

Phenology and Phenotypic Variation of *Thlaspi arvense* Along an Elevational Gradient

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Abstract

Thlaspi arvense, a non-native plant in the Brassicaceae family, was first introduced to the Gunnison valley of Colorado in the 1800s (Chew 1975). Long-term studies done at the Rocky Mountain Biological Laboratory suggest the presence of *T. arvense* has negative impacts on the fitness of native *Pieris* butterflies (Nakajima et. al 2013, Chew 1975). It may also negatively impact native plants in its introduced range; a closely related invasive mustard found in Northeast North America, *Alliaria petiolata*, is known to impede growth of native plants by reducing mycorrhizal colonization of plant roots through production of antifungal glucosinolates similar to those produced by *T. arvense* (Wolfe et. al 2008, Chumala and Suchy 2003). Current research is investigating the capacity of *T. arvense* to disrupt plant-mycorrhizal associations of surrounding native plants where it grows (Stinson lab unpub.). *Thlaspi arvense* shows a high degree of phenotypic plasticity for traits such as branching, fruit number and height, which may increase its competitiveness (Best and McIntyre 1974). In order to determine where *T. arvense* will spread and potentially dominate as climate changes, we measured phenology, specifically timing of seed set, and phenotypic variation of adaptive traits along an elevational gradient. At nine sites, we assessed phenology and phenotypic traits for a subpopulation of plants within consistent 1m² plots. While there was no significant relationship between change in phenology and elevation, there were significant relationships between phenotypic traits and elevation, with high elevation sites favoring shorter plants with fewer fruits and branches. Low elevation sites had denser populations of plants than medium and high elevation sites, and tended to have plants with more fruits and branches. Many factors that could impact trait plasticity of *T. arvense* vary predictably along the elevational gradient, including temperature, soil moisture and nitrogen availability (McCain and Grytnes 2010). Knowing that conditions at low elevations favor reproductive success of *T. arvense*, as climate changes and high elevation climates increasingly resemble current low elevation climates, *T. arvense* may be able to increase its range and persist at higher elevations.

Introduction

Thlaspi arvense, commonly known as field pennycress or stinkweed, is a flowering plant in the Brassicaceae family. While native to Eurasia, it has spread to North America, Australia, North Africa, Japan and most of the British Isles (Best and McIntyre 1974). Its success over such a large geographic area is due partly to its reproductive abilities; a single plant can produce up to 15,000 seeds, and those seeds can remain viable in the soil for up to 20 years (Best and McIntyre 1974). Where *T. arvense* has invaded, it has the potential to influence native species. Members of the Brassicaceae family produce glucosinolates, a class of secondary defense chemicals with anti-herbivore and antifungal properties (Halkier and Gershenzon 2006). *Alliaria petiolata* (garlic mustard), is a related invasive plant commonly found in the temperate forests of northeast North America. Garlic mustard disrupts mycorrhizal associations between native plant roots and mycorrhizal fungi by releasing glucosinolates into the soil, impeding the native plant's ability to uptake water and nutrients (Stinson et. al 2006). *Thlaspi arvense* synthesizes similar chemicals, and therefore may have the capacity to disrupt mycorrhizal mutualisms and native plant growth in a similar way (Chumala and Suchy 2003). Current research is investigating the effects of *T. arvense* presence on the diversity and composition of plant communities. In addition to the

secondary chemicals released, there are many other indirect ways for non-native species to impact the evolutionary pathways of native species, such as competitive exclusion, niche displacement and hybridization (Mooney and Cleland 2001). Non-native species become invasive when they exert substantial negative impact on native biota, ecosystem functioning or human health (Hellman et. al 2008). Non-native and invasive plants can impact native animals and insects as well as native plant species, and a changing global climate will increase these impacts (Hobbs and Huenneke 1992).

Climate change has the potential to exacerbate non-native plant invasions by allowing them to increase their ranges (Bradley et. al 2010). In some cases, higher ambient concentrations of CO₂ can increase the competitiveness of non-native plants relative to natives (Nagel et. al 2004). Other changes in local climate, as well as an increased chance of natural disaster and therefore increased disturbance frequency in some regions, could enable species historically restricted from an area to move in and dominate. Such climate-based disturbance will likely allow species such as *T. arvense* that thrive in disturbed areas to increase their ranges (Hobbs and Huenneke 1992). In order to predict where invasions will be most dense as climate changes, it is necessary to understand which factors favor the growth of certain non-native plant species. In the Gunnison basin of Colorado, where we will be studying *T. arvense*, it is likely that its establishment and proliferation are a result of human interference.

The presence of *Thlaspi arvense* was first detected in the Gunnison basin of Colorado in the 1800s (Chew 1975). Research at the Rocky Mountain Biological Laboratory over the years has found that there is a significant impact of *T. arvense* presence on native species in the area, namely the butterfly *Pieris macdunnoughii*, reducing calculated fitness for the native butterfly when eggs were laid on *T. arvense* instead of native mustard plants (Nakajima et. al 2013). Species of *Pieris* evolved to oviposit on mustard plants native to the southern Rocky Mountains of Colorado (Chew 1975). Glucosinolates are found in many species of mustard plants, including *T. arvense*, and serve as an oviposition cue for the *Pieris* butterflies (Nakajima et. al 2013). Although none of the eggs laid on *T. arvense* survived to be observed as first instar larvae, there was no difference in the number of eggs laid on each plant across plant species (Nakajima et. al 2013). *Thlaspi arvense* presents an evolutionary trap for *P. macdunnoughii*, which continues to oviposit on this non-native plant, and continues to suffer losses in egg survival. As *T. arvense* moves into new environments within butterfly habitat, the chance of finding *Pieris* eggs on *T. arvense* plants will increase.

Phenotypic plasticity can aid the success of non-native plants in new environments, leading to the displacement of native species (Richards et. al 2006). Phenotypic plasticity refers to environmentally induced changes in an organism's physiology, morphology and behavior (Price et. al 2003). Plasticity of adaptive traits can aid plants in overcoming climactic constraints that would restrict certain phenotypes from surviving and reproducing. Stem height and branching of *T. arvense* show a high degree of plasticity in varying soil conditions; dry, shallow and poor soils favored short and unbranched stems, while plants grown in wetter, deeper and more fertile soils grew taller and branched more frequently (Best and McIntyre 1974). Increased plant size means increased photosynthetic area, and subsequently improved fitness for the plant (Falster and Westoby 2003). Taller, more branched plants will be energetically capable of producing more seeds, and therefore may increase populations of *T. arvense* in its introduced range.

High and low elevation environments present biotic and abiotic stressors that can influence the phenology and phenotypic plasticity of *T. arvense*, which may in turn impact native

plant success. Phenology, the timing of seasonal phenomena such as appearance, flowering and seed set, is closely linked with climate and climatic factors that vary predictably along an elevational gradient (Cleland et. al 2007). Change in climate may lead to changes in the timing of flowering and seed set, allowing plants at some elevations to mature faster. At high elevation, abiotic stressors such as limited nitrogen, shallow soil and shorter growing season may negatively impact the fecundity of *T. arvense*, represented by fruit number per plant. At low elevation, biotic stressors such as herbivory and increased competition with other plants may do the same (Lomolino 2001). By studying the phenology and phenotypic variation of *T. arvense* plants along an elevation gradient, we can predict which factors favor proliferation and growth of *T. arvense*, and hopefully mitigate the effects of its invasion in a changing climate. Due to environmental stress along the elevational gradient, I hypothesize that height, branching, and fruit number will be highest at low elevations, where snowmelt is earliest to allow for a longer growing season. High elevations may favor more rapid change in phenology to accommodate for the shorter growing season, while growth and development at low elevations are not as restricted by time of snowmelt.

Methods

Within the Gunnison basin we established 9 field sites along an elevational gradient: Almont (2434 m), Roaring Judy Fish Hatchery (2492 m), West Brush Creek (2731 m), Brush Creek Trailhead (2737 m), Lower Meander (2759 m), Mount Crested Butte (2767 m), Kettle Ponds (2873 m), Brush Creek/Deer Creek (2900 m) and Snodgrass Mountain Trailhead (2919 m) (Fig. 10). At each site, we haphazardly placed 2-3 1m² quadrats to intersect populations of *Thlaspi arvense*. A minimum of 30 plants were assessed at each site. Each week, we returned to these plots to record density, phenology (Table 1) and phenotypic traits for a random subpopulation of plants within the plot. Phenotypic traits recorded varied by phenological stage – for stage 1 plants we measured rosette diameter (mm) and for stage 2-5 we recorded stem height (cm) and branching (basal and lateral branches). Fruit number was recorded for adult plants. We used a GeoXT GPS device to record the location and elevation of plots at each site. Density data collected at sites for a related community survey project was used in analysis. For this project, transects were run through areas of *T. arvense* invasion, and quadrats were placed along the transect at the spot where *T. arvense* first occurred. We counted the number of plants in each quadrat as an estimate of population density.

Microsoft Excel and R Studio (3.4.1) were used for creation of graphs and data analysis. ANOVA and ANCOVA statistical tests were performed to determine effects of interactions between variables. For analysis, sites were grouped into 3 elevational classes: Low, Medium and High. Low sites were at elevations under 2500 m, medium sites were at elevations under 2800 m, and high sites were at 2800 m and above. Only adult plants (phenological stage 4.5 and above) were included in analysis of fruit number and height.

Results

Average phenology at each site, when plotted over time since the start of data collection (06/19/17), did not show a significant relationship with elevation (Fig. 1, $p=0.684$). Mean tallest branch height and branch number were correlated with an increased number of fruits per plant

(Fig. 2 $p=1.07e-11$, Fig. 3 $p=2.01e-5$, Fig. 4 $p<2e-16$). For the interaction of mean tallest branch height and fruit number, as well as lateral branches and fruit number, low elevation sites showed the strongest relationship between phenotypic variant and fruit number (Fig. 2, Fig. 4). For the interaction of mean basal branches and fruit number, high elevation sites showed the strongest relationship between factors (Fig. 3).

Branching, height, density and fruit number all varied significantly along the elevational gradient. For mean density per plot, plots at high elevations were significantly less dense than medium and low elevation sites, with low elevation sites having the greatest average plot density (Fig. 9, $p=0.00644$). Branching and fruit number showed similar patterns; number of basal branches, lateral branches, and fruits were greatest on average at low elevation sites and significantly lower at high elevation sites (Fig. 5 $p=2.59e-8$, Fig. 6 $p=1e-5$, Fig. 8 $p=1.9e-6$). Branch height, however, followed a slightly different pattern. Tallest branch height was greatest, on average, at middle elevation sites, and lowest at high elevation sites (Fig. 7, $p=0.000561$).

Discussion

The general phenotypic trends indicated that plants displayed greater fitness at low elevations, as low elevation sites favored greater branching and fruit number than both medium and high elevations. Medium elevations favored tallest branches. Factors vary predictably along the elevational gradient; some of these factors may explain the downward trend in branching, height and fruit number as elevation increases. At lower elevations, growing season is longer due to an earlier snowmelt; this additional growing time may allow plants to produce more branches and fruits, and grow slightly taller (McCain and Grytnes, 2010). There are also more available nutrients in soil at lower elevations due to greater biomass and nutrient cycling (McCain and Grytnes, 2010). *Thlaspi arvense* could be more capable of varying its phenotypic traits in areas with more available nutrients.

There are also stressors that plants must overcome at low elevations to reproduce successfully. Competition and herbivory tend to be greater at low elevations (Lomolino et. al 2001). In order to combat enemies, plants at low elevations may be capable of producing more defense glucosinolates. Further studies on plant leaf chemistry across an elevational gradient could examine this suggestion. At low elevations, water can also be a limiting factor. The success of *T. arvense* in branching and fruit production at lower elevations suggests it may be capable of tolerating water stress. To test this, seeds collected from different elevations could be grown in varying soil moisture conditions and monitored during growth to evaluate success in growing large and producing fruits.

The changes in biotic and abiotic factors we observe along the elevational gradient are general. In order to understand exactly which factors are responsible for the difference in trait plasticity and reproductive success, *T. arvense* could be grown in controlled conditions to simulate environments in which traits are influenced. Since change in phenology was not affected by elevation, it may be driven more by microsite factors, such as soil moisture and aspect. Future studies could examine variations in soil moisture, aspect and snowmelt on a smaller, more specific scale. Phenology and phenotypic variation could also be genetically based. Seeds from populations of *T. arvense* at varying elevations could be grown in a common garden experiment to separate genetic effects from environmental effects.

This study showed that high elevation environments can be limiting for factors like branching, fruit production and height. However, as climate warms, high elevations may become more like low elevations in terms of precipitation and growing season, and plants historically restricted from high elevations will be able to increase their range (Beniston et. al 1997). If high elevations can increasingly facilitate *T. arvense*, and support its branching and fruit production, invasions may become increasingly dense at high elevations, and push out native species. Monitoring the spread of *T. arvense* as climate changes will be essential to preserving native biodiversity, especially if *T. arvense* has a negative impact on soil microbe and plant communities (Cousins, unpublished data).

Table 1: Phenological categories. Plants were assigned a phenological category based on their life cycle stage. Any plant under 1.0 cm in height was classified as a rosette. Plants were also categorized in between stages (e.g. 2/3, 4/5) to indicate they were transitioning between stages.

Category	Stage
1	Rosette
2	Bolting
3	Flowering
4	Going to seed, flowers remain
5	Fully senesced, no flowers remain

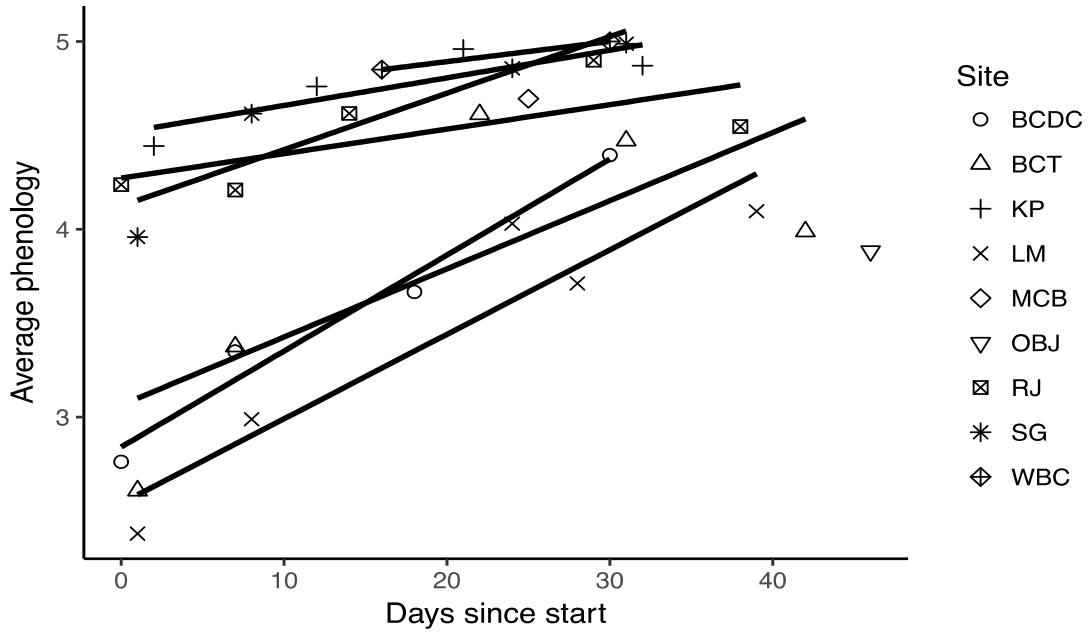


Figure 1: Average phenology of all plants at a given site over time. Start date is 06/19/17. While all sites showed a significant increase in average plant phenological stage, there was no difference between elevational categories (low, medium, high). ($F_{1,29}=0.169$, $p=0.684$)

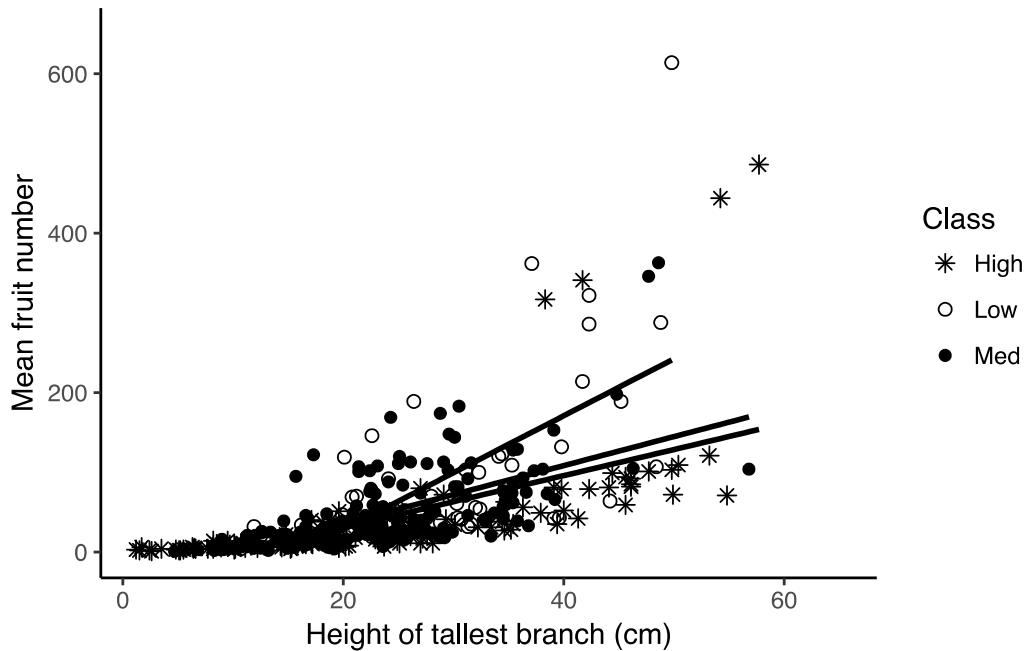


Figure 2: Mean tallest branch height (cm) by mean fruit number, separated by elevational category (Low, Med, High). Taller branch height is correlated with increased fruit number for all categories, but low elevation sites showed a closer relationship between height and fruit number. ($F_{2,370}=27.06$, $p=1.07e-11$)

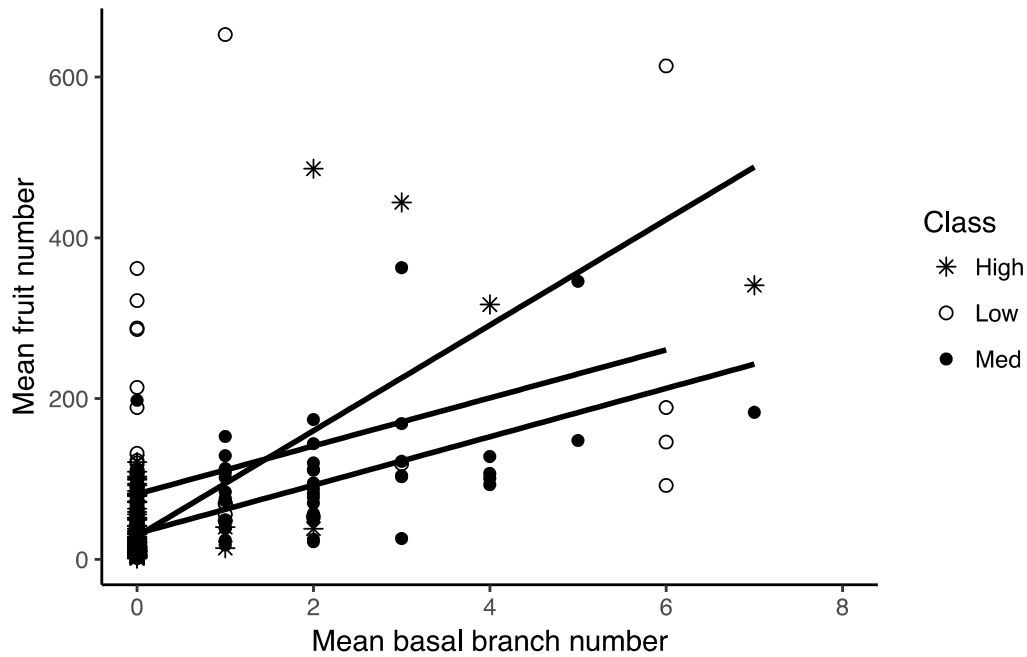


Figure 3: Mean basal branch number per plant by mean fruit number, separated by elevational category. For plants at high elevations, fruit number is most strongly correlated with fruit basal branches. ($F_{2, 370}=11.14$, $p=2.01e-5$)

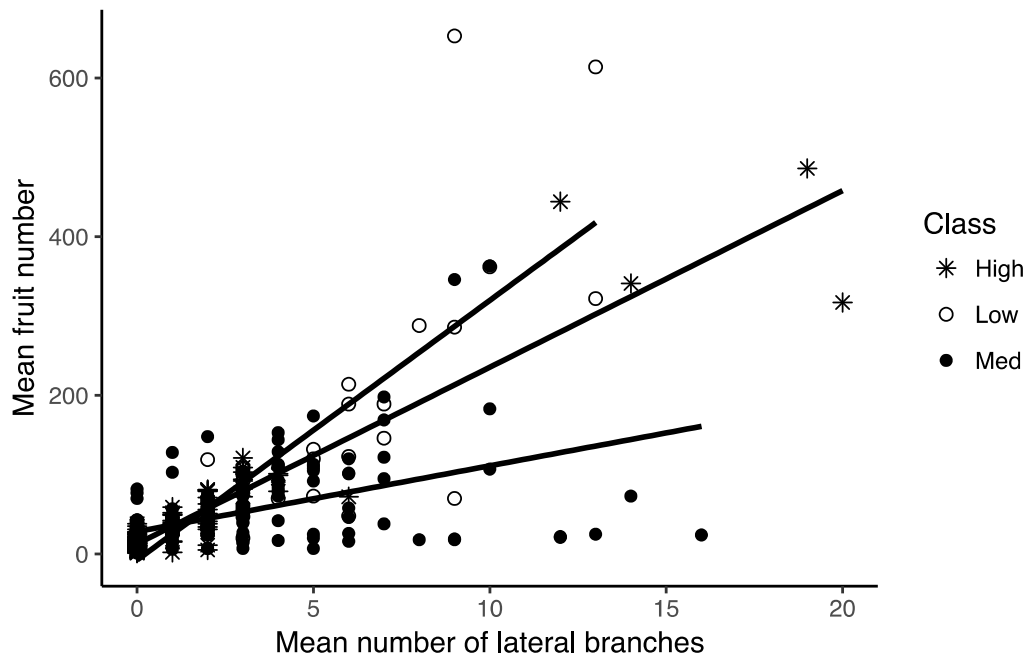


Figure 4: Mean lateral branch number per plant by fruit number, separated by elevational category. ($F_{2,370}=77.40$, $p<2e-16$)

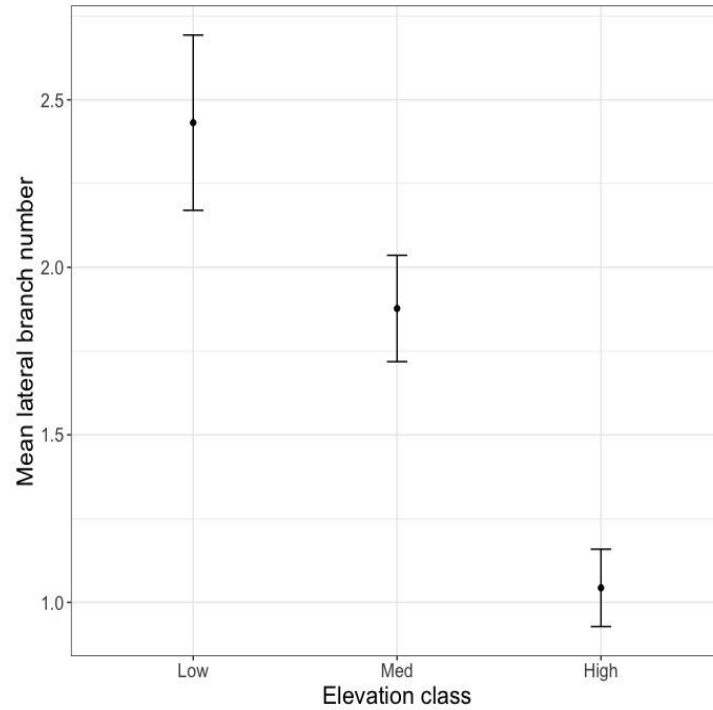


Figure 5: Mean lateral branch number for plants at each elevational category. Plants at high elevations had significantly fewer lateral branches than medium and low elevations. ($F_{1,959}=31.52$, $p=2.59e-8$)

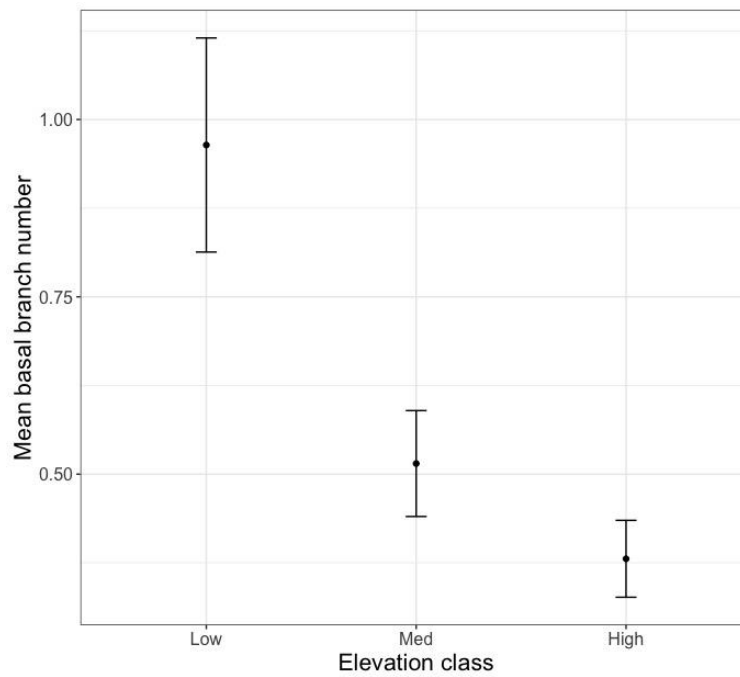


Figure 6: Mean basal branch number for plants at each elevational category. Plants at high elevations had significantly fewer basal branches than plants at low elevations. ($F_{1,959}=19.72$, $p=1e-5$)

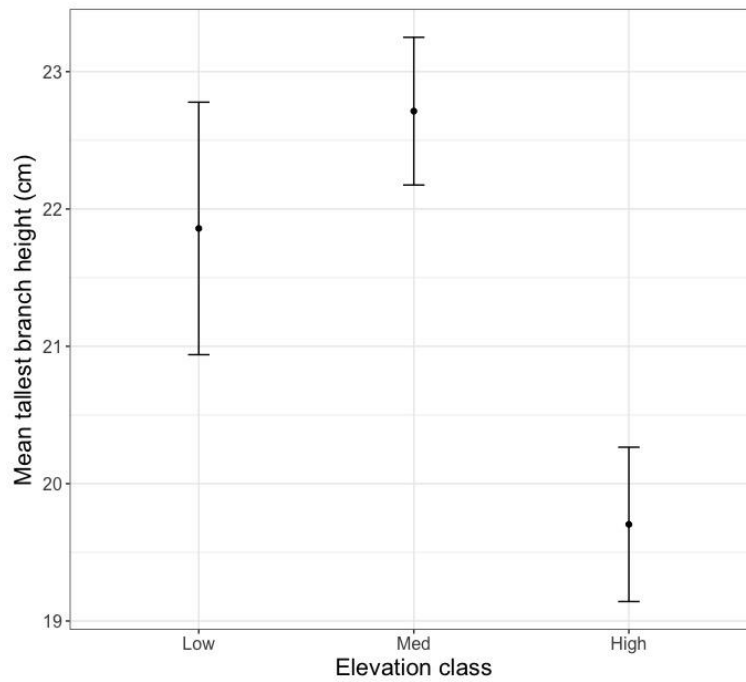


Figure 7: Mean tallest branch height for adult plants at each elevational category. Plants at high elevations had significantly shorter tallest branch height than medium and low elevations. Plants at medium elevations on average had tallest branches. ($F_{1,958}=11.7$, $p=0.000651$)

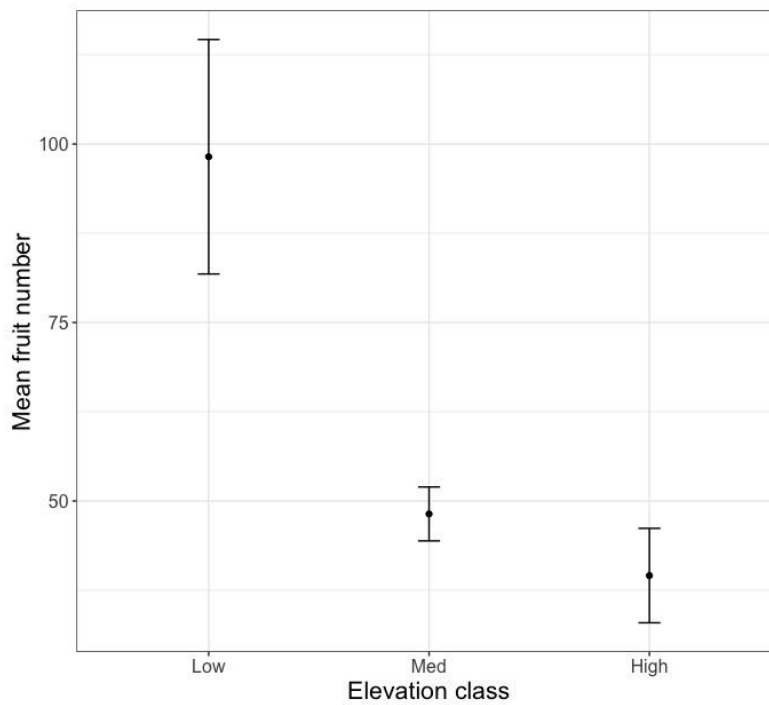


Figure 8: Mean fruit number for plants at each elevational category. Plants at medium and high elevations had significantly fewer fruits than plants at low elevations. ($F_{1,374}=23.42$, $p=1.9e-6$)

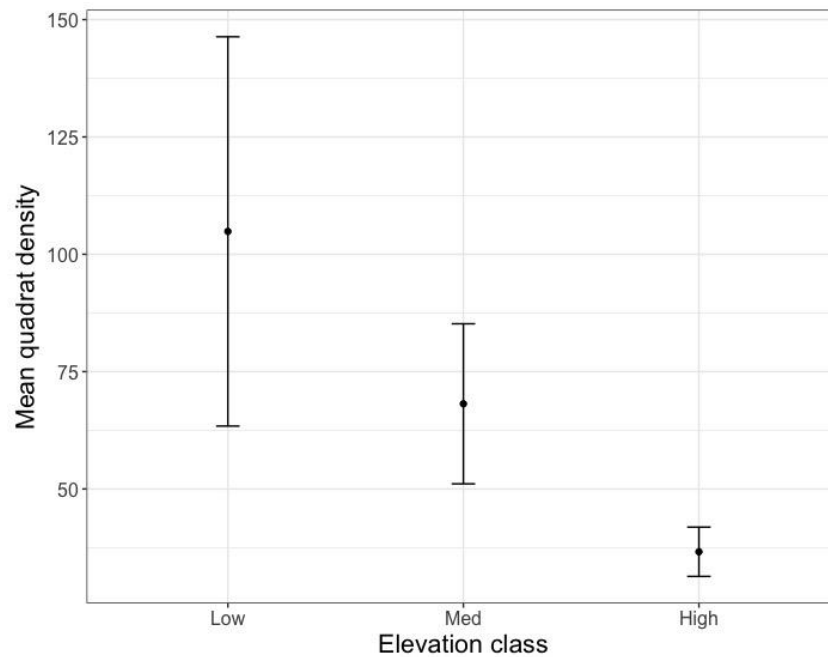


Figure 9: Mean quadrat density for plots at each elevation category. Plots at low elevations tended to be most dense, while plots at high elevations tended to be least dense. ($F_{1,58}=7.99$, $p=0.00644$)

Thlaspi arvense Research Sites 2017

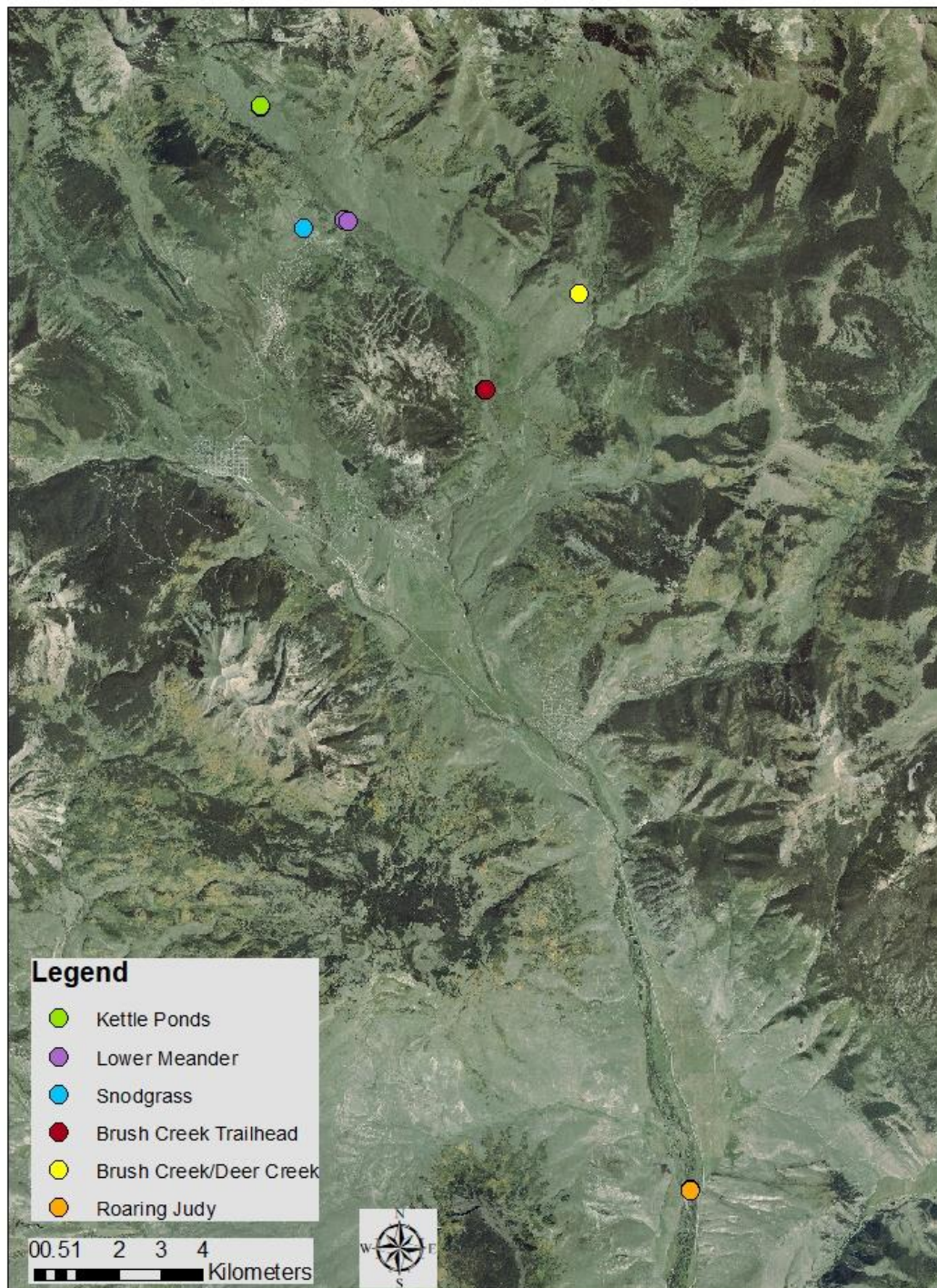


Fig. 10: Map of research sites in the Gunnison Valley in 2017.

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