

¹ fuelinex draft manuscript

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⁵ **Abstract**

⁷ **1 Introduction**

⁸ **2 Materials and Methods**

⁹ **2.1 Species selection**

¹⁰ Acma, Alru, Bepa, Prvi, Quma were purchased from Peel's nursery and arrived on *** at Totem
¹¹ Field Studios (**coordinates). Alru is a fast growing species and they already arrived taller than
¹² the other species. We decided to cut a segment from the saplings and plant them in bare soil. See
¹³ below for more info. Acne, Pist, Poba, Segi were species that were purchased in 2022 for 2023
¹⁴ Phaenoflex's experiment. We selected randomly these tres. At the time, these species were not used
¹⁵ because:

- ¹⁶ • Acne: root system very small (they came in carots compared to other trees that came in pots)
- ¹⁷ • Pist: smaller than other species
- ¹⁸ • Poba: after they flushed, then they were repotted and lost their leaves.
- ¹⁹ • Segi: smalller than other species

²⁰ Again, for the same reason as the Alru, we took cuttings from Poba and replanted them in soil with
²¹ the following methodology. The cuttings were stored in climate chambers with the corresponding
²² temperature (see Hobo loggers) from February 13, 2024 to Feb 20, 2024. The tree cuttings were
²³ planted at that time.

²⁴ Notes from Justin: Assuming that all the information prior to planting is already noted down
²⁵ (e.g. what temperature the cuttings were stored at etc.) 30 cm long shoot tip cuttings of both red
²⁶ alder and balsam poplar were soaked at the cut wound for 15 minutes in a solution of 20 mL indole-
²⁷ butyric acid 0.4% (Wilson Liquid root stimulator) diluted in 2 litres of warm tap water. (0.004%

28 concentration). 180 1-gallon pots were filled up to 1 inch from the lip with pre-moistened peat-based
29 potting mix containing large pumice chunks. Soil was pressed firmly to compact. Cuttings were
30 placed into the soil at the depth such that pre-drawn paint lines could still be visible just above
31 the soil surface.

32 **2.2 Tree measurements**

33 The following measurements were performed from Feb 7, 2024 to Feb 11, 2024. 1. Using red paint,
34 we marked the trees on their trunk at 1 inch from the soil. 2. The diameter was measured at the
35 top of that marked. 3. Height measurements were also done using these marks.

36 Shoot elongation: To facilitate the shoot elongastion monitoring, paper ruler was attached on the
37 following species Acne, Bepa, Poba and Quma. We used A3 RiteIntheRain paper so they would
38 survive rain. We also taped the end of each ruler with packaging tape for 2 reasons: 1. Increase the
39 the fusion of the tape with the ruler and 2. Increase the durability of the fixation to the tree. We used
40 Band Aid medical tape to fix the paper rulers to the trees in order for the trunk to be able to breath.
41 Prior to the installation, using red pain, we marked where the reference point for the measurement.
42 This is the bottom of the new-year apical shoot. For species on which we couldn't install the paper
43 rulers, manual measurements of the shoot elongation was performed each Wednesday.

44 **2.3 Fertilizer**

45 Using fertilizer premix from UBC's garden, I diluted by half to make it less concentrated. Dilution
46 factor: 1:2. Then I added 125mL to all trees on Friday, June 7, 2024. Another nutrient addition
47 was performed to maintain the nutrient availability in the soil on 6 July 2024. See git issue #14 for
48 more details.

49 **2025 :** On Friday 11 April 2025, 125mL of undiluted liquid fertlizer (same as 2024) was added to all
50 trees, excluding the nitro boost treatment replicates. The latter didn't get any nutrients in spring
51 and will get some only later on in the summer. Since we dropped the nitro treatments for the segi,
52 all of these replicates got fertilizer.

53 **2.4 Hobo loggers**

54 Hobo loggers (Temp/humidity) were set up in the climate chambers at the beginning of the Cool
55 Spring treatments. They were then transferred to Totem Field at different locations and hidden
56 behind a white sheet of paper to avoid the sun from hitting them directly.

57 On June 7, 2024, Hobo loggers (Temp/light) were placed in 3 different blocks at Totem Field.
58 They were placed at the top of PVC pipes at a height of 1m from the ground. They were placed
59 in a position where the foliage covers of the trees would not shade them. I set 6/block. This was
60 performed after I notice that there will be a big light difference. The plants that are the farthest from
61 the greenhouse door receive far less light then the one closest to the door. They were configured on
62 Sunday June 9, 2024. I also installed 4 loggers on the greenhouse roof in case the ones positionned
63 at 1m above the soil don't record the light properly.

64 **2.5 Spring and Fall treatments**

65 The Cool Spring treatment consisted of placing the CS replicates in climate chambers to delay the
66 start of their growing season on March 6 2024. The WS replicates remained at Totem Field studios

67 The Warm Fall treatment consisted of placing WS/WF, CS/WF and WSWF_nitro treatments in
68 the climate chambers on 4 September 2024. The photoperiod was set every week on Wednesday to
69 fit the local sunrise and sunset and was ramped until it reached full light. The temperature was set
70 to fit the mean 30 years daily maximum temperature of one prior month. E.g. the the temperature
71 for the first week of September was set to the temperature regime of the first week of August. The
72 CF treatments remained at Totem Field Studios.

73 For both climate chamber treatment, the trees were rotated and watered weekly to minimize the
74 effects the climate chambers could have on the trees.

75 **2.6 Senescence monitoring**

76 Every week, starting on September ***, senescence was monitored by two methods. The first being
77 a visual assessment of the remaining green leaf cover. We used a systemic aproach to estimate
78 what percentage of green leaf cover was remaining by comparing to what would be 100% of cover..
79 From September **** to September 25, we used a chlorophyll content meter ***. On October 2,
80 because of device failure, we switched to SPAD-502DL Plus (Konica Minolta) from Loren Riese-
81 berg's lab. To calibrate the two instruments to values that are comparable, we used****. check:
82 <https://nph.onlinelibrary.wiley.com/doi/full/10.1046/j.0028-646X.2001.00289.x>

83 **2.7 Shoot elongation measurements**

84 In 2024 and 2025, shoot elongation measurements were conducted using two distinct methods. But
85 for both methods, the following were conducted: in 2024, we selected the shoot coming off the apical
86 meristem when possible. If the bud died or if the shoot snapped off, we selected the closest lateral
87 shoot. In the case of Prvi on which there are no obvious apical shoot (sometimes there will be 2
88 branches of equal height) we selected the highest one and if that one died, we selected the other.
89 Then we went on the lateral shoot if both died.

90 Then using rain paint, we marked the base of the chosen bud.

91 In 2025, we preferably chose the continuous shoot that was measured the previous year. If that
92 shoot died, we chose another shoot acording to the previous criterias.

93 Two methods:

94 1. Paper rulers using rite in the rain paper and printed a 38cm ruler. For the species on which there
95 was trunk/branch space on which we could safely install a paper ruler, we installed one. The species
96 were: Acne, Bepa, Poba, Quma. Since the ruler was positioned at the bottom of the red mark, we
97 didn't have to adjust it every time we measured shoot elongation. Then the shoot elongation was
98 measured at the botttom of the apical bud.

99 2. Metal rulers: using a metal scientific ruler, we measured the shoot from the bottom of the red
100 mark to the bottom of the apical bud for deciduous species. We measured until the top of the apical

¹⁰¹ meristem for Pist.

¹⁰² Shoot elongation was measured weekly for all species. For determinate growth species, after
¹⁰³ two weeks of little or no change in elongation, we started monitoring them every second week. For
¹⁰⁴ indeterminate growth species, they kept on being monitored every week.

¹⁰⁵ **2.8 Leaf count**

¹⁰⁶ In order to determine whether nutrient addition treatments in the fall affected leaf primordia for-
¹⁰⁷ mation, we counted the leaves on 27 May for the determinate growth species only i.e. Acne, Prvi
¹⁰⁸ and Quma. We counted the leaves only for the shoot coming out of the apical meristem. We always
¹⁰⁹ counted all the leaves on the current-year shoot measured for shoot elongation measurements.

¹¹⁰ **2.9 Biomass collection**

¹¹¹ In the fall of 2025, when all the individuals from a species have lost all their leaves, we proceeded to
¹¹² remove the trees from their pots, remove the dirt by shaking them first and washing off the dirt off
¹¹³ the roots gently with regular water gun. Then, we moved the trees, 1 bloc at a time in the drying
¹¹⁴ ovens where they were left to dry for 72 hours at 70C

¹¹⁵ **2.10 Experimental Design**

¹¹⁶ **2.11 Statistical Analysis**

¹¹⁷ 'sectionResults

¹¹⁸ **3 Discussion**