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Developing a standardized method to assess drought stress responses in *Betula pendula* (silver birch)

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Abstract

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Abstract:

Drought stress is a major environmental factor influencing tree growth and survival, yet standardized methods for assessing tree responses to water deficit remain limited. This study focuses on *Betula pendula* (silver birch), a widely distributed and ecologically significant species, to develop a reproducible experimental framework for evaluating drought stress responses under controlled conditions. Two experimental setups were used: an automated phenotyping platform (NaPPI) and a conventional greenhouse, both designed to investigate the developmental and physiological adaptations of birch seedlings to varying levels of water deficit (WD). By measuring key growth parameters—stem elongation, internode development, stomatal conductance, and stem diameter as a proxy for cambial activity—this study provides insights into how silver birch responds to different WD treatments. Results from Experiment A (NaPPI) indicate that mild and moderate WD did not significantly impact growth, whereas severe WD led to a decrease in stem elongation and stomatal conductance. In contrast, Experiment B (greenhouse) did not reveal notable differences between treatments, likely due to seasonal timing, suggesting that factors such as temperature, day length and light availability may influence tree responses to WD. These findings highlight the developmental plasticity of *B. pendula* and reinforce the importance of experimental timing and environmental factors when assessing drought stress responses. This study contributes to a better understanding of tree adaptation to water deficit and give directions for more robust experimental protocols to assess tree developmental plasticity.

Abbreviations

AP4: Model of Porometer (Delta-T Devices)

Ctrl: Control

CV: Coefficient of Variation

DAT: Days After Transplant

NaPPI: National Plant Phenotyping Infrastructure

rH: Relative Humidity

RGR: Relative Growth Rate

RWC: Relative Water Content

tC: Temperature (Celsius)

WD: Water Deficit

WD01, WD02, WD03: Water Deficit Treatments 1, 2, and 3

WD20, WD30: Water Deficit Treatments 20, and 30

WHC: Water Holding Capacity

Contents

1 Introduction

1.1 Background

Trees play many important roles in forest ecosystems as they provide a wide range of ecological and economic benefits. They serve as the foundation of complex habitats, support biodiversity, regulate climate through carbon sequestration, and contribute significantly to the global economy through timber production and other forest products (Ritonga et al. 2021; McDowell et al., 2008; Petrokas & Manton, 2023; Cabon et al., 2022; Oksanen, 2021). When studying trees, significant progresses have been made in understanding biological processes - such as internal physiological, biochemical, and molecular changes - and environmental interactions (Ritonga et al. 2021; Tikhomirova et al 2022). However, there is a lot we do not understand about the mechanisms that drives and control their developmental plasticity (meaning the tree's he ability to alter their developmental trajectory in response to varying environmental conditions) and the combined effects of the environment on them (Bolte et al. 2016; Kojs et al 2023). This gap needs to be addressed especially in how environmental stresses, like water deficit (WD), influence tree growth and resilience. Trees like *Betula pendula* (silver birch) constitute an excellent model species for studying these mechanisms, due to their ecological and economical importance in northern Europe (Oksanen, 2021; Salojärvi et al. 2017), but also due to their fast growth in early development (Oksanen, 2021). The main aim of this work is to generate standardized methods to assess the drought stress responses of *Betula pendula*, with a particular emphasis on its developmental and physiological adaptations under varying water deficit (WD) conditions in controlled environments. This is done by investigating the settings of two small scale greenhouse experiments - a phenotyping platform and a conventional greenhouse – to explore how birch trees respond to controlled WD conditions.

By assessing morphological and physiological traits such as stem elongation (internodes numbers and height), stomatal conductance, and cambial activity (stem diameter), this thesis explores the complex responses of birch to drought stress. In this context, it is important to clarify that stem diameter increments are not solely a direct measure of cambial activity, as they also involve the activity of the cork cambium and reversible stem swelling. However, in this study, we use stem diameter as a simplified proxy for secondary growth, which includes contributions from both the cambium and the cork cambium.

1.2 Developmental Plasticity

Developmental plasticity is the ability of an organism to modify its growth and development in response to environmental signals. This ability is particularly important for trees, which cannot move around and that experience many different environmental conditions throughout their lifespans. Developmental plasticity allows trees to optimise their growth, survival, and reproduction in response to factors such as light, water availability, temperature, nutrient availability, and even mechanical stress (Kojs et al., 2023; Tomasella et al., 2017; Aspelmeier & Leuschner, 2004). In the context of trees, developmental plasticity manifests in a variety of ways, such as reaction wood formation, asymmetric cambial growth, changes in cell wall ultrastructure and chemical composition, or hydraulic safety mechanisms (Andriantelomanana et al., 2024; Fischer et al., 2019). In the context of this thesis, I will focus on the vascular cambium and its hydraulic acclimation.

Vascular Cambium

The vascular cambium is a layer of undifferentiated meristematic cells capable of dividing and differentiating into various cell types (Fischer et al., 2019; Mäkilä et al., 2023). Found in dicots and gymnosperms, it plays a crucial role in the secondary growth of plants, particularly in trees (Mäkilä et al., 2023). When active, this lateral meristem is responsible for producing xylem tissues, which are critical for water transport, nutrient distribution, and structural support in trees, and phloem tissues responsible for the transport of photo assimilates (Fischer et al., 2019). Cambial activity responsible for the thickening of stems and roots, known as secondary growth, which allows trees to increase in diameter and support (Fischer et al., 2019; Mäkilä et al., 2023). This contrasts with primary growth, which occurs at the tips of roots and shoots, and is responsible for organ elongation (Fischer et al., 2019). It has previously been described that during water deficit, cambial activity decreases, leading to narrower xylem vessels that enhance hydraulic safety but reduce overall water transport efficiency (Balducci et al., 2013). This adjustment allows trees to produce narrower xylem vessels, which are less likely to develop air bubbles (embolism) when water is not easily available. By reducing the risk of embolism, trees can maintain water flow and avoid hydraulic failure, which is one of the main factors inducing tree mortality during drought (Andriantelomanana et al., 2024).

Hydraulic Acclimation

Trees can modify their hydraulic systems, such as xylem vulnerability and leaf hydraulic conductance, to cope with drought (Meinzer et al., 2013; Tomasella et al., 2017). For example, in response to drought, trees can increase their resistance to embolism (the formation of air bubbles) in the xylem that can block water transport (Andriantelomanana et al., 2024; Tomasella et al., 2017). Several mechanisms contribute to this increased resistance. These include the formation of smaller vessels, an increase in conduit wall reinforcement, and changes in pit architecture (Tomasella et al., 2017). Additionally, hormonal interventions play a significant role. For instance, abscisic acid (ABA) promotes an increase in vessel number while reducing their cross-sectional area (Rosso et al., 2023), and ethylene promotes cambial cell division, enhancing the growth and development of xylem tissues (Fischer et al., 2019). Trees also regulate transpiration rates through stomatal regulation to limit water loss, further mitigating the effects of drought (Rosso et al., 2023). Finally, increased root growth helps maintain a balanced water status, supporting the tree's overall hydraulic function (Rosso et al., 2023).

In the spring, birch trees use root pressure to replenish embolised vessels caused by wintering (Strati et al., 2003). Their deep root systems help them access moisture from deeper soil layers, which helps them cope with severe water stress and reduces the risk of embolisms (Kang et al., 2023). Furthermore, birch trees may change their xylem structure by increasing the number and strengthening the walls of conduits, similar to adaptations observed in European beech (Rosso et al., 2023; Tomasella et al., 2017) and poplars, which increase vessel density while reducing their diameter (Zhang et al., 2017).

Additionally, they can adjust their stomatal conductance. Stomata are regulated leaf pores that control carbon dioxide intake and evapotranspiration, to conserve water by regulating water loss. (Meinzer et al., 2013; Rosso et al., 2023; Sellin et al., 2014). Stomatal conductance in trees is influenced by a number of factors, including soil water availability. In drying soils, conductance is reduced in order to conserve water (Meinzer et al., 2013; Sellin et al., 2014). Another factor is the atmospheric vapor pressure deficit (VPD), According to Meinzer et al, (2013) diffuse-porous species, shows significant responsiveness by tightening stomatal. Birch being a diffuse-porous species, this could also apply here. According to Sellin et al, 2014, air humidity is indeed an important factor, with birch trees acclimated to high humidity exhibiting steep declines in conductance under water deficits.

Wood Formation

Trees can adjust the amount and type of wood they produce in response to environmental conditions (Arend and Fromm., 2007; Kojs et al., 2023; Balducci et al., 2013). For instance, under drought conditions, trees may produce denser wood with smaller xylem vessels, which makes them more resistant to embolism (Kojs et al., 2023; Meinzer et al., 2013; Rosso et al., 2023). On the other hand, in wetter conditions, trees may produce less dense wood with larger vessels, which allows for greater water transport (Kojs et al., 2023; Meinzer et al., 2013).

1.3 Birch as a model species

Betula pendula, commonly known as silver birch, stands out as an excellent model species for investigating tree adaptation due to its ecological and economic significance, as well as its advantageous biological characteristics.

Ecological Importance

As a dominant deciduous tree in the northern boreal forests, birch thrives across diverse environments in Europe and Asia, making it highly adaptable to various climates and soil types (Aspelmeier & Leuschner, 2004; Oksanen, 2021). As a pioneer species (Hynynen et al., 2009; Oksanen, 2021; Salojärvi et al. 2017), it rapidly colonises disturbed areas, contributing significantly to biodiversity and soil stabilisation (Hynynen et al., 2009; Oksanen, 2021). It thrives in diverse climates and soil types, ranging from fertile forest sites to abandoned, and contaminated fields (Aspelmeier & Leuschner, 2004; Hynynen et al., 2009).

Economic Importance

Silver birch is highly valued in the timber and paper industries thanks to its fast growth and lean stem form (Hynynen 2009; Oksanen, 2021; Ritonga et al. 2021). Its wood is used for various purposes, such as plywood, fibreboard, and furniture production (Oksanen, 2021; Ritonga et al. 2021).

Biological Advantages

Silver birch exhibits rapid growth in its early years, making it suitable for experiments that require observable results within a shorter period (Hynynen 2009; Tenkanen et al 2019). This rapid growth

allows for quicker assessments of the impacts of environmental factors and/or genetic modifications. Silver birch also presents a relatively small genome - size of 440 Mb – simplifies genomic analysis, making it easier to identify and study genes related to adaptation, growth, and stress response (Salojärvi et al. 2017). Advancements in birch genomics, including the sequencing of its genome have provided valuable resources for researchers (Salojärvi et al. 2017; Oksanen, 2021). This adaptability makes it a valuable model for studying the genetic and physiological mechanisms that underlie this resilience to diverse environmental conditions (Oksanen, 2021). Prior research advancements in birch genomics and its responses to various environmental stressors, such as drought, temperature changes, and high air humidity, have laid a solid foundation for further investigation (Salojärvi et al. 2017; Oksanen, 2021; Ritonga et al. 2021; Sellin et al. 2014). This thesis builds upon these findings to further explore the mechanisms driving developmental plasticity in silver birch and how this plasticity contributes to its adaptation to a changing environment.

1.4 Stem elongation

Stem elongation is sensitive to water availability and can vary across developmental stages (Serra-Maluquer et al., 2018). During early growth, trees will prioritize development in height (stem elongation) to compete for light. Later, once the tree has developed sufficient structural integrity and resource allocation to support further growth it will switch its focus from vertical growth to prioritize secondary growth (diameter) (Fransson et al., 2020). Under drought conditions, stem elongation is one of the first processes to be inhibited as a result of a reduction in cell turgor pressure (Hannus et al., 2020). These findings are consistent with the observation that growth responses are genotype-dependent in birch, with drought tolerant genotypes maintaining elongation for a longer period of time than sensitive ones (Sellin et al., 2014). In birch related species, like alder and beech, stem elongation responses to drought depend on root depth and access to soil water content. Studies indicate that younger trees with shallower root systems are more vulnerable to drought, than older trees which have deeper roots to reach water reserves, thus delaying the effect of WD stress (Zapater et al., 2011; Balducci et al., 2013).

1.5 Developmental stages of the tree

The germination stage and the emergence of the radicle and plumule, is a highly drought-sensitive stage, requiring adequate moisture, amongst other factors to maintain growth or avoid seedling death (Osório et al., 2012). This is followed by the juvenile stage, which is the period starting when the seedling emerges up to when it develops strong root system and a large crown, this can last up to a few years or until the first flowers are formed. During the early juvenile stage, the trees are very sensitive to drought due to their limited root system which is relatively close to the soil surface (Beikircher et al., 2024). Mature trees, having reached their full height and developed extensive root systems and crowns, generally exhibit greater drought tolerance than younger trees (Au et al., 2022; Zhang et al., 2017). However, sensitivity to drought varies depending on species traits, such as wood density, and environmental factors, with prolonged or severe droughts still posing risks of reduced growth, increased vulnerability to pests and diseases, and even mortality (Au et al., 2022; Kang et al., 2023; Rosso et al., 2023; Zhang et al., 2017).

1.6 Scope of the study

This study investigates the physiological and developmental responses of Betula pendula (silver birch) trees to water deficit (WD) conditions in controlled greenhouse environments. Two distinct experimental setups were employed: the National Plant Phenotyping Infrastructure (NaPPI) platform and a conventional greenhouse. These setups simulate varying degrees of drought conditions to explore the developmental plasticity, with a focus on secondary growth.

In the NaPPI experiment, automated phenotyping technology enabled detailed monitoring of growth responses under different WD treatments. By contrast, the greenhouse experiment offered a more conventional approach to validate findings under simpler experimental conditions.

Predictions/hypothesis

- Increased severity of water deficit will lead to detectable changes in key developmental traits (stem diameter, height, internodes numbers) and in physiological parameters (stomatal conductance)
- The more severe the water deficit, the more physiological changes will be observed, especially in parameter such as stem diameter and stomatal conductance.

Objectives

- Assess the physiological and developmental responses of Betula pendula trees to varying levels of WD, with a specific interest on secondary growth.
- Establishing a reproducible experimental protocol for studying tree developmental plasticity under controlled WD conditions.
- To compare the outcomes of NaPPI experiments with conventional greenhouse methods, and to evaluate the reliability and scalability of different experimental approaches.

2 Material and Methods

This study was conducted in two different greenhouse settings: one at the National Plant Phenotyping Infrastructure (NaPPI) and one in a conventional greenhouse. The two experiments were conducted at different times of the year, with Experiment A running from March to June 2024 and Experiment B from June to September 2024. This section will first present the common bases of both experiments conducted and subsequently present a more detailed account of each experiment.

2.1 Common settings to experiment A and B

The two experiments were conducted with identical manual measurements, the data analysis approach, and most parts of the preparation phase settings. These aspects will be explained in the following sections.

2.1.1 Plant material and Growth conditions

The plant material used is a non-GMO line of *Betula pendula* that has been clonally propagated in-vitro at the University of Helsinki's Viikki campus laboratories. This particular cultivar is designated V5834. The trees used in these experiments are propagated using identical invitro conditions. The propagation process uses clones of birch trees that are approximately one month old and stored in *in vitro* boxes (Magenta box). The procedure is conducted within a sterile laminar flow hood; Trees are cut just under two nodes, in the middle of the stem. They are then transferred to new *in vitro* boxes containing cooled growth media. Up to eight plantlets are cultivated per box. The detailed

protocol for preparing the growth media presented in Annex 1 was adapted from Lemmetyinen et al. (1998); Alonso-Serra et al. (2020). After a period of seven days boxes were checked for contamination. After twenty days, propagation should be successful, and long, well-established roots should be visible. The plantlets were then ready for transplanting to soil in 8x8cm pots, within 20 to 30 days or as soon as they were reaching the top of the box. The soil mix was composed of forest peat, sand, and vermiculite, in a 6:2:1 volume ratio, respectively. The soil mix was prepared manually for small volumes or using a concrete mixer for larger batches. The pots are filled to the brim, watered, and trees can then be transferred. All trees are then kept in mini greenhouses or a growth chamber to maintain high moisture levels during the acclimation period. After a few weeks, the trees are transferred to 3L pots using the same soil mix as described before.

2.1.2 Timeline

Note that A and B experiments have been conducted at different times (Figure 1). Experiment A was conducted according to the space allocated to our team on the phenotyping platform during the spring season. Experiment B was conducted in a standard greenhouse at the end of the summer and start of autumn.

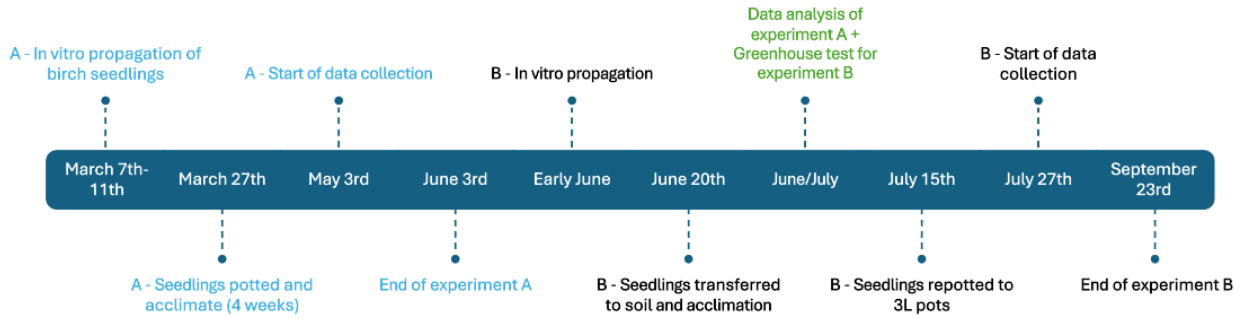

Figure 1: Timeline of the two experiments main events. Text in blue represent the experiment A – NaPPI. Text in black represent the experiment B – Greenhouse. Text in green represent the shared tasks of both experiments.

Further details about timing on each experiment can be found in Annex 2 for experiment A and Annex for experiment B.

2.1.3 Measurements

The manual parameters measured in both experiments A and B are identical, despite Experiment A being conducted phenotyping platform, which can offer more in depth and precise analysis. Those

parameters are not of concern in this study. The focus was on manual measurements that can be done also without a phenotyping platform.

The objective of this study was to focus on specific developmental and physiological traits:

1. Stem diameter as a proxy for cambial activity.
2. Height and Internodes numbers to assess stem primary growth.
3. Stomatal conductance to assess plant water status.
4. Total weight (pot, soil, water, and plant) to ensure that experiment is running in accordance with the plan.

The diameter of the stem was measured at a fixed stem height. To do so, the initial measurements the trees' height was taken, after which a mark was made on the stem at mid-height, using a regular black marker pen. This mark represents the zone of measurement for the stem diameter throughout the experiment. The height was measured from the collar of the trees to the top. The weight was recorded as the total weight of the seedling, soil, water content, and the pot. The number of internodes were counted from bottom to top; an internode was counted from the moment that an expanding leaf can be distinguished by naked eye and is detached from the apex meristem. The stomatal conductance was measured using the AP4 porometer from Delta-T Devices, and ideally from the third fully developed leaf.

2.1.4 Data analysis

In this study, the data was collected manually and recorded into Microsoft Excel spreadsheet for later analysis. The relative growth rate (RGR) in percentages was calculated using the following formula:

$$RGR = \left(\frac{(\ln(z2) - \ln(z1))}{(t2 - t1)}\right) \times 100$$

where z1 and z2 represent the measurement of a single trees at time t1 and t2, respectively.

This calculation allowed for a more uniform comparison of the growth rates across different time periods and individuals. In some cases where the dispersion of the data was big, we also analysed the coefficient of variation (CV). The formula for CV in percentages used was as follows:

$$CV = (Standard\ deviation/Average) \times 100$$

Where the Standard deviation and the Average were automatically calculated using excel.

Further data analysis was done using the publicly accessible PlotTwist software (Goedhart, J. 2020), facilitating the visualization of growth trends and statistical assessments of the results. All statistical analyses were done using the default settings of the PlotTwist software, unless otherwise specified.

2.1.5 Ethics

This study was conducted following the ethical guidelines for plant research and is in accordance with the institutional and national regulations. The plants used in these experiments were grown in vitro, and no genetically modified organisms (GMO) were used. All possible precautions were taken to follow sustainable practices. A slow-release fertilizer was used to reduce the potential nutrient runoff and prevent contamination or any environmental impacts. After completion of the sampling process, all plant material and soil were composted to contribute to sustainability practices. Plants used in this study were also shared with other research groups within the University of Helsinki. All users followed the same ethical protocols for plant care and disposal.

Throughout the entirety of the study, full transparency and data integrity were a priority. All experimental procedures and data collection were carefully documented to ensure that the research process could be reviewed and/or replicated. No data was fabricated, falsified, or selectively reported. Plant material used were carefully labelled to ensure traceability.

The data generated from this study has been openly shared with collaborating researchers and other master's students to ensure transparency in the use and interpretation of results.

Since this study did not involve endangered species, fieldwork, or external communities, no special permits or access agreements were necessary.

2.2 Soil weight and water content test

The objective of this test was to investigate the maximum water content capacity of the soil mix (Section 2.1.1), and to determine how this capacity evolves depending on the number of drips (water input pipes). This test was conducted without trees, on pots containing the soil mix. A total

of 30 pots of 3L were filled with soil mix. The mix was prepared manually, with no water was added during the mixing process. Each pot was filled with 1400g ± 5g of soil mix.

The watering system was composed of multiple drip tubes connected to a main pipe which was linked to the greenhouse's main water source. This automated watering system was set-up to start at 8a.m and 8p.m for a duration of 3min per event. This gave 600mL ± 5ml per day when using three drips. The water flow rate tested was of 6 mL per 10 seconds for a single drip. This flow rate was determined by measuring the volume of water released in a 50ml Falcon tube over a 10-second period, and from drips located at ten different points along the pipe.

The weight of the pots was recorded at three-days intervals over a period of 16 days. The relative water content (RWC) is then calculated using the following formula:

$$RWC = \left(\frac{(W1 - W2)}{1}\right)$$

Where $W1$ represents the new weight of the day and $W2$ represents the initial weight, of 1400g.

To determine the effect of varying quantities of water on the soil mix and the rate at which it reaches its maximum moisture content, three treatments were formulated: 4 drips, 2 drips, and 1 drip. Each condition containing ten pots. In this test, it was not possible to control the amount and timing of irrigation due to the use of a fully automated greenhouse irrigation system. Once the maximum moisture content was determined, it was used in the calculation of the water deficit (WD) treatments for Experiments A and B.

2.3 Experiment A – NaPPI

The data collection for this experiment was conducted from May 3rd to June 3rd , 2024, at the National Plant Phenotyping Infrastructure (NaPPI), located in the greenhouses of the University of Helsinki, on the Viikki campus. The infrastructure is autonomous, although most measurements required in this study were all done manually, with the exception of watering, which is automated and adjusted on a daily basis. The plants were watered twice a day, at twelve-hour intervals, in the morning and evening. Manual measurements were taken every three to four days. Refer to Annex 2 for the precise schedule.

For this experiment A – NaPPI, the trees were propagated invitro between the date of 7 – 11 March 2024. The trees were then potted on 27 March 2024, approximately two weeks after the propagation. Subsequently, the trees were acclimated to their new environment for a four-weeks period. During the first two weeks, the trees were maintained in closed mini greenhouses with high humidity. The two last weeks, the trees were grown in the conventional greenhouse environment.

For this experiment, 40 trees were divided into four treatments, with 10 replicates per treatment. The four treatments were as follows: Control (Ctrl), which was maintained at a full water capacity, and Water Deficit 1 (WD01), Water Deficit 2 (WD02), and Water Deficit 3 (WD03), corresponding to relative water contents (RWC) of 70%, 50%, and 30% of the control, respectively.

Each plant was given an identification as follows: Research group ID – Tree number – Treatment applied – Replicate group number.

Example: JBr – 001 – WD01 – B01

2.4 Experiment B - Greenhouse

The data collection for this experiment was conducted over a period of 6 weeks, between 27th of July to the 23rd of September 2024, in a conventional greenhouse, on the Viikki campus. The methodology of this experiment was adapted according to the preliminary results obtained from Experiment A – NaPPI.

The plant material used in the experiment was propagated and cultivated in-vitro at the beginning of June, then transferred to soil mix in 8x8cm pots on 20th of June. Subsequently, the trees were placed in a growth chamber with a 16/8hour of day/night cycle and a temperature of 23°C. The trees were then transferred to the conventional greenhouse for five days to facilitate adaptation before being transplanted into 3L pots on the 15th of July. The trees remained in the conventional greenhouse, which was also the location for data collection, to ensure better adaptation before data collection began on the 27th of July.

In order to ensure the reliability of the results, 18 plants that were as similar as possible in term of appearance and health status were selected. Three treatments were applied with six replicates per

treatment. The treatments are as follows: Control (Ctrl), which was maintained at an optimal water level, and Water Deficit 1 (WD30), Water Deficit 2 (WD20), corresponding to relative water contents (RWC) of 30%, and 20% of the control, respectively. These severe WD were selected to assess whether a significant difference would be observed in comparison to Experiment A.

The irrigation system was built with three independent drip lines. Each line was equipped with a single outlet automated timer from the Fiskars brand. The automated timers permitted the regulation of the timing and duration of irrigation for the trees based on the treatment applied to each line.

3 Results

3.1 Soil weight and water content test

The results of the watering system test indicate a flow rate of 0,6 ml/sec for duration of 6 minutes, which equates to 36 mL/min. and per drip. Thus, the volume of water dispenses by the system over a three-minute period and for three drips, is calculated to be 324 mL twice a day. Which is about 50mL over the initially predicted amount. The system was tested with one, two, and four drips (Figure 2).

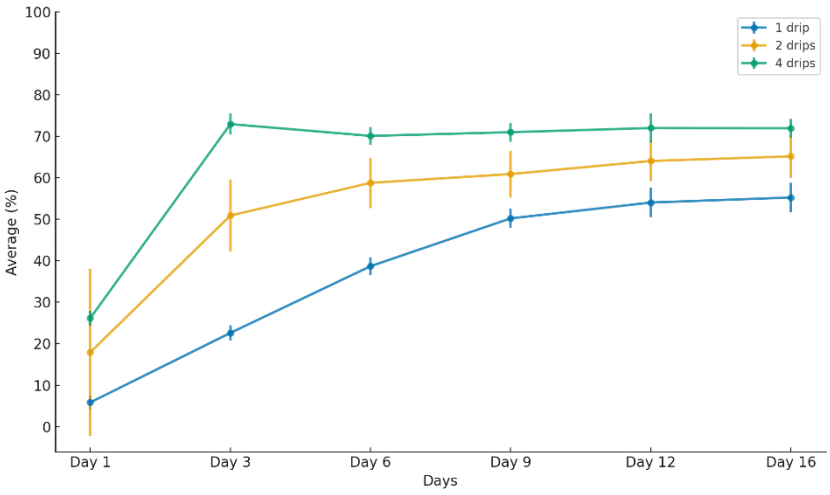

Figure 2: Pots weight average relative water content (RWC) (%) in relation to the number of drips.
Blue line is the 1 drip treatment. Orange line in the 2 drips treatment. Green line is the 4 drips treatment. Error bars represent the standard deviation. Start weight was 1400g ± 5g. Soil mix is a ratio of 6:2:1 forest peat, sand, vermiculite. No plant material was used in this test.

The pots with four drips reached their maximum water content within three days, stabilizing at 70-72% of their dry weight (Figure 2). These pots were irrigated with 860mL of water per day. In

contrast, pots with two drips did not reach their maximum RWC. After 16 days of continuous watering; the RWC was around 65% and still increasing very slowly. Theses pots received 430mL/day. Pots with one drip showed a slower increase in RWC, reaching 55% after 16 days with 215mL of water per day.

The treatments with two and one drip did not reach stabilization. However, the maximum RWC for this soil mix was reached in the four drips treatment. Using this maximum RWC we were able to calculate the water amount and soil RWC to be used during the experimental phases with the trees.

3.2 Experiment A - NaPPI results

The schedule followed in this experiment is as presented earlier (Section 3.1.2, and Annex 2). At the end of the experiment all trees were checked for abnormal phenotypes. Only "JBr_030_WD01_B08" was excluded from the data set due to a double stem formation.

The desired target weights per pot for each treatment (Table 1) were obtained using the calculations from the soil weight and water content test presented earlier (Section 2.2). The field capacity, represented here by the control treatment, is the maximum amount of water the soil can hold after excess water has drained away.

Table 1: Summary of the water content and pots weights per treatment used in the NaPPI experiment.

	Planned water level	Relative Water Content (%)	Target weight of pots during experiment (g)
Control	100	70	3500 ± 5
WD01	70	49.0	3014 ± 5
WD02	50	35.0	2736 ± 5
WD03	30	21.0	2457 ± 5

Soil water content

The water holding capacity (WHC) of the pots, which refers to the soil's ability to retain water, was measured daily before and after watering (Figure 3). The WHC is related to soil moisture levels, serves as a baseline for calculating the Field Capacity. The pots were weighted and watered accordingly to their treatment settings. All measurements were made automatically, and the data recorded gave an accurate evolution of the WHC during this experiment.

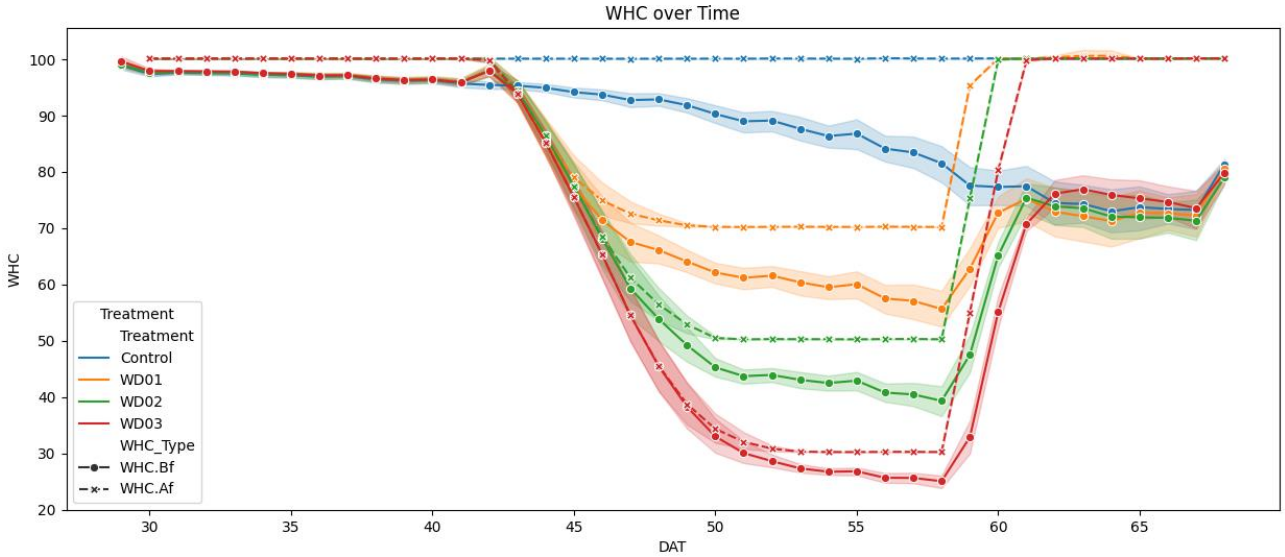

Figure 3. Water holding capacity (WHC) over time for four treatments.
Control, WD01, WD02, and WD03. The WHC in Y axis is shown in % across different days after transplant (DAT), with two WHC average types: WHC.Bf (before watering) full doted line, and WHC.Af (after watering) dashed line with crosses. The shaded areas are the confidence intervals.

The predicted drop in water content, as indicated in Table 1 was observed (Figure 3). This confirm that the experiment was functioning as intended. The water content of all treatment groups including control, exhibited a gradual decline throughout the experiment. All WD treatment present a steady drop even after reaching their designated WD levels. WD03 remains the closest to its set water content of 30%. WD02 frequently shows a 10% reduction in water content compared to anticipated levels. WD01 which was set to reach 70% water content, declines to 55% by the end of the drought phase of the experiment. However, these lower values represent the weights before watering (WHC-Bf) and were adjusted daily to the planned level after water watering (WHC-Af).

Manual measurements

From the progression of the four growth parameters measured across the experiment WD01 and WD02 do not show any significant change in any of the measured characteristics, in comparison to the Control condition (Ctrl) (Figure 4 A-D). Height RGR indicates a drop in RGR across all treatments, which coincide with the trees reaching the end of their fast growth phase at DAT 52 (Figure 4-D).

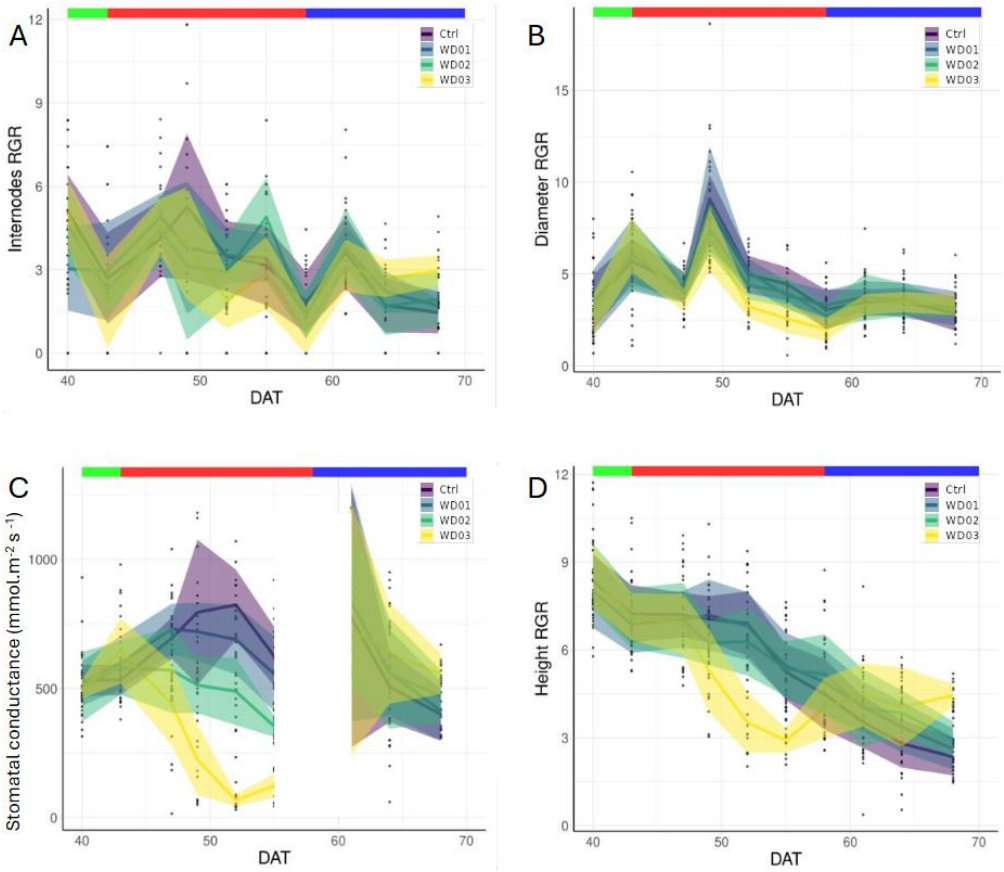

Figure 4. Relative growth rates (RGR) and stomatal conductance under different treatments (Ctrl, WD01, WD02, WD03) over time (DAT: Days After Transplant).
Panels A, B, and D show the RGR in Y-axis in %, of internodes (A), stem diameter (B), and plant height (D), respectively. Panel C show the raw values of Stomatal conductance in mmol.m^{-2} s $^{-1}$. Replicate per treatment n=10. The bar on top Green, Red, and Blue represents the stages of the experiment corresponding to well-watered, drought, recovery, respectively. The lines are the mean of each treatment. Shaded areas are the 95% confidence intervals. All figures were generated with PlotTwist, (Goedhart, J. 2020).

From Internodes and Diameter RGR show that the differences between treatments are minimal to non-existent, with WD03 showing slight difference compared to the control (Figure 4-A, 4-B). Stomatal conductance displays a gap between DAT 55 to DAT 61 due to a malfunction in the porometer, which resulted in a significant portion of the data being out of range (Figure 4-C). According to the AP4 user manual the reading ranger of stomatal conductance is 5.0 -1200 mmol m-2 s -1. Data exceeding this range were filtered out, leaving 353 usable data points out of 440 recorded (80.23%), with 87 points (19.77%) excluded as outliers. Nevertheless, a difference between Ctrl and WD03 is visible in the first half of the measurements, before DAT 58.

When comparing Ctrl to WD03 a clearer difference was detected in all four parameters (Figure 5).

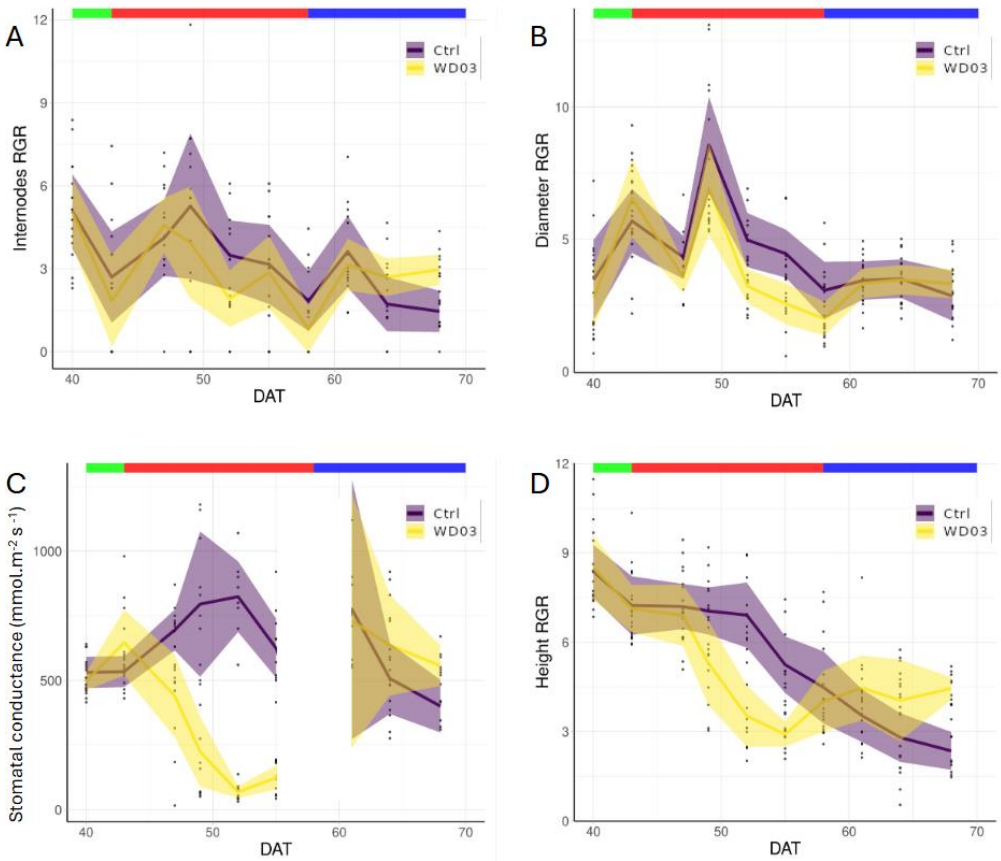

Figure 5. Growth Parameters comparison Ctrl vs WD03 Treatments over time DAT (day after transplant). Panels A, B, and D show the RGR in Y-axis in % of internodes (A), stem diameter (B), and plant height (D), respectively. Panel C show the Stomatal conductance in mmol.m^{-2} s $^{-1}$. Replicate per treatment n=10. The coloured line on top represents the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. The lines are the mean of each treatment. Shaded areas are the 95% confidence intervals. All figures were generated with PlotTwist, (Goedhart, J. 2020).

In Stomatal conductance, diameter and Height RGR, we can now observe a difference between Ctrl and WD03 treatments (Figure 5-B, 5-C, 5-D). Compared to Ctrl, WD03 exhibit a decrease, in the three previously cited parameters, during the drought phase. This decrease is less pronounced in the internodes results (Figure 5-A). During the recovery phase, WD03 demonstrate a higher recovery rate in both internodes and height RGRs compared to Ctrl (figure 5-A, 5-D). While a similar recovery trend is observed in stomatal conductance and diameter RGR, these differences are not statistically significant enough to draw conclusions (Figure 5-C, 5-B).

Environmental conditions

The temperature and hygrometry inside NaPPI platform were automatically recorded (Figure 6). The x-axis represents the time in hours, corresponding to the entire duration of the experiment. The data shows an increase in temperature over time, with a maximum of 35°C during the day and a minimum of 18 °C at night. Elevated temperatures (30°C and above) were affecting the trees

across all conditions. Signs of stress, such as drooping leaves, were observed even in the control treatment.

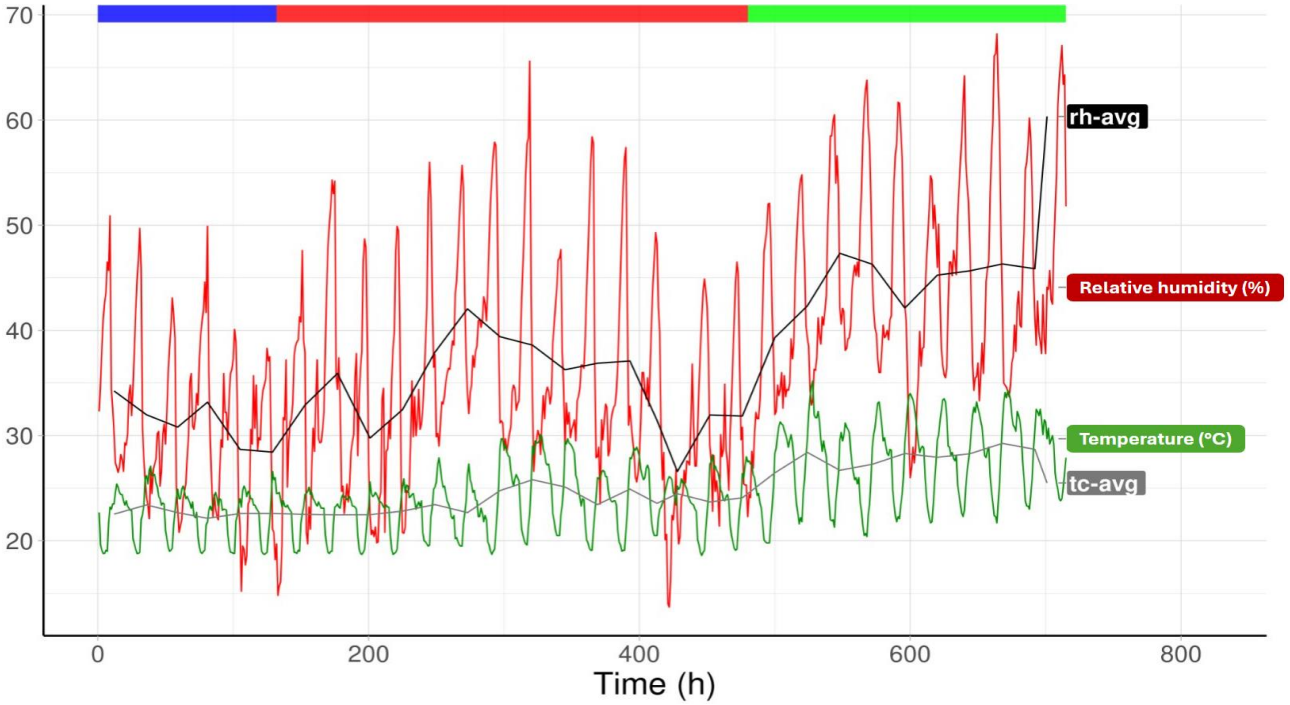

Figure 6. Temperatures and hygrometry changes over time (hours).
Temperatures in degrees Celsius (°C), in y-axis. Hygrometry in percentages (%) in y-axis. Green line (tC) represents the temperatures variations. Red line(rH) represents the hygrometry variations. Black line (rh avg) is the relative humididy average per day (%). Grey line (tc avg) is the temperature average per day (°C). The coloured line on top represents the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. Figure generated with PlotTwist, (Goedhart, J. 2020).

The hygrometry was highly variable, ranging from less than 30% to 65% on some days. This instability is closely linked with the temperature inside the facility. Water sprinklers, used to cool the environment during elevated temperatures, simultaneously increased the hygrometry levels.

Height variation

At the start of this Experiment A, the trees displayed height variations despite efforts to select individuals of similar developmental stage and size. To better understand how initial height influenced growth responses under drought stress, we compared the relative growth rates (RGR) of the three shortest and three tallest individuals from the Control (Ctrl) and severe water deficit (WD03) treatments (Figure 7). The classification was based on height measurement taken at the

start of the experiment, on May 3rd, 2024. Since the WD01, and WD02 group presented similar growth trend to the control group, they were excluded from this analysis.

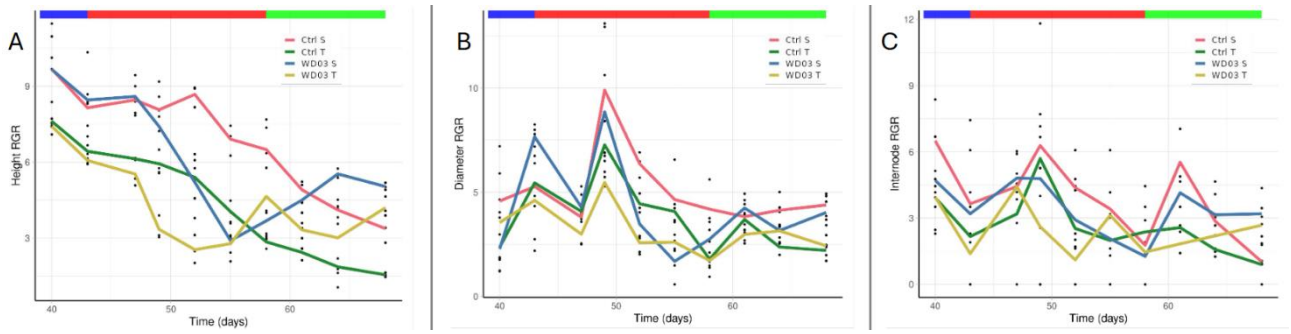

Figure 7. Ctrl and WD03 smallest vs tallest trees RGR average for Height, Diameter and Internodes.
Y-axis in %. Comparison of the RGR results depending on the start height. Panels A, B, C present respectively the RGR in Height, Diameter, and internodes. Panels display, from top to bottom: Ctrl small (red line), Ctrl tall (green line), WD03 small (blue line), and WD03 tall (yellow line). The lines represent the means. The bar above each treatment shows the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. All figures were generated with PlotTwist, (Goedhart, J. 2020).

Analysis of height RGR indicates that, throughout the experiment, smaller trees exhibited a higher growth rate than taller trees, irrespective of treatment (Figure 7-A). This trend was consistent across both the Control and WD03 groups. While all trees followed a general growth pattern, taller trees had consistently lower RGR values. The WD03 treatment display a similar behaviour for both tall and small with a drop during the drought phase and regain in RGR from the recovery phase. However, small trees in WD03 still maintained higher RGR than their taller counterparts throughout the experiment. A similar trend was observed in diameter RGR and internode RGR (Figure 7-B, 7-C). Control small displayed a higher RGR in all measured parameters, and WD03 tall exhibited the lowest values.

The coefficient of variation (CV) analysis further illustrates differences in growth stability between groups (Figure 8). For height tall trees in the water deficit group (WD03 T) presented the highest CV, while trees in the control group (Ctrl T) remains relatively stable (Figure 8-A). The highest peaks are observed at the end of the drought phase (red bar) and during the recovery phase (green bar).

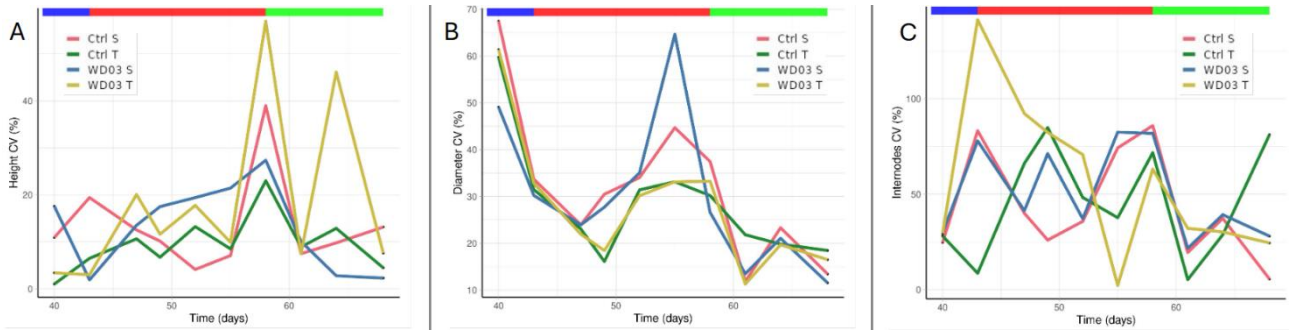

Figure 8. Ctrl and WD03 smallest vs tallest trees Coefficient of variation (CV) in %.
Each panels display results for Ctrl small (red line), Ctrl tall (green line), WD03 small (blue line), and WD03 tall (yellow line). The lines represent the means. The bar above shows the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. All figures were generated with PlotTwist, (Goedhart, J. 2020).

Diameter CV shows the clearest differences, with small trees (Ctrl S and WD03 S) exhibiting the strongest variability compared to both tall conditions (Figure 8-B). In contrast internodes CV displayed the highest fluctuations specifically in tall trees under the WD03 treatment, while small trees in both control and drought conditions followed more stable trends (Figure 8-C). We observe that both small treatments show trends that appear to oppose those of their tall counterparts (Figure 8).

Analysis of tree architecture

To further investigate how height influenced growth responses, we examined changes in tree architecture over time (Figures 9, 10, and 11). Trees were categorized by treatment (Ctrl and WD03) and initial height at the first measurement on May 3rd, 2024. These trees are the smallest and tallest of their treatment groups. The width and height of 4 trees – Ctrl small, Ctrl tall, WD03 small, WD03 tall - were compared in Figure 9. High-resolution images taken by the NaPPI system provided further insights into morphological differences over time (Figures 10 and 11). Measurement calculations for height and width were done using these images.

Regardless of treatment, taller trees consistently exhibited greater width compared to smaller trees (Figure 9-A). In addition, control trees (both small and tall) developed wider shapes than their WD03 counterparts. Height growth followed a similar trend, with control trees maintaining greater overall growth than WD03 trees (Figure 9-B).

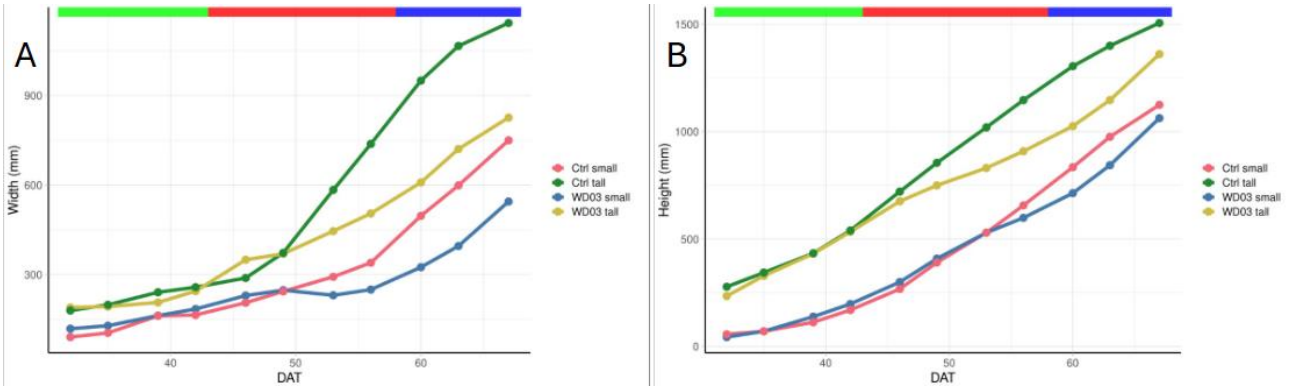

Figure 9. Width and Height of the Small and Tall trees from Ctrl and WD03, over time (DAT).
A: Width in mm. B. Height in mm. Each panels display results for Ctrl small (red line), Ctrl tall (green line), WD03 small (blue line), and WD03 tall (yellow line). The lines represent the means. The bar above shows the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. Measurements done from pictures using the scale 1pixel=0.54mm. All figures were generated with PlotTwist, (Goedhart, J. 2020).

The width and height measurements coupled with morphological differences observable to the naked eye provides further elements to the analysis. In tall trees, both treatments began developing side branches starting from DAT 46 (Figure 10). However, differences in architecture were observed between the treatments.

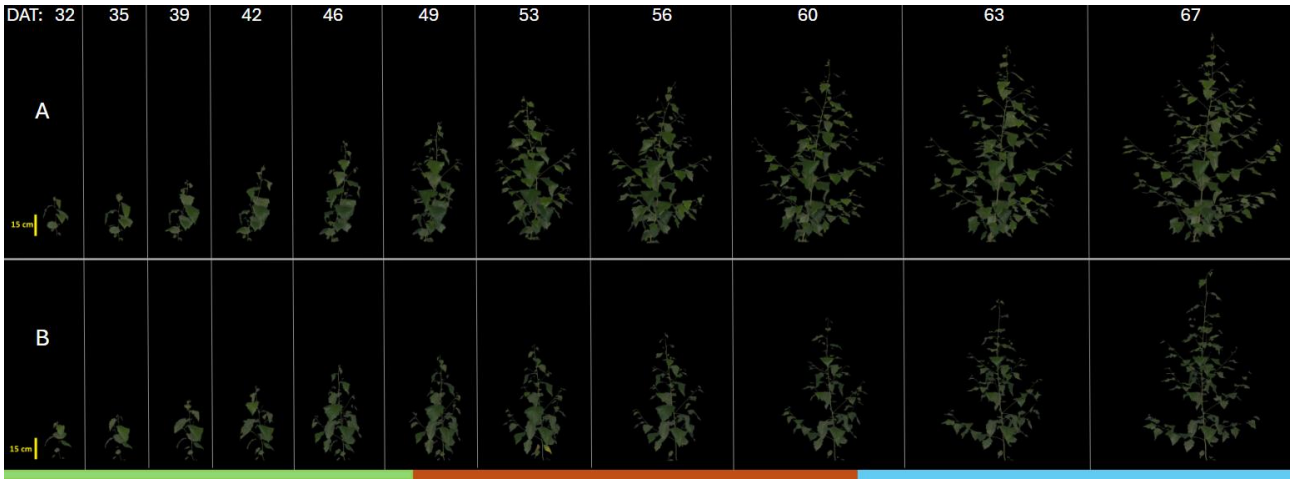

Figure 10. Visual comparison of tall trees from Ctrl and over time (DAT).
A: Ctrl condition. B: WD03. Scale: Yellow vertical bar, 15 cm (1pixel=0.54mm). Pictures taken automatically by the NaPPI system. The coloured line at the bottom represents the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery.

The tree under WD03 treatment is visibly thinner and smaller than the one in control treatment (Figure 10). Small trees display a similar developmental pattern (Figure 11). However, the small trees developed side branches between DAT 49 and 53 (Figure 11).

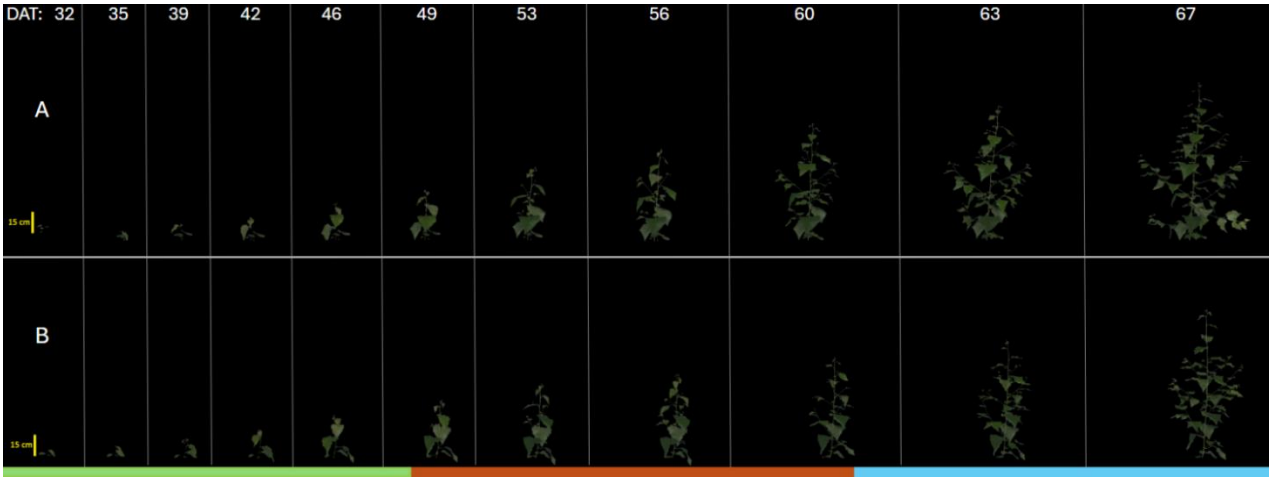

Figure 11. Visual comparison of small trees from Ctrl and WD03, over time (DAT).
A: Ctrl condition. B: WD03. Scale: Yellow vertical bar, 15 cm (1pixel=0.54mm). Pictures taken automatically by the NaPPI system. The coloured line at the bottom represents the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery.

The analysis of trees architecture evolution over time shows a difference in size and developmental stage between Ctrl and WD03 treatment, but also between tall and small (Figure10 and 11, respectively). The tall trees, regardless of their initial size, remain larger throughout the experiment compared to the smallest trees. Additionally, tall trees began developing side branches 3 to 5 days earlier than the small ones.

3.3 Experiment B - Greenhouse results

The timeline followed during this experiment is as presented earlier (Section 2.1.2, and Annex 4). As previously done in experiment A – NaPPI we used the previously obtained measurements on soil water content (Section 3.1) to obtain the target pot weight of each treatment. The target pot weights obtained were as follows: Ctrl: 2100 ± 10g, WD30: 1390 ± 10g, WD20: 1290 ± 10g.

Throughout this experiment there were no excluding characters or visuals differences. Tutors were added to each tree in the beginning of the experiment to keep a homogenous shape.

Water content

The evolution of weight throughout the experiment shows a drop in pot weight at DAT 53, along with a disturbance in the watering system at DAT 63 (Figure 12). System fixes were implemented, and the watering duration of the Ctrl was increased by 1 minute, which explains the increasing

weight afterward. WD30 and WD20 experienced an unplanned increase in water during the drought phase, delaying the expected weight evolution by approximately a week. Despite this, the weights of WD30 and WD20 dropped to targeted levels during the applied water deficit phase and recovered as planned during the recovery phase. The Ctrl treatment maintained a stable weight throughout the experiment**.** These observations shows that the experiment was functional as intended.

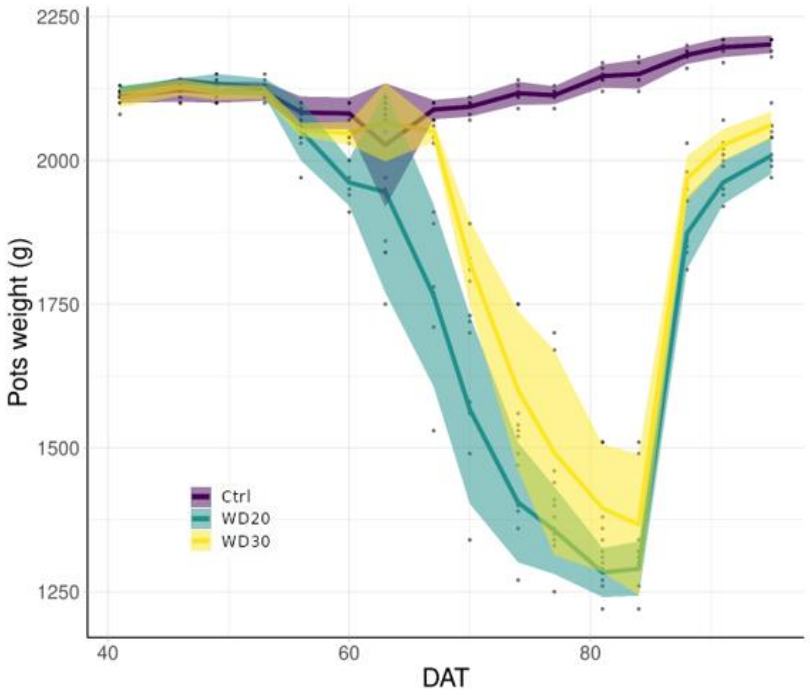

Figure 12. Trees weights (g) evolution for three treatments over time.
Ctrl, WD30, WD20. The weight is shown across different days after transplant (DAT). The shaded areas are the Confidence Intervals, and the lines are representing the mean. This Figure was generated with PlotTwist, (Goedhart, J. 2020).

Manual measurements

The manual measurements result for experiment B, are showing the progression of the four growth parameter measured during the experiment, depicted as subpanels A to D (Figure 13). No significant difference between treatments is observed for any of the measured parameters. In addition, similar trends for each parameter were observed in all water conditions.

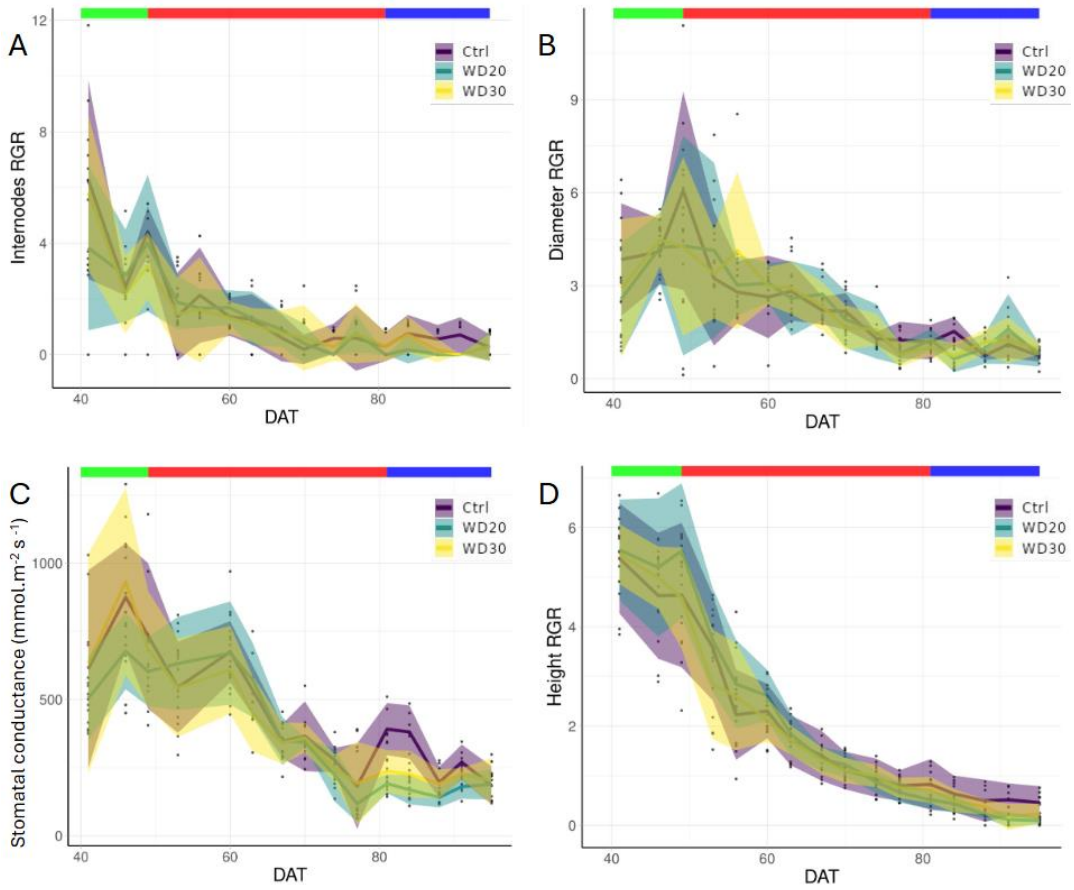

Figure 13. Relative growth rates (RGR) and stomatal conductance under different treatments (Ctrl, WD30, WD20) over time.
DAT: Days After Transplant. Panels A, B, and D show the RGR in % of internodes (A), stem diameter (B), and plant height (D), respectively. Panel C Stomatal conductance in mmol.m^{-2} s $^{-1}$. Replicate per treatment n=6. The coloured line on top represents the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. The lines are the mean of each treatment. Shaded areas are the 95% confidence intervals. All figures were generated with PlotTwist, (Goedhart, J. 2020).

However, Stomatal conductance present a difference between DAT 77 and 84, the WD treatments exhibited lower values compared to the control treatment (Figure 13-C). This period corresponds to the end of the applied drought phase. Despite no notable differences in RGR or stomatal conductance, all treatments are following the same trends regardless of the parameter, suggesting that other environmental cues may have influenced the trees.

The similarity among all replicates from the beginning to the end, combined with low number of replicates, did not allow for a comparison between smallest and tallest trees as it was the case in Experiment A.

Environmental Aspects

Temperatures and hygrometry in the greenhouse were relatively stable throughout the experiment (Figure 14). A slow drop in temperature over time is observed, with decreasing number of days above 25°C, and temperature dropping to 15°C during the recovery phase.

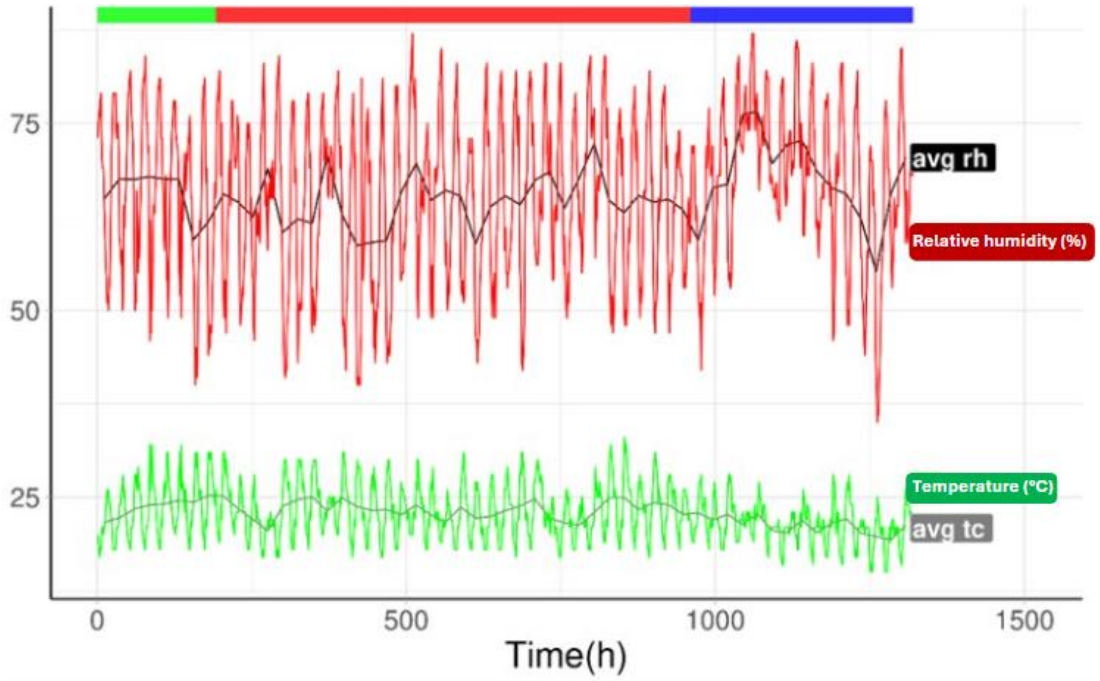

Figure 14. Temperatures and hygrometry changes over time in the Greenhouse.
Temperatures in degrees celsius (green line, tc). Hygrometry in % (red line, rh). The black and grey lines are the means of hygrometry and temperature, respectively. The coloured barre on top represents the stages of the experiment: Green = well-watered, Red = drought, Blue = recovery. Data provided by the greenhouse staff, located in Viikki campus at the Universiy of Helsinki. All figures were generated with PlotTwist, (Goedhart, J. 2020).

4 Discussion

This discussion examines the findings, experimental limitations and problems encountered, and what the implications for future research could be. The results from both experiments provide insights into the responses of Birch trees under varying water deficits (WD) conditions. Experiment A was conducted in NaPPI facility, which is an automated phenotyping platform. Experiment B was conducted in a conventional greenhouse setting.

4.1 Experiment A – NaPPI

Effect of Water Deficit Treatments on Growth Parameters

Looking at the varying WD treatments applied, the results show that birch trees were differentially affected in terms of the parameter we measured, especially for stomatal conductance (Figure 4).

The trees did not show major changes under mild and moderate WD conditions, (WD01 and WD02), but did show a significant stress response under the severe water deficit (WD03). Across the growth parameters measured (stomatal conductance, and relative growth rates (RGR) of internode number, stem diameter, plant height) WD03 showed the most pronounced drops and differences in comparison to the control condition, at the exception of internode number, which stayed relatively close to Ctrl albeit slightly lower. This suggests that while Birch trees can maintain growth under moderate WD, severe WD provoke growth restrictions (Kang et al., 2023) and probable changes in wood formation structure (Arend & Fromm, 2007; Rosso et al., 2023), likely as resources shift toward essential survival functions (Arend & Fromm, 2007; Rosso et al., 2023; McDowell et al., 2008), such as maintaining water transport, and keeping a positive carbon balance for energy, growth, and defences (Rosso et al., 2023; McDowell et al., 2008)

The height RGR data indicates first a stable growth (DAT 43 to 52) followed by a sharp but constant reduction across all treatments, including Ctrl, starting at DAT 52 (Figure 4-D). This reduction is likely due to the trees reaching a natural end of the fast growth phase rather than being a direct result of WD conditions. As seen in the chronological pictures of the trees' development, the plants start growing more side branches at the same time as the height RGR drops (Figure 9, 10, 4D). Thus, confirming a change in growth pattern. Future studies might benefit from timing experiments to avoid this fast growth phase transition, which could obscure WD effects and complicate data interpretation. As presented by Beikircher et al, 2024, trees are vulnerable to drought to limited roots, small water reserves, and prioritization of rapid growth. As they mature, developmental and anatomical changes, enhance drought tolerance by prioritizing survival over growth. It could, therefore, be interesting to repeat the experiment before and after this transition phase, to understand if a WD affects birch trees differently depending on their early developmental stage.

Stomatal Conductance Challenges

Stomatal conductance, measured by a porometer (model AP4 Delta-T), proved challenging to record reliable data in the NaPPI facility due to quick and strong variations in environmental conditions. According to the AP4 Delta-T user manual, accuracy is within +10 to -5°C of the calibration temperature, and < ±2.5°C temperature difference between leaf and the sensor cup. Even though the rapid temperature changes in the greenhouse, between 10 a.m. and noon when measurements were taken, did not exceed the calibration temperatures accuracy limits, we

observed highly variable results and out of range readings. However, accuracy limits between the leaf and the sensor cup were often exceeded. With calibration performed at the start of the measurement period, the constant rise of temperatures was the main cause to the data gaps, the high variability of the results observed and unreliability of the data starting at DAT 55. To address this limitation, future experiments should consider shorter measurement intervals or consistent recalibration throughout the measurement process.

Trees Acclimation

After in vitro propagation, the initial acclimation of the Birch trees was suboptimal due to issues in the mini-greenhouse settings. Excess water was added to the tray bases of closed mini-greenhouse, thus creating a high moisture environment. As presented by Osório et al. (2012) when trees grown in vitro are transferred to a lower-humidity ex vitro environment, such as a greenhouse or field, acclimation becomes critical for their survival. A significant challenge in this transition is the development of adequate stomatal control to effectively regulate water loss. However, a too high hygrometric levels created prevented the trees from acclimating to lower humidity levels used later in the experiment. And so, the abrupt transition from closed mini-greenhouses to open general greenhouse conditions, caused visible stress symptoms such as dry leaves and discoloration, as well as hidden impacts like underdeveloped root systems, that was discovered only during repotting. These stresses and especially the underdeveloped root systems due to high hygrometry (Parts et al., 2013) may have affected the trees behaviour during the experiment. For future research, a more gradual transition from a high-humidity mini-greenhouse to the open general greenhouse could help prevent similar acclimation issues, allowing roots and the vascular system to develop adequately before exposure to WD treatments.

Temperature Effects

Elevated temperature changes in the NaPPI facility created additional stress that may have exacerbate the effects of WD treatments. Daytime temperatures frequently reached 30°C and above, a level which likely adds to the WD effects by accelerating transpiration (McDowell et al., 2008; Rosso et al., 2023) and reducing water-use efficiency (Aspelmeier & Leuschner, 2004). All treatments, including control trees, showed signs of heat-related stress, such as wilting leaves. Future studies might consider conducting similar experiments under less severe temperature settings to isolate drought effects more accurately.

Trees Size and Developmental Stage

The variation in initial trees size introduced another layer of complexity to the results. Despite our best efforts to standardise the developmental stage and size of the trees, we still saw differences in growth patterns, and which likely influenced growth variability across the experiment. Smaller trees showed a higher RGR means than taller ones, suggesting a greater growth capacity among smaller trees (Figure 7 to 11). This section discusses the implications of the findings related to relative growth rates (RGR), coefficients of variation (CV), and architectural variations observed under different treatments.

The results suggest that smaller trees exhibited higher RGR in height and diameter compared to taller ones across both the control (Ctrl) and the severe drought treatment (WD03). This is consistent with the concept that smaller individuals may allocate more resources toward rapid vertical and radial growth to compete for light and other resources (Beikircher et al., 2024). On the other hand, taller trees grew more steadily, which might mean they were saving resources (Kang et al., 2023). It's interesting to note that there were no significant differences in internode RGR, which suggests that internode formation at the shoot apical meristem may be less sensitive to initial size and treatment. The CV analysis support these findings, showing how the drought and recovery phases affected the trees. Smaller trees under WD03 treatment exhibited pronounced variability in height and diameter, suggesting they were more sensitive to the environmental stress. This fits with the hypothesis that smaller plants have less resilience against drought-related stress (Beikircher et al., 2024; Balducci et al., 2013), potentially due to limited resource reserves (Zhang et al., 2017). Taller trees, displayed more stable growth patterns, indicating greater resilience to changing conditions (Aspelmeier & Leuschner, 2004; Serra-Maluquer et al., 2018). The pronounced variability during recovery suggests that some trees are better at making the most of the recover conditions than others. The elevated CV observed in smaller trees during these phases highlights their vulnerability, yet also shows their capacity for rapid compensatory growth once the stress is gone as presented by Kang et al. (2023)

The analysis of trees' architecture over time reveals clear morphological differences between control and WD03 treatments, as well as between tall and small trees (Figures 9, 10, 11). The WD03-treated trees consistently displayed restricted growth and lower width compared to the control, regardless of initial size. These differences show how severe drought affects growth and

development. Taller trees started to develop side branches earlier than the smaller ones, which was seen in both treatments. This suggests that taller trees may be at a more advanced developmental stage. Despite experiencing a temporary lag in branch development, the smaller trees ultimately demonstrated that growth trends may be initially different under stress, but growth can still resume and even improve during recovery (Kang et al., 2023).

4.2 Experiment B – Greenhouse

This experiment was carried out to test if we could replicate Experiment A without relying on the phenotyping platform. In fact, NaPPI facility poses limitations in height which is problematic when studying trees. However, experiment B also aimed to explore how different water content levels can impact the growth parameters in a controlled environment. While this experiment was designed based on experiment an early result to create water deficit conditions (WD30 and WD20 treatments), the results showed little to no differences in growth responses throughout all treatments. Unlike Experiment A, the analysis of trees' architecture over time was did not reveal observable variations and/or differences between the treatments (Ctrl, WD30, WD20), indicating that the drought treatments applied may not have been severe enough to induce noticeable growth changes noticeable to the naked eye within the timeframe of the experiment.

Water content

The weight of the samples for each treatment was maintained at the targeted levels (Ctrl: 2100 ± 10g, WD30: 1390 ± 10g, and WD20: 1290 ± 10g) during the drought phase, which confirms that the experimental setting was working according to our planification (Figure 12). The pot weight variation across the drought and recovery phases, show for an effective management of watering (Figure 12). This suggests that while the watering system faced a minor disruption between DAT 53 to 63, the setup successfully regulated water deficit overall. Despite the accurate pot weight maintenance, the lack of observable impact on growth parameters measured, suggest that there were deficiencies to induce measurable stress responses in growth. These deficiencies could be attributed to duration, degree of water deficit, or another external factor such as season change.

The drop in weight from DAT 53 to 63 could be explained by several independent factors. First, the drainage holes of the pots were not covered resulting in a minor soil loss by the drainage holes. Secondly the growing roots took up more space, leaving less space for the soil. Finally, we noticed

that during acclimation the pots were overwatered resulting in nutrient and soil leaching. Together, these factors may have lowered the soil water retention potential, resulting in the observed variations (Figure 12).

Growth parameters results

The growth parameters measurement results reveal that all treatments grew following the same trends (Figure 13). The only observable difference was in stomatal conductance during the drought phase (DAT 77-84), where the WD groups exhibited lower values than the control. While the stomatal response may reflect on an adaptive reaction to water deficit, the fact that other growth parameters did not seem impacted could suggests a certain degree of drought tolerance or recovery capacity in Birch depending on the season at which a drought phase is applied. Aspelmeier & Leuschner (2004), state that *Betula pendula* exhibits indeterminate leaf growth throughout the growing season, which could suggest that a drought occurring during periods of active growth, such as spring or early summer, might have a greater impact than one occurring during periods of slower growth, such as late summer or autumn. Furthermore, the observed drop in RGR for all treatments, including Ctrl, could be explained by the timing at which experiment B was conducted (Figure 13). In Finland Autumn starts at the end of August. Temperatures are dropping but what impacts more the plants is the quick decrease in daylight both in length and intensity (Li et al., 2003). Supporting the hypothesis postulated earlier that, seasonality and the effect of water stress may be linked as trees prepare for winter dormancy, the impact of severe water deficit on development at this stage of their lifecycle appears to be minimal.

Greenhouse environmental conditions were relatively stable but showed a gradual cooling trend over time (Figure 14). The drop in temperature during the recovery phase, along with the stable relative humidity, probably helped to keep the growth trends consistent across treatments. Lower temperatures can reduce evapotranspiration demand, which might help to offset the effects of the drought treatments (McDowell et al., 2008).

Unlike Experiment A, where height variation allowed for a comparison of growth dynamics between tall and small trees, the uniformity of trees in Experiment B limited the scope of analysis. The lack of significant differences in RGR and visual morphology in Experiment B serves to reinforce the importance of experimental conditions, including environmental consistency, drought severity and the timing of the experiment, in influencing plant responses.

4.3 Controlled vs Natural conditions

Studying *Betula pendula*'s responses to WD in controlled environment like greenhouses and phenotyping platform (NaPPI) has its advantages and disadvantages. Controlled experiments like those conducted in this study provide a precise and repeatable setting to test specific hypotheses, but they do not capture the full range of environmental complexities present in natural conditions.

In controlled settings like those of this study, factors like soil, humidity, temperature, and water were regulated to isolate the effect of WD. The obtained results may not fully mirror how birch responses are in a forest, where multiple environmental stresses affect the trees. For example, changes in humidity and temperature in natural environments may amplify drought effects on transpiration rates, as seen in studies of temperate forests areas (McDowell et all., 2008). During drought, trees may maintain stable leaf transpiration rate to keep cool while increasing the effort to pull water from increasingly drier soil. This is observed in many different tree species like *Fagus sylvatica* (European beech) and *Picea abies* (Norway spruce). It shows how trees prioritize transpiration and cooling at the expense of hydraulic stability (Tomasella et al., 2018; Meinzer et al., 2013). Furthermore, recent studies carried on Birch show that genotypic differences influence drought tolerance, with populations originating from wetter climates showing less resilience to water scarcity than those from drier regions (Sellin et al., 2014; Hannus et al., 2020). Other species from the Betulaceae family, like Alder (*alnus glutinosa*), show different hydraulic responses, with root depth and water use strategies having an important role. Zapater et al. (2011) found that hydraulic lift in sessile oak (*Quercus petraea*), is redistributing water to upper soil layers to benefit shallow-rooted species like European beech (Fagus sylvatica). Such interaction could also apply to birch in forest areas. Additionally, controlled settings often lack pests and microbial influences, which affect trees' health and tolerances. Microbial symbiosis in nature can make trees more drought tolerant by helping them with nutrient improvement and better water uptake (Smith & Read, 2008).

Some studies and part of the results of this thesis have suggested that trees react differently to WD depending on developmental stage. For example, in studies of European forests, birch and other Betulaceae species like hazel and alder showed variable growth responses based on local soil water retention capacity and canopy cover (Bolte et al., 2016). Field studies, or semi-controlled

setups could be done in association with fully controlled experiments to broaden our understanding. For example, controlled water deficits in outdoor plantation studies could show how plants interact with each other while still being able to control the experiment. This was done with Populus species, where field trials where field trials revealed adaptive mechanisms not observed in laboratory settings (Monclus et al., 2005).

All these interactions highlight the complexity of forest ecosystems, which controlled environments cannot fully replicate. However, controlled environment allows for targeted studies of mechanisms that we do not fully understand yet, and that are inaccessible to be studied in the forest context.

5 Conclusion

This thesis focuses on developing standardized methods to assess the drought stress responses of *Betula pendula* (silver birch), with a particular emphasis on its morphological and physiological adaptations under varying water deficit (WD) conditions in controlled environments. By implementing two experimental setups - an automated phenotyping platform (NaPPI) and a conventional greenhouse - this study aimed to investigate how different drought levels influence growth parameters such as cambial activity through stem diameter, stem elongation through height and internode development, and stomatal conductance.

The findings highlight the resilience of silver birch under mild and moderate WD conditions, as observed in Experiment A (NaPPI), where growth parameters showed little to no differences from control groups under WD01 and WD02 treatments. However, severe WD (WD03) led to significant growth restrictions, particularly in stem elongation and stomatal conductance, demonstrating the species' physiological changes towards survival strategies under severe drought stress. The overall results of experiment A emphasize the role of the vascular cambium and secondary growth adjustments in drought resilience.

Experiment B didn't show much difference between the treatments, probably due to the seasonal timing of the experiment and all the associated factors. The results suggest that environmental factors such as temperature, humidity, and seasonality play critical roles in influencing the growth responses of birch trees to drought.

While controlled experiments allow for targeted analysis of developmental mechanisms, the findings also show the limitations of such setups in controlling all environmental aspects, but also in replicating the complex interactions present in natural environments.

This work contributes to the growing knowledge on tree developmental plasticity and provides a foundational protocol for further investigations of birch adaptation mechanisms in the context of environmental stressors. The findings of this study can help us uncover new ways of managing birch in forestry and conservation in the face of changing environmental conditions.

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8 Annexes

8.1 Annex 1 – Growth media preparation for invitro culture of Birch

- Autoclave the Magenta boxes for the propagation of trees.
- Growth media for birch (*Betula spp.*) in invitro culture.
    - In a 500 mL bottle add:

MS with vitamins	1.65g
MES	0.5g
Sucrose	10g
Plant agar	3.5g
MiliQ water	500mL

    - Adjust the pH to 5.7 with KOH (2M)
    - Autoclave
- In a laminar hood, add 100µL of 1mg/mL IBA (Indole-3-butyric acid) stock solution, to the 500mL of previously autoclaved media.
- In a laminar hood pour 100mL of liquid media per Magenta box.
- Store the magenta boxes in cold room at 4°C, if not used right away.

IBA stock solution at (1mg/mL):

- In a 2mL Eppendorf tube, weight 10mg of Auxin powder and write down the exact amount.
- Calculate the volume of MilliQ water to add to obtain the desired concentration.
- Add a couple of NaOH (1M) drops to dissolve the Auxin.
- Transfer to a 15mL falcon tube and fill with the previously calculated MiliQ volume.
- Sterilize by passing the solution through a sterile Whatman filter (0.2µM)
- Aliquot in 1.5mL Eppendorf tubes
- Store at -20°C

8.2 Annexe 2 – Experiment A precise measurements planning

Date	DAT	Planned Events	Status	Observation	Measurements
25.4.2024	29	Pot loaded in NaPPI RGB1&2 (3h)	Well-Water	Pot SC Avg: 3490g RefWeight Set to 3500g	
26.4.2024	30		Well-Water		
27.4.2024	31		Well-Water		
28.4.2024	32	RGB1&2 @07:00	Well-Water		
29.4.2024	33	Randomization	Well-Water		
30.4.2024	34		Well-Water		
1.5.2024	35	FC+RGB1&2 @07:00	Well-Water		
2.5.2024	36	Randomization	Well-Water		
3.5.2024	37		Well-Water		Stem D/H/IN & T
4.5.2024	38		Well-Water		
5.5.2024	39	RGB1&2 @07:00	Well-Water		
6.5.2024	40		Well-Water		Stem D/H/IN & T
7.5.2024	41		Well-Water		
8.5.2024	42	FC+RGB1&2 @07:00	Well-Water to Drought	Change RefWeight	
9.5.2024	43	Randomization	Drought		Stem D/H/IN & T
10.5.2024	44		Drought		
11.5.2024	45		Drought		
12.5.2024	46	RGB1&2 @07:00	Drought		
13.5.2024	47		Drought		Stem D/H/IN & T
14.5.2024	48		Drought		
15.5.2024	49	FC+RGB1&2 @07:00	Drought		Stem D/H/IN & T
16.5.2024	50	Randomization	Drought		
17.5.2024	51		Drought		
18.5.2024	52		Drought		Stem D/H/IN & T
19.5.2024	53	RGB1&2 @07:00	Drought		
20.5.2024	54		Drought		
21.5.2024	55		Drought		Stem D/H/IN & T
22.5.2024	56	FC+RGB1&2 @07:00	Drought		
23.5.2024	57	Randomization	Drought		
24.5.2024	58		Drought		Stem D/H/IN & T
25.5.2024	59		Recovery	Change RefWeight	
26.5.2024	60	RGB1&2 @07:00	Recovery		
27.5.2024	61		Recovery		Stem D/H/IN & T
28.5.2024	62		Recovery		
29.5.2024	63	FC+RGB1&2 @07:00	Recovery		
30.5.2024	64	Randomization	Recovery		Stem D/H/IN & T
31.5.2024	65		Recovery		
1.6.2024	66		Recovery		
2.6.2024	67	RGB1&2 @07:00	Recovery		Stem D/H/IN & T
3.6.2024	68		Recovery	Final measurements and sampling	Stem D/H/IN & T

DAT: Days after Transplant; D: Diameter; H: Height; IN: Internodes; T: Stomatal conductance

8.3 Annexe 3 – Soil weight and watering content test data

				Day 1		Day 3		Day 6		Day 9		Day 12		Day 16	
	Treatment	Pot n°	start wieght (g)	After automatic watering	RWC	After automatic watering	RWC	After automatic watering	RWC	After automatic watering	RWC	After automatic watering	RWC	After automatic watering	RWC
date			4/2/2024	4/3/2024	4/3/2024	4/5/2024	4/5/2024	4/8/2024	4/8/2024	4/11/2024	4/11/2024	4/14/2024	4/14/2024	4/18/2024	4/18/2024
	1 DRIP	1	1400	1491.1	6.51%	1742.1	24.44%	1975	41.07%	2079	48.50%	2127	51.93%	2145	53.21%
		2	1400	1506.1	7.58%	1761.1	25.79%	1991	42.21%	2088	49.14%	2124	51.71%	2132	52.29%
		3	1400	1538.4	9.89%	1750	25.00%	1973	40.93%	2077	48.36%	2114	51.00%	2134	52.43%
		4	1400	1476.7	5.48%	1721	22.93%	1951	39.36%	2099	49.93%	2150	53.57%	2174	55.29%
		5	1400	1468.3	4.88%	1706	21.86%	1928	37.71%	2059	47.07%	2104	50.29%	2114	51.00%
		6	1400	1465.3	4.66%	1688.9	20.64%	1905	36.07%	2155	53.93%	2241	60.07%	2254	61.00%
		7	1400	1467.1	4.79%	1689	20.64%	1905	36.07%	2102	50.14%	2144	53.14%	2166	54.71%
		8	1400	1469.0	4.93%	1705.4	21.81%	1935	38.21%	2089	49.21%	2134	52.43%	2154	53.86%
		9	1400	1466.4	4.74%	1699.3	21.38%	1927	37.64%	2126	51.86%	2188	56.29%	2205	57.50%
		10	1400	1469.5	4.96%	1701	21.50%	1926	37.57%	2157	54.07%	2239	59.93%	2257	61.21%
AVERAGE				**1481.8**	**5.84%**	**1716.4**	**22.60%**	**1941.6**	**38.69%**	**2103.1**	**50.22%**	**2156.5**	**54.04%**	**2173.5**	**55.25%**
	2 DRIPS	11	1400	1558.4	11.31%	2058	47.00%	2205	57.50%	2241	60.07%	2298	64.14%	2308	64.86%
		12	1400	2452.0	75.14%	2457	75.50%	2422	73.00%	2413	72.36%	2436	74.00%	2431	73.64%
		13	1400	1571.0	12.21%	2109	50.64%	2276	62.57%	2320	65.71%	2276	62.57%	2382	70.14%
		14	1400	1554.5	11.04%	2060	47.14%	2198	57.00%	2238	59.86%	2309	64.93%	2322	65.86%
		15	1400	1556.4	11.17%	2061	47.21%	2163	54.50%	2205	57.50%	2260	61.43%	2253	60.93%
		16	1400	1565.8	11.84%	2092	49.43%	2273	62.36%	2313	65.21%	2364	68.86%	2373	69.50%
		17	1400	1562.8	11.63%	2081	48.64%	2203	57.36%	2245	60.36%	2301	64.36%	2312	65.14%
		18	1400	1565.0	11.79%	2064	47.43%	2112	50.86%	2125	51.79%	2175	55.36%	2180	55.71%
		19	1400	1565.7	11.84%	2087	49.07%	2202	57.29%	2224	58.86%	2293	63.79%	2298	64.14%
		20	1400	1557.3	11.24%	2057	46.93%	2176	55.43%	2204	57.43%	2256	61.14%	2266	61.86%
AVERAGE				**1650.9**	**17.92%**	**2112.6**	**50.90%**	**2223**	**58.79%**	**2252.8**	**60.91%**	**2296.8**	**64.06%**	**2312.5**	**65.18%**
	4 DRIPS	21	1400	1759.6	25.69%	2406	71.86%	2384	70.29%	2398	71.29%	2445	74.64%	2449	74.93%
		22	1400	1755.9	25.42%	2383	70.21%	2347	67.64%	2353	68.07%	2379	69.93%	2380	70.00%
		23	1400	1835.1	31.08%	2428	73.43%	2372	69.43%	2384	70.29%	2395	71.07%	2392	70.86%
		24	1400	1754.7	25.34%	2439	74.21%	2411	72.21%	2429	73.50%	2447	74.79%	2440	74.29%
		25	1400	1754.5	25.32%	2460	75.71%	2423	73.07%	2442	74.43%	2457	75.50%	2457	75.50%
		26	1400	1746.1	24.72%	2374	69.57%	2341	67.21%	2359	68.50%	2384	70.29%	2382	70.14%
		27	1400	1765.2	26.09%	2389	70.64%	2354	68.14%	2360	68.57%	2395	71.07%	2394	71.00%
		28	1400	1766.6	26.19%	2424	73.14%	2392	70.86%	2399	71.36%	2305	64.64%	2410	72.14%
		29	1400	1769.3	26.38%	2488	77.71%	2421	72.93%	2430	73.57%	2469	76.36%	2366	69.00%
		30	1400	1754.4	25.31%	2423	73.07%	2370	69.29%	2386	70.43%	2405	71.79%	2406	71.86%
AVERAGE				**1766.1**	**26.15%**	**2421.4**	**72.96%**	**2381.5**	**70.11%**	**2394**	**71.00%**	**2408.1**	**72.01%**	**2407.6**	**71.97%**

RWC = Relative Water Content

8.4 Annex 4 – Experiment B precise measurement planning

Precise planning of Experiment B, with the three phases of the experiment, what happened, when, and changes applied.

Date	Measurements day (DAT)	Planned Events	Status	Observation
	0	Transfer to soil	Well-watered	
	20	Transfer to GH for acclimatation	Well-watered	
	25	Transfer to 3L pots and setup to the automatic watering system	about 600ml water/day	water 2 times a day. 8am - 8pm
	35		about 600ml water/day	
	39	1st trees measurements	about 600ml water/day	
	41		about 600ml water/day	
	46		about 600ml water/day	
	49	Drought started at 8 pm	about 600ml water/day	
	53		drought	
	56		drought	
	60		drought	
	63		drought	Ctrl 1' water increase
	67		drought	WD30 + WD20 water amount decreased by 1'
	70		drought	
	74		drought	
	77		drought	
	81	Recovery start 8pm	drought	
	84		Recovery	
	88		Recovery	
	91		Recovery	
	95	Last measurement	Recovery	