

The Relationship between Soil Properties and Variation in the Morphology and Phenology of non-native  
mustard plant *Thlaspi arvense* in Subalpine Meadows

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## Abstract

The ability of soil to retain moisture can significantly impact the severity of droughts on plants. Deep, loamy soils retain moisture from snow melt into the dry season more efficiently than shallow, sandy soils that lose water to runoff and percolation. Soil depth and texture are thus significant in determining how plants respond to climatic challenges. Similarly, soil composition can impact the number of leaves, branching behaviour, and stem height of plants. Overall, there are many factors impacting the growth of plants and how they respond to challenges. *Thlaspi arvense*, an invasive mustard, has demonstrated success in diverse microhabitats in the subalpine meadows of Gunnison County. To determine what qualities of a microhabitat favour the growth of *T. arvense*, edaphic properties including moisture, pH, conductivity, organic content, and soil type were investigated. Distance from the road (as a proxy for disturbance) and elevation were also considered. These environmental characteristics were paired with morphological and phenological observations to understand how they might influence the behaviour of *T. arvense*. We recognize that genetic variation in our populations could influence conclusions made regarding the impact of environmental conditions on *T. arvense*. To understand these potential genetic effects, a common garden experiment was performed. Flowering time varied within genetically different populations sourced from opposite ends of the elevational gradient and grown in the same environment.

## Introduction

Invasive species are initially restricted to the biotic and abiotic conditions reflecting their native habitats. However, if populations experience rapid evolution and display high phenotypic plasticity after introduction, their invasive potential can increase and allow them to expand their ranges (Sultan et al. 2013).

During range expansion, populations on the expanding front experience evolutionary pressure for higher rates of dispersal and reproduction, since there are less conspecifics available for mating (Phillips et al. 2010). A study on invasive reed canarygrass demonstrated that populations had higher genetic diversity and heritable phenotypic variation in their invasive range than in their native range (Lavergne & Molofsky, 2007).

High genetic diversity and the ability to survive in unpredictable or challenging environments, self-fertilize, and reproduce large numbers of offspring allows many invasive plants to thrive in disturbed areas.

*Thlaspi arvense*, a non-native Eurasian mustard, has successfully persisted in disturbed areas of Gunnison County since the late 1880s (Chew 1975). It occupies patches for 5 to 7 years before being outcompeted by native plants and cropping up elsewhere. *T. arvense* produces glucosinolates, secondary chemicals typical of the Brassicaceae family that have the potential to disrupt belowground mutualisms between native plants and soil biota, negatively impacting native plant communities (Stinson et al. 2006). Additionally, the glucosinolate content of *T. arvense* is an evolutionary trap for native butterflies. Glucosinolates in native mustards signal oviposition, but the larvae cannot digest the leaves of *T. arvense* despite the similar cues (Nakajima and Boggs 2015).

Populations of *T. arvense* exist in various habitats along an elevational gradient, but we lack an understanding of how these habitats influence phenotypic and genetic variation, or which environments are particularly favourable to rapid population expansion. Previous greenhouse studies have tested the impacts of soil quality and composition on the morphology of *T. arvense*. Best and McIntyre (1975) demonstrated that an increase in the number of leaves and lateral branches can result from an increase in nitrogen supply. It is possible that the large number of additional branches would increase capacity for seed production. In greenhouse experiments, seeds collected from uncultivated farmland have

demonstrated high phenotypic plasticity, resulting in varied stem height and branching behaviour when grown in varying soil types. Specifically, plants may only grow 1.0 cm tall when grown in infertile, arid, or shallow soil, but in deeper, fertile soil without significant competition, the stems may grow 80 cm tall and produce many flowering lateral branches (Best and McIntyre 1972).

It is important to note that these morphological observations are from controlled garden experiments. Along an elevational gradient, many factors that may influence the plasticity of *T. arvense* change. These include temperature, nutrient availability, and soil moisture (McCain & Grytnes 2010). As a result, the ability of *T. arvense* to increase its range and persist at higher elevations with new conditions is currently unknown. Abiotic stresses including limited nitrogen, shallower soil, and later snowmelt may reduce fecundity (measured as fruit number per plant) at higher elevations. In contrast, biotic stress is more abundant at lower elevations, from herbivory or competition (Lomolino 2001). There are more accessible nutrients in soil at lower elevations as a result of larger biomass and nutrient cycling (McCain & Grytnes 2010). Previous work involving the dynamics of community structure demonstrates that the allocation of biomass is different within the same species existing in different habitats (Tilman 1988). Where strong competition for light occurs, there is more allocation to leaves and stems. Species with high specific leaf area (SLA) are prominent in soils with high concentrations of nitrogen. (Lambers & Poorter 1992) In contrast, species growing in nutrient-deficient soils tend to allocate more biomass to roots.

Changes in local climate and increased chances or frequency of natural disaster could enable currently restricted species to expand their range (Hobbs and Huenneke, 1992). It is necessary to understand the environmental conditions that favour *T. arvense*'s current growth habits so that projections can be made of its future success as climate change provides greater opportunity for encroachment.

To investigate the relationship between environmental factors and *T. arvense*, several null hypotheses were tested.

*Null hypothesis 1:* There is no significant variation in soil properties in the different microhabitats *T. arvense* occupies around Crested Butte, Colorado. Therefore, differences in morphology and phenology across sites are the result of other factors.

*Null hypothesis 2:* Similar soil properties do not correlate with similar plant phenology or morphology across sites. For example, sites with the same soil composition have plants with significantly different characteristics.

*Null hypothesis 3:* Morphological and phenological variation in populations of *T. arvense* does not have a significant genetic basis. When seeds from different sites, of known lineages, are grown in a common garden in which all other factors are kept the same, phenology and morphology do not vary appreciably.

## **Methods**

### *Study Sites*

We had ten study sites (Figure 1) spanning various microhabitats. They included the Snodgrass trailhead (2934m), Deer Creek Trail/Kettle Ponds (2873m), Brush Creek area (2899m, 2868m, 2739m, 2793m), East River Pump House area (2762m), Oh Be Joyful campground (2735m), Mount Crested Butte (2770m), and Hunter Hill (2840m). Triplicate 1m line transects were established across each invaded area. About 25 to 30 individuals were flagged at each site by choosing plants nearest to a random point along a given transect. Using a 1m<sup>2</sup> quadrat, density counts were taken weekly along each transect.

Distance from the road was measured as a way of quantifying disturbance. Sites less than 2m from a road were considered highly disturbed.

### *Morphological/Phenological Variation Among Sites*

We revisited each site weekly and recorded the following morphological traits: height, rosette diameter, number of basal and main branches, and fruit number (if applicable). To ensure we had a sufficient sample size, and to combat high rosette mortality, we replaced rosettes that died within the first three weeks with the next closest plant and recorded mortality of the original plant.

We also scored phenological stage on a scale of 1 to 5 (Table 1).

Plants in-between stages received a partial score. For example, seed set on the main branch but only flowering on the lateral branches was considered a 3.5. Scoring the plants allowed us to assess differences in the timing of life events at different sites, such as flowering time. The score was also used for collecting leaf samples at the appropriate stage for each plant.

At flowering, we removed a leaf below the flower buds for analysis of leaf area. Removing a leaf from the same spot of each plant ensured the leaves were all at about the same point in the plant's life history. This mitigated a difference in area being the result of one leaf being older or bias in choice of leaf. Leaf scans were taken in the field using a portable flat bed scanner, then scans were analyzed in Image J. All seeds, both aborted and realized fruit, were counted at reproductive maturity. If seeds appeared to be missing due to damage or seed dispersal, pedicels were counted as a proxy for the seed pod that would have been there.

### *Soil Properties across Sites*

We collected soil samples from each site. To extract the samples, a bulb planter was used to extract a core with a depth of 10cm. A 50mL conical tube was run through the core of the bulb planter to reduce the diameter of the soil core to 4cm. The smaller tube enabled collection of an appropriate sample size that still accounted for differences in composition with depth. Soil was collected at least 5 cm away from plants to avoid damaging lateral roots. Five soil samples were taken at each site, making sure to include the perimeter and central areas of the site. Samples were combined to produce one, approximately 250mL, composite sample representing the average soil properties of that site. Samples were stored in tightly sealed Whirl-Pak bags until they were taken back to the lab for analysis. Bags were kept in a fridge at 3°C to prevent decomposition and loss of soil moisture. Soil sampling occurred at the beginning of the season, the week of June 18-23, 2018, proceeding a weekend of rain, and in the middle of the season, July 2-6 2018, after two weeks without rain. Moisture, organic matter, pH, conductivity, and mineral composition were analysed for each sample.

Soil moisture was calculated using gravimetric analysis. First, rocks greater than 2mm in diameter and most roots were removed from the soil. The wet mass was then weighed in a tin and placed in a Lab-Line L-C Oven for four days at 105C. (Lab-Line Instruments, Inc. Designers and Manufacturers Melrose Park, ILL). The samples were removed from the drying oven and immediately weighed. Dry mass was subtracted from wet mass and divided by the wet mass to get the percent moisture of the soil.

Soil samples were subsequently analyzed for organic matter using the RMBL Warming Meadow Soil Carbon Protocol (2005), established by Dr. John Harte. A Fisher Scientific IsoTemp Programmable Mobile Furnace (Made in Thailand) was used. The same samples from the gravimetric analysis were transferred from the drying oven to the muffle furnace, so that the change in mass would not be affected by moisture evaporating from the soil. Change in mass resulted from combustion of organic compounds,

leaving only the mineral components of the soil remaining. To burn the soil, the oven was preheated to 200C. Samples were processed in groups of five. The furnace temperature increased at 19C/ minute from 200C up to 430C, where it was held for five minutes. Samples were then allowed to cool and re-weighed. Organic content was calculated by subtracting the post-combustion mass from the dry mass, and divided by the dry mass.

To determine soil composition, 15mL of undried soil was removed from every Whirl-Pak collected the week of June 18-23. Each soil sample was separated into its three basic mineral fractions: sand, silt, and clay. Rocks greater than 2mm in diameter and most roots were removed from the soil. A 15mL soil sample was combined with 1mL of LaMotte texture dispersing reagent, then diluted to 45mL with tap water. The tube was capped and shaken for two minutes, then allowed to sit undisturbed. After 30 seconds, the supernatant was decanted and allowed to settle. After 30 minutes, the supernatant was again decanted. Since clay is colloidal in solution, the portion of clay was calculated by adding the sand and silt fractions and subtracting their sum from the initial volume of soil. The first tube contained the sand fraction, the second silt, and the third clay. Percent composition was calculated and the soil type identified using the NRCS soil texture triangle.

Conductivity and pH were measured using a VWR H3OPC0 Handheld Multimeter with Multiprobe, VWR part #89231-702 (Made in Thailand). All sites were analyzed twice. Falcon tubes were filled with 10mL of de-ionized water and 10mL of undried, unfiltered soil. The tubes were capped and shaken for one minute, then allowed to settle for three minutes. The multiprobe was rinsed with DI water before each use, then placed in the soil solution. Conductivity and pH were recorded.

### *Common Garden*

A common garden experiment was conducted in Gothic, CO, USA to assess genetic variation among populations of *T. arvense*. Seeds were from known maternal lines previously established from the Brush Creek and Snodgrass field populations in a greenhouse at the University of Massachusetts, Amherst. Ten plants were collected from each site. 25 F1 offspring from each field plant were raised in the greenhouse to remove maternal effects, for a total of 250 F1 offspring from each site. Seeds were collected from each F1 plant, and a subset of 25 F1 offspring from 6 successful mothers per site were selected for the common garden experiment. 75 seeds were sown from each F1, for a total of 3,750 seeds. These F2 seeds were sown into flats in a Weatherport in Gothic with soil-less potting mix (Miracle Gro potting mix, Marysville, OH, USA). After three weeks, 1200 germinants were transplanted to a common garden. The germinants represented 50 F1 plants of 12 maternal lines from the two field populations. The germinants were transplanted into a randomized array of 24 trays with 50 plants per tray, in wells that measured 2x2x5 inches (SureRoots 50 deep cell plug trays, Greenhouse Megastore, Daville, IL, USA) and submerged into divots in the common garden. The trays contained the roots for later measurement and prevented the release of phytochemicals into the soil. Hoop-houses were placed over the plants to prevent pollination, though herbivory was still possible. The common garden consisted of four blocks. Each block contained six trays arranged in a row. Trays were rotated weekly within blocks to minimize edge effects. Trays were watered as needed, typically in the morning or at noon daily to prevent excessive wilting.

Rosette diameter and height of each plant were measured at transplant and at flowering time. Leaves were collected as in the field. Additionally, total leaf counts were taken on a subset of 250 plants at flowering time to better understand resource allocation. Percent herbivory was estimated on each plant at flowering. This was recorded as the percentage of total leaf area that had been damaged.

## *Data Analysis*

An Analysis of Variance (ANOVA) was used to compare properties of the field sites, and Tukey's HSD test was used to identify the source of the differences. Linear regressions were used to test for correlation between soil traits, elevation and plant characteristics. RStudio (3.5.0) was used to create all graphs and perform tests.

For the common garden, Welch's Two Sample t-test was performed to understand differences between the Snodgrass and Brush Creek populations in the common garden.

## **Results**

### *Soil Properties across Sites*

Loam, loamy fine sand, sandy (clay) loam, sandy clay, and sandy loam were observed (Table 2). Soil composition was not related to elevation (Fig. 2).

There was a significant decrease in soil moisture across all sites in the two week period without rain (Fig. 3, Welch two sample T-test  $p < 0.001$ ). The highest soil moisture was 27.467% early season. Maximum moisture dropped to 5.689% later in the season.

There was significant variation in pH (ANOVA,  $p = 3.039e-05$ ) across sites, ranging from 5.7 to 7.29. The pH at sites did not change significantly between sampling periods.

Clay fraction and soil moisture had a significant positive relationship at time point 1 (Fig 4A). A higher fraction of clay in the soil resulted in greater retention of moisture in the early weeks following snowmelt ( $R^2 = 0.5048$ ,  $p = 0.01281$ ). However, later in the season, soil moisture was not strongly affected by the clay fraction (Fig 4B).

Moisture did not significantly increase with elevation at either time point. (ANOVA,  $p = 0.2236$  for time point 1, ANOVA  $p = 0.7522$  for time point 2).

There was significant variation in conductivity among sites early season, at timepoint one, (ANOVA,  $p = 0.01361$ ) but not at time point two. Most sites increased in conductivity (Fig 5), which weakly correlated with a decrease in moisture. (linear regression,  $p = 0.09669$ )

Organic matter did not vary significantly across sites (ANOVA,  $p = 0.1352$ ), but when grouped by disturbance (low, medium, and high) based on distance from the road, the sites closest to trails had significantly lower organic matter. (ANOVA,  $p = 0.01124$ ).

### *Soil Properties and *T. arvensis* Characteristics*

Flowering time differed across soil types. (Fig 6, ANOVA,  $p = 0.029$ ). There was a significant difference in flowering time between sandy (clay) loam, averaging 17 days to flowering, compared with loam sites taking 30 days to flowering (Tukey's HSD,  $p = 0.038$ ). Average flowering time for each soil type is shown in Table 3.

There was no relationship between soil type and fruit count (ANOVA,  $p = 0.5321$ ), nor did fruit count did not vary significantly across site (ANOVA,  $p = 0.2013$ ). Branch number did not vary significantly across sites.

Density of plants at flowering time varied significantly by soil type (ANOVA,  $p=2.2e-16$ ). Loam sites had much greater densities compared to other sites. (Tukey's HSD,  $p<10e-7$  compared to all other soils)

### *Common Garden*

Flowering time varied significantly between seeds sourced from the high elevation (Snodgrass) and low elevation (Brush Creek) sites (Fig 7, Welch Two Sample t-test,  $p=2.464e-05$ ). Flowering time also varied by mothers of the low (Fig 8A, ANOVA,  $p=0.0003622$ ) and high (Fig 8B, ANOVA,  $p=2.2e-16$ ) elevation sites. The Snodgrass population demonstrates greater genetic variation than Brush Creek in terms of flowering time, though the experiment is ongoing and additional traits of mature plants could not yet be evaluated.

Leaf area did not vary significantly by maternal elevation (Welch two Sample t-test,  $p=0.9663$ ). Leaf area positively correlated with plant size. Plants with larger leaves had greater rosette diameters for both Brush Creek and Snodgrass (linear regression,  $R^2=0.328$ ,  $p\text{-value}<2.2e-16$ ).

Plants with a greater leaf area had less leaves  $R^2=0.09745$ ,  $p\text{-value}:8.245e-05$ ).

Taller plants had more leaves (linear regression,  $R^2=0.1656$ ,  $p=1.221e-09$ ) and greater rosette diameters (linear regression,  $R^2=0.2378$ ,  $p=9.556e-14$ ).

## **Discussion**

Overall, there were notable differences in soil properties at the different microhabitats. The first null hypothesis, that there is no significant variation in soil composition, is rejected. Additionally, there were several relationships between morphology and phenology that were influenced by differing environmental factors. Thus, the second null hypothesis was also rejected.

Soil moisture, soil type, and flowering time were not related to elevation. These factors may not change greatly due to the limits of our elevational gradient, which only spans the 200m of subalpine meadow where *T. arvense* is present. Both sandy loam and loam, the most frequent soil types, occur near the minimum and maximum elevation sites. Therefore, it is unlikely that elevation was a covariate in the variation observed in plant traits, and the influence of soil texture seems to be more significant to flowering time and soil moisture than elevation at small changes in altitude.

Differences in soil moisture among sites, influenced by soil type, played a role in the characteristics of *T. arvense*. Early in the season, sites with a higher fraction of clay had more water available to them. Soils consisting of more clay do not dry or warm up as quickly in the spring since the smaller granules trap water more effectively (Reid 2014, p.63). The sites with soil types consisting of higher portions of sand (loamy fine sand) lost water sooner, likely because sandy soils lose water from snow melt to percolation and runoff. In typical conditions, this would be advantageous as the soil warms up sooner and is exposed to the surface, allowing earlier germination. However, in a drought year such as 2018, and following a substantially lower snowpack than normal, this would have been a disadvantage. Less moisture availability could delay flowering and stunt growth.

Later in the season, soil moisture was not strongly affected by the clay fraction. It is likely that most available water would have been used up by plants or evaporated, so temporary drought tolerance provided by a higher clay fraction would become less relevant to plant growth. Factors not measured, such as slope and aspect, could have also played a role in soil moisture. Sites on steeper or south-facing slopes normally have less moisture, as water is lost to runoff or evaporation.

Changes in soil moisture between timepoints 1 and 2 likely were the source of increased conductivity at most sites, as evaporation leaves behind salts in the soil. A study on *Arbutus unedo* (Navarro et al, 2016) demonstrated that an increase in the salinity of soil causes a decrease in the intercellular spaces of the mesophyll, impairing both stomatal and mesophyll conductance of carbon dioxide. Additionally, increased salinity in soil decreases osmotic pressure due to the soil being more hypertonic, which can make it harder for the roots to take in water, exacerbating the effects of drought and potentially stunting growth and delaying timing of life events.

Soil pH varied across sites, though remained within the range of 6 to 7 that plants prefer. It did not vary significantly with plant traits and was probably not a cause of differences exhibited by *T. arvense*.

More disturbed sites seem to have lower organic content. Disturbed sites are typically less productive since fewer organisms can survive under conditions including compacted soil and regular damage to organs such as flowers, stems, or leaves. This results in less biomass deposition year after year. Disturbed sites can also experience more erosion since less roots are available to keep the soil in place, causing further loss of organic matter.

Loam sites had the greatest densities at flowering time, although they had the longest average flowering time. This indicates greater competition for resources could have prolonged flowering time. *T. arvense* may have generated more foliage or grown taller instead of putting resources into fruits or flowers to combat competition. Loam soils generally hold the most plant-available moisture due to ideal fractions of sand, silt, and clay, and have good capacity to retain nutrients. Often, plants grow too well in these soils and create more foliage than fruits or flowers (Reid 2014, p.64).

### *Common Garden*

The third null hypothesis states that genetics are not a highly influential factor in variation present in populations of *T. arvense*. The null hypothesis was rejected. Seeds from various moms grown in the common garden exhibited significant differences in flowering time, indicating substantial genetic variation both between sites and within sites. There was a greater difference in flowering time for the Snodgrass population than the Brush Creek population. The natural Snodgrass population experiences regular disturbance by foot traffic and large mammals, causing compaction of the soil. It also has a shallow layer of soil and the lowest organic content of all the sites (3.901% compared to the average 7.25%). Overall, Snodgrass experiences more stressful conditions compared to the more fertile, less disturbed Brush Creek populations, which could explain pressure for greater genetic variation in the Snodgrass population to combat year-to-year variation in environmental challenges. The influence of soil conditions on flowering time suggests that environmental differences can outweigh population level genetic differences on significant early life traits.

The plants in the common garden had more and larger leaves than plants in the field. Rosette diameters were successfully measured for all plants that flowered in the common garden, while the rosettes typically shriveled in the field and the plants allocated resources more towards flowers and seed count instead of leaf area. The common garden provides more nutrients, more protection from herbivory, and a regular supply of water, which seems to favour a response toward much greater leaf area or more leaves. *T. arvense* demonstrates high plasticity even when grown under common conditions. The plants mirrored the behaviour of those growing around them. Trays were randomly sorted by genotype, but some trays had a much greater average height, while other trays had much larger leaves in response to light competition. These highly plastic traits contribute to its invasive potential.

## *Future Directions*

From field and common garden observations, roots of *Thlaspi arvense* seem to vary in length by site. Generally, plants growing in sandier soils divert more resources into roots in the search for extra water and nutrients. Thus, the glucosinolate impact on soils at different sites may vary with root size.

Contrasting leaf area and height with plant fitness would contribute to understanding how these plants allocate resources in different environments. It would also be interesting to grow seeds from the same population a couple years from now, and contrast them with new plants to understand if genetic variation increased or decreased at the Snodgrass site as a result of environmental pressure.

Sampling soil moisture more frequently could indicate why some sites differed in rosette mortality. Repeating this study in a wet year and seeing if there is a shift in sites that flower first could support the idea that in drought years, soils with more clay are more beneficial, while in years with greater snowpack, sandy loams flower faster.

Comparing fruit count, leaf area, and height between the common garden and the field could indicate where plants are likely allocate resources. Looking at the fruit/abort ratio of each site could indicate which environments favour reproduction. Our data suggested that abortions were higher at more disturbed sites, but these results were not significant.

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## Figures and Tables

*Table 1. Phenological Stage Rankings*

| Phenological Stage | Phenological State Description              |
|--------------------|---------------------------------------------|
| 1                  | Rosette                                     |
| 2                  | Bolted (main stem taller than 1cm)          |
| 3                  | Flowering                                   |
| 4                  | Producing seeds (plant predominantly green) |
| 5                  | Senescence (plant brown)                    |

*Table 2. Various Soil Types Observed across Sites*

| Soil Type         | Frequency |
|-------------------|-----------|
| Sandy Clay        | 1         |
| Sandy (clay) Loam | 1         |
| Loamy (fine) sand | 1         |
| Sandy Loam        | 6         |
| Loam              | 2         |

*Table 3. Average Flowering Times by Soil Types*

| <i>Soil Type</i>         | <i>Average Flowering Time (days)</i> |
|--------------------------|--------------------------------------|
| <i>Loam</i>              | <i>30.06667</i>                      |
| <i>Loamy fine sand</i>   | <i>25.50000</i>                      |
| <i>Sandy (clay) loam</i> | <i>17.10000</i>                      |
| <i>Sandy clay</i>        | <i>21.72000</i>                      |
| <i>Sandy loam</i>        | <i>25.88496</i>                      |

Figure 1. Map of Study Sites 2018

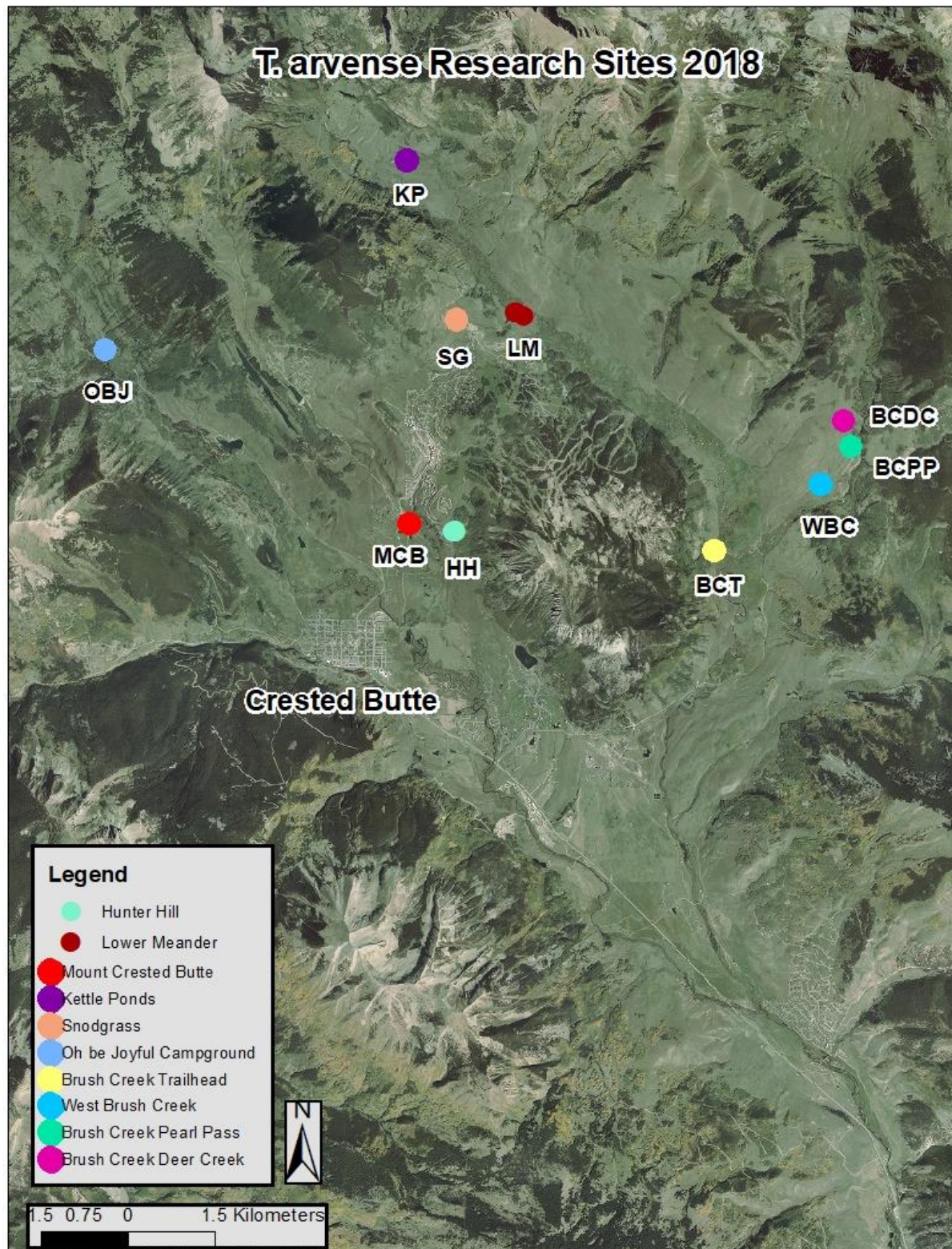


Figure 2. Soil type is not related to elevation along a 200m range

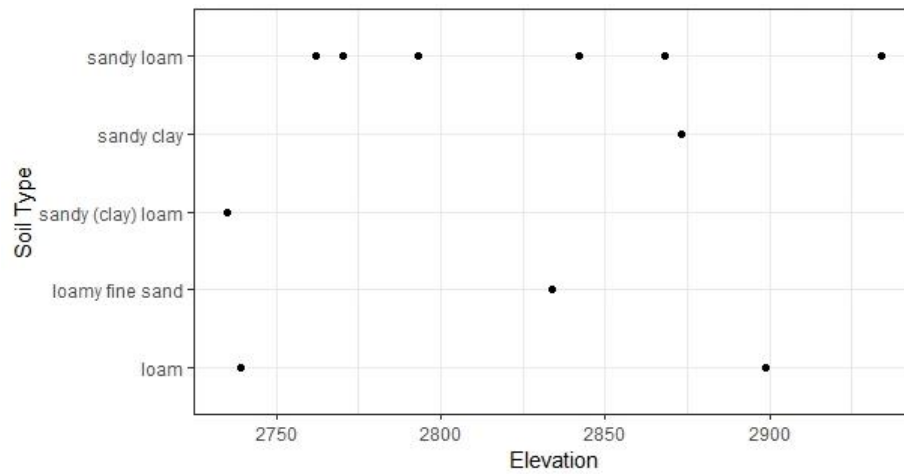


Figure 3. Soil % Moisture Early and Mid Season (Welch two sample T-test  $p < 0.001$ ) There was a significant change in soil moisture between early and mid season.

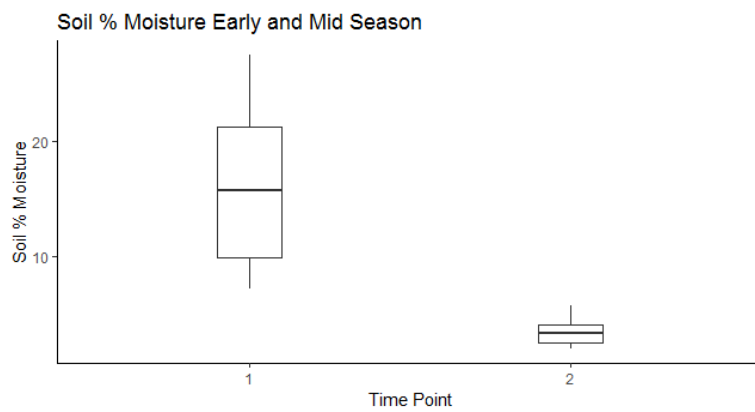


Figure 4. The Relationship between Clay Fraction and Soil Moisture (time point 1,  $R^2 = 0.5048$ ,  $p = 0.01281$ ). Soils with a greater clay fraction had higher moisture at time point 1, but not at time point 2.

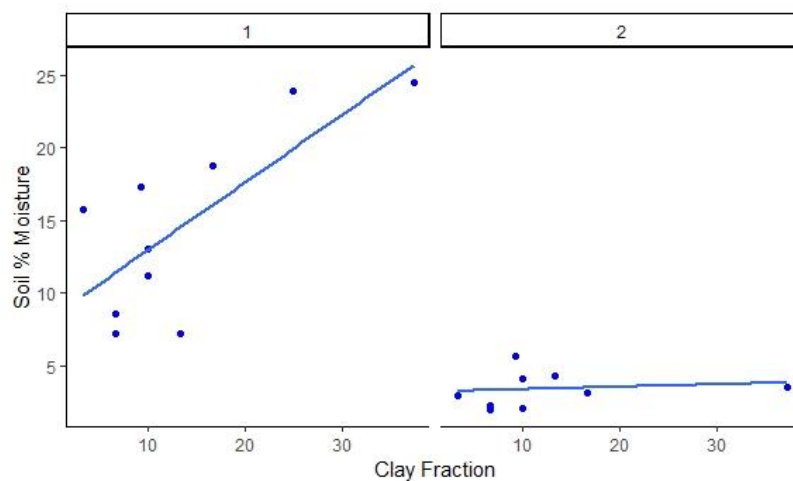


Figure 5. There was an increase in conductivity over time at most sites.

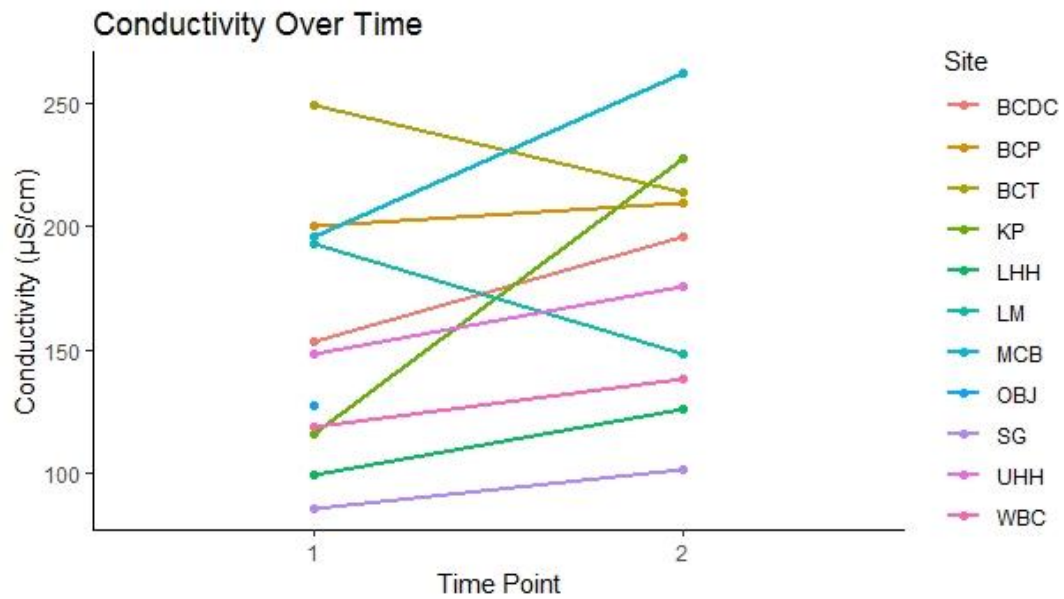


Figure 6. Soil composition significantly affected flowering time (ANOVA,  $p=0.029$ )

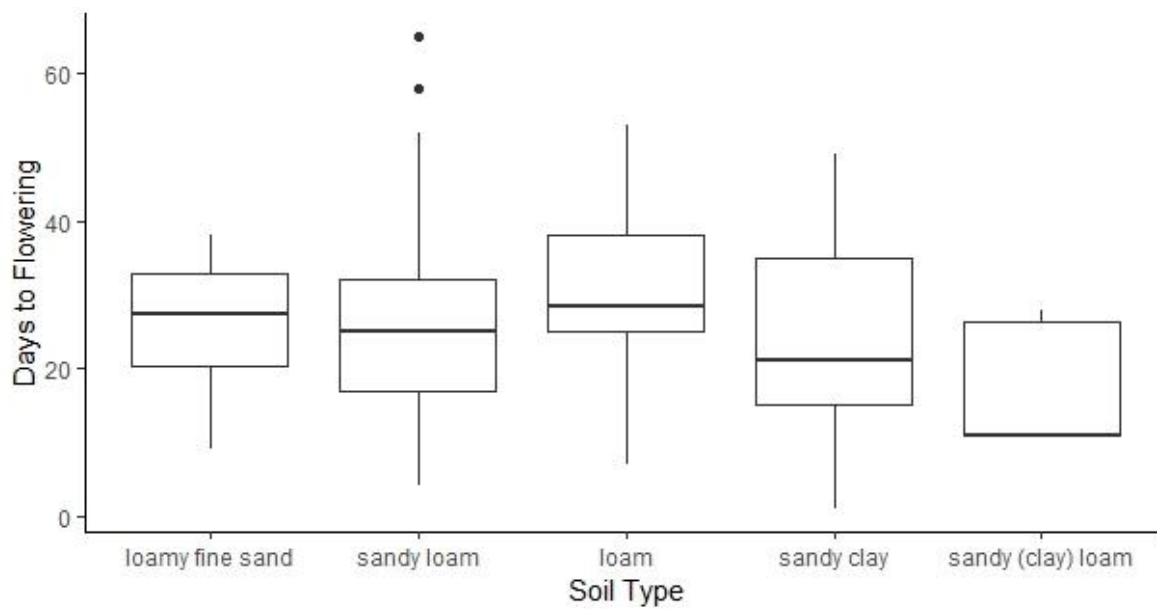


Figure 7. Flowering time varied significantly between seeds sourced from the low and high elevation sites. (Welch Two Sample t- test,  $p= 2.464e-05$ )

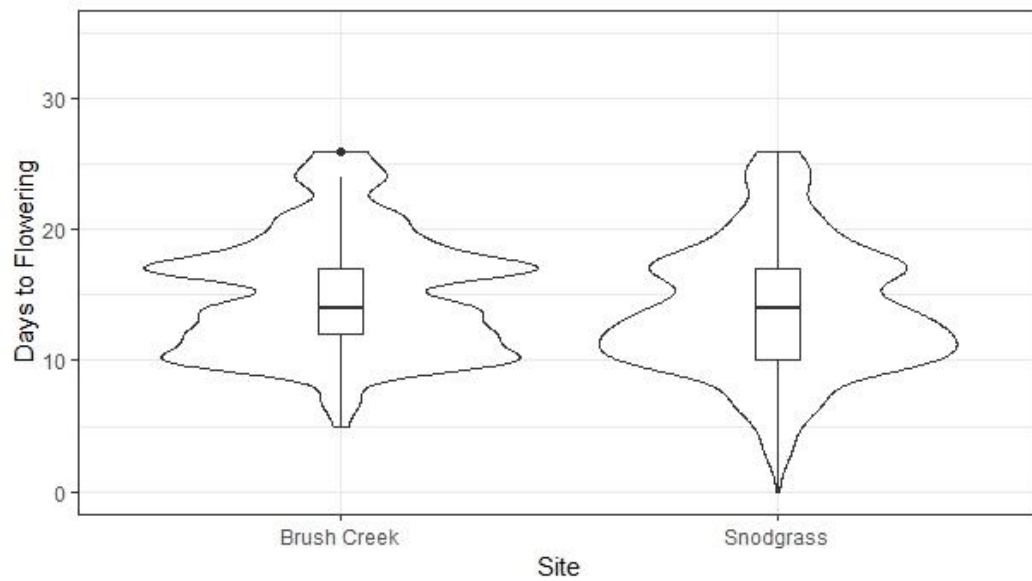


Figure 8. There was a significant variation in flowering time by maternal source (ANOVA,  $p=0.0003622$ )

