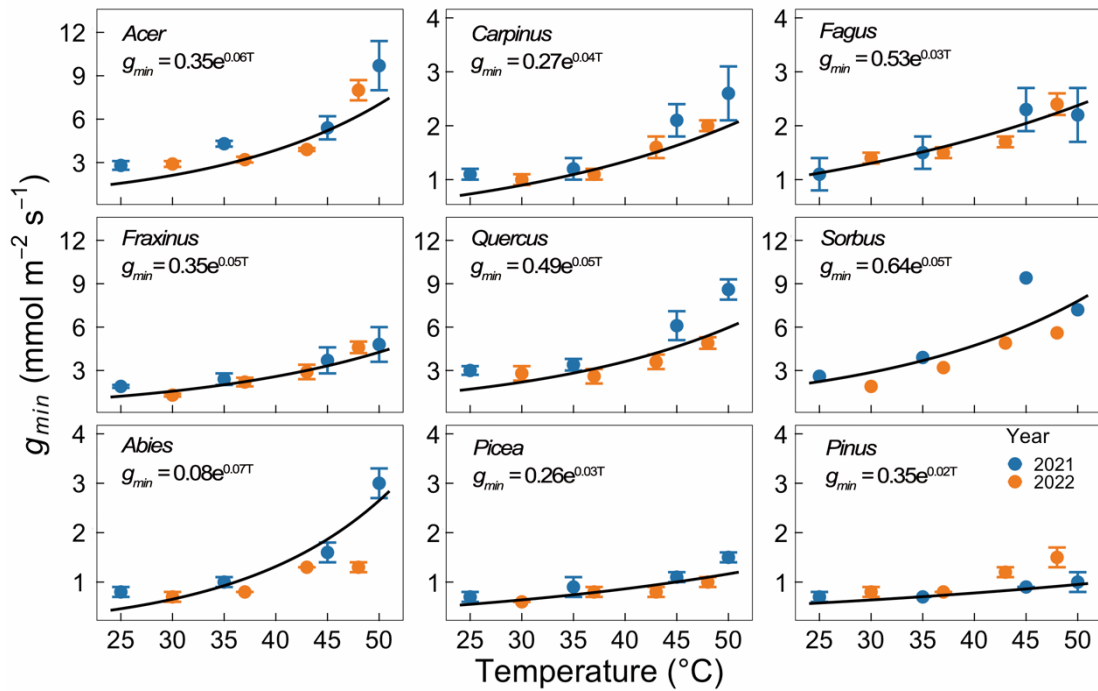


**Figure 2.** Relationships between  $g_{min}$  measured in the 2021 growing season at 25 °C and stomatal density (SD) (a), stomatal size (SS) (b), the fraction of epidermis allocated to stomata ( $f_{gc}$ ) (c), the maximum stomatal conductance ( $g_{max}$ ) (d), total cuticular thickness ( $CT_{total}$ ) (e) and specific leaf area (SLA) (f). Each point represents the mean value for a species ( $n = 2 - 4$ ), and the error bar represents the SE. The black lines indicate the fitted regression lines. Pearson correlation coefficients are shown; ns, nonsignificant; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ .

### ***The relationship between $g_{min}$ and stomatal and cuticular traits across species***

$g_{min}$  measured at 25 °C in the 2021 growing season was strongly negatively correlated

with SS (Figure 2b). Besides that, a strong positive correlation was also observed between  $g_{min}$  and  $g_{max}$  (Figure 2d). We found a significant difference in cuticular thickness between the adaxial (upper) and abaxial (lower) leaf sides, the former being twice as thick as the latter (see Figure S6 available as Supplementary Data at Tree Physiology Online).  $g_{min}$  was positively correlated with both  $CT_{total}$  and  $CT_{adaxial}$  (Figure 2e, Figure S5a), but not with  $CT_{abaxial}$  (Figure S5b). There was also a strong correlation between  $CT_{total}$  and SLA (Figure S5c), but there was no significant correlation between  $g_{min}$  and SLA (Figure 2f). And there was no significant correlation between SD and SS (Figure S5d).

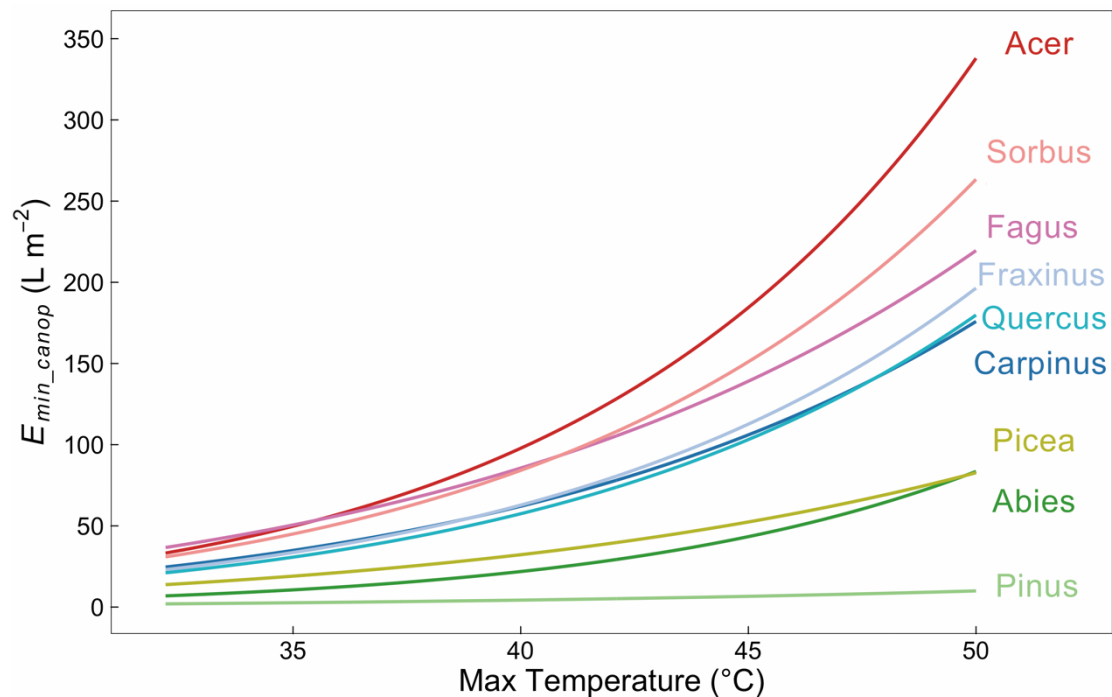


**Figure 3.** Temperature response of  $g_{min}$  for nine species. Each point is the mean value ( $n = 2 - 4$ ), and the error bar represents the SE. Blue and orange colors indicate the different sample years. Solid black lines and equations illustrate the significant exponential models for pooled data. Please note  $n = 2$  in *Sorbus*, only the mean values are shown without SE.

### ***The temperature response of $g_{min}$***

With temperature increasing from 25 to 50 °C,  $g_{min}$  measured in the 2021 and 2022 growing seasons increased across all studied species (Figure 3, also see Table S1

available as Supplementary Data at Tree Physiology Online). In deciduous broadleaf species, the strongest temperature response of  $g_{min}$  was observed in *Acer*, where  $g_{min}$  increased by a factor of 3.5 from 25 to 50 °C (2.8 to 9.7 mmol m<sup>-2</sup> s<sup>-1</sup>, Table S1). By contrast, *Fagus* was the least responsive to increasing temperatures, with an approximate double increase in  $g_{min}$ . In conifer species, *Abies* showed a stronger temperature response than *Picea* and *Pinus*, with its'  $g_{min}$  increasing by a factor of 3.8 from 25 to 50 °C. (0.8 to 3.0 mmol m<sup>-2</sup> s<sup>-1</sup>, Table S1). By applying segmented linear regression, significant phase transition temperatures were determined only in *Acer* (Table S1, 43.4 ± 0.9 °C,  $p < 0.001$ ), *Carpinus* (38.4 ± 3.1 °C,  $p < 0.001$ ) and *Fraxinus* (40.6 ± 2.7 °C,  $p < 0.001$ ).



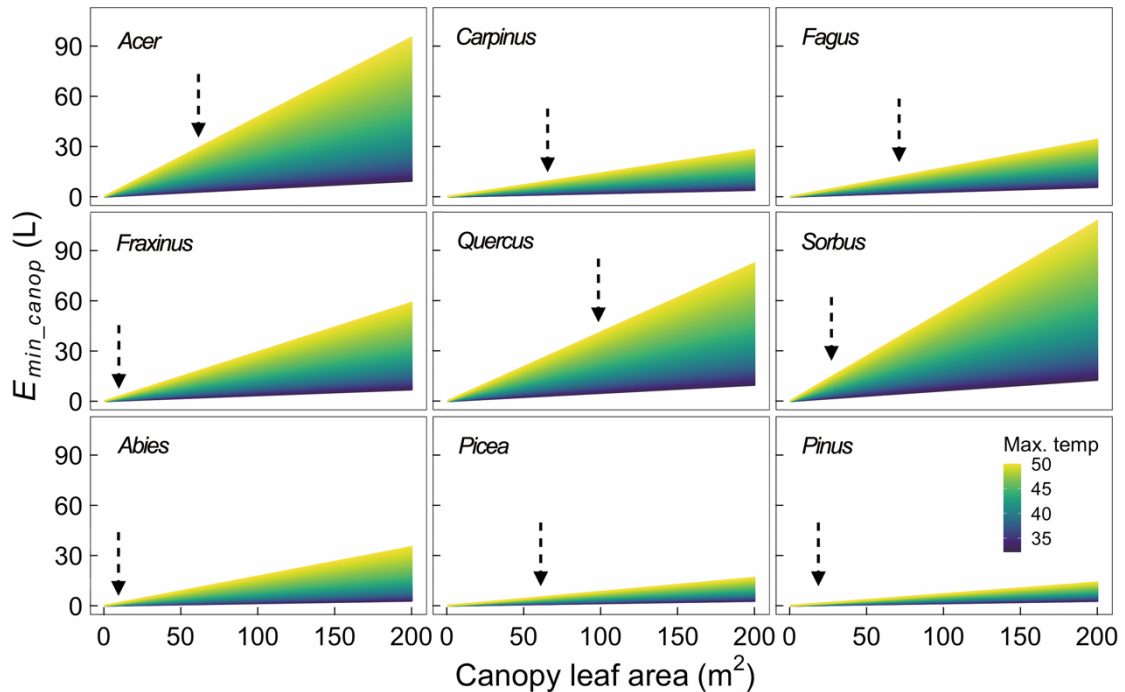
**Figure 4.** Estimation of residual whole tree canopy transpiration at full stomatal closure ( $E_{min\_canop}$ ) at different air temperatures standardized to tree size by stem basal area for nine tree species during a day of the 2022 summer drought.

#### ***Residual whole tree canopy transpiration at full stomatal closure ( $E_{min\_canop}$ )***

Using environmental data that were recorded during a summer drought in 2022, we determined the residual whole tree canopy transpiration at full stomatal closure during



a single hot day by calculating the amount of water transpired by a tree canopy in terms of square meters of stem basal area. Under the natural temperature condition, namely a maximum temperature of 32.2 °C, *Fagus* had the highest  $E_{min\_canop}$  of 36.7 L m<sup>-2</sup>, followed by *Acer* (33.3 L m<sup>-2</sup>) and *Sorbus* (30.9 L m<sup>-2</sup>) (Figure. 4, Table 2). The lowest  $E_{min\_canop}$  was observed in *Pinus* with about 2.0 L m<sup>-2</sup>. As we increased the temperature in our simulation, these nine species showed varying degrees of increase in  $E_{min\_canop}$ . After the maximum temperature was beyond 35.5 °C,  $E_{min\_canop}$  of *Acer* became higher than that of *Fagus* (Figure 4). Similarly, *Fraxinus* showed higher  $E_{min\_canop}$  than *Carpinus* when the maximum temperature was above 39.0 °C. Compared to other species, *Acer* and *Abies* exhibited the strongest temperature responses of  $E_{min\_canop}$  when the maximum temperature raised from 32.2 to 40.0 °C, with an almost threefold increase (Table 2).



**Figure 5.** Non-standardized estimation of daily water use at full stomatal closure ( $E_{min\_canop}$ ) for nine tree species as a function of canopy leaf areas and temperature settings during a day of the 2022 summer drought. Black arrows indicate the average canopy leaf area for a single adult tree at the Swiss Canopy Crane II (SCCII) research site.

**Table 2.**  $E_{min\_canop}$  ( $L\ m^{-2}$ ) and actual canopy water loss (L) for single trees of the 9 studied species under three different maximum temperature conditions at the study site (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus sp.*, *Sorbus torminalis*, *Abies alba*, *Picea abies*, *Pinus sylvestris*).

Species	$R$	$E_{min\_canop}$ ( $L\ m^{-2}$ )			$E_{min\_canop}$ (L)		
		standardized by basal area			for single trees at SCC II site		
		$T$ ( $^{\circ}C$ )	32.2	35	40	32.2	35
<i>Acer</i>	611.0	33.3	49.8	97.8	3.3	5.0	9.8
<i>Carpinus</i>	1046.6	24.6	34.9	62.1	1.5	2.1	3.7
<i>Fagus</i>	1161.3	36.7	50.5	85.6	2.2	3.0	5.1
<i>Fraxinus</i>	379.5	23.1	33.6	62.8	0.7	1	1.9
<i>Quercus</i>	401.9	21.1	30.7	57.5	5.3	7.7	14.4
<i>Sorbus</i>	406.3	30.9	45.0	84.2	2.8	4.1	7.6
<i>Abies</i>	274.2	6.9	10.6	21.9	0.3	0.4	0.9
<i>Picea</i>	836.8	13.8	19.0	32.3	1.0	1.3	2.3
<i>Pinus</i>	96.1	2.0	2.7	4.3	0.4	0.5	0.8

$T$  indicates three different maximum temperature conditions in the simulation.  $R$  means the ratio of the average canopy projected leaf area to the average stem basal area for each species, data from Kahmen *et al.* (2022)

The non-standardized amount of canopy transpiration at 32.2 °C and full stomatal closure for an adult tree during a single hot drought day was the highest in *Quercus* (Table 2), about 5.3 L at our research site, followed by *Acer* (3.3 L) and *Sorbus* (2.8 L). By contrast, *Abies* only lost 0.3 L, which was lower than *Pinus* (0.4 L) and *Picea* (1.0 L). Additionally, canopy water loss increased two to three times for all species, when the maximum temperature increased from 32.2 to 40.0 °C (Table 2, Figure 5).

## Discussion

Our study shows that  $g_{min}$  measured at ambient temperatures of 20 °C varied considerably among the nine studied species, ranging from 0.8 to 4.8 mmol m<sup>-2</sup> s<sup>-1</sup>. This large interspecies difference was associated with leaf cuticular and stomatal traits. Although seasonal variations of  $g_{min}$  were observed in a few species, most species kept rather stable  $g_{min}$  values throughout the growing season.  $g_{min}$  exhibited strong temperature responses in all studied species and it generally increased by a factor of two to four in a temperature gradient of 25 to 50 °C. Based on our estimation,  $E_{min\_canop}$  standardized by stem basal area ranged from 2.0 to 36.7 L m<sup>-2</sup> during a hot drought summer day with a maximum temperature of 32.2 °C and increased to 4.3 to 97.8 L m<sup>-2</sup> when the simulated maximum temperature was 40 °C. At 32 °C, an adult tree at our research site transpired between 0.3 and 5.3 L day<sup>-1</sup> which increased to 0.8 and 14.4 L day<sup>-1</sup> at 40 °C. Regarding the risk of canopy water loss caused by high  $E_{min\_canop}$  under compound hot drought, *Acer* was the most vulnerable and *Pinus* the most resistant tree among the species that we investigated.

### *Seasonal variation of $g_{min}$*

Knowledge of the seasonal variation of  $g_{min}$  is scarce in the literature. Hauke and Schreiber (1998) found that *Hedera helix* had a constant cuticular conductance throughout the growing season after the first 30 days of leaf development. On the contrary, Hadley and Smith (1994) observed that  $g_{min}$  of *Picea engelmannii* increased by 40 % in the current-year needles after the first winter in the Central Rocky Mountains, USA, and Heinsoo and Koppel (1998) found a rapid decline of  $g_{min}$  in *Picea abies* in the first weeks after budbreak.

There is multiple evidence that the leaf cuticle typically forms early during leaf ontogeny and then keeps a constant water permeability during the rest of the season (Hauke and Schreiber 1998, Sachse et al. 2010, Kahmen et al. 2011, Gamarra and Kahmen 2016). At our research site, all deciduous tree species fully unfolded their leaves in early May 2020 (Zahnd et al. 2023). Our fieldwork started in the middle of

June when leaves were already fully developed. Although we observed statistically significant monthly variations of  $g_{min}$  in *Fagus* (Figure 1), these fine-scale fluctuations were unlikely to have profound effects on canopy water loss. Accordingly, we conclude that after leaf maturation, most deciduous tree species have a stable  $g_{min}$  throughout the growing season.

Spring bud break of the three evergreen conifer species commenced in late April (*Abies* and *Picea*) and in late May (*Pinus*) 2020 (Zahnd et al. 2023). We observed a rapid decline in  $g_{min}$  of the current-year needles early in the growing season and found significant differences in  $g_{min}$  in different needle-age groups, with older needles having a slightly higher  $g_{min}$  than fully matured current-year needles. Similarly, Heinsoo and Koppel (1998) observed a fast decline of  $g_{min}$  in Norway spruce during the first weeks of growth, followed by a gradual increase in  $g_{min}$  with needle age (up to 5 years). Although, in the present study, the differences of less than  $1 \text{ mmol m}^{-2} \text{ s}^{-1}$  in  $g_{min}$  between the current-year and 1-year-old needles will only make a physiologically insignificant difference for canopy water loss at full stomatal closure, we think the potential changes of  $g_{min}$  with leaf age should be kept in mind when considering the drought vulnerability of evergreen tree species.

### ***Interspecies variation of $g_{min}$ and its' association with leaf morphology and anatomy***

The  $g_{min}$  values of the nine temperate European tree species reported here are within the range of previously published values for other species (see data review of Schuster et al. 2017, Duursma et al. 2019). Our data therefore confirm that  $g_{min}$  is about two orders of magnitude smaller than the maximum stomatal conductance of trees and approximately 1% to 2% of  $g_{max}$  of the species at our study site (Figure 2d, Table 1). At the same time, we observed a large variability in  $g_{min}$  among the nine studied species. The highest  $g_{min}$  values were detected for *Acer* and *Sorbus* with about  $4.8 \text{ mmol m}^{-2} \text{ s}^{-1}$ , which were more than six times higher than the lowest  $g_{min}$  observed for *Pinus*. We also found a trend that gymnosperm trees had a generally lower  $g_{min}$  than angiosperm species, but we found no significant difference between these two functional groups. This result is in concert with the findings of Schuster et al. (2017), who showed no

significant difference in  $g_{min}$  among 11 life form groups, including deciduous trees and evergreen conifers. In our study,  $g_{min}$  was very low in some deciduous species (e.g., *Carpinus*,  $1.2 \text{ mmol m}^{-2} \text{ s}^{-1}$ ), similar to that of conifers. By contrast, in a total of 54 tropical tree species, Machado et al. (2021) and Slot et al. (2021) found that  $g_{min}$  was higher in deciduous than in evergreen tree species.

We found a strong positive correlation between  $g_{min}$  and  $CT_{total}$  (Figure 2e). At first glance, this may come as a surprise as it is very common to hypothesize that thicker cuticle can reduce water permeability, and thus lead to lower  $g_{min}$ . Yet, different lines of evidence suggest a weak role of cuticular thickness in the control of cuticular water permeability (Riederer and Schreiber 2001, Bueno et al. 2020, Machado et al. 2021). Rather, the leakiness of the cuticle may rest on the integrity and chemical composition of the wax layer of the cuticle, instead of the wax amounts (Jetter and Riederer 2016, Grünhofer et al. 2022). In a recent study, Grünhofer et al. (2022) demonstrated that an up to 12.5-fold higher wax coverage was unable to reduce the residual water loss in detached leaves of *Populus × canescens*. Additionally, it is worth noting that in our study  $g_{min}$  was correlated to  $CT_{adaxial}$  rather than  $CT_{abaxial}$  (Figure S5), and that there was an average two-fold difference between  $CT_{adaxial}$  and  $CT_{abaxial}$  (Figure S6). So, the positive correlation between  $g_{min}$  and  $CT_{total}$  may imply the necessity of increasing wax amounts on leaf adaxial surface to enhance the defense of the leaf against harsh canopy microclimates. The negative correlation between  $CT_{total}$  and SLA also indicated this carbon investment in leaf construction (Figure S5c).

Previous studies have reported that higher  $g_{min}$  values were associated with higher SD,  $f_{gc}$ , and smaller SS (Muchow and Sinclair 1989, Machado et al. 2021). In this study, we detected indeed a negative correlation between SS and  $g_{min}$ , but no significant correlations between SD,  $f_{gc}$ , and  $g_{min}$  (Figure 2). For the poor statistical performance in the latter, one of the potential explanations was due to the small number of investigated tree species and a large interspecies variation in stomatal morphology among these tree species. We also explored the correlation between gas exchange capacity and  $g_{min}$  and found that  $g_{max}$  was tightly and positively correlated with  $g_{min}$ . This result was in agreement with those reported by (Machado et al. 2021), in which

$g_{max}$  was estimated by the theoretical link between stomatal conductance and stomatal morphological traits. It is therefore plausible that a trade-off exists, in which canopy leaves have a higher  $g_{min}$  and bear higher dehydration risk while increasing the gas exchange rate.

### ***Temperature response of $g_{min}$***

Temperature response of  $g_{min}$  has been shown for plant species across different lifeforms from contrasting climates, including tropical broadleaf tree species (Slot et al. 2021), temperate evergreen conifers (Billon et al. 2020), cool-temperate evergreen angiosperms (Hartill et al. 2023), and desert vine (Bueno et al. 2019). All temperate tree species that we tested in this study also showed a significant increase in  $g_{min}$  with rising temperatures, as well as a large difference in the thermal sensitivities of  $g_{min}$ . According to Schuster et al. (2016), the temperature response of  $g_{min}$  can be possibly ascribed to the change in the structure of either the polymer chains in the cutin matrix or the crystalline wax barrier, resulting in increasing cuticular water permeability and thus higher  $g_{min}$ . To a certain degree, this mechanism may shed light on the highly species-specific thermal sensitivities of  $g_{min}$  that we found in the study. Additionally, it is worth noting that only sunlit leaves from the south-exposed side of the upper canopy were investigated in our study, considering that the structure of the forest canopy at the site is rather open, with a basal area of 24.6 m<sup>2</sup> ha<sup>-1</sup> and an average leaf area index of ca. 2.2 (Zahnd et al. 2023). There are remaining uncertainties in the temperature response of  $g_{min}$  for shade leaves at lower canopy positions, and they deserve closer scrutiny. For example, Slot et al. (2021) found sun leaves generally had significantly higher  $g_{min}$  than shade leaves for some tropical tree species, but there was no evident interaction between the temperature response of  $g_{min}$  and the position of leaves in the canopy.

Although sampling in two years with different approaches had minor effects on the temperature responses of  $g_{min}$  for the studied tree species, all data showed a strong exponential increase in  $g_{min}$  with temperature. We can, however, not identify, if these differences are caused by the different methods we used or if these differences are

because samples originated from different years and thus reflect year-to-year variability in  $g_{min}$ . According to our study of  $g_{min}$  in a provenance trial, both  $g_{min}$  and its' thermal sensitivity can exhibit strong phenotypic plasticity with changing hydroclimate in some tree species (unpublished data), which may hint toward year-to-year differences in  $g_{min}$  as a reason for the observed variability.

Several studies also revealed a phase transition temperature in the thermal sensitivity of  $g_{min}$ , suggesting the dramatic changes in the structure of cuticular wax under certain hot temperatures (Schuster et al. 2016, Bueno et al. 2019, Billon et al. 2020, Slot et al. 2021, Hartill et al. 2023). This temperature threshold was determined by different approaches, such as by data transformation (Arrhenius plot, Billon et al. 2020) or directly using a segmented bi-linear function (Slot et al. 2021). For our nine studied tree species, similarly, phase transition temperatures could also be determined in three deciduous broadleaf tree species by using the bi-linear function, ranging from 38.4 to 43.4 °C (see Table S1 available as Supplementary Data at Tree Physiology Online). Ecologically, however, these different methods all yielded a similar core knowledge of the thermal sensitivity of  $g_{min}$ , namely the non-linear increase in  $g_{min}$  under compound hot drought. In the study, temperature responses of  $g_{min}$  all showed significantly exponential changes under hotter temperatures.

### ***Residual whole tree canopy transpiration at full stomatal closure ( $E_{min\_canop}$ )***

The time a tree can remain hydrated with complete stomatal closure after the dysfunction of the capillary continuum from soil to the root is strongly influenced by  $E_{min\_canop}$  (Blackman et al. 2019, Billon et al. 2020, Challis et al. 2022, Lemaire et al. 2021). We estimated  $E_{min\_canop}$  for the nine tree species at our research site for a hot day during the 2022 Central European hot drought. According to monthly predawn and midday canopy leaf water potential measurements during the growing season 2022 at the research site (unpublished data), all nine studied species experienced severe soil water shortage in July and August, at which their stomates were typically closed (Dietrich et al. 2018, Peters et al. 2023). Based on our estimation, the maximum difference in  $E_{min\_canop}$  between nine studied tree species was about 18-fold either when

standardized by stem basal area or considering the actual water loss of individual species for trees at our research site. *Fagus*, *Acer* and *Sorbus* had relatively high  $E_{min\_canop}$  values and they may thus encounter a higher risk of canopy desiccation than other species under severe droughts, once water supply from the soil has become extremely limited. However, there are significant differences in root water uptake depth among the nine tree species that we studied at our research site (Brinkmann et al. 2019, Kahmen et al. 2022), which might compensate for high values of  $E_{min\_canop}$  in some species, including *Acer*, *Quercus* and *Fraxinus*. Others, including *Fagus*, *Carpinus* and *Picea*, mainly rely on shallow root water uptake (Kahmen et al. 2022). In the peak of the 2022 summer hot drought (July and August), the mean minimum predawn branch water potentials ( $\Psi_{min}$ ) were  $-1.98 \pm 0.36$  (mean  $\pm$  SD,  $n=12$ ) and  $-2.02 \pm 0.3$  ( $n = 7$ ) MPa for *Quercus* and *Acer*, respectively (unpublished data). At the same time, these values were  $-2.49 \pm 0.2$  ( $n = 12$ ) and  $-2.89 \pm 0.42$  ( $n = 13$ ) MPa for *Picea* and *Fagus*. It is worth noting that the mean  $\Psi_{min}$  was lower than  $\Psi_{12}$  in *Fagus* ( $-2.74 \pm 0.16$  MPa). Thus, the water leakiness caused by high  $E_{min\_canop}$  in *Acer* and *Quercus* may be compensated by access to deep soil water during a drought. In contrast, the combination of shallow root water uptake depth and high  $E_{min\_canop}$ , as observed in *Fagus*, is likely to accelerate the risk of this species being severely impacted by drought as observed across Europe in recent years (Arend et al. 2022, Frei et al. 2022, Kahmen et al. 2022).

Under severe drought conditions, the demand of stem water flux for transpiration will dramatically decrease because of the full stomatal closure (Körner 2019, Duursma et al. 2019). At our research site, the water consumption of trees at full stomatal closure during a hot dry summer day can be as low as 0.3 L but may be as high as 5.3 L (Table 2), depending on the species. Importantly, our simulations demonstrate that hotter temperatures can lead to an exponential increase in  $E_{min\_canop}$  for all studied tree species, due to the simultaneous increase in VPD and  $g_{min}$ . Our results suggest that the resistance to canopy water loss decreased to varying degrees with increasing temperature in different tree species. For instance, when the maximum temperature was below 35.5 °C, *Fagus* exhibited higher  $E_{min\_canop}$  than *Acer*, despite the higher  $g_{min}$  of *Acer*, indicating the large contribution of more leaves associated with each square meter



of the basal area to  $E_{min\_canop}$  in *Fagus* (Table 2). Beyond 35.5 °C, however, *Acer* showed a higher  $E_{min\_canop}$  than *Fagus*, which was caused by the stronger temperature response of  $g_{min}$  in *Acer*. Similarly,  $E_{min\_canop}$  of *Fraxinus* exceeded that of *Carpinus* when the maximum temperature was beyond 39 °C, implying the changing risks of canopy desiccation among these nine tree species under hotter environments. Thus, with the increasing severity of compound hot droughts, interspecies drought vulnerabilities may become more elusive, as some tree species that are considered drought-tolerant (with lower  $E_{min\_canop}$ ) in current climatic environments may become more vulnerable than other species.

Although standardizing  $E_{min\_canop}$  based on the ratio of canopy leaf area to the basal area in the study allowed us to compare  $E_{min\_canop}$  among the nine studied species, some caveats should be considered. Firstly, the ratio of canopy leaf area to basal area used in the standardization will have a strong influence on  $E_{min\_canop}$  and it might change in different forests due to differences in tree size, stand density, leaf area index, growing conditions and forest management practices (Leuschner and Ellenberg 2017). Secondly, there are few reports on the temperature response of  $g_{min}$  in tree species, especially on the genetic variation and environmental plasticity of the thermal sensitivity of  $g_{min}$  (Cochard 2021). For instance, the variation of  $g_{min}$  across the canopy gradient, which we discussed earlier, may lead to biased estimates of  $E_{min\_canop}$  and influence the assessment of canopy desiccation vulnerability in different tree species, and thus warrant future investigations.

## Conclusion

Our study demonstrates that  $g_{min}$  varies considerably among nine studied species and is tightly associated with leaf cuticular and stomatal traits. The seasonal variation in  $g_{min}$  was observed only in few species, and such slight changes are unlikely to cause profound influences on leaf water loss. Importantly, our study shows that  $g_{min}$  responds to the instantaneous change in air temperature and increases strongly with rising air temperature. Ultimately, there were as large as 18 times differences in the  $E_{min\_canop}$

among these species during the peak of hot drought 2022 and the resistance of different tree species to  $E_{min\_canop}$  was decreasing at varying degrees under hotter temperatures. Our present study emphasizes the importance of  $g_{min}$  and its temperature response as one of the dominant variables that are responsible for species differences in  $E_{min\_canop}$ . Our data show that even when stomates are fully closed during a hot drought, different tree species need substantial amounts of water to remain hydrated. The amounts of water needed vary among species and can, in combination with other traits (root water uptake depth, tissue desiccation tolerance and capacitance) contribute to understanding species differences in drought vulnerability.

## **Data and materials availability**

Data that support the findings of this study will be made available upon request.

## **Supplementary data**

Supplementary data for this article are available at Tree Physiology Online.

## **Conflict of interest**

None declared.

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## Authors' contributions

A.K. and G.H. designed the research. S.W. and G.G. built Drought Box. S.W. performed the research and collected data. S.W., A.K. and G.H. analyzed data, and S.W. wrote the manuscript with contribution from A.K. and G.H.

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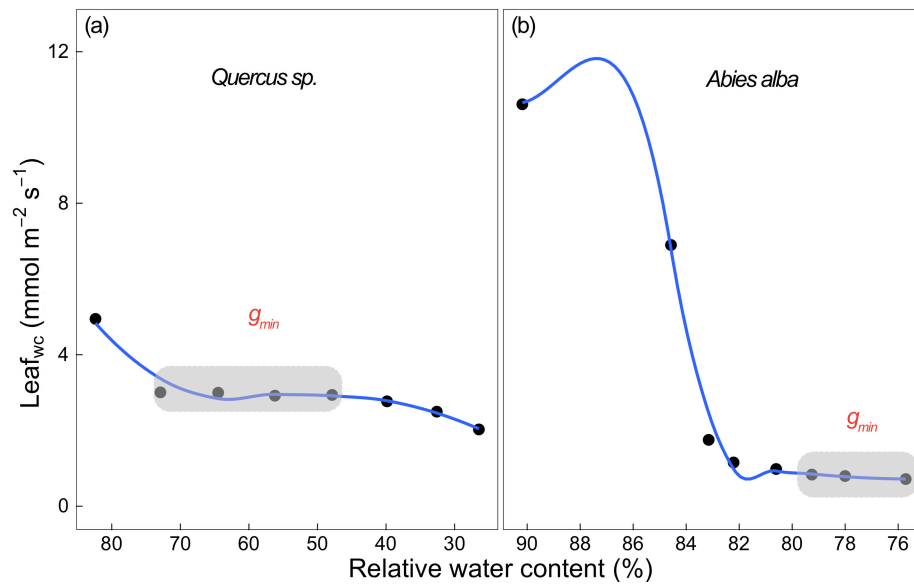
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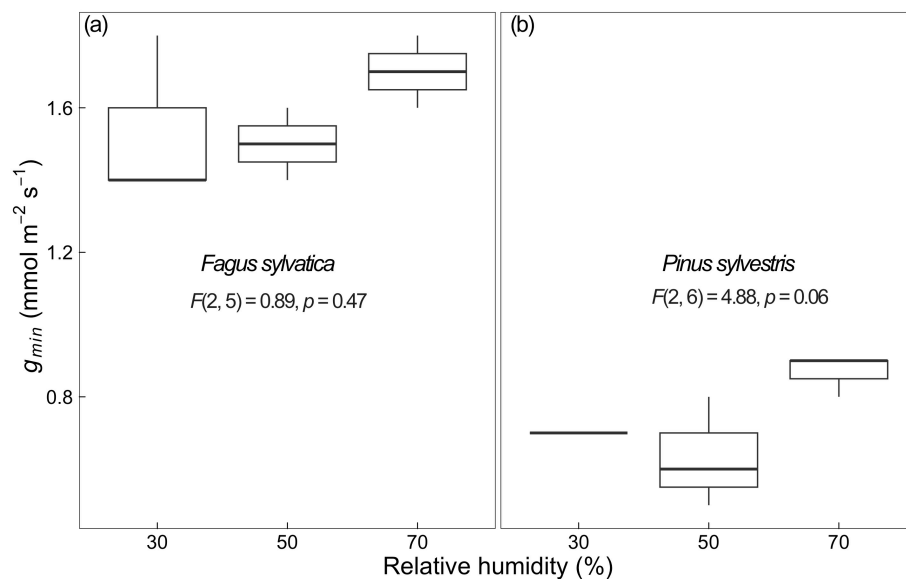
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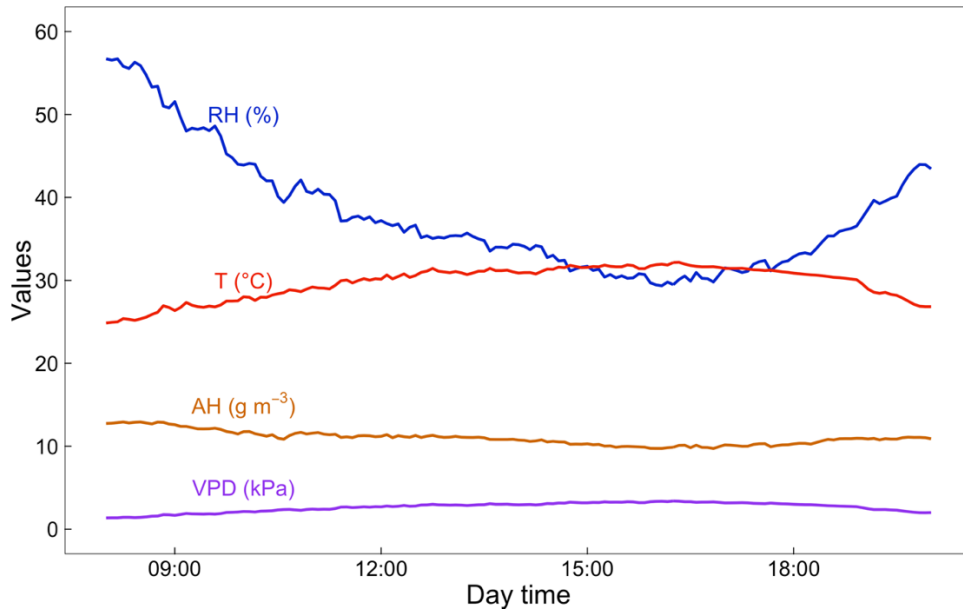
## Chapter I – Supporting Information



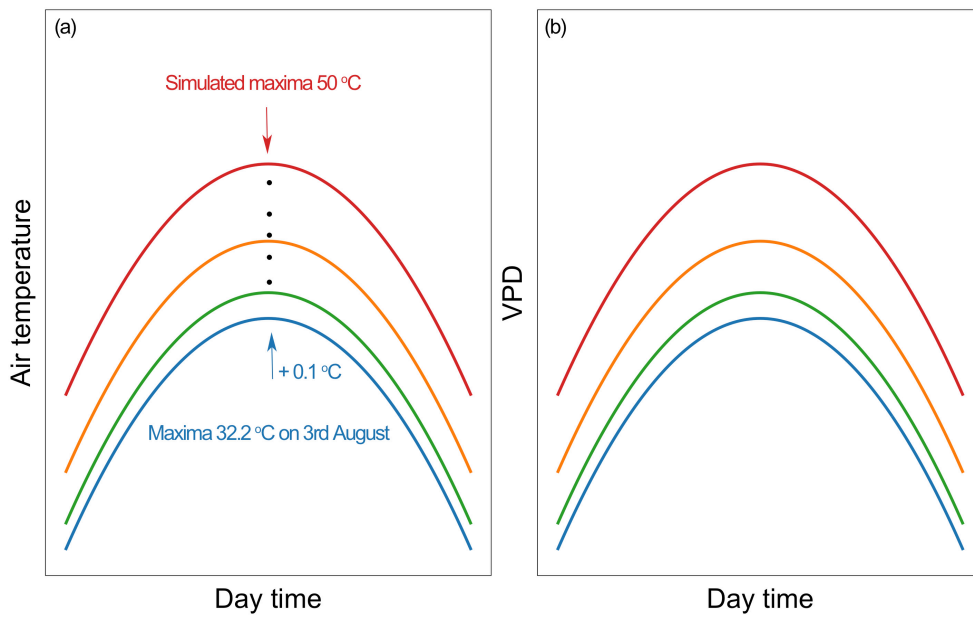
**Figure S1.** Leaf water conductance ( $g_{min}$ ) as a function of the relative water content for two species (a: *Quercus sp.*, b: *Abies alba*). Each point represents an individual measurement at 20 °C. A smoothing curve based on generalized additive mode is fitted to aid the eye. Shade area indicates the occurrence of  $g_{min}$  during dehydration process, and in which the mean of measured values was used for  $g_{min}$ .



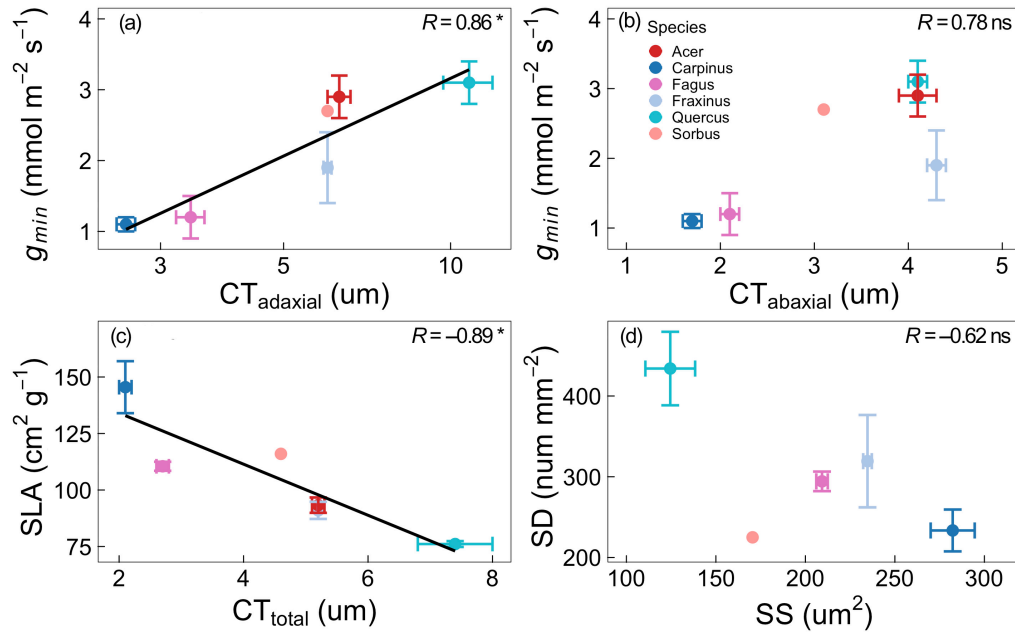
**Figure S2.** Comparison of leaf minimum conductance ( $g_{min}$ ) under 40 °C with different relative humidity conditions for 2 tree species (a: *Fagus sylvatica*, b: *Pinus sylvestris*). No significant humidity effect on  $g_{min}$  was observed.



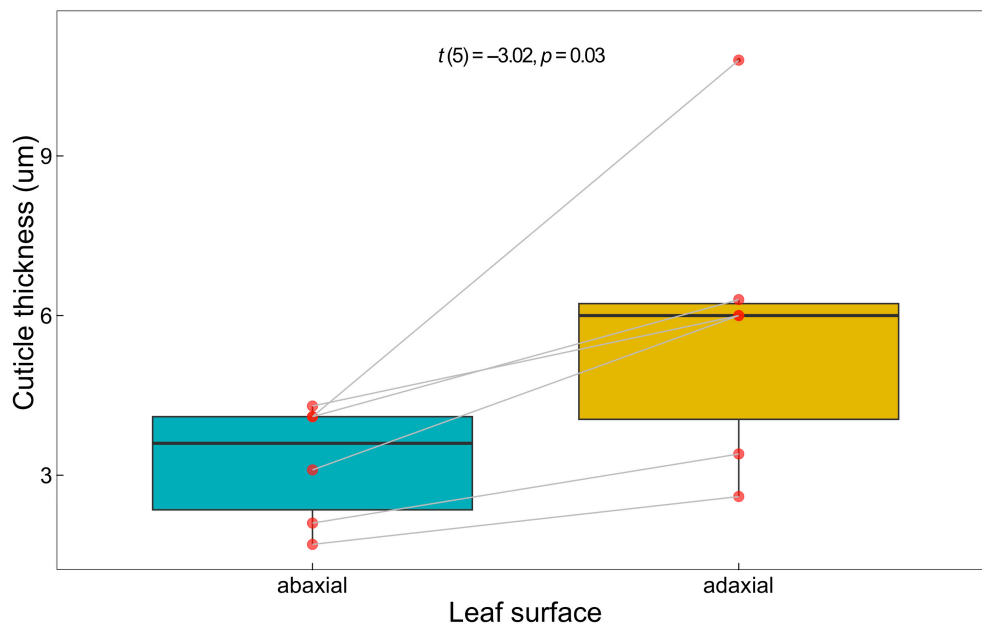
**Figure S3.** The dynamic pattern of the canopy climate at the research site between 8:00 and 20:00 on 3<sup>rd</sup> August 2022. Blue line is relative humidity (RH, %), red line is air temperature (T, °C), brown line is absolute humidity (AH, g m<sup>-3</sup>), purple line is vapor pressure deficit (VPD, kPa).



**Figure S4.** The schema of the estimation of the canopy climate under hotter and drier circumstances. (a): based on the dynamic pattern of the canopy climate from 8:00 to 20:00 on 3<sup>rd</sup> August 2022 (blue line), the temperature curve is elevated by 0.1 °C to that maximum of from 32.2 °C on 3<sup>rd</sup> August to 50 °C. (b) vapor pressure deficit (VPD) is covarying with elevated air temperature.



**Figure S5.** Relationships between (a) leaf minimum conductance ( $g_{min}$ ) and adaxial cuticular thickness ( $CT_{adaxial}$ ), (b) leaf minimum conductance ( $g_{min}$ ) and abaxial cuticular thickness ( $CT_{abaxial}$ ), (c) specific leaf area (SLA) and total cuticular thickness ( $CT_{total}$ ), (d) stomatal density (SD) and stomatal size (SS). Each point represents mean value for each species ( $N = 2 - 4$ ), and the error bar represents the SE. The black lines indicate the significant fitted regression line, and Pearson correlation coefficients are shown; ns, nonsignificant; \*,  $p < 0.05$ .



**Figure S6.** Comparison of thickness between abaxial and adaxial cuticle for 6 deciduous broadleaf species.

**Table S1.**  $g_{min}$  ( $\text{mmol m}^{-2} \text{s}^{-1}$ )  $\pm$  standard errors for nine studied species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus sp.*, *Sorbus torminalis*, *Abies alba*, *Picea abies*, *Pinus sylvestris*) at the different temperature levels and phase transition temperature ( $T_p$ ) value for each specie

Temperature (°C)	$g_{min}$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )								
	<i>Acer</i>	<i>Carpinus</i>	<i>Fagus</i>	<i>Fraxinus</i>	<i>Quercus</i>	<i>Sorbus</i>	<i>Abies</i>	<i>Picea</i>	<i>Pinus</i>
25	2.8 $\pm$ 0.3	1.1 $\pm$ 0.1	1.1 $\pm$ 0.3	1.9 $\pm$ 0.1	3.0 $\pm$ 0.3	2.6	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1
30	2.9 $\pm$ 0.2	1.0 $\pm$ 0.1	1.4 $\pm$ 0.1	1.3 $\pm$ 0.1	2.8 $\pm$ 0.5	1.9	0.7 $\pm$ 0.1	0.6 $\pm$ 0.0	0.8 $\pm$ 0.1
35	4.3 $\pm$ 0.2	1.2 $\pm$ 0.2	1.5 $\pm$ 0.3	2.4 $\pm$ 0.4	3.4 $\pm$ 0.4	3.9	1.0 $\pm$ 0.1	0.9 $\pm$ 0.2	0.7 $\pm$ 0.0
37	3.2 $\pm$ 0.2	1.1 $\pm$ 0.1	1.5 $\pm$ 0.1	2.2 $\pm$ 0.3	2.6 $\pm$ 0.5	3.2	0.8 $\pm$ 0.0	0.8 $\pm$ 0.1	0.8 $\pm$ 0.0
43	3.9 $\pm$ 0.1	1.6 $\pm$ 0.2	1.7 $\pm$ 0.1	2.9 $\pm$ 0.5	3.6 $\pm$ 0.5	4.9	1.3 $\pm$ 0.0	0.8 $\pm$ 0.1	1.2 $\pm$ 0.1
45	5.4 $\pm$ 0.8	2.1 $\pm$ 0.3	2.3 $\pm$ 0.4	3.7 $\pm$ 0.9	6.1 $\pm$ 1.0	9.4	1.6 $\pm$ 0.2	1.1 $\pm$ 0.1	0.9 $\pm$ 0.0
48	8.0 $\pm$ 0.7	2.0 $\pm$ 0.1	2.4 $\pm$ 0.2	4.6 $\pm$ 0.4	4.9 $\pm$ 0.4	5.6	1.3 $\pm$ 0.1	1.1 $\pm$ 0.1	1.5 $\pm$ 0.2
50	9.7 $\pm$ 1.7	2.6 $\pm$ 0.5	2.2 $\pm$ 0.5	4.8 $\pm$ 1.2	8.6 $\pm$ 0.7	7.2	3.0 $\pm$ 0.3	1.5 $\pm$ 0.1	1.0 $\pm$ 0.2
$T_p$ (°C)	43.4 $\pm$ 0.9	38.4 $\pm$ 3.1	N.A.	40.6 $\pm$ 2.7	N.A.	N.A.	N.A.	N.A.	N.A.

$T_p$  in the temperature response of  $g_{min}$  was determined by segmented linear regression analyses. N.A. indicates no significant braking points in segmented linear regression

**Table S2.** Summary of the linear regression models. T is temperature (numeric), Species is tree species tested in the study (categorical), and Sample indicates the leaf and shoot samples used in 2021 and 2022, respectively (categorical). SS is the sum of squares, DF is the degree of freedom. Significant p values are shown in bold.

Model	Effects	SS	DF	F value	P-value
	T	7.49	1	316.07	<b>&lt;0.001</b>
	Species	27.93	8	147.25	<b>&lt;0.001</b>
	Sample	0.79	1	33.32	<b>&lt;0.001</b>
$\ln(g_{min}) = T * \text{Species} * \text{Sample}$	T : Species	0.48	8	2.55	<b>0.026</b>
	T : Sample	0.03	1	1.46	0.24
	Species : Sample	0.65	8	3.42	<b>0.005</b>
	T : Species : Sample	0.20	8	1.03	0.43

**Table S3.** Summary of linear regression between temperature, VPD and  $g_{min}$  for 9 studied tree species (*Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus sp.*, *Sorbus torminalis*, *Abies alba*, *Picea abies*, *Pinus sylvestris*).  $g_{min}$  was log transformed.  $R^2$  indicates the adjusted R-squared.

	Ln( $g_{min}$ ) ~ Temperature		Ln( $g_{min}$ ) ~ VPD	
	$R^2$	$p$	$R^2$	$p$
<i>Acer</i>	0.75	<0.01	0.22	0.14
<i>Carpinus</i>	0.80	<0.01	0.21	0.14
<i>Fagus</i>	0.89	<0.001	0.39	0.06
<i>Fraxinus</i>	0.81	<0.01	0.35	0.07
<i>Quercus</i>	0.60	<0.05	0.11	0.41
<i>Sorbus</i>	0.71	<0.01	0.11	0.22
<i>Abies</i>	0.66	<0.01	0.16	0.33
<i>Picea</i>	0.64	<0.01	0.12	0.40
<i>Pinus</i>	0.51	<0.05	0.93	<0.001

## General discussion

In an illuminating commentary on the mechanisms of drought-induced tree mortality, Körner (2019) stated that ‘A dripping tap (residual transpiration) will eventually empty the pipe system (xylem conduits) and associated reservoirs (tree water capacitance) to the extent that gravity or other constraints permit.’ Indeed, there is increasing recognition that residual transpiration plays a vital role in determining a tree’s surviving time after full stomatal closure under hot droughts (Billon et al., 2020; Blackman et al., 2016; Cochard, 2021; Duursma et al., 2019; Martin-StPaul et al., 2017). Nevertheless, quantifying residual transpiration is challenging, mainly because of the considerable variation in  $g_{min}$  and  $g_{bark}$ . In this thesis, we conducted a comprehensive survey of the variability of  $g_{min}$  and  $g_{bark}$  in nine ecologically and economically important European temperate tree species using a new research tool, the Drought Box. With the implementation of the project, we have found some exciting novel results. In the following sections, I will present our main findings, discuss them in a broader context of the research field, and conclude by pointing out new research questions we identified in our work.

Firstly, we assessed whether the Drought Box is a reliable tool for determining  $g_{min}$  and  $g_{bark}$ , and explored  $g_{bark}$  thermal sensitivity in eight temperate European tree species. Our results show that the Drought Box can create and maintain stable environments at different set points. Meanwhile, it can accurately and continuously measure plant water loss. At a given temperature of 25 °C,  $g_{min}$  varied considerably among the eight studied tree species, ranging from 0.7 to 3.0 mmol m<sup>-2</sup> s<sup>-1</sup>. At the same time, the range of  $g_{bark}$  values was very close to the range of  $g_{min}$ , from 1.7 to 2.8 mmol m<sup>-2</sup> s<sup>-1</sup>. Notably, most of the studied tree species kept relatively stable  $g_{bark}$  when the temperature increased from 25 to 45 °C. Therefore, given the small surface area of bark compared to leaves in twigs, measuring  $g_{min}$  on twigs in the Drought Box is recommended, and the resulting values are not flawed by  $g_{bark}$ . The Drought Box can significantly improve the efficiency of  $g_{min}$  and  $g_{bark}$  studies on a wide range of tree species, especially the temperature response of  $g_{min}$  and  $g_{bark}$ .

Consistent with previous studies, both  $g_{min}$  and  $g_{bark}$  showed considerable interspecies variation, implying many constraints in assessing forest residual transpiration. Therefore, applying the Drought Box can significantly facilitate future research of  $g_{min}$  and  $g_{bark}$  for various tree species. Despite the significant interspecies variation, the range of  $g_{min}$  and  $g_{bark}$  values was similar for the studied tree species. Based on the data review by Duursma et al. (2019),  $g_{min}$  values in 127 woody species were between 0.1 and 25.0 mmol m<sup>-2</sup> s<sup>-1</sup>. To date, only a few studies examined  $g_{bark}$ , including for *Pinus halepensis* (1.46 – 1.96 mmol m<sup>-2</sup> s<sup>-1</sup>, Lintunen et al., 2021), 18 tropical tree species (0.86 – 12.98 mmol m<sup>-2</sup> s<sup>-1</sup>, Loram-Lourenço et al., 2022; Wolfe, 2020), five temperate broadleaf tree species (5.0 – 27.3 mmol m<sup>-2</sup> s<sup>-1</sup>, Wittmann & Pfanz, 2008). These studies, together, suggest that  $g_{min}$  and  $g_{bark}$  have similar interspecies variability in woody plants. At the same time, Loram-Lourenço et al. (2022) showed a strong positive correlation between  $g_{min}$  and  $g_{bark}$ . Moreover, they suggested that the variability in  $g_{min}$  and  $g_{bark}$  across tree species and functional groups may be related to the tree growth strategies, especially the trade-off between water conservation and carbon economics. This significant ecological relationship between  $g_{min}$  and  $g_{bark}$  may facilitate systematic estimation of minimum water use in forests, while more available data on  $g_{min}$  and  $g_{bark}$  are needed.

A critical novel knowledge gained from this study is that  $g_{bark}$  responds weakly to temperature increases in the investigated tree species. Some studies have revealed that the effective waterproof barrier of the bark mainly depends on phellem in the outer bark, especially the hydrophobic waxes (e.g., suberin) in the phellem cell wall (Leite & Pereira, 2017; Lenzian, 2006; Loram-Lourenço et al., 2022; Rains et al., 2018; Wittmann & Pfanz, 2008). On the other hand, many studies have demonstrated that phellem plays a crucial role in protecting tree stems from wildfires, implying the extraordinary heat resistance of phellem (Hoffmann et al., 2012; Lawes et al., 2013; Pausas, 2015; Schafer et al., 2015). In particular, in some Mediterranean *Quercus* species, hydrophobic waxes in the phellem cell wall can tolerate 200 °C without significant degradation (Şen et al., 2012). Therefore, our study implies that the excessive bark water loss during hot droughts is mainly ascribed to elevated driving



force VPD rather than increased  $g_{bark}$ , which may allow a simplified model parameterization when estimating the whole tree residual water loss.

Given that both leaf cuticle and stomata can respond significantly to changes in environmental factors, especially soil water availability (Duursma et al., 2019), we investigated genotypic and phenotypic plasticity of  $g_{min}$  and its thermal sensitivity for four temperate tree species in three well-established common garden trials with contrasting soil water environments. Significant genotypic plasticity of  $g_{min}$  was only found in *Fagus*, with tree provenances from wet regions displaying higher  $g_{min}$  values than those from dry regions. Nevertheless, the absolute differences in  $g_{min}$  between *Fagus* provenances resulting from genotypic plasticity were slight. At the same time, the other three tree species (*Acer*, *Picea*, and *Pseudotsuga*) showed little genotypic plasticity in  $g_{min}$ . Surprisingly, all species displayed significant phenotypic plasticity of  $g_{min}$ , with higher  $g_{min}$  values in the wet trial than in the moderate and dry trials. However, the absolute differences in  $g_{min}$  between trials were minimal in conifer species, which suggests that phenotypic plasticity would only lead to physiologically non-significant differences in  $g_{min}$  and (or) canopy residual water loss. All studied species exhibited strong temperature responses of  $g_{min}$ , with a two- to seven-fold increase in  $g_{min}$  when temperature increased from 30 to 50 °C. Interestingly, we also found significant phenotypic plasticity in the thermal sensitivity of  $g_{min}$  in *Acer*, with  $g_{min}$  responding more strongly to temperature in the dry trial than in the wet trial.

Many studies have demonstrated that  $g_{min}$  varies widely across genotypes for crop species, such as peanut (Rosas-Anderson et al., 2014), cotton (Fish & Earl, 2009), soybean (James et al., 2008), and rice (Saito & Futakuchi, 2010). For woody species, Challis et al. (2021) found that *Corymbia calophylla* saplings from warm-dry origins had significantly lower  $g_{min}$  values than saplings from cold-wet origins. However, most of our studied species showed little evidence of genotypic plasticity of  $g_{min}$ , suggesting that the hydroclimates of the provenances of these species play little selective role in shaping intraspecies variation of  $g_{min}$ . The causal mechanisms leading to the absence of genotypic plasticity in functional traits remain unclear. Several processes, such as weak molecular variation, genetic constraints, and canalized traits, may be responsible for

this phenomenon (Lamy et al., 2011). Based on our findings, replanting seeds from dry origins during reforestation may hardly improve the drought tolerance of the four studied tree species by forming low  $g_{min}$  values. However, foresters may be able to find “low- $g_{min}$ -adapted” genotypes in tree populations growing at the dry trailing edge of species distribution.

Different lines of evidence have shown that plants can decrease their  $g_{min}$  values when soil water availability becomes limited (Cavender-Bares et al., 2007; Duursma et al., 2019; Villar-Salvador et al., 1999; Warren et al., 2011). For instance, for four *Eucalyptus* and two *Acacia* tree species,  $g_{min}$  in one-year-old seedlings decreased by 40% to 70% after eight weeks of drought treatment (Warren et al., 2011). Consistent with previous studies, our results demonstrate that trees grown in the dry trial had significantly lower  $g_{min}$  values than those grown in the moderate and wet trials. At first sight, this finding suggests that  $g_{min}$  is able to adapt to future soil water deficits and thus enhance the drought tolerance of the studied tree species. However, upon closer examination, the absolute differences in  $g_{min}$  for evergreen conifers are limited between different soil water trials. According to a meta-analysis of 168 plant species, including herbs, woody deciduous, and woody evergreens, phenotypic plasticity in leaf morphology and physiology is positively correlated with leaf specific area, suggesting a trade-off between leaf economics spectrum and phenotypic plasticity (Stotz et al., 2022). Specifically, resource-conserving species (e.g., *Picea* and *Pseudotsuga* in our study) are characterized by higher tissue construction costs and lower growth and turnover rates, and they tend to maximize survival rather than growth, and therefore, compared to resource-acquiring species (*Acer* and *Fagus*), they exhibit lower phenotypic plasticity. Unexpectedly, we found no significant difference in leaf cuticular and stomatal traits between provenances or soil water trials, implying that the genotypic and phenotypic plasticity of  $g_{min}$  in our study was driven by other factors (e.g., the composition and structure of leaf cuticle). For instance, Grünhofer et al. (2024) showed that a large accumulation of dimeric wax esters and an increase in the weighted mean carbon chain led to a twofold increase in cuticle transpiration in a *Populus × canescens cer6* mutant line.

To further determine the amount of canopy residual water loss after stomatal closure during drought, we conducted a comprehensive investigation of the variation of  $g_{min}$  in nine temperate European tree species at our SCCII research site. With this newly obtained insight, we calculated the whole tree canopy residual transpiration ( $E_{min\_canop}$ ) in the nine species for one day during the 2022 Central European hot drought. The results show that most species kept relatively stable  $g_{min}$  values throughout the growing season, and only a few species (mainly evergreen conifers) exhibited seasonal variations in  $g_{min}$ , but the absolute differences were very small. When averaged across the growing season,  $g_{min}$  ranged from 1.2 to 4.8 mmol m<sup>-2</sup> s<sup>-1</sup> for the six temperate deciduous broadleaf tree species and from 0.8 to 1.5 mmol m<sup>-2</sup> s<sup>-1</sup> for the three evergreen conifers. Therefore, our results are consistent with previously published ranges of  $g_{min}$  values for the corresponding plant life form groups (Duursma et al., 2019; Schuster et al., 2017). Furthermore, our results suggest that the interspecies variation of  $g_{min}$  was closely related to leaf cuticular and stomatal traits. Specifically, tree species with smaller leaf stomata and thicker leaf cuticle had higher  $g_{min}$  values. At the same time, all studied tree species showed strong temperature responses of  $g_{min}$ , with a two- to four-fold increase in  $g_{min}$  over a temperature gradient of 25 to 50 °C, which keeps in line with previous studies on tropical broadleaf tree species (Slot et al., 2021), temperate evergreen conifers (Billon et al., 2020), cool-temperate evergreen angiosperms (Hartill et al., 2023), and desert vine (Bueno et al., 2019). Ultimately, according to our estimation,  $E_{min\_canop}$  standardized by stem basal area ranged from 2.0 to 36.7 L m<sup>-2</sup> during a hot drought summer day with a maximum temperature of 32.2 °C and increased to 4.3 to 97.8 L m<sup>-2</sup> when the simulated maximum temperature was 40 °C. Non-standardized  $E_{min\_canop}$  at 32.2 °C for an adult tree at our research site transpired between 0.3 and 5.3 L day<sup>-1</sup>, which increased to 0.8 and 14.4 L day<sup>-1</sup> at 40 °C.

Large adult trees can consume hundreds of liters of water per day during the peak growing season when soil moisture is abundant. For example, Čermák et al. (2007) found that an old-growth Douglas-fir (*Pseudotsuga menziesii*), 57 m tall and with a crown length of 31 m, transpired 150 to 300 L day<sup>-1</sup> on some fine summer days. Moreover, they estimated that the water stored in the whole tree could maintain this

large transpiration for a week. In the third chapter, we demonstrate that although  $g_{min}$  was only approximately 1% to 2% of the maximum stomatal conductance, there were substantial amounts of water leakiness from adult trees even after full stomatal closure under severe drought. It is noteworthy that  $E_{min\_canop}$  varied considerably among tree species, with a maximum difference of 18-fold. This significant interspecies variation is not only caused by  $g_{min}$ , but also related to the stand characteristics at our research site (i.e., the ratio of canopy leaf area to basal area in our study). A typical example is that although *Fagus* had significantly lower  $g_{min}$  values than *Acer* and *Sorbus*, these three species showed similar  $E_{min\_canop}$ . This is because the ratio of canopy leaf area to basal area is much higher in *Fagus* than in *Acer* and *Sorbus* at our research site. When combined with other hydraulically relevant functional traits (e.g., root water uptake depth, leaf water potential, and stem cavitation resistance),  $E_{min\_canop}$  partly explained the different drought responses among the nine studied species at our research site (Brinkmann et al., 2019; Kahmen et al., 2022; Peters et al., 2023). Some species (e.g., *Acer*, *Quercus* and *Fraxinus*) could maintain leaf and stem hydraulic safety margins in recent summer droughts due to their deeper root water uptake depths, despite their high  $E_{min\_canop}$  values. In contrast, for other tree species that rely on shallow root water uptake (e.g., *Fagus*, *Carpinus* and *Picea*),  $E_{min\_canop}$  led to evident leaf and stem embolism, canopy water deficit and dieback (Arend et al., 2021, 2022; Kahmen et al., 2022). To our knowledge, this is the first study to quantify  $E_{min\_canop}$  in mature forests. Given that stand characteristics might vary across forests due to differences in tree size, stand density, leaf area index, growing conditions and forest management practices (Leuschner & Ellenberg, 2017), the next important step is to validate our findings in other forests, especially considering the standardized  $E_{min\_canop}$ , which will provide a better understanding of the canopy desiccation risks among tree species. At the same time, sap flow or eddy covariance data may help provide independent estimates of tree canopy residual water loss.

A critical topic that runs through all three studies presented in this thesis is the temperature response of  $g_{min}$  and  $g_{bark}$  in temperate European tree species. One of the most critical mechanisms by which hot temperatures increase the mortality risk of

drought-stressed trees is the increase in tree minimal water use after full stomatal closure. Trees will likely suffer lower leaf and stem water potentials, higher xylem cavitation, more cellular electrolyte leakage, and more pronounced canopy dieback under compounded hot drought than under a single drought stress (Gong & Hao, 2023; Marchin et al., 2022; Morais et al., 2022; Ruehr et al., 2016). Ecologically, hot temperatures often lead to higher VPD during drought, and physiologically,  $g_{min}$  and  $g_{bark}$  may also respond to increasing temperatures, and ultimately all three factors act together to increase the amount of tree minimal water use. Unexpectedly, our survey in **Chapter 1** has shown that  $g_{bark}$  in most studied species had little response to increasing temperatures. This novel finding may help simplify modelling tree water consumption during hot droughts. In contrast, the temperature response of  $g_{min}$  has been reported in some tree species from different vegetation types, including tropical broadleaf tree species (Slot et al., 2021), temperate evergreen conifers (Billon et al., 2020), and cool-temperate evergreen angiosperms (Hartill et al., 2023), and these findings collectively suggest that the thermal sensitivity of  $g_{min}$  is highly species-specific. Consistent with previous studies, we found that  $g_{min}$  responded strongly to increasing temperatures in young trees about 12 years old in the common garden trials (**Chapter 2**) and mature trees over 100 years old at our SCCII research site (**Chapter 3**). For a total of ten tree species studied in these two chapters,  $g_{min}$  increased nearly two to seven times along an ascending temperature gradient from 25 to 50 °C, thus also making the case for the considerable interspecies variation in the thermal sensitivity of  $g_{min}$ . The temperature response of  $g_{min}$  can significantly affect trees' surviving time under hot drought (Billon et al., 2020; Cochard, 2021). Our estimates of  $E_{min\_canop}$  have demonstrated that tree resistance to canopy water loss decreased to varying degrees with increasing temperature in different tree species, suggesting that the increasing hot droughts may exacerbate differential survival among temperate European tree species.

An important aspect not examined in detail in this thesis is the amount of water loss due to bark transpiration. There is comparatively little research on bark water loss, partly because bark water loss accounts for only a small fraction of whole-tree water loss when leaf transpiration is functioning properly. According to the estimation of Oren

and Pataki (2001), bark transpiration accounted for only approximately 5% of total annual water loss in deciduous broadleaved tree species. However, bark transpiration may become physiologically important under prolonged drought conditions, especially in maintaining stem hydrated. According to the estimation by Lintunen et al. (2021), bark water loss of *Pinus halepensis* trees in a semi-arid environment accounted for 64 – 78% of the total water loss of trees after stomatal closure. At the same time, Wolfe (2020) showed that stem water deficit during drought was strongly correlated with  $g_{bark}$  in eight tropical tree species, with stem dehydration being more severe in trees with higher  $g_{bark}$ . Nevertheless,  $g_{bark}$  is strongly affected by outer bark thickness and lenticel density, with thinner outer bark and more lenticels resulting in higher  $g_{bark}$  values (Loram-Lourenço et al., 2022). At the same time, these two anatomical and morphological traits vary significantly with tree height (Wilms et al., 2021). For example, Konôpka et al. (2022) showed that bark thickness dramatically decreased with increasing distance from the stem base. In our study, some  $g_{bark}$  values were determined using sunlit branches of young understory trees, and the variation of  $g_{bark}$  along the stem and the total bark area of adult trees remains unclear. Although we did not quantify the bark transpiration at our SCCII research site, we revealed that most studied tree species kept stable  $g_{bark}$  under hot temperatures, which implies that the main reason for the increase in bark transpiration during hot droughts is elevated in VPD. In many canopy-closed temperate deciduous forests, ratios of total bark surface area (including branches and stems) and leaf area to ground-surface area were respectively estimated at 2.0 – 2.2 and 4.0 – 6.0 (Whittaker & Woodwell, 1967). However, the larger the tree, the greater the proportion of the aboveground tree's total surface area occupied by the stem and branch (Hölttä et al., 2013; West et al., 1997). Therefore, when assessing tree drought vulnerability, especially for large mature trees,  $g_{bark}$  should also be considered a critical physiological trait that determines a tree's hydraulic safe time.

Over recent decades, advancements in our understanding and modelling of plant drought response have demonstrated the paramount importance of accounting for canopy and bark residual transpiration to the drought vulnerability of trees (Billon et al., 2020; Blackman et al., 2016; Cochard, 2021; Körner, 2019; Loram-Lourenço et al.,

2022; Wolfe, 2020). However, the rates of residual transpiration during severe droughts and how these rates vary across species are rarely quantified, mainly due to the considerable variation in  $g_{min}$  and  $g_{bark}$ . This thesis provides a clear panoramic view of the variability of  $g_{min}$  and  $g_{bark}$  in nine ecologically and economically important temperate European tree species. Our findings show that  $g_{min}$  and  $g_{bark}$  varied considerably among tree species but displayed a similar range of values overall. Based on our data, interspecies variation of  $g_{min}$  was associated with leaf cuticular and stomatal traits, but the cause of intraspecific variation is unclear. At the same time, we have revealed the remarkable acclimation of  $g_{min}$  to soil water availability in deciduous broadleaf tree species but not in evergreen conifers. These findings have important implications for European forest management. We further show that the seasonal variation of  $g_{min}$  was very limited in the studied species and, thus, unlikely to cause an ecologically significant change in tree drought tolerance. At the same time, our findings also highlight the remarkable response of  $g_{min}$  to increasing temperatures, providing novel insights into the mechanisms underlying rapid tree dieback under hot droughts. Finally, we confirm that even when stomates are fully closed during a hot drought, different tree species need substantial amounts of water to remain hydrated. Through in-depth studies in three chapters, we conclude that  $g_{min}$  and  $g_{bark}$  display a large amount of variation among tree species and strong acclimation to the changes in soil water availability and temperature. Accordingly, tree residual transpiration, mainly dependent on  $g_{min}$  and  $g_{bark}$ , may further amplify differences in drought vulnerability between tree species, and simplified modelling of residual transpiration currently appears to be challenging.

Finally, I want to point out a topic that particularly caught my interest after completing this project, and that is leaf dehydration tolerance. Our present studies have suggested that  $g_{min}$  and  $g_{bark}$  are significant causes of tree dehydration after stomatal closure under serious droughts. Yet, the mechanism by which residual water loss leads to tree mortality remains controversial. Over the past several decades, most studies addressing the vulnerability of the hydraulic system of trees have focused on hydraulic failure due to cavitation and suggested percentage loss of conductance (PLC) thresholds

for mortality (50% and 88% for conifers and angiosperms, Anderegg et al., 2016; Brodribb et al., 2020; Choat et al., 2012; McDowell et al., 2022). Recently, however, several studies have shown that some tree species can recover from levels of water stress imposed at P50 or P88, suggesting that these hydraulic thresholds may not be precise enough to predict tree death under drought (Dietrich et al., 2018; Hammond et al., 2019; Johnson et al., 2022; Mantova et al., 2021). Furthermore, different lines of evidence have demonstrated that leaf cellular dehydration and damage can significantly weaken the capacity of tree recovery after drought, with cellular resistance to dehydration varying greatly across tree species (Mantova et al., 2021, 2023; Vilagrosa et al., 2010). Given the strong temperature dependence of canopy residual transpiration, future research should focus on quantifying the physiological and temporal coordination between stomatal closure, residual transpiration, lethal levels of tissue dehydration, and loss of hydraulic conductivity in the conducting tissue under different magnitudes of hot droughts, which will contribute to our breakthrough understanding of drought-induced tree mortality.

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# Songwei Wang | Curriculum Vitae

## Contact

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## Education

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02/2020 – Ongoing Universität Basel: Doctorate studies in Botany. Title: «The role of leaf minimum water conductance in tree response to global-change-type drought», supervised by Prof. Dr. Ansgar Kahmen.

09/2016 – 06/2019 Yunnan University, China. Master studies in Botany. Title: «Phenological studies of evergreen broadleaf *Quercus pannosa* in the upper and lower range limits in Hengduan Mountains», supervised by Prof. Dr. Yang Yang & Prof. Dr. Christain Körner.

09/2012 – 06/2016 Nanjing University of Information Science & Technology, China. Bachelor studies in Applied Meteorology. Title: «Effects of drought on fluorescence parameters of tomato», supervised by Prof. Dr. Yang Zaiqiang

## Research area

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Plant ecology, ecophysiology, plant hydraulics, plant water relations; climate change, hot drought stress, stomatal control, tree mortality, plant acclimation.

## Teaching Experience

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2021-2023: teaching assistant, University of Basel, Basel, Switzerland

Assisting in the bachelor's block course on field techniques and supervising one-week student research projects (yearly).

## Skills

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**Programming:** R (advanced), C++(basic);

**Lab and field work:** commonly used methods of plant hydraulics (e.g., water potential,  $g_{min}$ , xylem resistance, anatomy, photosynthesis, NSC), canopy crane working

## Publications

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**Wang, S. W.**, Hoch, G., Hopf, S., Kahmen, A. Genotypic variability and phenotypic plasticity of leaf minimum conductance. (in preparation)

**Wang, S. W.**, Hoch, G., Grun, G., Kahmen, A. (2024). Water loss after stomatal closure: Quantifying leaf minimum conductance and minimal water use in 9 temperate European tree species during a severe drought. *Tree physiology*. 2024. DOI.org/10.1093/treephys/tpae027.

**Wang, S. W.**, He, X. F., Chen, J. G., Sun, H., Körner, C., & Yang, Y. (2021). Elevation-specific responses of phenology in evergreen oaks from their low-dry to their extreme high-cold range limits in the SE Himalaya. *Alpine Botany*, 131, 89-102. DOI: 10.1007/s00035-020-00245-4.

He, X. F., **Wang, S. W.**, Körner, C., & Yang, Y. (2021). Water and nutrient relations of mistletoes at the drought limit of their hosting evergreen oaks in the semiarid upper Yangtze region, SW China. *Trees*, 35, 387-394. DOI: 10.1007/s00468-020-02039-x.

He, X. F., **Wang, S. W.**, Sun, H., Körner, C., & Yang, Y. (2021). Water relations of “trailing-edge” evergreen oaks in the semi-arid upper Yangtze region, SE Himalaya. *Journal of Systematics and Evolution*, 59(6), 1256-1265. DOI: 10.1111/jse.12696.

## Posters and presentations

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**EGU General Assembly 2024** | April. 2024 - Vienna, Austria. Presentation title: “genetic variation and phenotypic plasticity of leaf minimum water conductance”

**SwissForestLab Science Days** | October. 2023 - Zürich, Switzerland. Presentation title: “Tree’s dehydration: from  $g_{min}$  to canopy residual water loss”

**GfÖ (Gesellschaft für Ökologie von Deutschland, Österreich und der Schweiz) Annual Meeting** | September.2023 - Leipzig, Germany. Presentation title: “Leaf minimum conductance and residual water use in 9 European tree species during a hot drought”

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