
¹ Aim

² As climate continues to warm, ecosystems are facing more extreme heat and drought waves. At the
³ same time the potential growing season of temperate and boreal latitudes extends. To which degree
⁴ plants and forests adapt and indeed prolong their photosynthetic activity in spring and autumn is
⁵ currently under heavy debate. Not only may soil moisture resources limit plant activity and overall
⁶ performance but also internal growth control mechanisms could limit further Carbon uptake from the
⁷ atmosphere. Therefore, this experimental study aimed to provide evidence how longer climatic growing
⁸ seasons translate into increased biomass production in relation to the negative impacts of drought and
⁹ heat events.

¹⁰ Methods

¹¹ Study species and study site

¹² 3 year-old saplings of 6 species, each representing a different family were selected to get a wide range of
¹³ possible tree responses including coniferous evergreen and broad leaved deciduous species. All species
¹⁴ selected occur naturally along the Pacific west coast of USA and Canada. The studied deciduous trees
¹⁵ were *Prunus virginiana* L., *Acer macrophyllum* Pursh., *Betula papyfera* Marsh. and *Quercus garryana*
¹⁶ Dougl.; evergreen trees were *Pinus contorta* Dougl., and *Sequoia sempervirens* (D. Don) Endl. In the
¹⁷ following we refer to their genus name only.

Table 1: Species information

Species name	Family	Initial height	drought tolerance	remarks
<i>Prunus virginiana</i> L.	Rosaceae	x	x	x
<i>Acer macrophyllum</i> Pursh.	Sapindaceae	x	x	x
<i>Betula papyfera</i> Marsh.	Betulaceae	x	x	x
<i>Quercus garryana</i> Dougl.	Fagaceae	x	x	x
<i>Pinus contorta</i> Dougl.	Pinaceae	x	x	x
<i>Sequoia sempervirens</i> (D. Don) Endl.	Cupressaceae	x	x	x

¹⁸ The study was conducted on the campus of the University of British Columbia (Totem Field; 49.2572
¹⁹ N, -123.2503 E) located in Vancouver, Canada. The climate is oceanic characterized by the proximity of
²⁰ the Pacific with a mean annual temperature around 10°C (18°C in July and 4°C in January). Together
²¹ with an annual precipitation of c. 1500mm this climate supports a typical temperate rain forest along
²² the west coast.

²³ Experimental setup

²⁴ Saplings arrived in Winter 2023 and were, still dormant, repotted using a medium for perennials con-
²⁵ sisting of 50% peat, 25% crushed pumice and 25% crushed bark (www.westcreekfarm.com). The low
²⁶ water-retention capacity of this potting medium allowed to accelerate and intensify the effects of the
²⁷ drought treatments. Soil volume was adjusted for each species, specifically doubled in volume com-
²⁸ pared to the previous container to minimize limitations later in the season (final pot volume: 4.5l for
²⁹ *Sequoia* and *Pinus*; 9l for *Quercus* and *Betula*; 18l for *Acer* and *Prunus*).

³⁰ After potting, saplings received 2g of slow-release NPK fertilizer (osmocote plus) to meet natural con-
³¹ ditions.

³² On 31 March 2023, saplings were transferred to cooling chambers set at 4°C with ambient photoperiod

33 conditions to prolong dormancy for one month (until 30 April 2023). The only exception was the
34 saplings designated for growing season extension, which remained at the experimental site.
35 Saplings were then arranged in three blocks, each containing a subset of all treatments. Two blocks
36 were sheltered from rain by an open-walled and well ventilated polytunnel greenhouse to protect sen-
37 sitive electronics. All saplings were attached to a drip irrigation system (40 PVC frame from Netafilm
38 with a Toro controller) that ensured saturated soil moisture conditions throughout the experiment
39 (120±6 ml water every 6 hours; except for the drought treatment duration).

40

41 Study design and treatments

42 The whole study design is depicted in Fig. 8 for an overview. Saplings were subjected to a) a growing
43 season extension, b) one out of 3 drought timings, c) one out of 3 defoliation events and d) a heat event,
44 resulting in eight treatments plus control. 15 replicates were randomly assigned to each treatment (8
45 treatments plus control à 15 replicates = 135 saplings/species).

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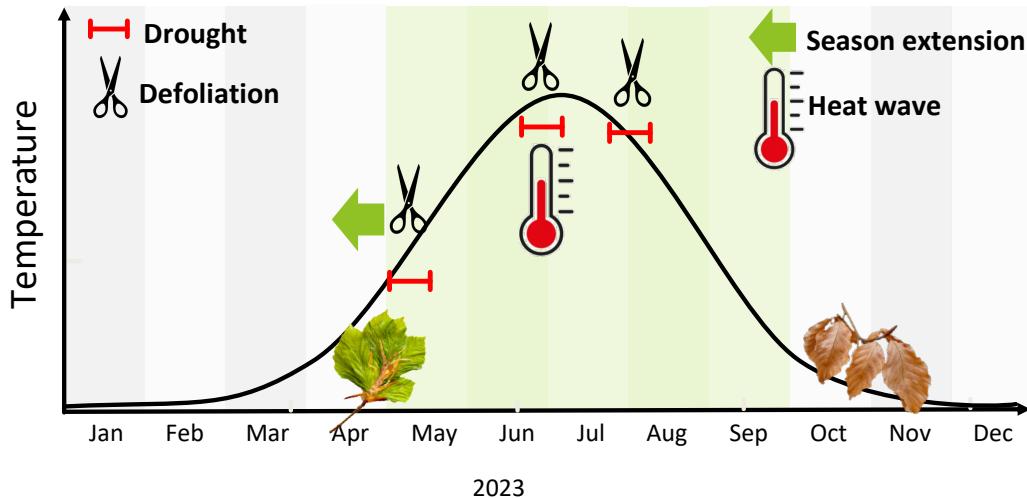


Figure 1: Schematic overview of the average temperature curve at the field site with the vegetation season in green. Depicted are all types and timings of treatments during the year 2023

47 *Growing season extension*

48 Growing season extensions were achieved by prolonging dormancy of all other treatments for a month
49 (see above). Hence sampling of this treatment accumulated xxx more growing degree days (GDD) by
50 being exposed to ambient conditions. Depending on species this ‘warming’ advanced budburst by X
51 to Y days (see XX).

52

53 *Drought treatments*

54 Drought treatments were conducted in climate chambers (TPC-19, Biochambers; Canada) at close
55 proximity to the experimental site (Faculty of Forestry, UBC). Drought conditions were simulated
56 with temperatures set to 30°C during the day and 20°C at night. These temperatures rose and fell
57 at the same time every day, corresponding to the photoperiod at Vancouver’s summer solstice (i.e.
58 photoperiod: 16h and 15min). The photoperiod was adjusted weekly, to the current ambient sunrise
59 and sunset time. The first drought treatment started species-specific once leaf-out reached stage 4 (i.e.
60 leaves fully unfolded). Second and third drought treatment were started on a fixed date, namely 23
61 June and 31 July 2023. Subsequent drying of the pots was monitored by measuring whole pot weight

62 (balance accuracy 0.1g) as well as volumetric water content (VWC, Fieldscout TDR 150). Saplings
63 were released from drought stress on species-specific dates, marked by the first signs of desiccation, such
64 as curled or discolored leaves, and soil moisture levels approaching the wilting point. Saplings were
65 again weighted under field capacity and then transferred back to the experimental site and plugged
66 into the irrigation system.

67

68 *Defoliation treatments*

69 The defoliation treatments were intended to simulate leaf loss due to frost, browsing, hail or overheating.
70 As these scenarios cause different physiological reactions (e.g. release of defence substances), we
71 cut off each fully unfolded leaf (stage 4) halfway up the petiole using pruning scissors. Younger stages
72 were left intact to prevent accidental damage to the meristem. The leaf area was reduced to 0% for
73 all deciduous species. For pines, all needles older than 1 year were removed by hand by tearing them
74 delicately in the direction of the apex. The current year needles were preserved in the first defoliation
75 treatment since they were less than 1cm in length and still developing. In the second and third de-
76 foliation event c. $\frac{3}{4}$ of the current-year needles were removed, which presumably contributed already
77 most to the total photosynthetic assimilation. All defoliation events coincided with the start of the
78 respective drought treatments, i.e. the first defoliation took place on the same day as the start of
79 the first drought treatment. In the following two weeks we continuously cut all newly emerging leaves
80 reaching stage 4 to suspend all assimilate supply. Subsequent recovery of saplings was assessed by eye
81 as the percentage of recovering leaf area compared to a control sapling.

82

83 *Heat treatment*

84 The simulated heat wave (walk in climate chamber, LTRB, BioChambers) aimed to bring saplings to
85 their upper temperature threshold where growth and photosynthesis ceases. The treatment started
86 together with the second drought timing (23 June) and lasted the same species-specific duration.
87 Temperature followed ambient photoperiod with temperature reaching up to 39°C during the day and
88 29°C during the night. Saplings were watered every day to saturation and relative humidity was around
89 90% to avoid cooling by transpiration.

90 **Phenological monitoring**

91 *Leaf emergence*

92 Bud development in spring was assessed by the same observer twice a week starting 24 April 2023
93 using a categorical scale depending on species. Deciduous species were scored on a four-stage scale
94 (see (Vitasse *et al.*, 2013)): stage 0 - dormant, stage 1 - bud swelling, stage 2 - bud burst, stage 3 -
95 leaf-out and stage 4 - leaf unfolded. Pine saplings were scored differently as follows: stage 1 - swelling
96 or elongation of shoot visible, stage 2 - green needle tips along the shoot visible, stage 3 - scales open
97 along the shoot and first needles become visible, stage 4 - green needles emerging away from the shoot.
98 Phenostages for Sequoia were limited to two stages because this species does not form buds: stage 1
99 - first signs of needles visible at the apical meristem but all bended inwards towards the center, stage
100 2 - needles start to grow and bend outwards from the center. For all species and saplings the day of
101 year was recorded as soon as 50% of all buds reached the newest stage.

102

103 *Bud set*

104 Cessation of bud development was monitored starting in early July 2023 until the apical bud was
105 dormant. Bud set was generally scored on a four-stage score as follows: stage 3 - ongoing shoot
106 growth/elongation, stage 2 - apical bud forms and remains as a light-green bud with the last (pair)
107 of leaves remaining small, stage 1 - first bud scales appear, stage 0 - bud turns dark red/brown and
108 hardens. In Acer only stages 3, 2 and 0 were distinguished and recorded. Bud set of Pinus and Sequoia
109 were not monitored, since shoot elongation was the best activity proxy of the shoot apical meristem.

110

111 *Leaf senescence*

112 Leaf senescence was monitored in weekly intervals between 1 Sept 2023 and 4 Nov 2023 i.e. until
 113 all leaves were shed. For each sapling and at every monitoring occasion the chlorophyll content was
 114 estimated using a leaf spectral index (LSI; mean of three representative leaves per replicate; MC-
 115 100, apogee instruments). Following (?) this value was weighted by simultaneous estimates of the
 116 percentage of remaining green leaves (by eye; 100% = all leaves remaining; 0% = all leaves shed). For
 117 example, a sapling with 50% remaining leaves and a mean LSI of 10 was rated with a total LSI of
 118 10 ($0.5 * 20$). A sapling was considered senescent once a value was below 50% of the maximum LSI value.
 119

Table 2: Phenological stages used for all deciduous species (Vitasse *et al.*, 2013), pine as well as Sequoia

Group	Scale	Phenostage	Description
<i>Deciduous species</i>			
	0	dormant	no bud development visible
	1	bud swelling	swollen and/or elongating buds
	2	budburst	bud scales open and leaves partially visible
	3	leaf-out	leaves fully emerged from bud but still folded, crinkled or pendant
	4	leaf unfolding	leaves fully unfolded
<i>Pine</i>			
	0	dormant	no signs of activity
	1	swelling	swelling or elongation of shoot visible
	2	budburst	green needle tips along the shoot visible
	3	leaf-out	scales open along the shoot and first needles become visible
	4	leaf-unfolding	green needles emerging away from the shoot
<i>Sequoia</i>			
	1	not active	first signs of needles visible at the apical meristem but all bent inwards towards the center
	2	active	needles start to grow and bend outwards from the center

120 **Soil moisture measurements**

121 Soil drying during drought treatments was documented with daily volumetric water content (VWC)
 122 measurements using a soil moisture meter (Fieldscout TDR 150; rod length 12cm or 20cm for small
 123 or large pots, respectively). In addition, and for a more integrated indicator of soil water loss near
 124 the wilting point, whole pots were weighed using a scale (accuracy $\pm 1g$). Replicates equipped with
 125 magnetic dendrometers were weighed at the start and end of the drought treatments only.

126 **Shoot apical growth**

127 Shoot growth activity of the apical meristem was measured on 10 replicates per treatment throughout
 128 the season in biweekly intervals. Water resistant measuring tapes were attached to the stem base under
 129 the terminal bud after budburst and subsequent shoot elongation was tracked on the tape.
 130

¹³¹ **Radial growth and tree-water deficit**

¹³² To track radial growth, magnetic dendrometers were installed that were designed to not injure the
¹³³ bark during installation and operate without friction (Clonch *et al.*, 2021). Devices were installed at
¹³⁴ the stem base avoiding branches and abnormalities using breathable bandage material (). Five control
¹³⁵ replicates were equipped permanently while drought treatments switched devices so that five replicates
¹³⁶ of every drought timing captured diameter fluctuations 1 week prior, during and 2 weeks after the
¹³⁷ respective drought treatment.

¹³⁸

¹³⁹ **Biomass assessment**

¹⁴⁰ Before budburst and after the growing season we measured diameter c. 2 cm above plant collar (digital
¹⁴¹ calliper; accuracy $\pm 0.1\text{mm}$) and height (graduated pole; accuracy $\pm 1\text{mm}$) of each sapling. Total
¹⁴² above-ground biomass was estimated following allometric equations provided by Annighöfer *et al.*
¹⁴³ (2016). Subtracting before from after season estimates revealed the calculated above-ground biomass
¹⁴⁴ increment.

¹⁴⁵ After entering full dormancy, all saplings were removed from pots in December 2023 to wash off the
¹⁴⁶ potting substrate. Whole saplings were dried at 80°C for 48h before tissue was separated into roots
¹⁴⁷ and shoots with the latter being further sorted into current year and past years tissue. All partial
¹⁴⁸ quantities were weighted to an accuracy of $\pm 0.01\text{g}$.

¹⁴⁹

¹⁵⁰ **Wood anatomy**

¹⁵¹ Prior to every drought and defoliation event, 10 replicates were pinned at a homogenous stem section
¹⁵² at the base using a needle and dyed with ethylene blue (Gärtner & Farahat, 2021). This pinning hole
¹⁵³ acted as a ‘marker in time’ that allowed to separate wood formation before and after treatment start.
¹⁵⁴ During harvest at the end of the growing season, a 2cm section containing the pinning hole was cut
¹⁵⁵ using an electric saw and stored in 35% ethanol solution.

¹⁵⁶ C. four stem sections per replicate were cut using a microtome (semi-automated Lab-microtome, WSL;
¹⁵⁷ thickness: $15\mu\text{m}$) to ensure the visibility of the anatomical structure around the pinning hole. Sections
¹⁵⁸ were double stained with Safranin (to color lignified structures red) and Astrablue (to color unlignified
¹⁵⁹ structures blue) following standard protocol (Gärtner & Schweingruber, 2013). Sampled where then
¹⁶⁰ fixed using UV-sensitive mounting medium (Eukitt UV, Fisher scientific) and dried under a commercial
¹⁶¹ nail dryer UV-lamp for c. two minutes. Samples were then scanned with a slide scanner () and then
¹⁶² processed with xxxx.

¹⁶³ **Data analysis and statistics**

¹⁶⁴ **Preliminary results**

¹⁶⁵ Preliminary results indicate that early successional tree species are more affected by defoliation and
¹⁶⁶ drought events than late successional species. Defoliation and particularly late drought events substantially
¹⁶⁷ reduced their total biomass accumulation compared to control saplings. This finding is surprising
¹⁶⁸ since pioneer species can be expected to be more plastic and may cope better with stress. However,
¹⁶⁹ many early successional species pursue an indeterminate growth strategy. This means that only a small
¹⁷⁰ fraction of their leaf and stem tissue was already preformed and overwintered in buds. Consequently,
¹⁷¹ they rely on conditions in the current year to form new tissue (neogrowth atop preformed tissue) which
¹⁷² make them more sensitive to current year conditions. The degree of determinism defines how much
¹⁷³ a tree species invests already into next years growth. With increasing frequency of stress events but

¹⁷⁴ also with the potential of extending the growing season length this trait seems to be crucial for our
¹⁷⁵ mechanistic understanding of how trees will respond in a future climate

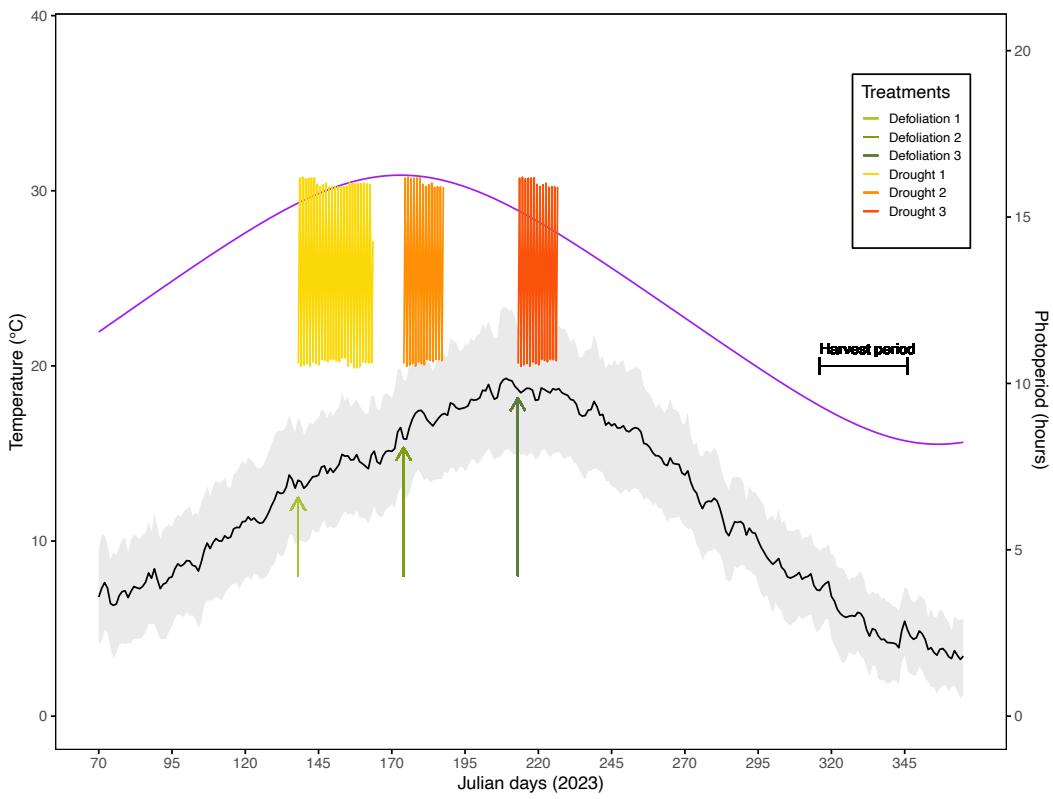


Figure 2: Daily mean (solid black line) and min/max (shaded area) temperature as well as photoperiod at the experimental site at UBC. Temperature during the three drought treatments are shown in yellow, orange and red. Arrows indicate defoliation events.

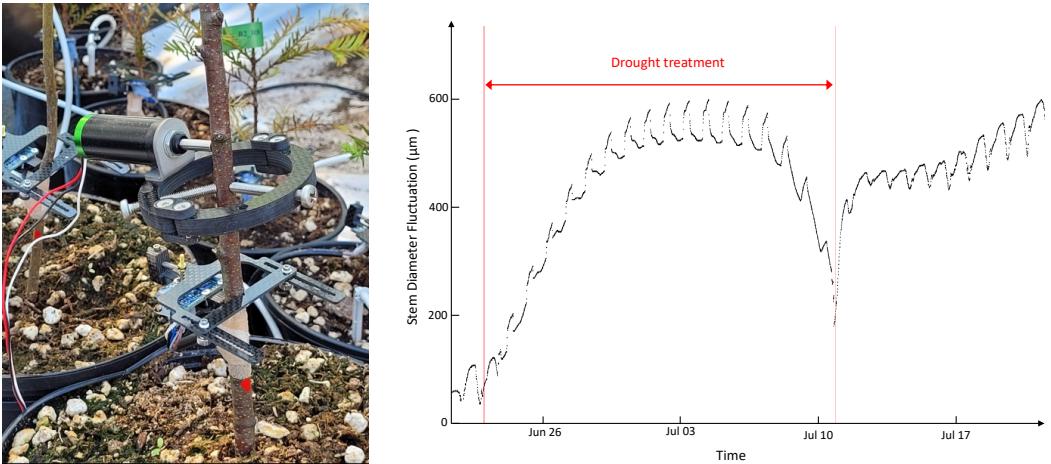


Figure 3: Left: Newly developed magnetic dendrometer (at the stem base) were used to monitor radial growth in a 15 min interval. Their performance was compared to classical point dendrometers (upper part of the stem). Right: Example of the changes in stem diameter of a Bigleaf maple sapling during a drought treatment and subsequent recovery phase.

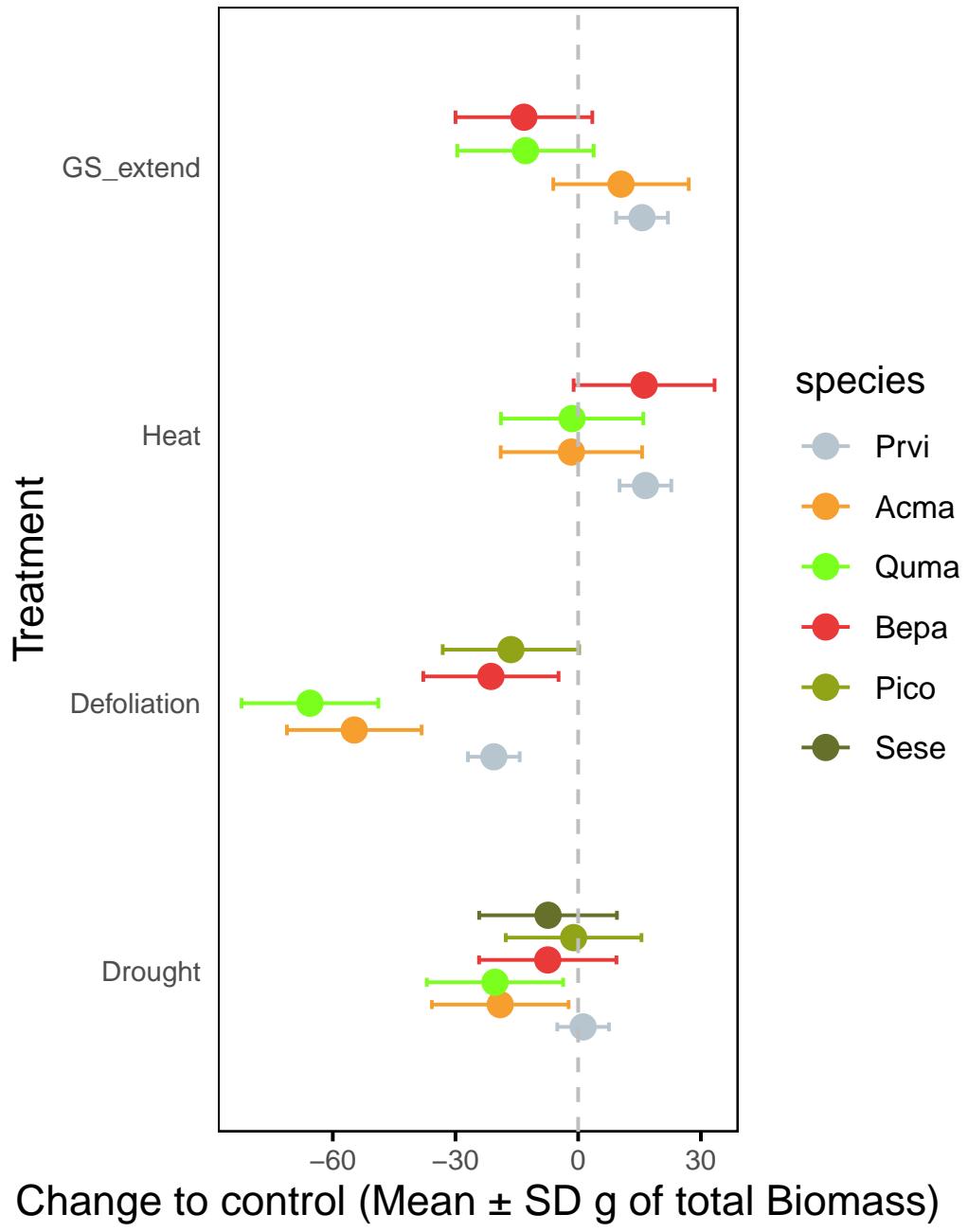


Figure 4: Effect size in g of biomass compared to control sapling when exposed to an extended growing season (GS_extend), heat, defoliation or drought event. Colors represent the six study species.

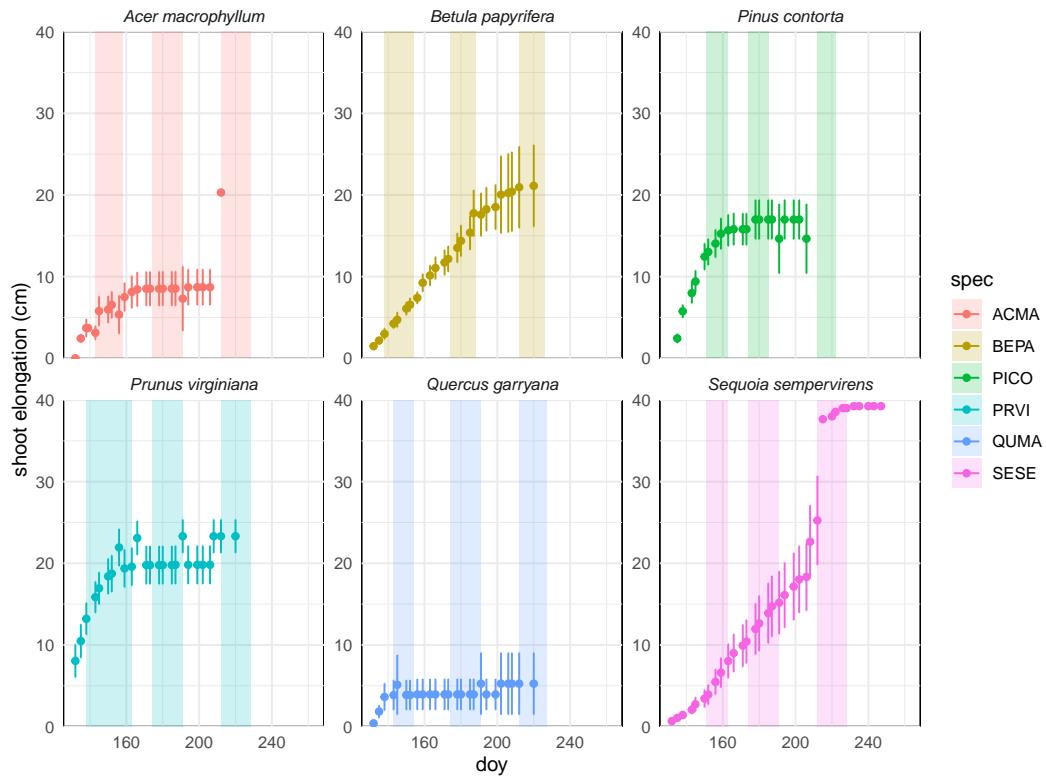


Figure 5: Shoot extension over the growing season 2023 for the six study species. Note the species-specific differences in absolute growth and in growth phenology with Quercus stopping first and Sequoia elongating until the very end of the season.

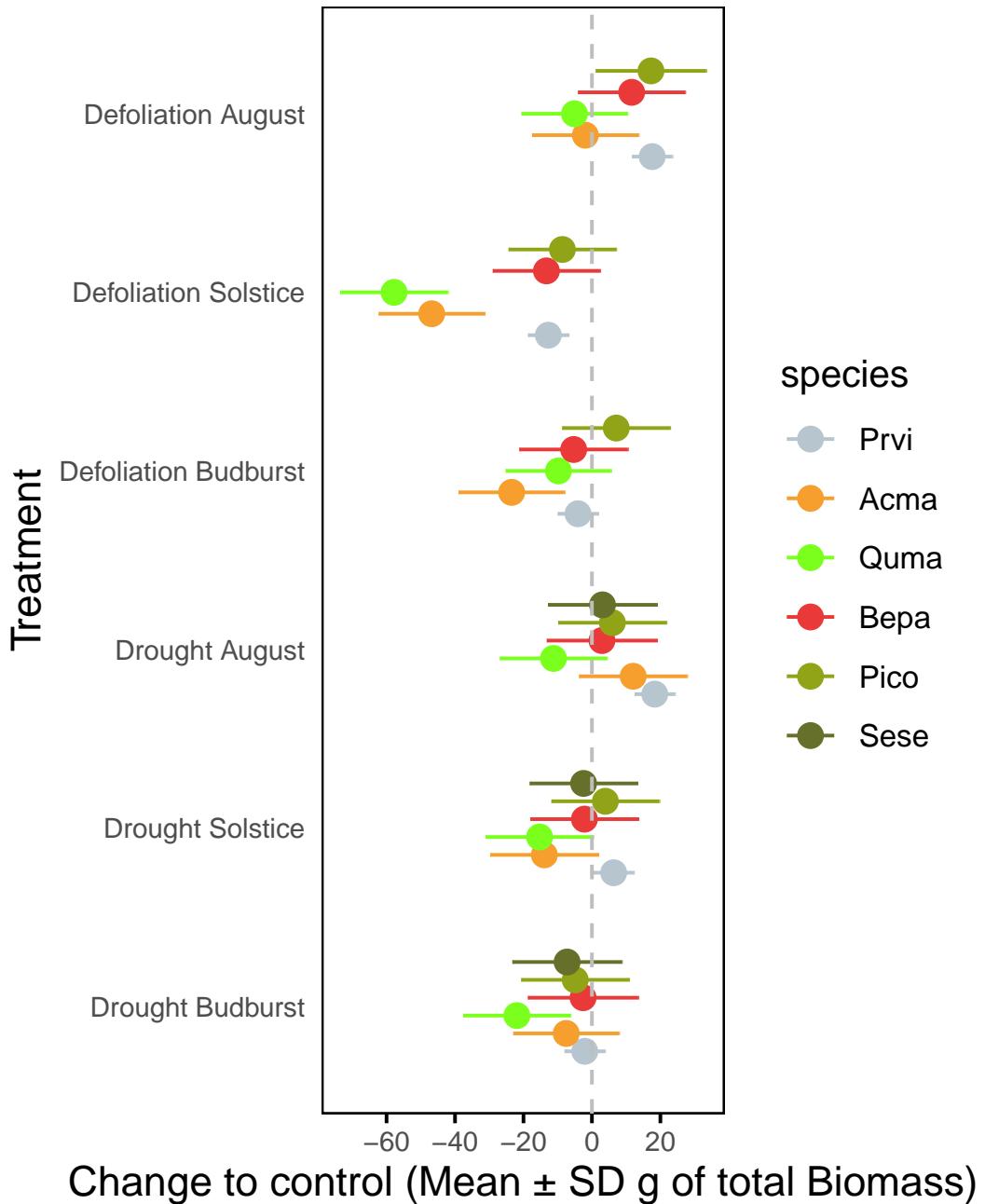


Figure 6: Effect size in g of biomass compared to control sapling when exposed to defoliation or drought treatments on 3 occasions. Colors represent the six study species.

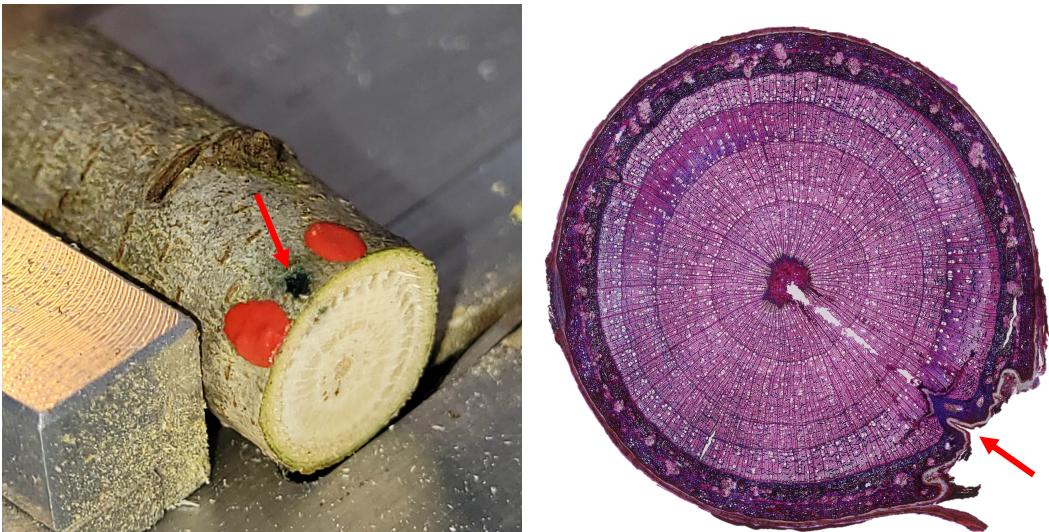


Figure 7: Left: stem section cut with an electrical saw close to the marked pinning hole. Right: 15µm cross-section of *Betula papyrifera* double stained with Astrablue and Safranin. Pinning holes are indicated with the arrow

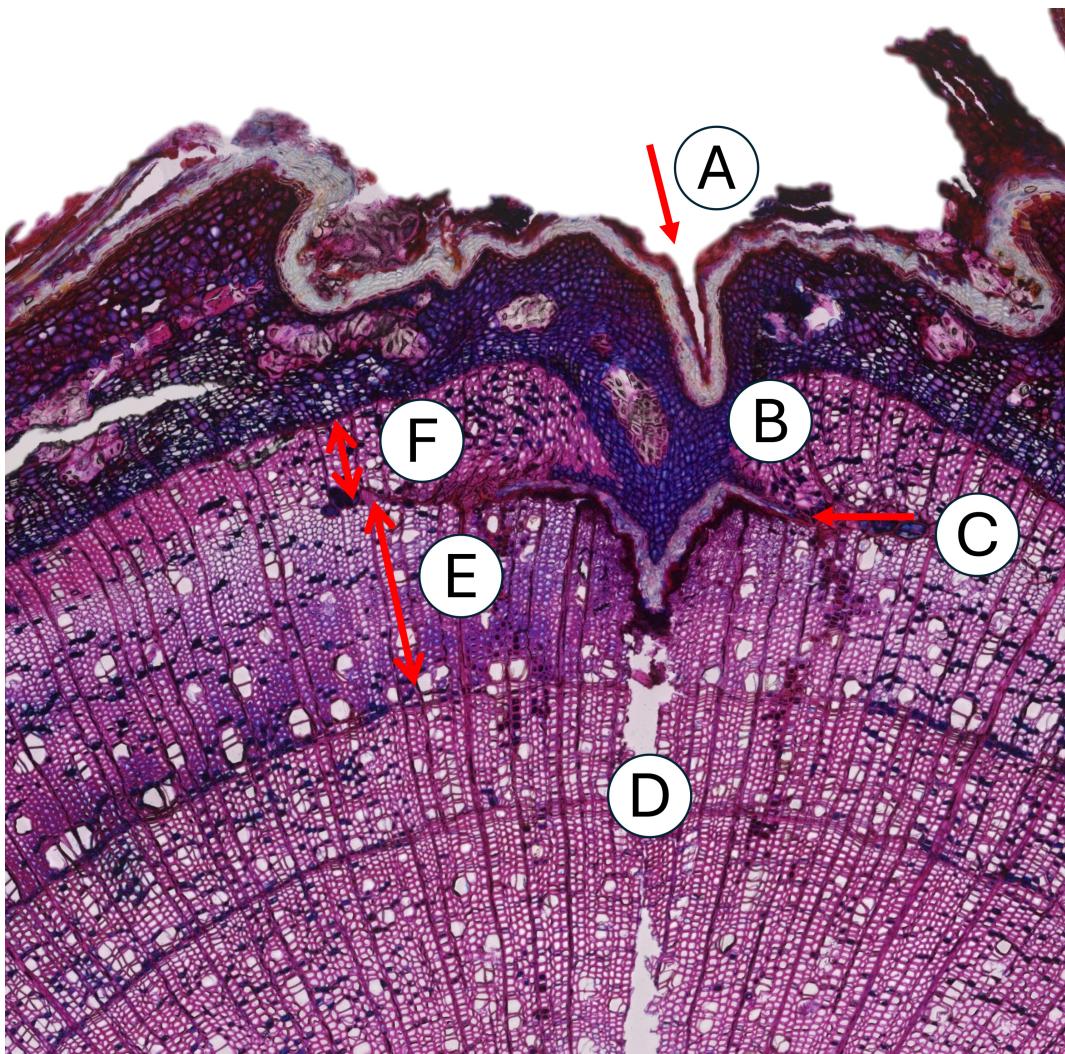


Figure 8: Cross-section of *Quercus garryana* depicting the reaction caused by the pinning. A: pinning hole with bark and phloem cells; B: zone of irritated cambial cells surrounding the pinning hole (callose tissue); C: border of cambium at the time of pinning; D: Pinning hole penetrating into the xylem cells that were already formed at the time of pinning; E: xylem cells formed prior to pinning; F: xylem cells formed after pinning.

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