Drought tolerance phenotyping using thermal imaging

High throughput phenotyping of *Pinus Sylvestris*

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Abstract

Climate change is an emerging problem in the world. One of the many challenges that comes with it is an increase of droughts. Droughts cause stress for plants, which impact the growth. An important stage for trees is the seedling stage, and an important tree in the forest is the Scots pine (*Pinus Sylvestris*), therefore this study will focus on these.

High throughput phenotyping is a tool that can help identifying drought tolerance. In this paper the use of thermal imaging as a way to achieve high throughput phenotyping in *Pinus sylvestris* for transpiration and its links to drought tolerance was evaluated. Another possible phenotyping method, used in this study, is if drought tolerance can be evaluated by their divergence from an average growth.

Experiment took place during the summer of 2022 and plants evaluated started growing that same year. It took place inside a greenhouse and with a drought/control treatment. The results revealed differences between natural and bred plant material, and also between northern and southern plant material. With bred and southern plants expressing lower stress compared to the others. A strong negative association between total volume and canopy temperature for drought treated plants suggest there is potential for using infrared light to evaluate drought tolerance. Also consistent patterns between the transpiration and divergence from average growth supports the idea that thermal imaging could be used for high throughput phenotyping.

Keywords: Scots pine, *Pinus sylvestris,* drought tolerance, thermal imaging, high throughput phenotyping

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Abbreviations

CWSI	Crop Water Stress Index
DTI	Drought Tolerance Index
C/R	Canopy and Root Relation
DAG	Days after Germination
RGB	Red, Green and Blue

Introduction

Threats to our forests are always present. Drought is one such threat, that stresses our plants. One important plant for Swedish forests and forestry is Scots pine (*Pinus sylvestris*). It one of the most common species in Sweden (SLU 2022). Securing its future is important, both biologically and economically. Seedlings are less stress tolerant than their full-grown counterparts and drought stress could therefore be one of the factor affecting seedling establishment of Scots pine (Castro et al. 2004), (Niinemets 2010). A plant exposed to a drier climate is more likely to be affected by drought and the stress from that drought will affect growth and also lead to it being more vulnerable to pathogens (Niinemets 2010). Securing a future for the plant and understanding the processes behind tolerance is necessary and can be alleviated with the use of high throughput phenotyping for stress tolerance. *P.sylvestris* is currently an important plant and for it to continue to be a staple of Swedish forests and forestry, more research around drought tolerant plants and understanding how the tolerance works is needed. Identifying drought tolerance using a fast selection method and further research the tolerance mechanisms could help produce more tools to adapt Swedish forests to climate change.

1.1 Background

Plants interact with the surrounding environment. These interactions depend on conditions in the environment. Conditions can be both positive and negative for the plant. This may affect the plants' development and the character of affecting factors can be both biotic and abiotic. In the last years, research is generating more knowledge about how plants interact with their environment. With the arrival of climate change, knowing how plants adapt to stresses can be a key knowledge to adapt for the future climates. This knowledge will help for management strategies and breeding strategies with different plant materials.

Stress is any disturbance that causes plant development to slow down (Jackson 1986). Plants respond to stress in a number of different ways but the most common result is generally a reduced growth (Jones 2013). As mentioned in the previous paragraph, development is affected by biotic and abiotic factors, which is also why stress is can be both biotic and abiotic. Biotic stress can be caused by living things such as pathogens or pests, while abiotic stress is a result of non-living factors, e.g.

temperature and water. Abiotic stress in the form of water deficits, often droughts, is an increasingly issue nowadays (Caretta et al. 2022).

Water is important for plants and used in many different processes, such as growth and transport of nutrients and internal signaling (Jones 2013). Drought comprises a limited availability of water to the plant. A water deficit in a plant therefore can lead to different kind of alterations in the plant physiology, with the most common being reduced growth (Jones 2013). The reduced growth is because of water being involved in growing and expanding processes such as: metabolism, cell expansion and turgor pressure (Fricke 2017). Turgor pressure being the driving force behind cell expansion and mechanical strength in plant cells (Fricke 2017)(Jones 2013). Plants will try to optimize their photosynthetic gain, while also minimizing water loss (Chaves et al. 2003). If water is limited they have to adapt to a loss of available water, because of the importance of water for plants.

Droughts can be defined as drier than normal conditions for an ecosystem (Douville et al. 2021). In other words a water deficit relative to an average for a time and place (Caretta et al. 2022). This mean they can have different regimes depending on the biome, a drought in a desert will have different characteristics from a drought in a forest (Douville et al. 2021). Different kinds of droughts exist and can be categorized depending on where in the water cycle the deficit occurs and the climate (temperatures) of the region. Some categories are meteorological(precipitation), hydrological(storage, flow and runoff) and ecological/agricultural(low soil water levels and evaporation) (Caretta et al. 2022). Looking at droughts where it gets to the point that plants suffer from it, might be more relevant to look at in this study. Many of the droughts lead to a reduced water supply. A reduced water supply often leads to a water deficit which can severely affect the plants' development. Plants have however developed some mechanisms to handle drought and some are better adapted than others, with differences between species but also between individuals of the same species. With the current trend of climate change, an increase of droughts is expected. In recent years droughts in Europe has led to losses of forests through wildfire, abiotic stress death and other means (Senf et al. 2020) (Anderegg et al. 2013). Seedlings are also sensitive to drought and found to be related to the number of dry days during planting (Sukhbaatar et al. 2020). The affect drought has on plants be disastrous both for agriculture and forestry if it is not mitigated somehow. One way to mitigate losses is to identify more drought tolerant plants, find out what make them better than others and plant tolerant plants in our forests.

Drought tolerance are the mechanisms involved with a plant's productivity and survivability during droughts (Jones 2013). In agriculture the most important part of a drought tolerant plant is often the ability of said plant to have a higher yield in drought conditions compared to a non-tolerant counterpart. In natural environment drought tolerance refer to a plant capable of survival and reproduction in dry environment (Jones 2013). Seedling establishment can be a bottleneck in forestry and during this period, tolerance to abiotic and biotic stresses is crucial. Also, due to trees' long life cycles, the ability to survive is of great importance in the forestry industry in order to minimize losses and guarantee future material (Polle et al. 2019).

Plant leaves have a lower temperature than their surroundings when they transpire, due to water evaporation. Temperature of plants decrease with increased transpiration and increase with lower transpiration, one of the regulating factors for transpiration is stomatal aperture (Jones 2013). Therefore, stomatal conductance can be estimated by measuring temperature in canopy of plants. If the plants get stressed their canopy temperature increases. This is because of stomatal closure in leaf to keep water by stopping transpiration. Although it is also a balance with keeping the water if it has little available (Hamanishi & Campbell 2011). In turn, stomatal conductance is an indicator for stress responses. These relationships allow for drought stress to be estimated from thermal radiation (Prashar & Jones 2014).

Plant phenotyping is an important step in identifying drought resistance, and the use of thermal imaging as a tool might be able to speed up the process in a large scale (Prashar & Jones 2014). Phenotyping can be defined as ”the set of methodologies and protocols used to measure plant growth, architecture, and composition with a certain accuracy and precision at different scales of organization, from organs to canopies” (Fiorani & Schurr 2013). Responses to changes in climate are often destructive and time consuming to monitor (Seidel et al. 2016). Which means a ”phenotyping bottleneck” occurs and the process is slowed down. Overcoming this bottleneck is important for high throughput phenotyping to be viable in the future. For the development of phenotyping it is important to find more efficient and non-destructive ways to phenotype plants. This is because of the possibility of scaling, ability to asses plant behavior during the development and repeated across populations (Yang et al. 2020). Canopy temperature as a way of measure the ”crop water stress” has been recognized for some time (Tanner 1963) and a way to normalize it is the ”Crop Water Stress Index”(CWSI)(Idso et al. 1981). CWSI uses references to get a different values adapted to the environment by using artificially dry and wet references.

Thermal imaging is one method for high throughput and non-destructive phenotyping (Fiorani & Schurr 2013). The evolution of thermal reading has gone from single point thermometers measuring with a laser to high definition thermal images with data for an entire scene. Images makes it possible to see what is being measured, exclude dead matter and focus on the living (Jones 2004). This evolution allowed for a more accurate and easy use of thermal data collection and analysis.

Forests play an important role in our society and come with several different ecosystem services: timber, biodiversity, carbon storage, etc. (Hansen & Malmaeus 2016). Many of these values are related to the trees in the forests. Commercial values are important for future utilization of the forests biomaterial and the change to a more sustainable consumption of goods. For the sake of the environment it is important that products are reusable, recyclable, carbon neutral and biodegradable, which many products made from trees are. Environmentally they have other functions, some being carbon storage and conversion of carbon-dioxide to oxygen. They are an important part of many ecosystems and can help mitigate climate change. Therefore, it is important to manage these resources for future usage and make sure can thrive and survive.

1.2 Problem and aim

Plants are adapted to certain conditions, even if they possess some plasticity they cannot thrive in all conditions. Drought is one of the stresses that will become more common in the future, and with it comes drastic changes to the conditions that the plants are adapted to (Caretta et al. 2022).With a warmer and drier climate, more plastic plants (i.e. drought tolerant plants) are necessary to ensure the productivity and survival of Swedish forests. Certain families or individuals can be more tolerant to drought (Seidel et al. 2016). Phenotyping is one you might be able to understand drought tolerance. Today the art of phenotyping is a drawn out and expensive process, usually requiring the damaging of the plants that are tested (Fiorani & Schurr 2013). Drought tolerance in plants has been shown to be related to the temperature of a plant. With the use of a thermal camera, temperatures of several plants can quickly be registered and this possibility allows for analysis of multiple plants at once from a single measurement occasion. Making it possible to maybe overcome the phenotyping bottleneck due to the ease of scaling (Pineda et al. 2021). *P.sylvestris* is a tree important in Swedish forests and therefore a good subject for a study. The aim for this study is to see if a high throughput phenotyping process is possible to assess drought tolerance responses of *P.sylvestris* in a greenhouse experiment, using thermal imaging as a tool to achieve these goals.

The research questions for this study are:

-Are there differences between individuals/families regarding drought tolerance?

-Can the drought tolerance of an individual be estimated using infrared light?

-Can analysis of thermal radiation be a way to achieve high throughput phenotyping?

1.3 Theory

When plants are faced with drought stress, they can respond in different ways. One of the ways they try to mitigate the drought stress is through the stomatal regulation. Gas and water exchange between plant and aboveground environment occurs primarily through stomata (Lawson & Matthews 2020). Plants have evolved to optimize their photosynthetic processes to be as efficient as possible without losing too much water (Chaves et al. 2003). These stomatal changes will affect the leaf temperature difference with the environment because of the effect of the reduction in temperature due to water evaporation. With stomatal closure the transpiration from the plant decreases and with it the evaporative cooling, leading to increased leaf temperatures (Raschke 1960).

All matter emits certain amounts of thermal radiation, if they have a temperature above the absolute zero. They emit radiation following Stefan-Boltzmann's law in a range of wavelengths defined by the Planck's law of black body radiation. Infrared range used for measurement of thermal radiation is between 3 and 20 µm of wavelength. As water vapor absorbs a lot of radiation in this region a span of 8 - 14 µm is common for sensors to minimize disruptions caused by water vapor differences (Jackson 1986). This radiation is capable of being captured by sensors called microbolometers. Microbolometers works by absorbing infrared light leading to an increase of the temperature of material in the microbolometer. This changes the resistance of a material which can be electrically transferred to a circuit capable of processing it (Bhan et al. 2009).

The stomatal regulation and ability to record the temperature of the plant makes it possible to indirectly estimate transpiration of plants. With thermal imaging, the transpiration of the plants then quickly measured. This measurement can be an indicator of drought tolerance mechanisms and how the plants regulate the transpiration during the drought stress (Tanner 1963).

Crop water stress index was calculated using the following formula developed for analyzing thermal images in crops (Idso et al. 1981):

Where T_c is the temperature of the canopy obtained from images. T_{dry} is the highest temperature of a leaf with all its stomata closed and T_{wet} the lowest temperature of a fully transpiring leaf with all the stomata open. These conditions are hard to achieve in real leaves, and therefore artificial surfaces can be utilized (Möller et al. 2007)(Cohen et al. 2005). Both T_{dry} and T_{wet} is calculated averages from artificially wet and dry surfaces in each picture. Higher value indicates higher canopy temperatures and thus closure of stomata.

Drought tolerance is a complex trait (Passioura 1996), and several ways have been proposed to estimate it. A way used in this work to estimate a plant family's drought tolerance is by using something referred to as drought tolerance index (DTI) in this paper. It works by measuring the volume of biomass in several plants of a family in control and drought conditions (Blum 2005), a regression can be made for the average *P.sylvestris* plant (Figure 1). This regression is useful to correct for the growth rate differences, as is a population characteristic that changes among latitudes: northern adapted populations grow slower than southern adapted populations (Persson & Ståhl 1990). Using the family deviation from this linear model allows to compare between tolerances of families with different growth rates. It is to make sure that it is just not only a plant that happen to grow better but also continues to grow better under harsher climates. If a point is above the line it is better at handling drought, but if it is under it is worse. Values from deviation comes out as how much volume per day (mm^3/day) more or less than expected the plant grows. Some deviation from the line is expected, but for a at a certain level it is suspected to be something other than randomness.

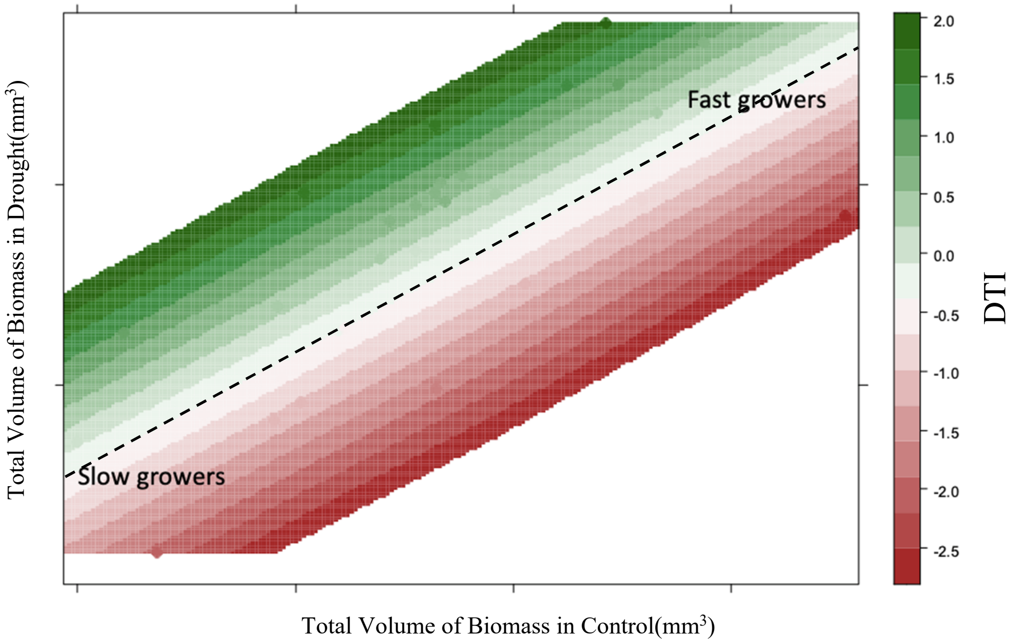


Figure 1: Figure showing how the average regression line of a plant might look and how the Drought Tolerance Index (DTI) is distributed around it. Slow growth plants are located more in the lower left corner and the further to the right they go, the faster they are growing. The more a family deviates to the green area, the better at tolerating drought it is, the opposite is true for plants in the brown area.

Material and methods

2.1 Material

2.1.1 Equipment

A thermal camera (Thermal capture 2.0, ThermalCapture)(Tau 2, Teledyne FLIR LLC) with a span of 7.5 µm to 13.5 µm was used. Resolution for the pictures were 640 x 512. The thermal resolution within pictures were capable of being 0.04°C at operating temperature and had a range of -25 °C and 135 °C . This means the camera is capable of registering temperatures in the span of the plant leaves. Emission coefficient was set to 1. It captured pictures at a rate of 60Hz. A handheld camera (EOS M50 Mark 2, Canon Inc.) capable of capturing light with the red, green and blue wavelengths (RGB camera) and then producing a colored picture, was also used to help find plant locations and later mark them in the thermal images.

2.1.2 Plant material

Plant material was Scots pine (*Pinus sylvestris*). 2880 Plants in total from 120 different families and 24 individuals from each family. Families does in this case mean that they at least share a mother. Half of the individuals from each family were later put in control and other half in drought. Not all plants however lead to a successful germination and ended up with 670 living plants from 78 families. The plant material was both natural from forests and bred form breeding programs. It came from different parts of Sweden, some more southern and some more northern, from forests around Arjeplog, Jokmokk and Östersund.

2.1.3 Reference surfaces

Reference surfaces where chosen to be artificially created, and included in each picture (Figure 2C). A reference surface is required for calculating CWSI and artificially creating them has been suggested to be one way to get these references (Möller et al. 2007). Reference surfaces was created by using cotton and either wetting it or leaving it to dry. Cotton was chosen because it is a plant material and thought to closely simulate a fully transpiring leaf when wet or a non-transpiring leaf when dry.

2.1.4 Software

To extract the temperatures from the thermal images a program is needed. The thermal camera had its own program for extracting the temperatures from the thermal images. Program used was called FLIR Tools 6.4 (Teledyne FLIR 2015). Excel was used to summarize the temperatures extracted from the images and for calculating CWSI for each of the plants. For the volume calculation the program RhizoVision Explorer (Seethepalli & York 2020) was used. The program R Statistical Software (R Core Team 2021) was used for all statistical analyses, and in the creation of some of the graphs.

2.2 Method

2.2.1 Growing

The growth period was during the summer of 2022 and occurred inside greenhouses. Planting began on the 15th of May. Plants were grown in a crate with a 18x11 grid, but the rows around the edges had no plants. Soil in each crate was a mixture of 2/3 of the volume being regular planting soil and the rest being sand. There were 5 blocks with 2 controls and 2 drought-affected crates in each block (Figure 2A). This comes to a total of 10 control plots and 10 drought plots. Location for the plantation of the plants was decided randomly, but done so equal amounts from each family were in both treatments. First plants grew for 3 weeks under normal conditions, given water and some nutrients via watering can. This period allows plants to develop roots acclimate to the conditions in the glasshouse. Half of the plants were after the period, on the 18th of June, exposed to a water deficit, or simulated drought. The other half were kept under normal conditions as control. Drought was induced using a system were the plants have different distance to the water (Marchin et al. 2020). Plant crates was placed in containers and on top of a foam brick of. Foam brick was able to absorb water to some degree, but with longer distances the amount decreased. For the drought the distance to the water table was between 19-20cm, and in control this was 3-4 cm. These containers allowed for a stable and adjustable water table. How intense the water deficit is, depends on the distance from plant to water table. Larger distances are equal to a bigger water deficit. This was supposed to simulate more closely how droughts are in nature, with the water table always being present, only distance to it being larger (Marchin et al. 2020). Depth to water table was de kept level and plants were not watered from above to avoid nutrient leakage, watering was done from the side. Markings for where the water table should be at were present in the containers, and watering was done up to those markings when it deviated from them. The growing period ended on the 26th of July.

2.2.2 Photography

Plants were recorded with an approximately minute-long video at regular intervals. Videos taken on the 29th of June(beginning date) and 22nd of July(ending date) were used for the base for data collection later. The recording was done manually holding the thermal camera over the plants and moving it slightly around to be sure all plants and reference plates were visible at one point. Coloured RGB-pictures of the plants was taken when the experiment was done and with a quality where plants were easily distinguishable from the soil. Pictures were taken approximately above 2 m from the plants.

2.2.3 Data collection

Thermal pictures were processed in with a digital images processing program. First pictures were greyscale with darker spots indicating higher temperatures. Pictures was looked through and then manually selected pictures that had the best quality. 3 images per block and for the beginning date and ending date, for a total of 30 pictures. Colour gradient of selected images was changed to a more easily distinguishable gradient called “Iron” with the yellow representing higher temperatures and purple representing colder (Figure 2C). RGB images were used as reference to see where plants were present. Each square with a plant in it was marked and later, using the box measurement tool each square was analysed to see the coldest point belonging to the plant canopy (Figure 2B). The coldest point was meant to represent the highest transpiration of the plant, and is often located in the centre of the canopy (Clawson et al. 1989). To ensure that the canopy was selected the RGB pictures thermal pictures were used together with the coloured pictures reference the to see that a plant was selected. For every picture the reference plate for each block was visible and selected with the circle tool (Figure 2C). After every plant and reference surface was marked the temperature was extracted and converted to an excel file.

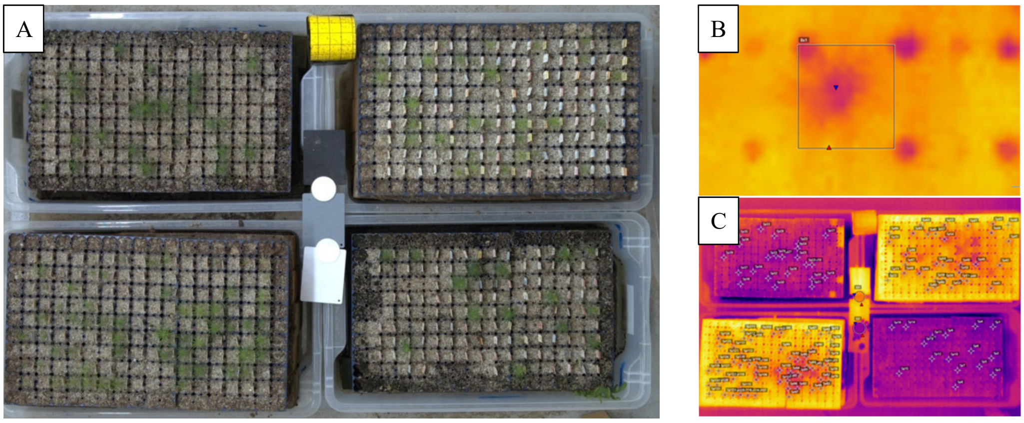


*Figure 2: Overview of temperature extraction of plants can be seen in the figure. In **A**, a picture of how the experiment was set up can be seen. It represent what one block looked like with drought treatment in top right and bottom left and the other two being control. Circles in the middle are dry reference(top) and wet reference(bottom). Picture **B** shows how the coldest point was identified using the box measurements tool. Blu triangle points to coldest point and red to hottest. Picture **C** Shows how the ending thermal picture might look with all plants marked. Cold reference can be seen as the circle at the bottom in the middle and warm reference above it.*

2.2.4 Data processing

Data was taken from the collection and compiled in excel documents. The wet temperature was calculated as an average of the wet reference surface and dry temperature as an average of the dry reference surface, this was done for every picture to get min and max values for each picture. Every block had the canopy temperature(T_c) recorded from the pictures chosen for that day. Using T_c, T_{wet}, and T_{dry} each plant´s CWSI was calculated. The average CWSI of each plant and for each date was then calculated as the average of the three pictures from each date. Those averages were later used to calculate CWSI for each family. Considering losses of plants during the experiment only families with 3 living individuals at the end of the experiment had their family average CWSI calculated. Another drought tolerance index was calculated by making a regression for the average tree growth, taking the volume for the drought affected plants and comparing them to the control plants. Values was calculated as deviance of families from the average. These values were also later divided into family averages in families with three or more individuals.

2.2.5 Statistical analyses.

Statistical analyses done were ANOVA, Post-Hoc, T-test, Pearson's corelation test and linear regression. ANOVA to see if source, region and family might have an effect on the CWSI. Also treatment, block and days after germination (DAG) as ambient factors were included in the ANOVA. Family and source were tested together with the ambient factors, while family was on its own. This is because including it would have divided the groups to much and lead to a lower statistical power in the analysis. When significance in the ANOVA was detected a post hoc (Dunnett's test) to see which groups differed. The CWSI values was estimated to be normally distributed, looking at histograms to see if it is reasonable. T- test to see if the CWSI for the beginning and ending date differed. Linear regression for the volumes relation to CWSI was done, and also linear regression for relation of canopy and root volume(C/R) to the CWSI. Both of these seemed to be affected by the treatment so linear regression divided for the treatment was done. Correlation between CWSI for families and DTI for families was done using Pearson's corelation test. Also different scatterplots with either volume or CWSI was done and heatmaps over these scatterplots were used to see whether pattern formed between DTI and CWSI. Boxplots were used to see how values might differ between groups. For statistical analyses the program R Statistical Software version 4.1.2 (R Core Team 2021) was used. Significance level was set to 0.05.

Results

Analyses was done both between individuals and between families. The first part of the results discusses results from data where data is separated to each individual, while the later part uses the average for a family to get the results.

For reference surfaces, the temperature should be the hottest and coldest the plants could get. Dry being the hottest and wet being the coldest. This was not always the case however, with some individuals being even cooler and hotter than the references. Leading to values above 1 and below 0.

3.1 Tolerance differences

Plants in drought blocks are expected to be more stressed than those in the control blocks. The higher values for CWSI in the drought blocks compared the controls for both dates also confirm this for this study (Figure 3). Drought stress is dependent on the treatment of the plant. This suggest that drought causes plants to be more stressed.

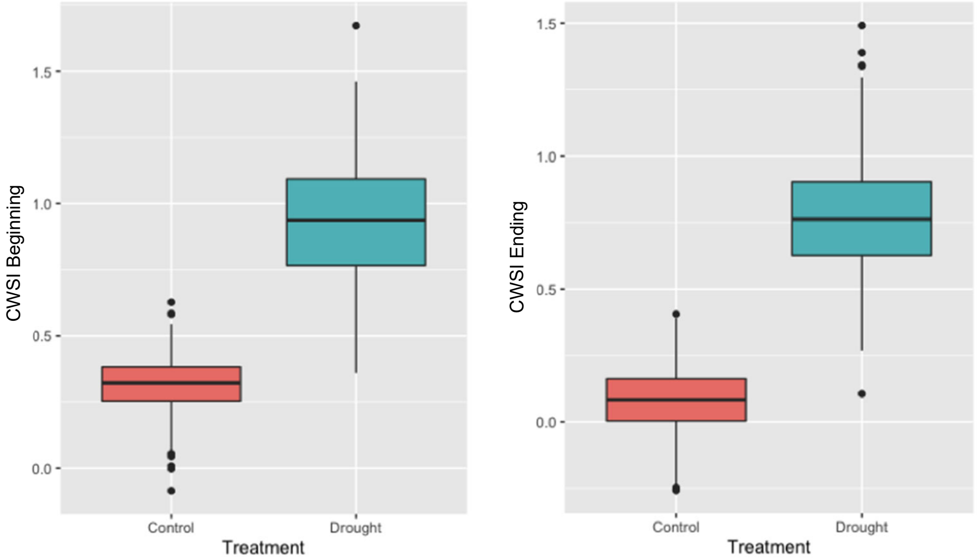


Figure 3: Boxplots for CWSI for the beginning date(left) and ending date(right) divided by the treatment. Red represents the control and blue the drought affected plants' CWSI.

A difference for the CWSI depending on the date could be observed (Figure 4). Lower CWSI on average on the ending date compared to the beginning. Doing a t-test revealed this difference to be significant(p: <0.001) (Table 1).

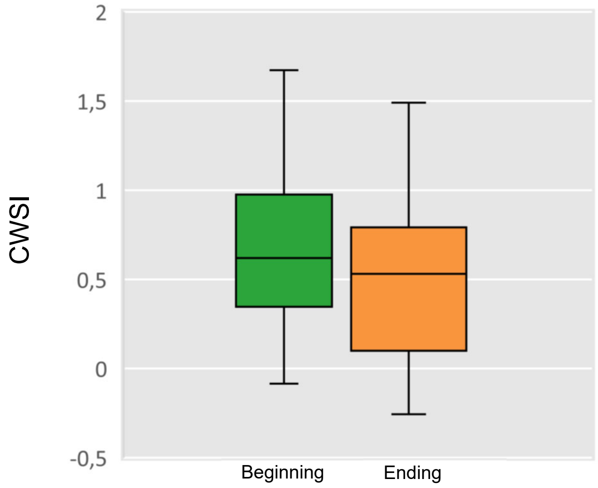


Figure 4: Boxplots for the CWSI for the plants on the beginning (green) and ending date(orange).

Table 1: Table showing results of a t-test between CWSI of the beginning and ending date. Revealing significance between the dates. Table has t-value, degrees of freedom(df) and p-value. Bold values indicate significance at 0.05 level.

CWSI Ending vs CWSI Beginning		
t-value	df	p-value
28.34	669	**< 0.001**

The plants ability to handle drought differed depending on the source of the plant (Figure 5) source in this case referring to whether material was taken from nature or a breeding program. ANOVA suggest there is an significant difference between the two sources average CWSI (Table 2). Plants from breeding programs had lower average CWSI than those from nature (Figure 5) Performing a Dunnett's post-hoc test also confirmed the statistical difference between these sources (Table 3), but only for the CWSI of the plants on the last date.

Table 2: Results of the ANOVA showing the results for the CWSI dependence on each date divided in two tests, one that used source(S) and region(R) and one for only families. Significance for all factors was shown. Bold values indicate significance at 0.05 level.

ANOVA	CWSI Beginning		CWSI Ending	
	S and R	Family	S and R	Family
Source	**0.039**	**-**	**< 0.001**	**-**
Region	**< 0.001**	**-**	**< 0.001**	**-**
Family	**-**	**< 0.001**	**-**	**< 0.001**
Block	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Treatment	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
DAG	**0.001**	**0.004**	**0.002**	**0.019**

Table 3: Results of the post hoc using Dunnett's test. Significance for all except source in beginning. Bold values indicate significance at 0.05 level.

Post hoc(Dunnett)	CWSI Beginning	CWSI Ending
Source	0.224	**0.0016**
Region	**0.016**	**< 0.001**

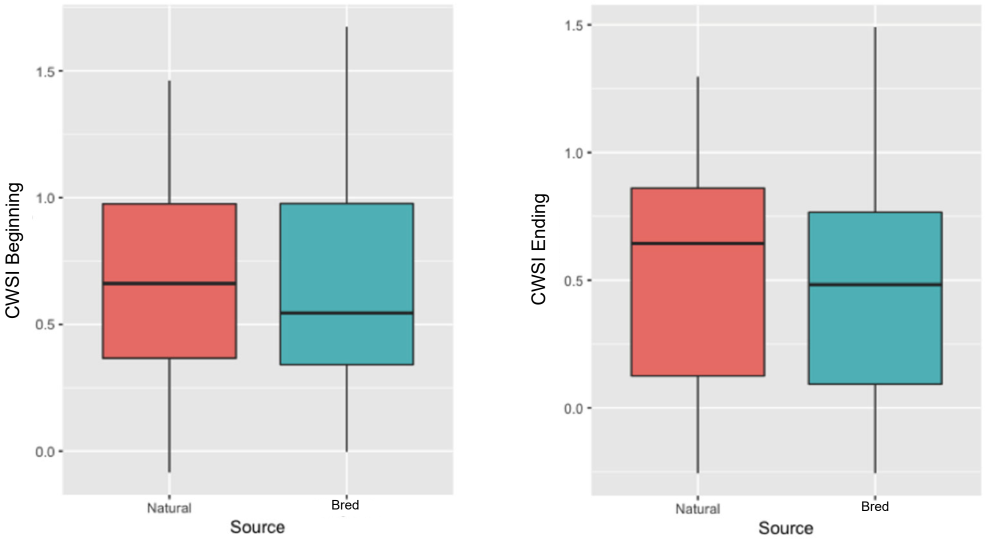


Figure 5: Box plot for CWSI beginning and ending date, divided by source. Lower values in the bred plants compared to the natural ones, suggesting higher transpiration. Red is natural and blue is bred.

Drought tolerance seem to have a significant difference between regions, with the southern parts having lower values for CWSI compared to the northern (Table 2; Figure 6). The factor region is referring to the whether plant material came from the north(Arjeplog, Jokmokk) or the south(Östersund). Dunnetts' test also reveals significance between north and south for both dates (Table 3).

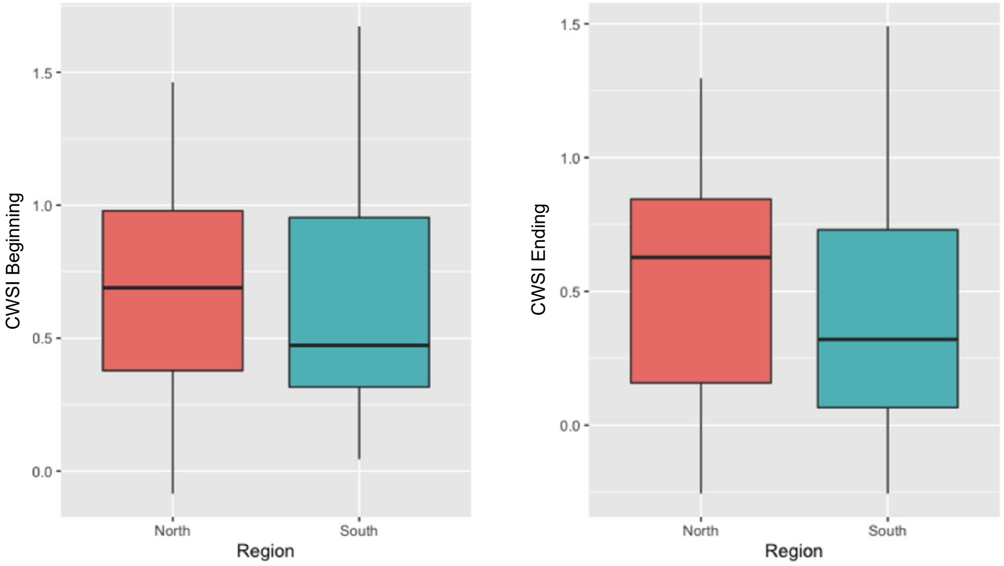


Figure 6: Boxplots for the CWSI of both dates looking at how the regions might differ. The southern parts seem to have lower CWSI on average. Southern in this case meaning plant material from Östersund and northern being both Arjeplog and Jokmokk.

From the ANOVA there could also be seen that some significant difference in the average CWSI depending on family, DAG, block and treatment (Table 2). No post-hoc was done for these factors. No post hoc for family because of the number of families being so high.

3.2 Drought tolerance and growth

Linear regression for relationship between CWSI and total volume of the plant or the canopy/root volume relationship(C/R) was done. For both also treatment was looked at, because it seemed to have a significant effect on the relation. Looking at Table 4 there seem to be no significant relation between the volume the and CWSI independent on the date, however the treatment and interaction are significant. Looking at figure 7A and 7B there seem to be a strong negative relation for volume and CWSI when the treatment is drought, however not so much if the treatment is control. A regression looking only at drought for the relation between CWSI and volume on the beginning date showed significance for the volume (estimate: -759, p-value: <0.001), same circumstances for the ending date gave similar results(estimate: -1073.79, p-value: <0.001). However when looking at the same relations in control for the beginning date (estimate: 6, p-value: 0.992) and ending date(estimate: 33, p-value: 0.945) the significance did not show.

Figure 7C and 7D show how the C/R was trending negatively with CWSI in control but no obvious trend in drought. Larger number in the C/R means more canopy in relation to the root. For the beginning there were significance in control (estimate: -2.33, p-value: <0.001) but not in drought (estimate: -0.06, p-value: 0.756). The ending date gave similar results with significance in control (estimate:

-2.34, p-value: <0.001) and not in drought (estimate: 0.257, p-value: 0.241). Negative correlation between correlation meaning that for higher CWSI you will get smaller volume, or smaller C/R relation.

Table 4: Table showing the results of linear regression for the entire CWSI of a date and either compared to the total volume of the plant or the canopy and root relation. Treatment was also included in the regression. Bold values indicate significance at 0.05 level.

Lin. Reg.	**CWSI Beginning**		**CWSI Ending**	
Variable	Total Volume	Canopy/Root	Total Volume	Canopy/Root
Intercept	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Volume	0.995	-	0.953	-
C/R Volume	-	**0.033**	-	**0.001**
Treatment	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Interaction	**<0.001**	0.2042	**< 0.001**	**0.001**

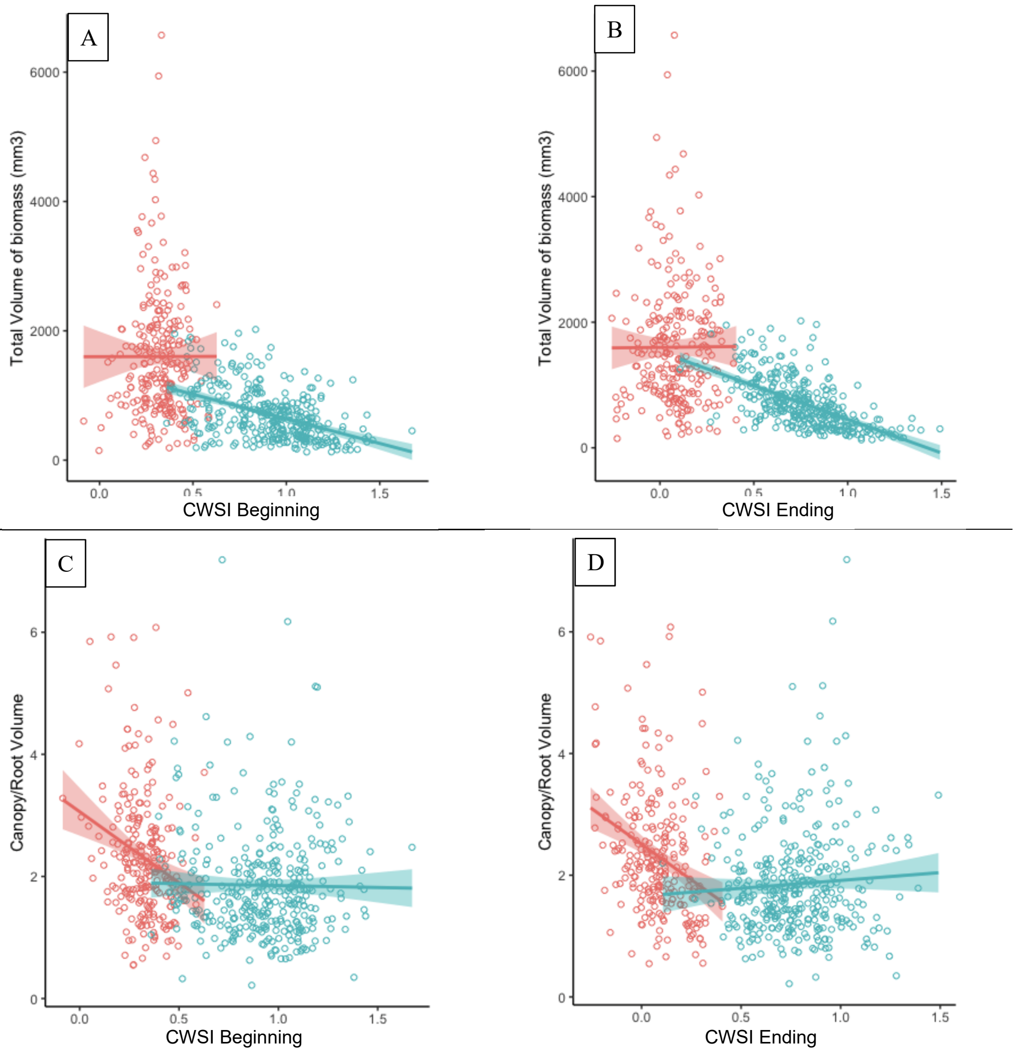


Figure 7: Graphs for total volume and canopy/root volume relation against CWSI. The red represent values from the control and blue the drought. The line for the relationships in each treatment can

be seen in each figure. In ***A*** *it is the relation between CWSI on the beginning date against volume.* ***B*** *has the CWSI of the ending against volume.* ***C*** *is the canopy/root relation against CWSI in the beginning. Lastly* ***D*** *is the canopy/root and CWSI in the ending date relation.*

3.3 Relationship between thermal indexes

CWSI for the beginning and ending date, in both the control and drought was checked for correlation with DTI using Pearson's correlation test (Table 5). No significance found except for CWSI of drought treatment in the end (cor: -0.337, p: 0.048), even if the p-value was close to being not significant. This only tests for linear correlation and there might still be some other kind of correlation between the two indexes. This only uses averages of families instead of all individuals.

Table 5: Results of a Pearson's correlation test between DTI and CWSI for both dates, divided by treatment. Bold values indicate significance at 0.05 level.

	CWSI Beginning				CWSI End			
Treatment	Control		Drought		Control		Drought	
	P-val	Cor	P-val	Cor	P-val	Cor	P-val	Cor
DTI	0.781	-0.048	0.304	-0.178	0.555	-0.103	**0.048**	**-0.337**

The DTI was calculated as the deviation from the average tree line for each of the families (Figure 8). Plants above the line are expected to be better at adapting to drought. Looking at the graph there seemed to be connection between fast growing plants and source of the plants, with southern plants seeming to grow faster (Figure 8). Deviation from line can be read as how much the volume gain per day (mm^3/day) differ from the expected volume gain per day.

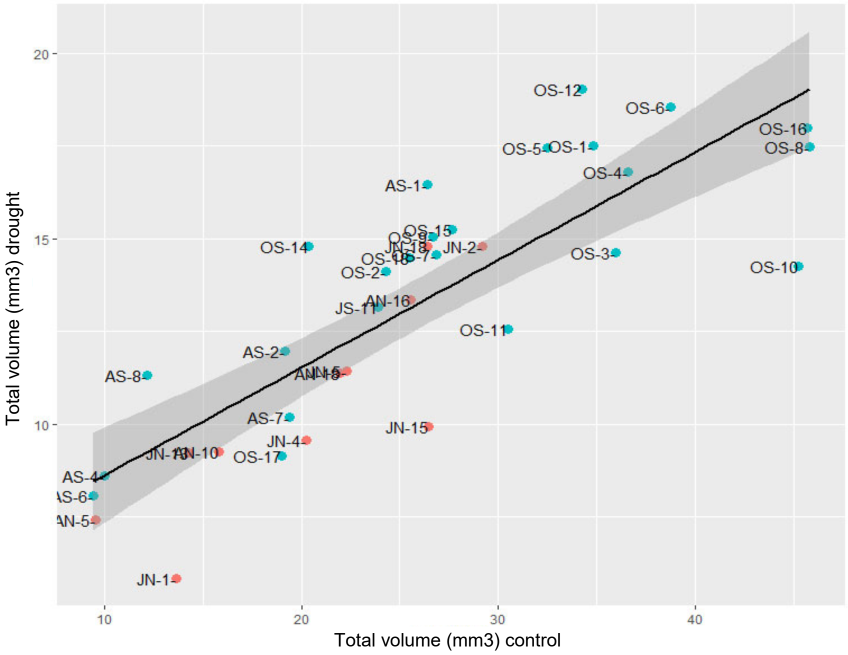
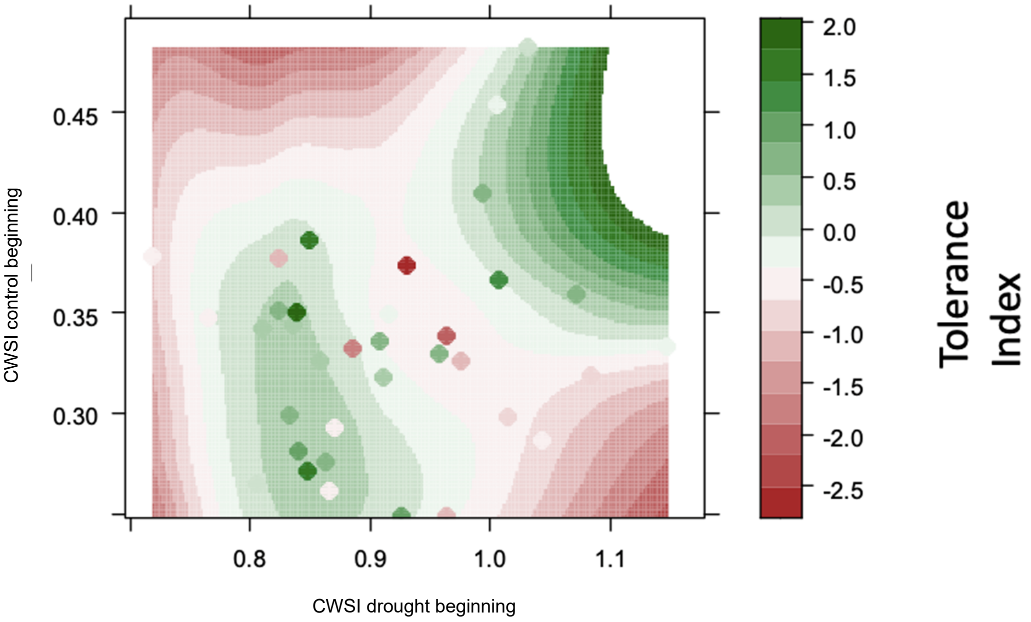


Figure 8: Graph showing how different families are spread out for the drought tolerance difference(DTI). DTI for a family is calculated as the family's deviation from the line. Blue dots are more southern families and orange dots are northern families.

Using a scatterplot between CWSI for the families in drought and control and then overlaying a heatmap of the DTI eventual patterns or relations between all three could be seen (Figure 7). One graph for each date was created. While not extremely clear there seem to be some kind of pattern forming in the bottom left corner having higher DTI for both dates.



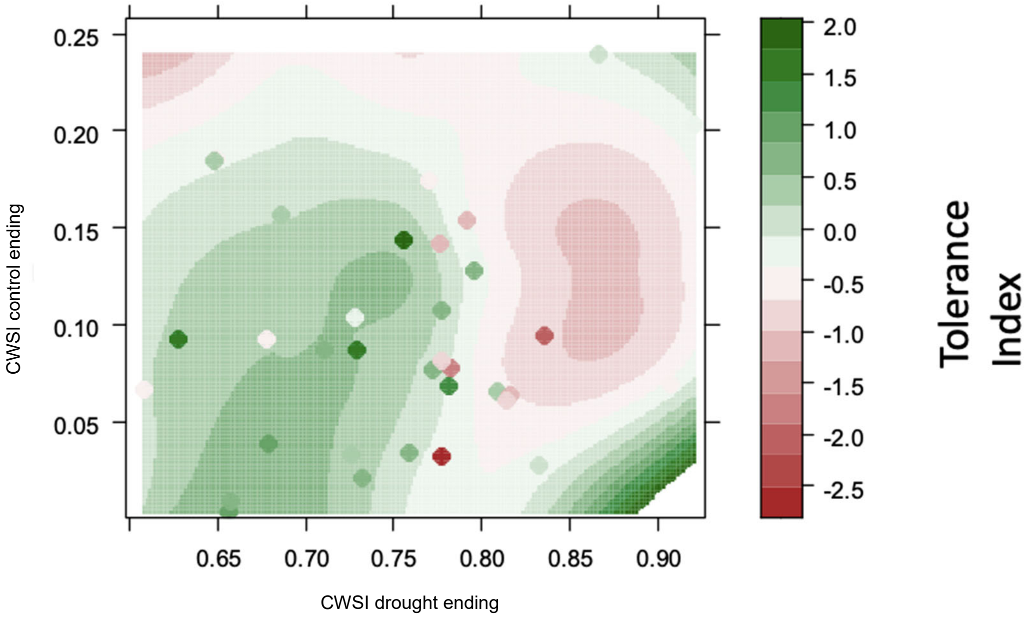


Figure 9: Scatterplots between CWSI in control and drought for both dates, with the DTI added as a heat map to show how these relate to one another. Top graph is for the beginning, bottom for the end. The green represents a higher DTI and brown a lower DTI. Please note the difference in scale for the CWSI for the both dates.

Taking the volume in drought and plotting it against the volume in control is how DTI was calculated, but overlaying a heatmap with each families CWSI can reveal interesting relation that might form. This makes it possible, for example, to see how a fast growing family with a high DTI might adapt, according to its CWSI. These graphs were done for both dates and for CWSI of both treatments. Different patterns could be seen depending on date and treatment. Further away form the dots the patterns of the heatmap are less confident, since fewer data points are used to form it.

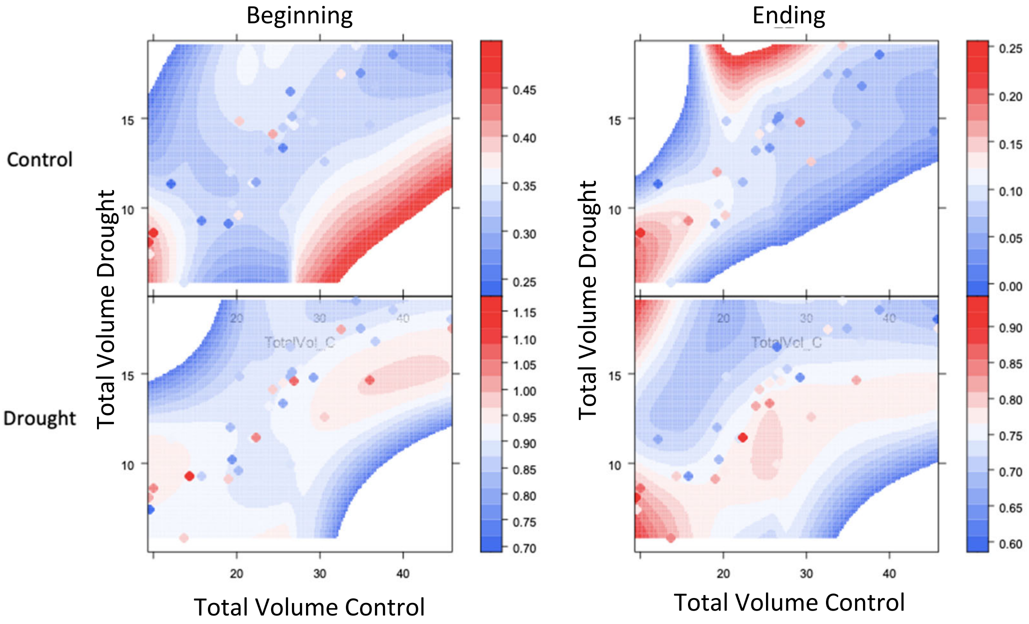


Figure 10: Graphs with volume for control and drought of families plotted against each other and the CWSI for each family on each dot as reference for a heatmap. CWSI of families in control in the upper graphs, bottom is drought. CWSI measurements from the beginning date in the left graphs, the right uses measurements from the end. Blue values represent a lower CWSI and red represents higher CWSI. Please note the difference in the scale in each graphs for the CWSI.

Discussion

4.1 Potential for phenotyping drought tolerance

There is potential for the use of thermal imaging in regards to drought tolerance. Estimating drought tolerance is hard and many ways of trying to estimate it have been tried. In this study two different indexes were used, CWSI and DTI. CWSI using transpiration and DTI using the volume of the plant. Even though no significant linear correlation between the two were found, except for the ending date and drought treatment($p = 0.048$, corelation= -0.337) (Table 5) there might still be some other correlation for the indexes. The correlation still being negative for all of them supports the idea that increasing transpiration and bigger plants are somehow connected.

Other relations between CWSI and DTI can be seen in Figure 9. Looking at Figure 9, when plotting the CWSI in the beginning and ending for each treatment a similar pattern can be seen. In both cases there seem to be a collection of families with both low CWSI in drought and control that also has high DTI. There is however some difference regarding the scale for CWSI depending on the date, meaning it is not a straightforward relationship. This might be because of the timescale of the drought, with longer droughts acting differently compared to shorter ones (Prajapati et al. 2021). Or it might be other ambient factors that differed and potentially affected the responses. Figuring out what might have caused it and then finding a way to adapt measurements to those cause might make it possible to find these relations later on.

Figure 10 also show patterns suggesting there might be a relation between the two indexes. Looking at all the graphs there seem to be a trend with all the slowest growing trees also having higher CWSI and therefore lower transpiration. This could be because of higher yielding plants also often being associated with better performance even under drought (Blum 2005) and CWSI being associated with transpiration and drought tolerance of a plant (Jones 2004). In the graphs for the droughts there seem to higher CWSI for plants with a lower DTI. It is not as clear in the graph for the beginning but the ending graph shows a pattern indicating it to be true. No obvious difference in the control is not a bad thing, since we are mostly interested in looking at the drought affected plants and see whether any responses because of stress from it happen. A response in the drought treatment not showing in the control may indicate that certain adaptions might happen because of drought. Figuring out specifics on how these patterns form might give a reasonable way for phenotyping drought tolerance.

Liner regression for the experiment showed promise when looking at the volume compared to the CWSI (Figure 7A and 7B). In drought conditions there seemed to be a strong negative correlation between them. Meaning bigger plants transpire more and therefore are more drought tolerant. This has been shown to be the case for other plants (Lopes et al. 2012), and higher yielding plants have been thought to handle drought better (Blum 2005). It not being as obvious in the control might be because of different factors affecting the growth more and stress not being a limiting factor. The phenotyping process is not really limited by this in regards to drought tolerance, since we are interested in how they respond to drought and what might contribute to these responses. All this pointing to thermal imaging being a possible way to phenotype drought tolerance.

The relation for CWSI and canopy/root showed negative correlation for control which indicates smaller canopy in relation to the root leads to worse transpiration and therefore drought tolerance (Figure 7B and 7C). In the drought no significant correlation could be seen, meaning some kind of change caused the pattern from control not appear in the drought. In drought it almost seem like the relation does not matter.

Difference in CWSI between dates might indicate an change over time in adaption of the plants. However the shown decrease in stress might not be expected (Seidel et al. 2016) and could be indicative of some shortcomings in this experiment. More dates to follow the CWSI more closely could have been beneficial.

Something that needs to be remembered is that this experiment was carried out in a greenhouse and might not translate to all other environment, especially natural ones.

4.2 High throughput phenotyping

The possibility of high throughput phenotyping for drought tolerance seem positive. Many correlations and patterns discussed in the previous paragraph show that drought tolerance had many significant and potential ways to asses it using the information gained from the infrared light. Because of the scaling capabilities of thermal imaging (Pineda et al. 2021) and the information you are able to get from just using it, high throughput phenotyping for drought tolerance using thermal imaging seems like it is feasible.

For the phenotyping to be as efficient as possible it could still improve some. For a better efficiency, the canopy selection need to be automated. Manually selecting plants is a slow process and comes with a risk of results becoming subjective (Leinonen & Jones 2004). Automated solutions has its own problems, especially in the field. If the solution is using thermal radiation to differentiate canopy from non-canopy the risk of not selecting the desired area increases if there is an anomaly. Using RGB pictures require that the thermal pictures are able to align and threshold of picture values do not exclude part of the plant (Leinonen & Jones 2004).

4.3 Performance

Source of the plant might matter because of the bred plants in general growing larger (Andersson et al. 2007). Bigger plants also have more transpiration which could contribute to the ability to mitigate water stress (Blum 2005). There not being any significant difference the first date for the source might be a sign that initial performance difference might not be too big. It being more relevant later could also be an indication that dynamics of a drought differ depending on how long it has been going on (Prajapati et al. 2021) and therefore adaptions might differ.

Similarly to the source there is also a difference in growth between plants depending on the region they originally come from. Difference in drought tolerance depending on region has been seen in other experiments (Seidel et al. 2016). The difference in regions might be because of more southern plants generally growing faster compared to the more northern (Persson & Ståhl 1990).

In this study which families was performed better was not evaluated. It was only seen that there seem to be a significant difference between families, where this difference occur is however unknown. This is interesting because it means it might be possible to identify these families in other studies and see what might make them better than others.

4.4 Improvements

There were some problems with pictures being blurry. A picture with too much motion blur can lead to the object appearing significantly cooler than it truly is (Oswald-Tranta 2018). This can lead to problem when selecting pictures for analysis. When selecting pictures, some were to blurry to see individual plants and in some cases, selecting a picture without any plant at least partly affected by motion blur was impossible. Number of blurry pictures was kept to a minimum, but the pictures selected was purely subjectively assessed to be the least blurry. The loss of details and potential alteration of the plants actual temperature have made the temperature registration part harder and could have affected the end results.

Use of RGB picture overlay on thermal images could have made it easier and certain that part of the plant was selected. There was a possibility that coldest part in a square were not part of any plant and because of a mistake was still selected to represent the plant in question. In some cases the plants' location was difficult to make out and without an RGB picture taken from the same position the location was guessed and coldest point chosen. These situations could have been avoided or at least limited with the option to toggle between infra-red and RGB view to see how plants were positioned. The RBG pictures that were available helped with identifying in which square plants were present, but it was unreliable in revealing the exact positions of the plants. This was because of a difference in the angle of the photographs making it impossible to line up perfectly (Zhou et al. 2021).

More dates to see how the plants changed over time could lead to a better understanding of differences between dates and possible changing adaptations over the course of the experiment. Instead of just looking at the beginning and end it might give some clues on how it changes over time.

4.5 Future

This test was done for a summer and a longer test period might be necessary to see if length of the drought, or repeating droughts could change which plants that are most tolerant (Bose et al. 2020). Will benefits persist in the future or will the previous stress be too much for the plant. As it stands now it only looks at how seedlings handle the stress from drought in a greenhouse environment.

As with most greenhouse experiments it is good to see if results can be replicated in the field. If it only is representative in a greenhouse results in the field could be different. The phenotyping might be still possible in a greenhouse, but the tolerances still need to be tested in the field to see if benefits persist.

There have been some experiments done with the relation between color of the leaves and the chlorophyll in them (Fawzy et al. 2022)(Riccardi et al. 2014). Because of the relation between chlorophyll and drought stress it could be possible to use a RGB camera to estimate the drought tolerance of a plant using only a colored picture (Riccardi et al. 2014).

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Publicering och arkivering

Godkända självständiga arbeten (examensarbeten) vid SLU publiceras elektroniskt. Som student äger du upphovsrätten till ditt arbete och behöver godkänna publiceringen. Om du kryssar i **JA**, så kommer fulltexten (pdf-filen) och metadata bli synliga och sökbara på internet. Om du kryssar i **NEJ**, kommer endast metadata och sammanfattning bli synliga och sökbara. Även om du inte publicerar fulltexten kommer den arkiveras digitalt. Om fler än en person har skrivit arbetet gäller krysset för samtliga författare. Läs om SLU:s publiceringsavtal här:

☒ JA, jag/vi ger härmed min/vår tillåtelse till att föreliggande arbete publiceras enligt SLU:s avtal om överlåtelse av rätt att publicera verk.

☐ NEJ, jag/vi ger inte min/vår tillåtelse att publicera fulltexten av föreliggande arbete. Arbetet laddas dock upp för arkivering och metadata och sammanfattning blir synliga och sökbara.