SELECTION FOR LOW CALCIUM TOLERANCE IN *BRASSICA RAPA*

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MASTER OF SCIENCE

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ABSTRACT

Ecosystems across a landscape can vary in their selection pressures and therefore can vary in the species that are able to survive there. Selection pressures applied on a species found in multiple ecosystems may lead to a divergence into different taxa adapted to different selective conditions. One such soil condition with strong selection pressures are serpentine soils. They are unique in that they have low levels of essential nutrients, specifically calcium, and high levels of heavy metals.

To examine the effect of serpentine-like conditions on a model plant species, I grew Brassica rapa in a low calcium hydroponic environment and selected the most tolerant individuals within a population. After three generations, life history variables didn't change in comparison to controls, except dry mass. This could indicate that this population is at the beginning of a longer term evolutionary divergence. More generations of selection are needed to confirm this idea.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

CHAPTER 1: ADAPTATION AND SELECTION IN SERPENTINE SOILS

"Nothing can be more abrupt than the change often due to diversity of soil, a sharp line dividing a pine- or heather-clad moor from calcareous hills."

—Alfred Russel Wallace (1858)

Both Wallace (1858) and Darwin (1859) argued that adaptation of organisms to new environments results in the origin of new species. An excellent example of this would be when a species encounters a relatively new soil environment with unique challenges for growing and reproduction. Previous research has shown that variations in soil properties have caused selection within a population (Kazakou, Dimitrakopoulos, Baker, Reeves, & Troumbis, 2008). One such community that has been heavily studied is the plant community growing on serpentine soils (Brady, Kruckeberg, & Bradshaw Jr., 2005; Kruckeberg, 1984; Selby, 2014).

There are three main patterns that are exhibited by plants in relation to serpentine soils: 1) the species are unable to grow on serpentine soils because they are not tolerant of the condition. 2) the species only grows on serpentine soils and cannot grow in any other location (endemic to serpentine). 3) the species are "indifferent" and have the ability to survive both on and off serpentine soils. I am interested in how plants evolve the ability to tolerate and grow on serpentine soils that have originated off serpentine environments. This knowledge can increase our understanding of plant evolution and adaptation to new environments.

Serpentine soils are created from ultramafic rocks, igneous rocks with high levels of magnesium and iron. They are located worldwide, focused around the 'ring of fire', and in North America they are primarily on the west coast. Serpentine soils are characterized, generally, by extremely low concentrations of calcium, a low Ca:Mg ratio, and high concentrations of heavy

metals that are toxic to plants (Alexander et al., 2007). The soil is located in patches across the landscape surrounded by 'normal' soil. Plants growing on serpentine soil must be tolerant of relatively low amounts of calcium and high levels of potentially deadly heavy metals (e.g. Nickel, Lead, Copper). There are many plants species that have adapted to survive both on serpentine soils and on normal soils (Brooks, 1987). Additionally, the soils often reside in locations that are particularly prone to erosion and drought. So, plants often have to not only be tolerant of the edaphic conditions, but of potential drought and erosion as well (Selby, 2014). Common garden experiments have shown that plants from serpentine and non-serpentine populations grew better in their original environment relative to the other environment (Selby, 2014). This suggests that plants adapt to serpentine soils but that there is a cost to this adaptation.

There are a few possible mechanisms by which some plants can survive on serpentine soils. The mechanisms include 1) tolerance to low concentrations of calcium, 2) tolerance to high concentrations of magnesium, and 3) hyperaccumulation of heavy metals (Kruckeberg, 1984). There is support for the hypothesis that plants growing on serpentine have primarily evolved a tolerance to low calcium. Studies have shown that non-serpentine plants could survive in serpentine conditions if and only if calcium was added back to the soil (Vlamis & Jenny, 1948; Walker, Walker, & Ashworth, 1955; Whittaker, 1954). Thus, low calcium may be the primary stressor to serpentine plants.

Higher than normal concentrations of magnesium is also characteristic of serpentine soil, high enough where they are potentially toxic to many species (Brooks, 1987). Some research indicates that magnesium poisoning as the primary hurdle for serpentine tolerant plants to overcome (Brooks, 1987; Brooks & Yang, 1984; Proctor, 1971, 1970). These studies found that non-serpentine plants were more susceptible to magnesium poisoning, but also that it relied on a

low concentration of calcium as well. This suggests that serpentine plants must tolerate low ratios of calcium to magnesium (Brady et al., 2005).

Finally, hyperaccumulation of heavy metals has also been hypothesized to be the main contributor to serpentine tolerance. Hyperaccumulation is the uptake of high levels of metals normally toxic to plants. Hyperaccumulation potentially benefits the plant by helping ward off herbivores, protection from pathogens, allelopathy, and heavy metal tolerance (Brady et al., 2005). However, most known hyperaccumulators are not known to exist on serpentine environments.

Preadaption or cross tolerance seems to be the most likely mechanism for colonizing serpentine soils. Preadaptation in regard to tolerating serpentine soils arise when individuals within a population would already contain genes that would allow them to survive and reproduce on serpentine soils. These surviving individuals would then reproduce and potentially create more tolerant generations. Cross-tolerance is similar in that plants would already have the ability to survive on serpentine soils before colonizing them, but plants would gain this ability from growing in conditions similar to that of serpentine (Brady et al., 2005).

We know that plants are adapting to these extreme edaphic conditions. This has been proven to occur independently on serpentine soils many times, even within the same species (Kruckeberg, 1984). As to what the mechanism of this adaption is still not exactly known. More work needs to be done that helps to tease apart how adaptation to these environments occurs.

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CHAPTER 2: SELECTION FOR LOW CALCIUM TOLERANCE IN *BRASSICA RAPA*

Introduction

Ecosystems across a landscape can vary in their selection pressures and therefore can vary in the species that are able to survive there. Multiple selection pressures can be applied on a species found in multiple ecosystems which may ultimately lead to a divergence of species into different taxa adapted to different selective conditions. These selection pressures can derive from habitat, predation, climate, and nutrition (Schluter, 2000).

Variation in selection pressures over space is one of the potential origins of speciation (Schluter and Conte 2009, Packard 2014). When natural selection occurs in a specific isolated environment, species can become locally adapted to those conditions. A study conducted showed that recolonization near a mine showed a gradient in plant tolerance to heavy metals as you moved farther away from the mine (Caisse & Antonovics, 1978a). Eventually, plants near the mine and far from the mine were not able to inter-breed and experienced reproductive isolation (Caisse & Antonovics, 1978a). This is an example of how divergence from other populations that have not adapted to that environment could lead to speciation. By adapting to the local environment species may not be adapted to other environments because of fitness tradeoffs (Bennington et al., 2012; O'Dell & Rajakaruna, 2011; Sarkissian & Harder, 2001).

All species are limited to a specific range of conditions and thus have limited ability to grow and reproduce in different conditions. Nonetheless there is variation in range among species. Some species have limited ranges while others are very widespread. Local adaptation

likely plays a role in determining the final range of a species. This raises the question of why some species adapt to a wide variety of environments and other species do not?

Species undoubtedly vary in their ability to respond to natural selection. The Breeders equation specifies that a phenotypic response (R) to selection is dependent on both the heritability (h^2) of traits and selection differentials (S) (Falconer, 1981).

$$R = h^2S$$

So, populations exposed to selection may vary in heritable variation and/or on the strength of selection. I am interested in understanding the potential for plants to respond to selection from specific soil conditions. I examined plant adaptation to extreme variation in soil nutrition.

If there is no heritable variation in a trait then even if there is strong selection for it to change, there will be no change and no adaptation. Likewise, if there is heritable variation but very weak selection, it is expected there would be no response to selection.

We know that some species are capable of adapting to a wide range of soil conditions (Schluter, 2001; Selby, 2014). Serpentine soils are a good example of soils with extreme nutritional qualities (see chapter one). Serpentine soils have relatively little calcium and contain high concentrations of heavy metals (Kruckeberg, 1984). It is assumed endemic species originated from a process of ecotypic adaptation where individuals had the ability to adapt and survive on the soil, and others did not. I am interested in the ability of the soil characteristics to provide a selection pressure for tolerance to the soil, and whether plants have enough heritable variation in important traits that determine the ability of a species to tolerate extreme serpentine soils.

Serpentine soils are unique in that they are toxic to most plants and have 1) very low levels of essential nutrients: Calcium (Ca), Nitrogen (N), Potassium (K), and Phosphorus (P) and

2) high levels of Magnesium (Mg) and heavy metals such as Nickel (Ni), Cobalt (Co), Chromium (Cr), and Iron (Fe). This type of environment exists because of the weathering of ultramafic rocks that originate from the Earth's mantle have been exposed at the surface (Alexander et al., 2007). Serpentine soils are usually found near continental plate subduction zones (e.g. the 'Ring of Fire') and are found in small patches that can be distinct from one another due to variation in weathering and parent material (Selby, 2014).

I used a model plant species (*Brassica rapa*) to assess the ability of plants to evolve tolerance to some of the nutritional characteristics of serpentine soils. Specifically, the experiment in this study attempts to establish 1) if low calcium soil conditions could select for phenotypic traits in a plant that would result in increased tolerance; and 2) how quickly tolerance in a species becomes prevalent in a population. My goal was to better understand how a specific environmental selection pressure could modify plant traits and potentially lead to adaptation and speciation.

Methods

I conducted two pilot studies and a selection experiment. The selection experiment was designed based on the results from both pilot studies. The overall objective was to determine if selection to tolerate a low calcium-magnesium environment would result in an increased tolerance to the environment over time. A complete description of the two pilot studies can be found in Appendix A. The objectives of the pilot studies were to 1) determine if the environment affected plant success and reproduction; and 2) determine what early life history variable correlated to plant fitness.

Study Species

The plant used in the experiment is *Brassica rapa (B. rapa),* commonly known as field mustard. *Brassica rapa* can be an annual or biannual that is self-incompatible. The plant originated in Eurasia but has been introduced to much of the world and is now widely cultivated for food. It is also a model species in that is it well studied and has a quick lifecycle (Williams & Hill, 1986). I studied the cultivated variety of *B. rapa* known as the Fast Plant®.

The Fast Plant® variety of *B. rapa* used was bred for quicker flowering time, fast seed maturation that doesn't require dormancy, and highly fertile females as well as being self-incompatible. At approximately 2 weeks after seed sowing, flowering begins and persists for approximately 5 days. Seed maturation is complete approximately 3 weeks after pollination.

Hydroponic Growth Method

The growth method used in the selection study is a modified version of the recommended hydroponic Fast Plant® growth method (University of Wisconsin, Madison, WI, USA). All plants were grown hydroponically in nutrient solutions with no soil. This allowed for greater control of the nutrients being delivered to the plants. Plants were grown on a lab bench under grow lights.

I used a rectangular plastic tub with a lid that holds three liters of solution. Twenty-four plants were grown per container in six Styrofoam quads that hold four plants each. Rock wool, a non-nutritive substance that is excellent at absorbing and holding water, was placed within each of the four chambers per quad. Rock wool was used instead of soil to provide greater control over the nutrients taken up by the plants. In each of the four chambers per quad the rock wool was wrapped around a knot in a small piece of nylon rope. Approximately fifteen centimeters of

the nylon rope was threaded through a hole drilled in the plastic top of the container and served as a wick for the solution.

Seeds were placed just under the surface of the rock wool with one seed per quad. The containers were placed under two 6400K, 54W bulbs. Lighting was provided 24 hours a day through the duration of the experiment. As the seedlings grew the lights were adjusted to between four and eight inches above the top of the plants.

The containers were initially provided 1.5 liters of nutrient solution. One liter of solution was added to each container weekly. The solution in each container varied by experiment and will be discussed within each section.

Nutrient Solution

Two different bulk nutrient solutions were initially prepared: a control solution, which was a quarter strength Hoagland's solution (Hoagland & Arnon, 1950), and a low calcium solution (Appendix B). The low calcium solution was identical to the control solution with the exception of the calcium nitrate and the magnessium nitrate. The low calcium solution contained 1% of the calcium of the control. To create a similar nitrate concentration between the control and the low calcium solutions, magnesium nitrate was added to the low calcium solution. As a result the ratio of calcium to magnesium was 4:1 in the control solution and 1.1:1 in the low calcium solution.

Selection Experiment

The basic experimental design of the selection experiment was to create two lines of plants: one of which experienced three generations of selection for tolerance to low calcium

growth solutions and one of which experienced no selection over three generations (Figure 1.1). The two experimental lines are referred to as control and low calcium, I grew four generations of plants within each line; the parent population (G0), second generation (G1), third generation (G2), and the fourth generation (G3). Both lines of plants originated from a single order of 100 Standard Wisconsin Fast Plants® grown in the hydroponic conditions described above. One hundred seeds for the parent generation of the Control population were grown with control calcium solution and 100 seeds were grown with the low calcium solution.

In the parent generation (G0) I measured the height of each plant at day 16 since planting as the initial measure of growth with this study. Height was measured as the length of the main stem of the plant from the rock wool surface to the top of the highest point. The height at first flower and the number of days to first flower was also recorded.

In the control line we removed all but 20 randomly chosen plants on day 16. In contrast, in the low Calcium line, the 20 tallest plants on day 16 were retained and all others were removed. On day 24, flower and bud numbers per plant were recorded as well as the height. The remaining plants in each population, were then hand crossed to other plants in the same line to produce the next generation. I pollinated a flower by removing 1-2 stamens from a donating flower with a pair of forceps. The anthers were then brushed against the stigma of the receiving flower which was marked with a jewelry tag. At least 100 unique pollinations were conducted within each line in the parent generation. Plants were chosen to be crossed if the stamen and pistil were mature and had yet to be paired.

The fruits were allowed to mature for the next 3 weeks. At the end of the 3 weeks the seed pods began to lose their green color and turn partially light brown. At this point the solution was drained from the containers to help dry out the seeds for one day. The next day, the pods

were cut from their plant and placed into a labeled coin envelope that was placed into a plastic bag put into the refrigerator and filled partially with silica gel to keep them dry.

The above procedures were repeated for the next three generations (G1, G2 and G3) with a few modifications. In the first offspring generation (G1) only 72 seeds were sown for each line because of limited fruit and seed production in the parent population. However, out of the 72 seedlings growing in each line twenty plants were still chosen in each line according to the rules described above. The number of starting seedlings in the G2 generation was 72 for the two lines and the number for generation G3 was 48 for the two lines. Thus, the intensity of selection varied from generation to generation.

After two generations of selection, the tolerance to growing in a low calcium was measured. I grew seeds from each line in low calcium solution and measured the growth characters of the seedlings (Figure 2.1).

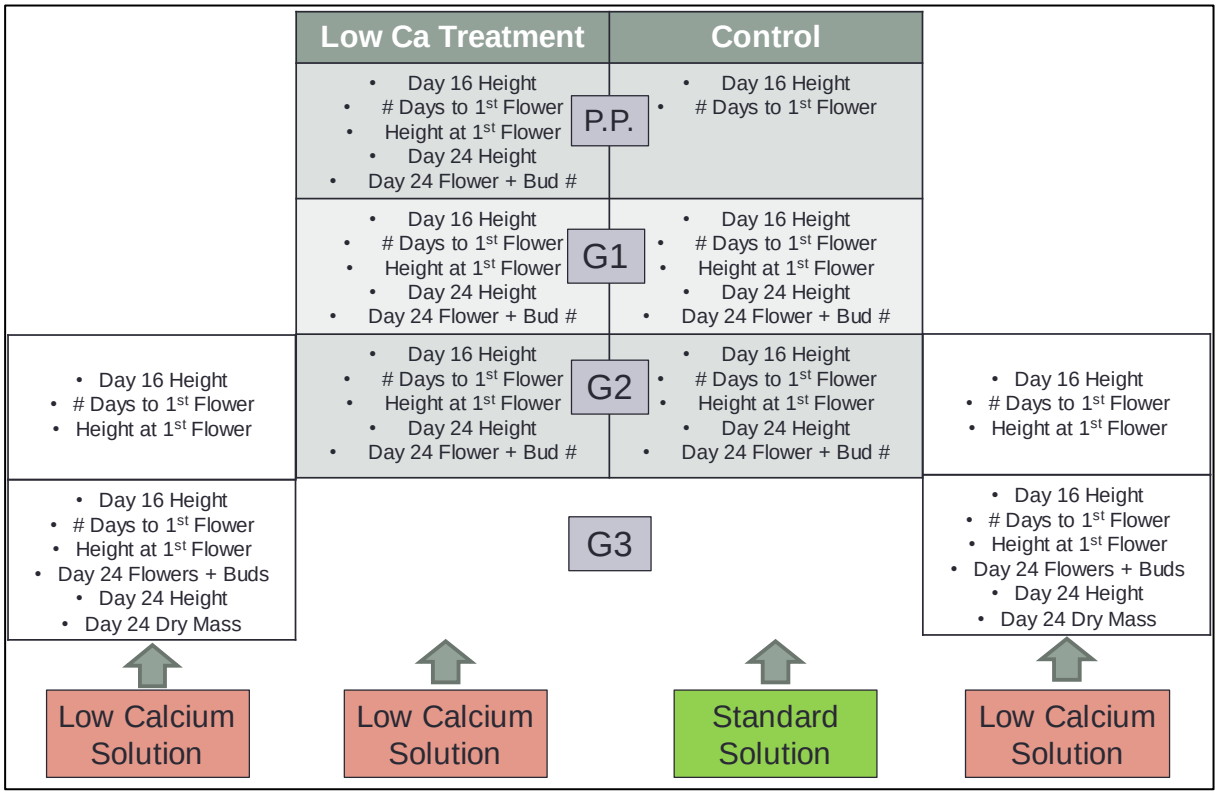

Figure 2.1. Experimental design of the selection experiment and all data collected from each population. The two middle lines were used for selection and reproduction with each population staying in the respective solutions. The two outside populations were created from the main lines, then both grown in low Calcium solution.

For the 3rd generation (G2) tolerance population, G2 seedlings from both lines were grown in low calcium solution for 16 days and height was recorded on that day. For the 4th generation (G3) tolerance population, the height of seedlings was recorded every other day. I also measured the number of days to first flower, height at the first flower, flowers at day 24, buds at day 24, and the dry mass of aboveground plant material after day 24. To measure dry plant mass, above ground material was placed in a paper bag and placed in a drying oven at 70°C for approximately two days.

Statistical Analysis

In the analysis of the results, specific statistical tests were used to answer questions about the data. All statistical tests were conducted using R-Studio (R Core Team, 2008) and JMP® (JMP, Version 14.).

1. Question: Was there a response to selection? Was the environmental selection pressure strong enough?

To answer this question, a two-way ANOVA was used to look for an interaction between the mean day 16 height and the mean days to first flower for both control and the selected population. This was run for the day 16 height and the number of days to 1st flower.

2. Question: Does three generations of selection on day 16 height lead to adaptation or a greater tolerance in a low calcium environment?

To answer this question, 6 t-tests were performed to determine if there were any significant differences between means of the control and selected populations growth variables. This analysis was conducted for the day 16 height, days to 1st flower, height at 1st flower, day 24 height, number of flowers and buds, and the dry mass.

3. Question: What life history variables have the greatest effect on the differences seen between the control and selected populations after three generations of selection?

To answer this question, a principal component analysis (PCA) was used to reduce the number of dependent variables. The PCA was conducted on the variables of the G3 tolerance population.

4. Question: Is there a relationship between the covariance matrices before and after selection? Does the correlation coefficient between life history variables change after selection has taken place?

To answer this question, a Mantel test was used to determine if there were changes in the correlation coefficients when looking at values before and after selection had taken place. Matrices from the control parent population, and G3 control and selected in low Calcium were used.

Results

Variables collected within the parent population (Figure 1.2) include the day 16 height (D16H), day 24 height (D24H), total flowers and buds (FplusB), and Dry Mass. In the parent population, there was a strong relationship between day 16 height and dry mass (R=0.67) as well as flowers and buds with dry mass (R=0.8: Figure 2.2) Thus we used day 16 height as our measure of a fitness correlate. Additional variables collected for the G3 population are the number of days to 1st flower and the 1st flower height.

The goal of the experiment was ultimately to understand changes in plant reproductive success as a measure of fitness with sequential generations of selection for tolerance to low calcium growth conditions. Because it was necessary to impose selection and cross plants prior to the measurement of lifetime growth and reproductive effort we required a variable that was highly correlated with these two components but measurable early on.

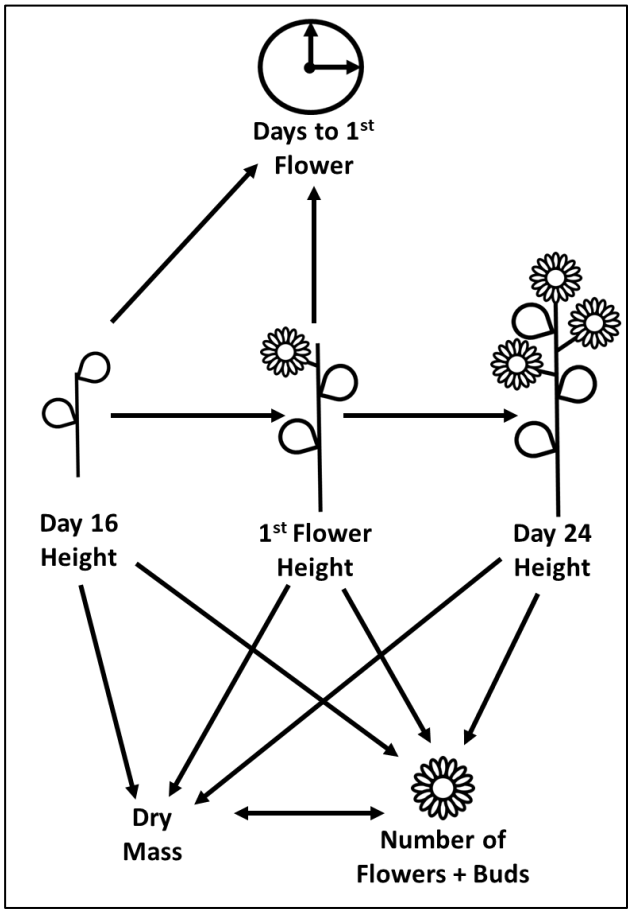

Figure 2.2. The relationship among all life history variables.

Treatment Effects on Growth in Different Environments

Results of the 2-way analysis of variance on the parental, G1 and G2 generations show a significant difference in day 16 height between the 2 different treatments ($P<0.001$) as well as among generations ($P<0.001$; Table 2.1). The Control populations were taller on day 16, except during the second generation where the low Calcium population was taller. In the parent and G2 generations the average height was nearly twice as large for plants grown in control solutions

compared to low calcium solutions (Figure 2.4). There was a significant interaction between treatment and generation as well ($P<0.001$).

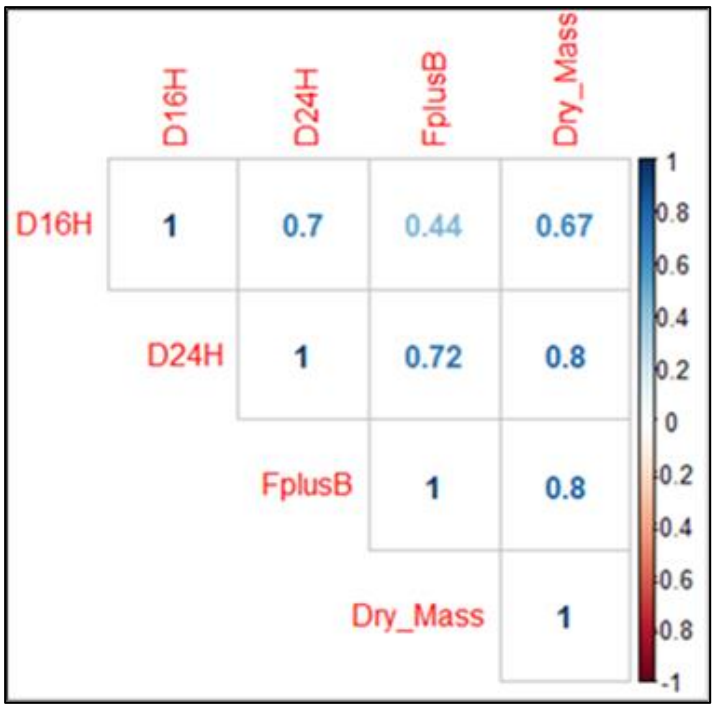

Figure 2.3. The correlation matrix of the parent population in normal solution. Values given are the correlation coefficients (R^2).

Table 2.1. Analysis of Variance table for the mean day 16 height between populations and treatments.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Generations	2	1604.9	802.46	57.265	< 2.2e-16
Treatment	1	1765.2	1765.23	125.972	< 2.2e-16
Gen:Treat	2	3434.1	1717.06	122.534	< 2.2e-16
Residuals	341	4778.4	14.01		

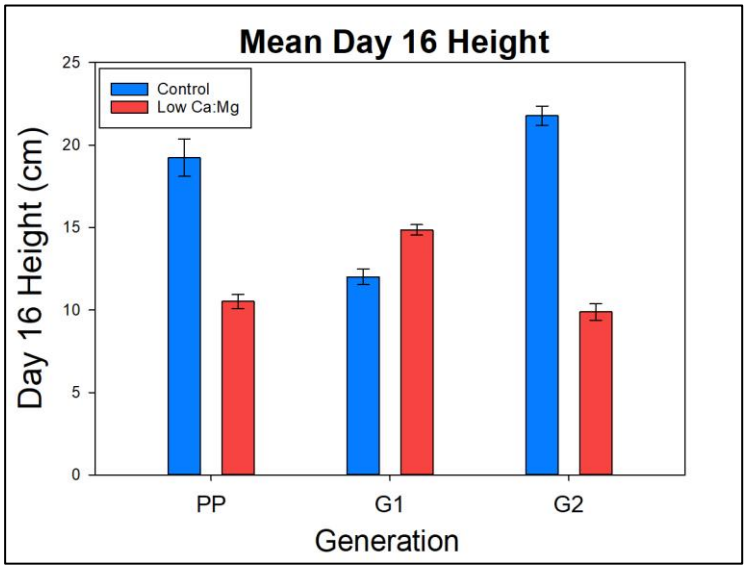

Figure 2.4. Mean day 16 height of the populations through 3 generations. The blue bars represent the control populations and the red bars represent the low Calcium populations.

The data for the number of days to first flower shows that the low Calcium population flowered slightly faster than the control population, except for the second generation. Results of the 2-way analysis of variance shows that there is a significant difference among the means when looking at the treatment (P=0.007) (Table 2.2) and the generation (P<0.001) (Figure 2.5). A significant difference was also found when considering the interaction among the treatment and the generation (P<0.001).

Table 2.2. Analysis of Variance Table for the mean days to 1st flower between populations and treatments.

	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Generations	2	562.1	281.06	171.096	< 2.2e-16
Treatment	1	12.1	12.08	7.352	0.00704
Gen:Treat	2	219.4	109.69	66.773	< 2.2e-16
Residuals	336	551.9	1.64		

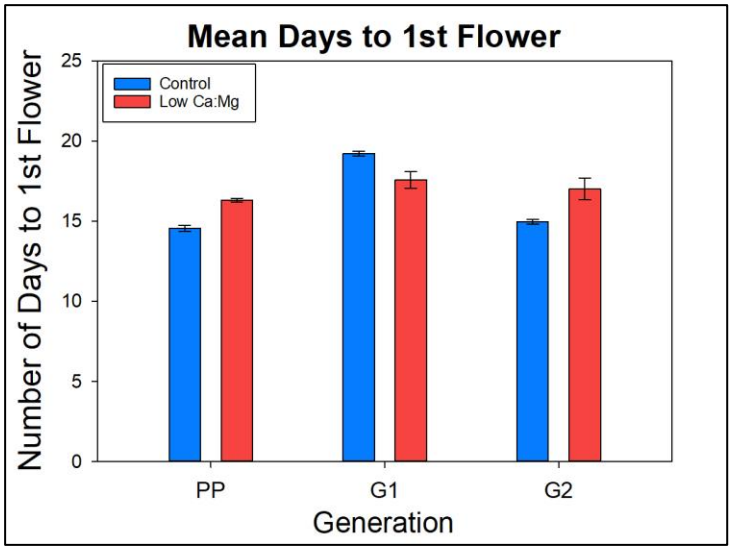

Figure 2.5. The mean number of days to 1st flower of the population through 3 generations. The blue bars represent the control populations and the red bars represent the low Calcium populations.

Treatment Effects on Measures of Low Calcium Tolerance

The day 16 height mean was similar between the control (9.5) and selected (9.49) populations (Figure 2.6); the t-test results show that they are not significantly different ($P>0.05$). Similarly, the average number of days to 1st flower was slightly higher for the selected population (16.9) than the control population (16.4; Figure 2.7). However, the t-test results show that they are not significantly different ($P>0.05$).

The days to first flower showed that the selected population took slightly longer to flower (16.9) than control (16.4; Figure 2.8). The t-tests showed that there was no significant difference between the two ($P>0.05$). The day 24 height appeared to be similar between the control (11.9) and the selected (11.4; Figure 2.9) populations and the t-test showed that they were not significantly different ($P>0.05$). The flowers plus buds also appeared to be similar between the control (18.8) and selected (19.4; Figure 2.10) populations, with similarly no significant difference between the populations ($P>0.05$).

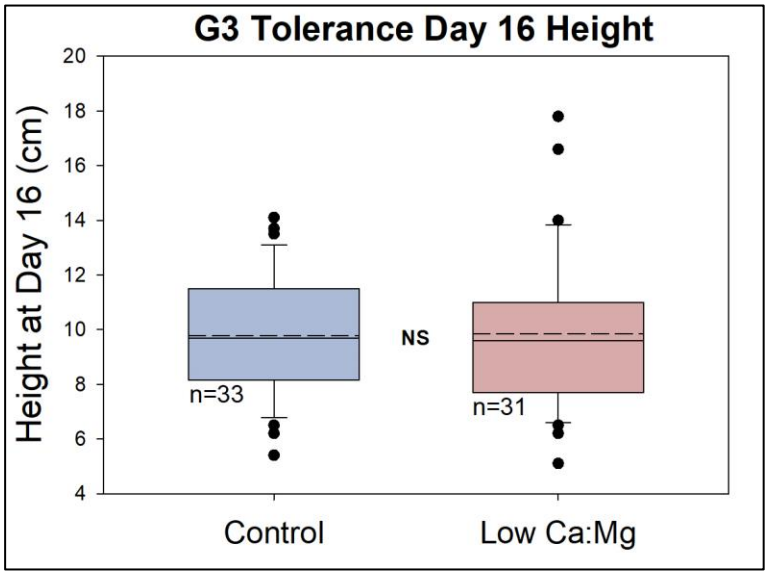

Figure 2.6. G3 tolerance population's control and selected day 16 height in low Calcium solution. The inter-quartile range represents the middle 50% of the data and 25% existing both above and below that range. The median is represented by the solid line within the box plot, and the mean is the dashed line. T-test: t= -0.11398, df= 55.417, and p= 0.9097.

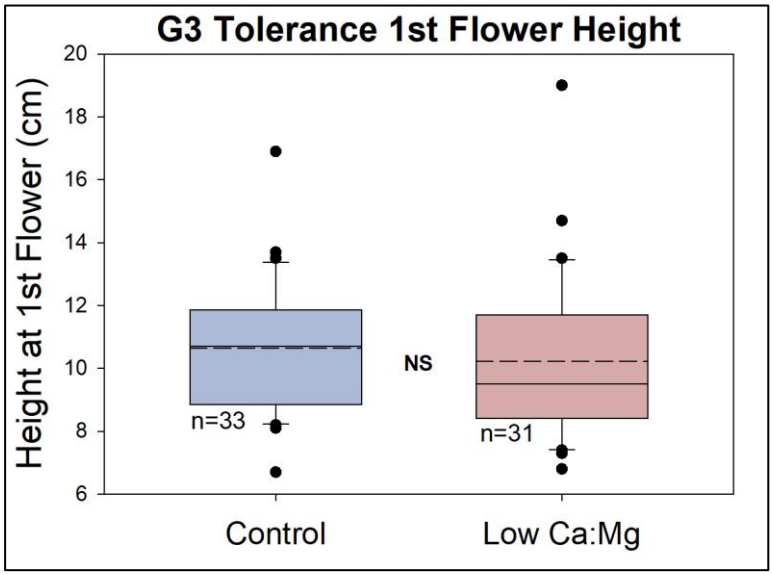

Figure 2.7. G3 tolerance population's control and selected 1st flower height in low Calcium solution. The inter-quartile range represents the middle 50% of the data and 25% existing both above and below that range. The median is represented by the solid line within the box plot, and the mean is the dashed line. T-test: t= 0.17228, df= 61.146, and p= 0.8638.

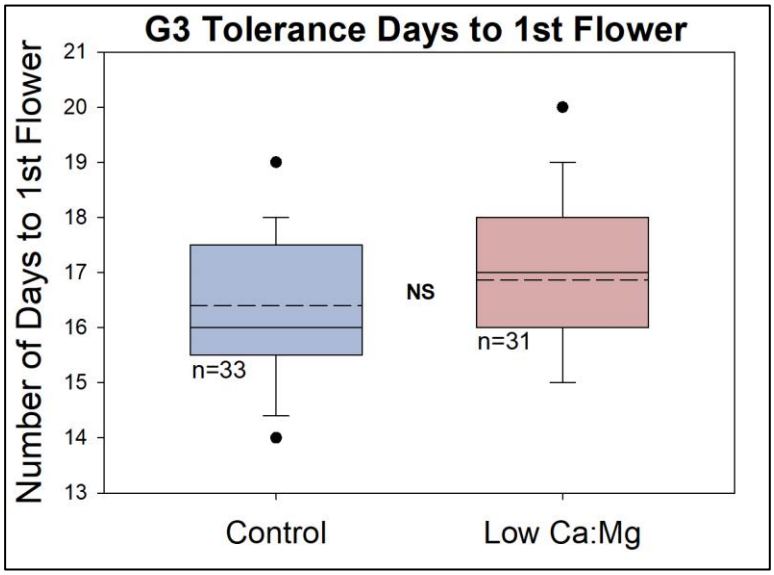

Figure 2.8. G3 tolerance population number of days to 1st flower in low Calcium solution. The inter-quartile range represents the middle 50% of the data and 25% existing both above and below that range. The median is represented by the solid line within the box plot, and the mean is the dashed line. T-test: t= -1.4217, df= 61.477, and p= 0.1602.

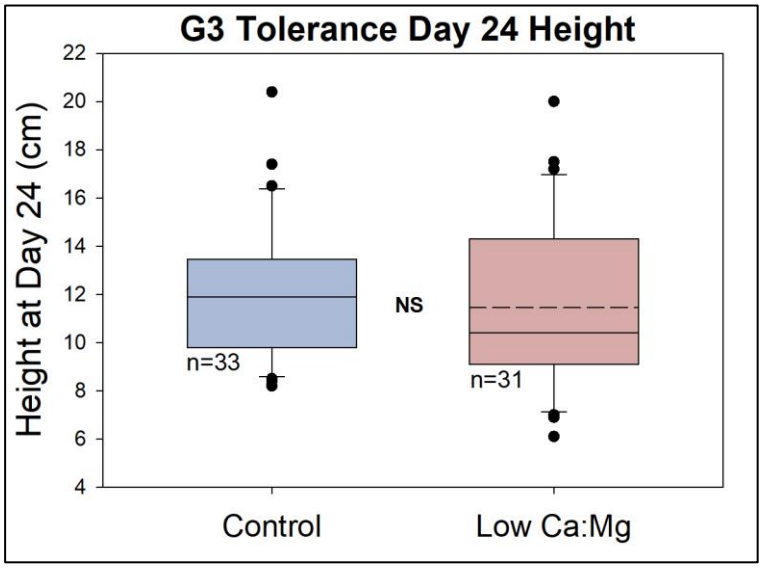

Figure 2.9. G3 tolerance population's control and selected day 24 height in low Calcium solution. The inter-quartile range represents the middle 50% of the data and 25% existing both above and below that range. The median is represented by the solid line within the box plot, and the mean is the dashed line. T-test: t= 0.55448, df= 57.013, and p= 0.5814.

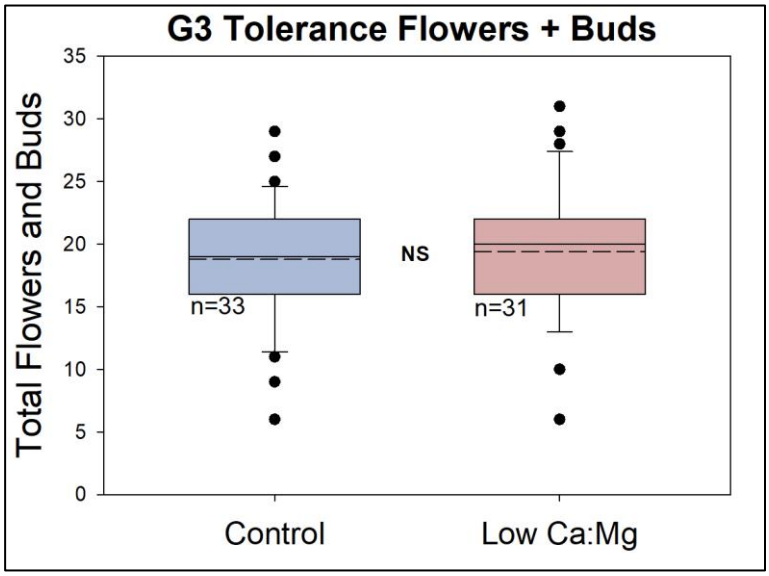

Figure 2.10. G3 tolerance population dry mass in low Calcium solution. The inter-quartile range represents the middle 50% of the data and 25% existing both above and below that range. The median is represented by the solid line within the box plot, and the mean is the dashed line. T-test: t= -0.48797, df= 61.033, and p= 0.6273.

Overall, there were no significant treatment effects on any variables except for dry mass. The dry mass of plants from the selected low calcium line was significantly greater than the dry mass of control plants when both were grown in low calcium (Figure 2.11).

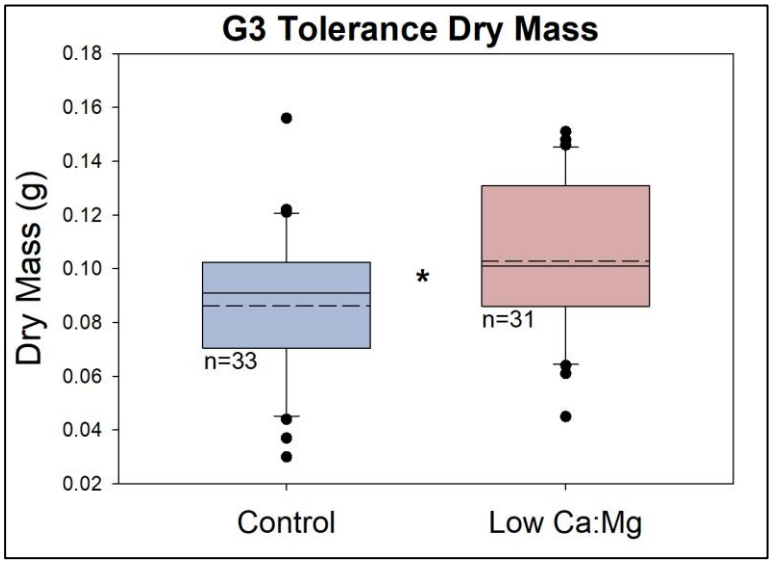

Figure 2.11. G3 tolerance population dry mass in low Calcium solution. The inter-quartile range represents the middle 50% of the data and 25% existing both above and below that range. The median is represented by the solid line within the box plot, and the mean is the dashed line. T-test: t= -2.3964, df= 61.211, and p= 0.01963.

Because the life history variables are closely related it is useful to collapse all of the variables into principal components and compare the values between the selected line and the control line when both plants are grown under low calcium conditions.

The first three principal components of the analysis performed on the G3 population explained a total of 81% of the variation (Figure 2.12). The first three principal components were determined to be significant based on the results of the Bartlett test (Table 2.3). Bivariate plots comparing each of the first three principal components (Figure 2.13;2.14;2.15) showed little variation between them. They all indicated a relatively large degree of overlap between plants from the two selection lines. The loading of principal component one was greatest on day 16 height (44% of the variation) with dry mass for principal component two (21% of the variation), and flowers plus buds for principal component three (16% of the variation).

Figure 2.12. Principal Component Analysis of G3 Selected and Control Populations

Importance of components:

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.6325	1.1129	0.9798	0.75004	0.60085	0.46123
Proportion of Variance	0.4442	0.2064	0.1600	0.09376	0.06017	0.03546
Cumulative Proportion	0.4442	0.6506	0.8106	0.90437	0.96454	1.00000

	PC1	PC2	PC3	PC4	PC5
Daysto1stFlower	0.3735282	-0.48079576	-0.09031732	0.75762189	-0.0850383
X1stFlow_Height	-0.4720777	-0.26582552	-0.32726538	0.15916025	0.7315465
D16H	-0.5635094	0.02453354	-0.05557835	0.05527425	-0.1751242
D24H	-0.5033603	-0.31282507	-0.07282129	0.10051539	-0.6255586
FandB	-0.2275240	-0.02228637	0.92063538	0.24744719	0.1836250
Dry Mass	-0.1222198	0.77408923	-0.16964303	0.57121659	-0.0435660

Table 2.3. Results of the Bartlett test of the principal component analysis. The test found that the first three principal components were significant.

Principal Component	Chi Square	DF	P-Value
1	121.622	14.621	<0.0001
2	57.092	12.903	<0.0001
3	35.791	8.840	<0.0001
4	13.569	5.326	0.0231
5	4.044	1.761	0.1075

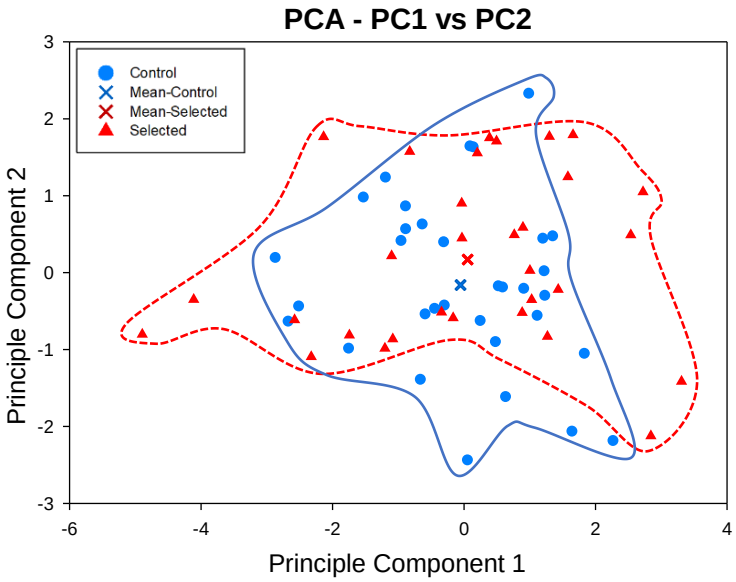

Figure 2.13. Scatter plot of principal component 1 against principal component 2 of the G3 tolerance population. The blue circles represent the control population and the red triangles represent the selected population. The blue X is the mean for the control population, and the red X is the mean for the selected.

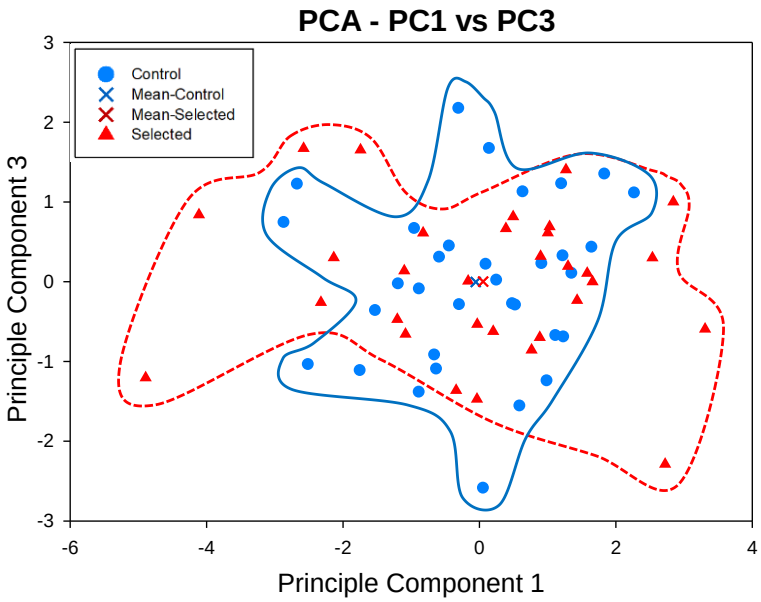

Figure 2.14. Scatter plot of principal component 1 against principal component 3 of the G3 tolerance population. The blue circles represent the control population and the red triangles represent the selected population. The blue X is the mean for the control population, and the red X is the mean for the selected.

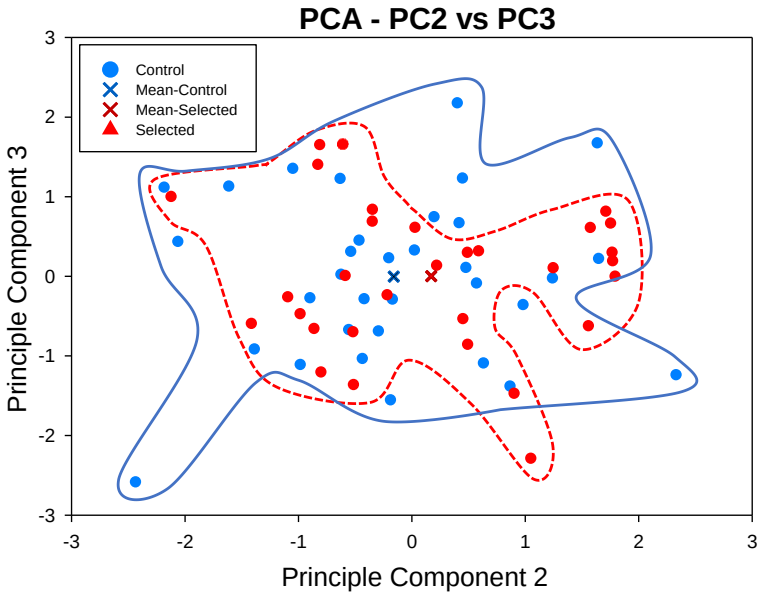

Figure 2.15. Scatter plot of principal component 2 against principal component 3 of the G3 tolerance population. The blue circles represent the control population and the red triangles represent the selected population. The blue X is the mean for the control population, and the red X is the mean for the selected.

Covariation in G3 Tolerance

In order to examine how the relationships among the variables differed for the two lines of plants under low calcium conditions, I calculated the correlation matrix among the variables for each line separately. In contrast to the original correlation matrix (Figure 2.2), the selected population's flowers and buds didn't show as strong of a correlation to dry mass (Figure 2.16). The control population's D16H didn't show as strong of a correlation to total Flowers and buds (Figure 2.17). There were also differences between the matrices of the two G3 populations. The selected populations had a much higher correlation between 1st flower height and day 16 height (Figure 2.16) than the control population (Figure 2.17).

Correlation Matrix of Life History Variables in G3 Selected

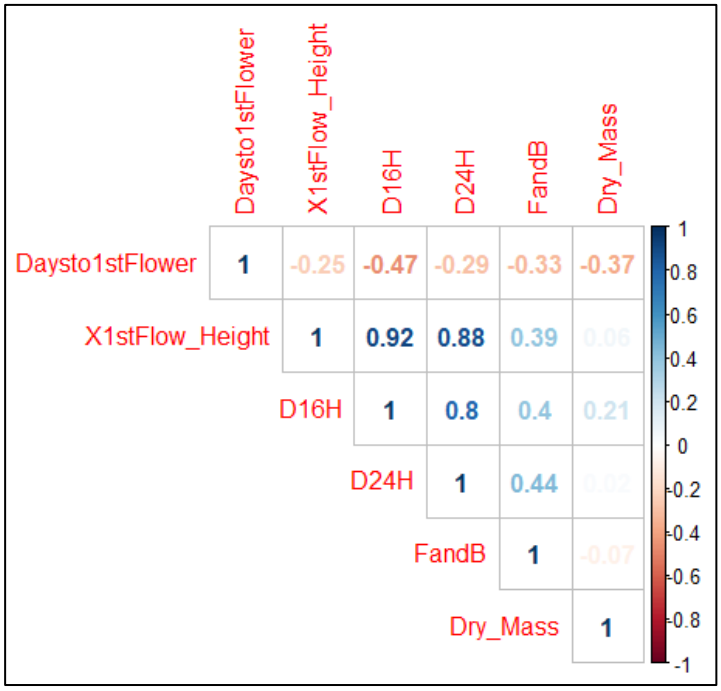

Figure 2.16. The correlation matrix of the G3 selected population in low Calcium solution. Values given are the correlation coefficients (R^2).

A Mantel test between the correlation matrices of the G3 control and the G3 selected populations, both in low calcium solution, found a significant correlation between the two ($p<0.05$) suggesting that there are no significant differences between the relationships among the biological variables from one population to the next (Figure 2.18, Figure 2.19).

Correlation Matrix of Life History Variables in G3 Control

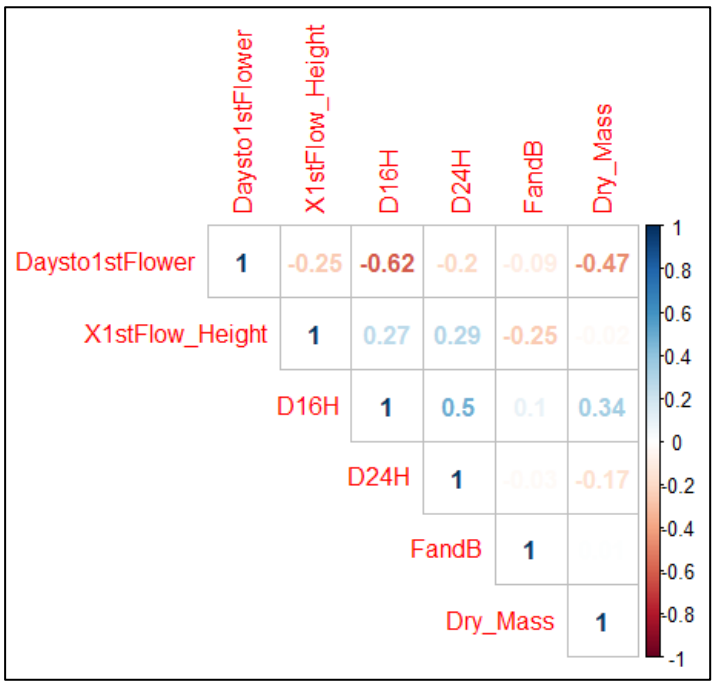

Figure 2.17. The correlation matrix of the G3 control population in low calcium solution. Values given are the correlation coefficients (R^2).

Figure 2.18. Mantel Test Comparing G3 Control and G3 Selected

Mantel statistic r: 0.7989

Significance: 0.0055556

Upper quantiles of permutations (null model):
90% 95% 97.5% 99%
0.515 0.620 0.712 0.754
Permutation: free
Number of permutations: 719

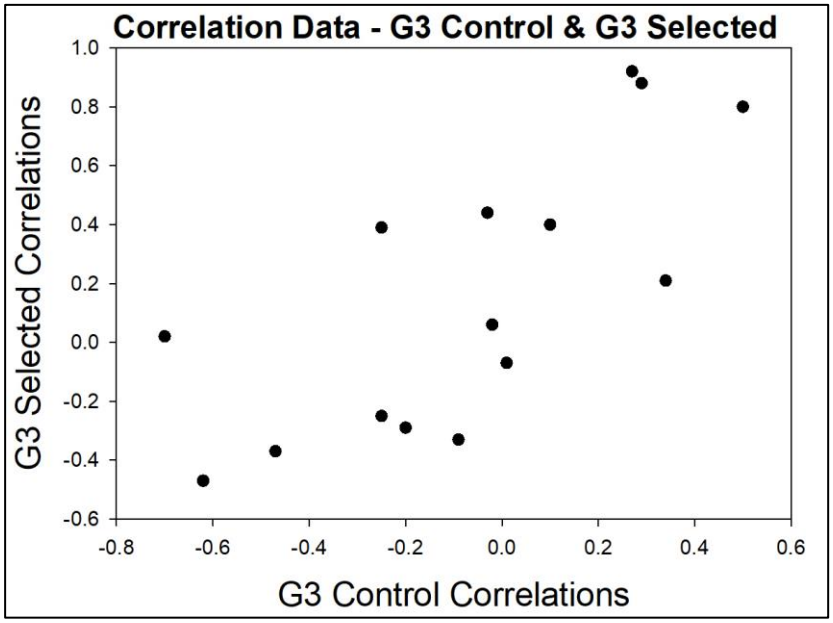

Figure 2.19. A scatter plot of the correlation coefficients of the G3 control populations and the G3 selected population.

However, the Mantel test between the correlation matrices of the G3 control population, in low calcium solution, and the G0 parent population, in normal solution, resulted in a p-value greater than 0.05. Thus, there is a significant difference in the relationships among the biological variables between these two (Figure 2.20, Figure 2.21). There was also a significant difference between the relationships between the variables between the G3 selected population and the G0 parent population (Figure 2.22, Figure 2.23).

Figure 2.20. Mantel Test Comparing G0 Control and G3 Control

Mantel statistic r: -0.2987

Significance: 0.70833

Upper quantiles of permutations (null model):

90%	95%	97.5%	99%
0.617	0.767	0.838	0.879

Permutation: free
Number of permutations: 23

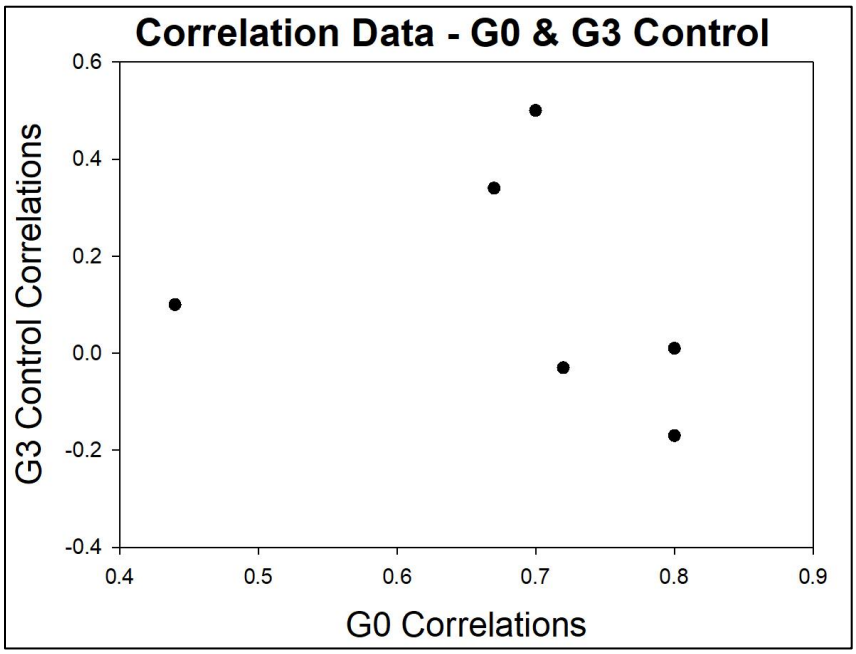

Figure 2.21. A scatter plot of the correlation coefficients of the G3 control populations and the G0 parent population.

Figure 2.22. Mantel Test Comparing G0 Control and G3 Selected

Mantel statistic r: -0.4062

Significance: 0.83333

Upper quantiles of permutations (null model):

90%	95%	97.5%	99%
0.648	0.757	0.787	0.801

Permutation: free
Number of permutations: 23

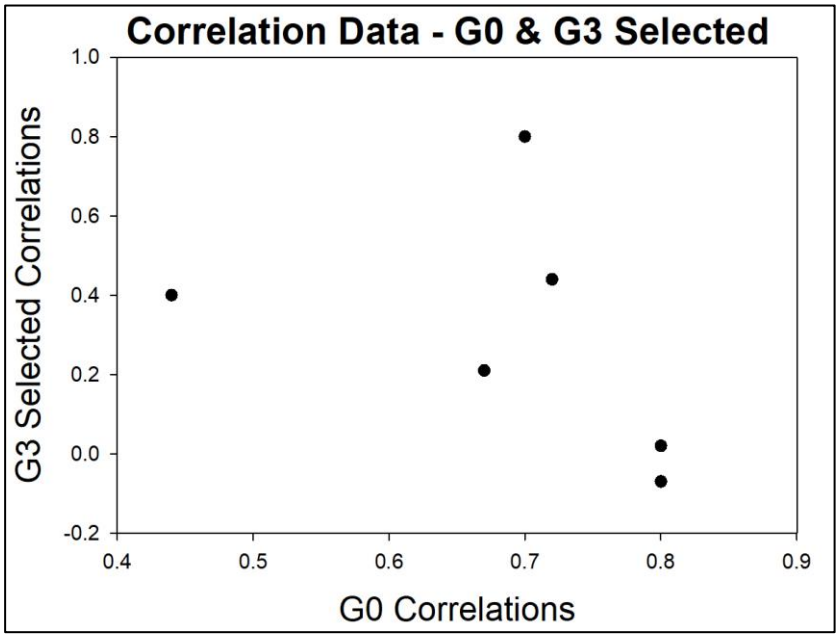

Figure 2.23. A scatter plot of the correlation coefficients of the G3 control populations and the G0 parent population.

Discussion

The main goal of this study was to determine if fast plants would respond to selection for tolerance to low calcium. However, it is first important to know if my experimental design provided significant selection pressure on the plants. Was there sufficient artificial selection on plant growth, flower production and plant mass? An important piece of evidence that there was sufficient selection pressure from my treatments is that when plants were grown in both a normal calcium solution and a low calcium solution, differences in growth rates were observed. On average, the plants grown in low calcium grew approximately 10% shorter than the normal calcium plants (Figure 2.4). Over three generations, on average plants in the selected lines showed reduced levels of growth relative to parental plants in control conditions (Figure 2.4).

On average over the three generations of the selection experiment, the control populations in normal solution grew approximately 30% taller by day 16 than selected

populations in low calcium solution. Two-way analysis of variance on day 16 height found a significant effect by the treatment on the populations (Table 2.1).

What then were the effects of this selection for more rapid growth in low calcium environments? If tolerance varied among individuals in the three generations, we would expect to see selected plants in G3 would have a higher fitness in low calcium than the control plants in low calcium. This would include selected plants having a greater height, and/or a greater number of flowers and fruits produced.

Neither of these happened. Day 16 height was not significantly different between the two populations (Figure 2.6), and day 24 was also not significantly different (Figure 2.9). There were also no significant differences between the control and the selected for the total flower and bud number (Figure 2.10).

However, there was one significant difference. The dry mass between the two populations in low calcium was significantly different. Selected plants were able to grow 18% more mass by the end of their lives than the control plants. When comparing both G3 control and selected in low calcium solution to G0 control in normal solution, the change in solution decreased the dry mass of the selected population much less than the control. The difference between the average G0 and the G3 control dry mass is 0.04 grams, while the difference between G0 and the G3 selected dry mass is 0.025 grams.

How did the selected plants gain more mass without getting taller? One possible answer is that it was the result of a change in plant architecture. Perhaps selected plants had thicker stems, increased number of leaves, increased leaf size, or increased stem branching.

I also observed that selected plants had more dry mass but not more flowers. If plants are increasing their branching, then you would expect an increase in flower number, which is not the

case. Plants could potentially be putting more energy into growth rather than height, which would result in a change in the plant mass to flower ratio. Control plants have 5 mg of mass for every flower while selected plants have 6 mg per flower. A possible fitness consequence of this shift is that it could potentially mean more energy was available for each flower which in turn could result in healthier seeds.

It's not clear what the long-term evolutionary consequences of a greater mass would be. It could potentially be related to a higher fitness through a variety of means; tolerating nutrient deficits, photosynthesis, herbivory, etc. Plants could produce more seeds per flower within each pod and/or have a higher seed fitness.

My results may indicate that the selected population is at the beginning of adaptation. The response to selection (R) has been described as the product of both heritable variation (h^2) for a trait and the strength of selection (S) on that trait in the breeder's equation (Falconer 1981).

$$R = h^2S$$

Why a difference was seen within the dry mass and no other traits could have been affected by multiple factors suggested by the breeder's equation: 1) Three generations simply could not be enough time for any other adaptations to occur within any other trait. 2) Selection to the low calcium solution may not have been strong enough. 3) There is low heritable variation in day 16 height. However, because of how the Fast Plants® were bred in the past and initially selected for, it is possible that there is more heritable variation for mass than there is for height, resulting in a change seen in mass and not height.

Based on my results, I conclude that the number of generations it would take for a population to adapt to a low calcium condition, needs to be more than three. It would take much longer in the wild for this to occur. An off-serpentine population would colonize a serpentine

environment and only the adapted would have the ability to survive and reproduce. The adapted population would then increase over time. There is no evidence from this study that populations will adapt very quickly to low calcium conditions.

Conclusion

This study has found that three generations of selection for low Ca tolerance in *B. rapa* is not enough time for adaptations to be seen in the population. No significant differences were seen between the control and selected G3 populations, except with dry mass, which could indicate the beginning of adaptation. A variety of reasons could have contributed to no adaptation being seen, from low generation numbers to too weak of selection.

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CHAPTER 3: A COMPARISON OF MIMULUS GUTTATUS GROWTH ON AND OFF SERPENTINE CONDITIONS

Introduction

Plants growing in different environments can be exposed to different selective pressures which could ultimately lead to phenotypic divergence, reproductive isolation and the creation of a new species. These selection pressures can originate from habitat, predation, climate, and nutritional sources (Schluter, 2000). Prior to the evolution of new species, new environments may lead to the evolution of "ecotypes" (Turesson, 2010). An ecotype is a step along the speciation process where plants are divergent due to an environmental pressure but are not yet a completely new species.

There are many examples of ecotypic adaptation across a wide variety of environments. A study of plants growing on different soils near a mine found that there was a gradient in plant tolerance to heavy metals as one moved farther away from the mine. Due to ecotypic adaptation, plants near the mine and far from the mine were not able to inter-breed and experienced reproductive isolation (Caisse & Antonovics, 1978b).

Adaptation has also been identified in serpentine soils (see Chapter 1). Serpentine soils are unique in that they are toxic to most plants and have 1) very low levels of essential nutrients: Calcium (Ca), Nitrogen (N), Potassium (K), and Phosphorus (P) and 2) high levels of Magnesium (Mg) and heavy metals such as Nickel (Ni), Cobalt (Co), Chromium (Cr), and Iron (Fe). This type of soil results from the weathering of ultramafic rocks that originate from the Earth's mantle that have been exposed at the surface (Alexander et al., 2007). Serpentine soils are usually found near continental plate subduction zones (e.g. the 'Ring of Fire') and are found

in small patches that can be distinct from one another due to variation in weathering and parent material.

Plants that have adapted to serpentine conditions fall into two categories: 1) endemic species that grow only on serpentine soils and 2) tolerant species that grow both on and off serpentine soils (Kruckeberg, 1984). Plants on serpentine must be tolerant of poor soil nutrition, and these species represent ecotypes.

Mimulus guttatus (*Phrymaceae*) is a yellow bee-pollinated plant that grows along seeps in western North America including regions characterized by serpentine soils (e.g. northern California). It is a very well-studied plant and was recently renamed *Erythranthe guttata*. If *E. guttata* growing on serpentine is evolving as an ecotype, then those plants should have evolved tolerance to low calcium conditions by natural selection favoring genetic variants better suited to low calcium.

I conducted a greenhouse study on *E. guttatus* collected from on and off serpentine soils to answer the following questions:

1. Does low calcium in the soil negatively affect growth or reproduction of *E. guttatus*?

2. Is there variation in how plants respond to growing in low calcium?

3. Do plants from serpentine populations demonstrate a reduced negative effect of growing on low calcium relative to plants from non-serpentine populations?

I predict that within this study, serpentine plants will grow better in the low calcium conditions, and non-serpentine plants will grow better in the control environment.

Methods

Study Species

The species used within this experiment was *Erythranthe guttata (*formerly *Mimulus guttata)* commonly known as monkey flower. It is native to western North America that has the ability to grow in Serpentine soil conditions. The species is known to have many different varieties that can be either perennial or annual. Individual plants produce a small yellow flower that is self-compatible. Seeds were collected from two different source populations in the Red Hill Management area near Keystone California. One population was on serpentine soil and the other one was from non-serpentine soil less than 5 km away from the first population.

Growth Method

I addressed the study questions by using a reciprocal transplant experiment combined with a hydroponic growth method. Twelve seeds from each home environment (serpentine population and non-serpentine population) were placed on rock wool (24 total) and kept moist for approximately 7 days at room temperature in a greenhouse with a light schedule set to 14-hour days. After 7 days of seedling growth, the plants and their rock wool were moved to the top of large planting tubes and grown in the same environment with daily watering until day 28.

At day 28 the 24 plants were assigned to one of two different nutrient treatments, a low calcium solution and a control (normal calcium) solution. Four plastic containers were each prepared for hydroponic growth by cutting holes in the plastic lids. Each container held 6 tubes with seedlings. Each container had three seedlings from the serpentine population and three from the non-serpentine populations. The plastic container was covered in aluminum foil to

block light from entering and to discourage algal growth. The containers were then filled with one of two solutions. The first was a standard quarter strength Hoagland's solution (Hoagland & Arnon, 1950), and the second was a low calcium solution that contained only 1% of the calcium concentration. A piece of nylon rope with a knot was placed within the middle of the rock wool and hung down through the bottom of the tube so that it would sit within the solution in the tub and wick it up into the rock wool. Plants were maintained in the greenhouse under 14 hour-a-day light schedule.

After day 60, plants were cut at the base and above and below ground tissue was separated. Tissue was dried for 1-2 days before above and below ground dry masses were recorded for each plant.

Results

Plants from normal source populations (non-serpentine) grew slightly better on average than did plants from serpentine source populations regardless of the nutrient treatment (Figure 3.1). The average above ground biomass of plants from normal populations was approximately 1 gram greater than plants from serpentine populations on average. The above ground biomass also tended to be higher on average for plants growing in serpentine solutions compared to normal solutions. However, neither of these two differences were statistically significant due to large standard errors around the means (Table 3.1).

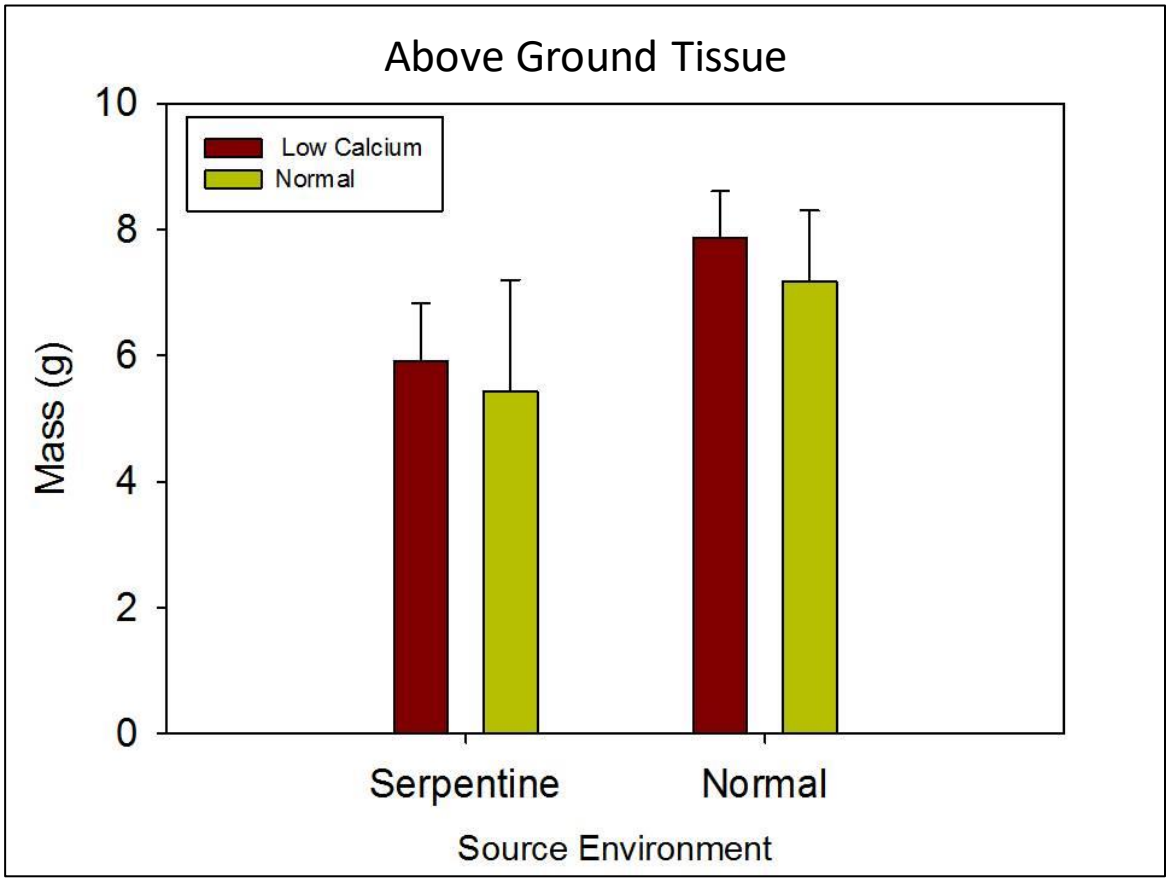

Figure 3.1. The mean above ground mass of each population from each source population in each treatment. Red bars indicate low calcium treatment, and yellow indicate a normal treatment.

Table 3.1. Analysis of variance for the mean above ground dry mass.

	F-value	P-value
Source Population	1.255	0.277
Treatment	0.026	0.874
Source Pop:Treat	0.04	0.844

The differences between populations and nutrient treatments were even smaller overall for the mean below ground dry mass. The plants from the serpentine source populations grew slightly more below ground mass on average than non-serpentine plants if they were in low calcium solutions (Figure 3.2). But once again, the 2-way analysis of variance showed that neither of the main effects was significantly different (Table 3.2).

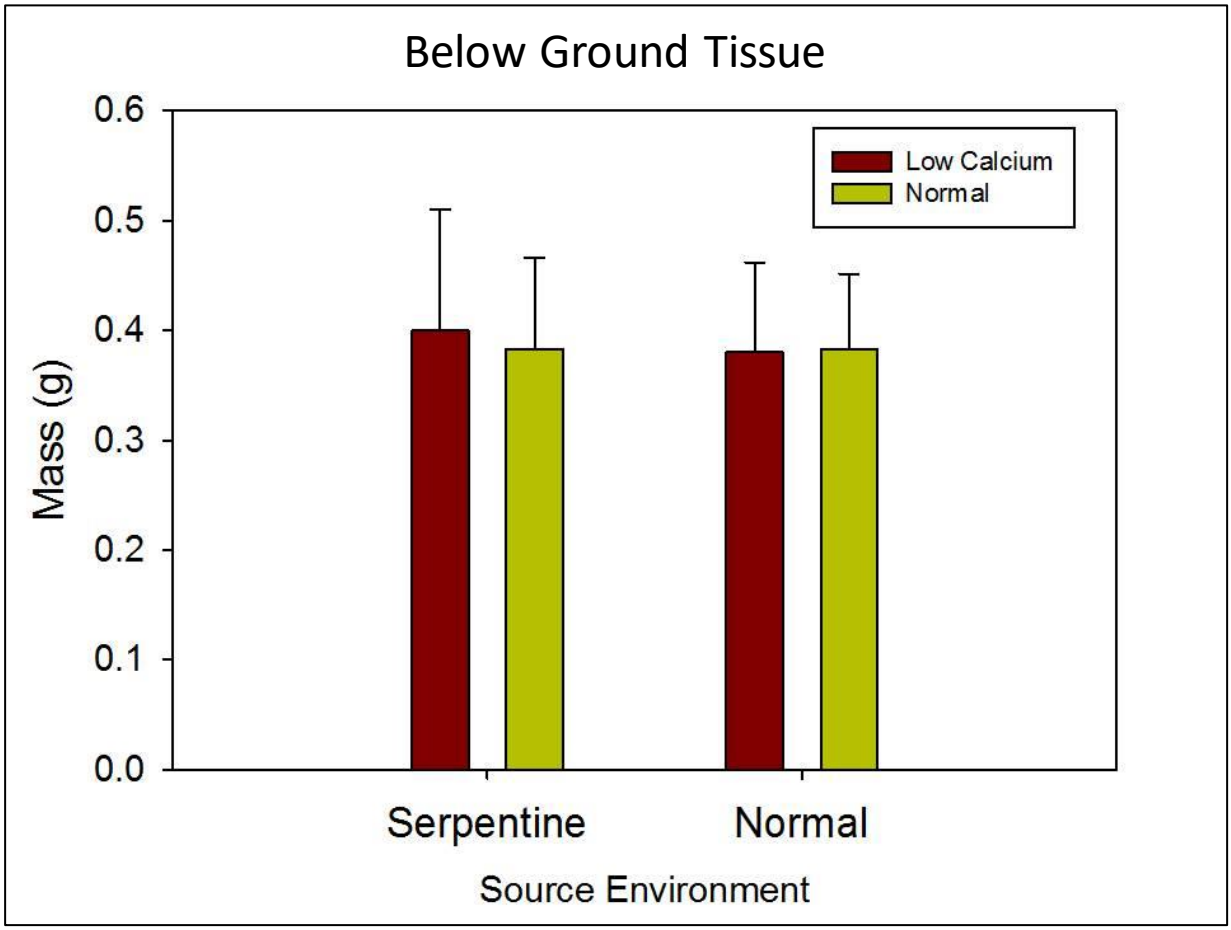

Figure 3.2. The mean below ground mass of each population from each source condition grown in each treatment. Red bars indicate low calcium treatment, and yellow indicate a normal treatment.

Table 3.2. Analysis of variance for the mean below ground dry mass.

	F-value	P-value
Source Population	0.01	0.92
Treatment	0.005	0.942
Source Pop:Treat	0.011	0.919

Discussion

In my experiment, the source of the seeds did not have a significant statistical effect on growth. If local adaptation to serpentine soils has led to ecotypic adaptation, we would expect the serpentine plants to grow better in the low calcium environment, and the non-serpentine plants to grow better in the control environment. We did not see this.

A. Does low calcium in the soil negatively affect growth or reproduction of *E. guttatus*?
B. Is there variation in how plants respond to growing in low calcium?
C. Do plants from serpentine populations demonstrate a reduced negative effect of growing on low calcium relative to plants from non-serpentine populations?

I found no evidence that low calcium significantly affected plant growth although there was much variation in how much plants grew in both low and normal calcium. I also found no significant reduction in negative effects of low calcium on plants from each source environment.

The lack of statistical significance for treatment and population effects could be due to a few factors. The population sizes used in the experiment were quite small with 24 plants total split between 2 source environments and 2 treatments. The large standard errors around the means indicate that adding more replication could uncover significant effects. Secondly, the low calcium treatment may not have been low enough. If a larger difference in calcium to magnesium was present between treatments I may have found significant effects. In this study, the Ca:Mg

ratio was 4:1 in the normal solution and 1.1:1 for the low calcium solution. In natural serpentine conditions, the Ca:Mg ratio ranges from 0.003 to 0.05 (Brooks, 1987).

And lastly, the separation of the plants into normal and low calcium treatments at 28 days may have been too late. The overall success of the plant may have already been largely determined before that day occurred. A previous study separated their populations into the treatments at day 10, and it found significance in their data (Selby, 2014).

Conclusion

I hypothesized that each source environment would grow better in their corresponding treatment. Results were trending towards this, but ultimately an analysis of variance showed there was no significant difference between the populations in each treatment. Low population size may have contributed to no significance being observed.

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APPENDIX A: PRELIMINARY STUDIES

Description of the methods and results of the two preliminary studies to the selection experiment conducted in Chapter 2.

Pilot Study Methods

The first pilot study's goal was to determine if the growth environment (calcium concentration) affects plant reproductive success, and to determine the minimum amount of calcium required for plant growth. The second pilot study's goal was to determine what early plant life characteristics could be used to predict a plant's reproductive success, while also determining the last day new buds are formed.

Pilot Study One

With the objective of this experiment being to determine if varying concentrations of Calcium affect plant growth, we used multiple solutions with decreasing calcium concentrations. 50 standard seeds were split between five different containers. The solutions used were created using the control and low Calcium solutions in different ratios, 1:0, 2:1, 1:1, 1:2, and 0:1, with the first value being the control solution and second being the low calcium solution. This was done to determine the minimum calcium concentration required for plants to grow and reproduce. Plants were given 1.5 liters of solution initially with 1 liter added every week. Daily height measurements were recorded for 14 days. The study found that there was indeed a variable response to calcium, with a significant drop off in plant growth and reproduction with the 0:1 solution.

Pilot Study Two

The objective of pilot study two was to determine what early plant life characteristics could be used to predict a plants reproductive success. Success needed to be predicted before the plants finished flowering, to still be able to pollinate them for the next generation. This information was used in the selection experiment and it will be elaborated upon.

Similar to the first pilot study, this experiment only included one population of plants. 72 seeds were initially placed across three containers, all containing a 1.5 liters of normal calcium solution with one liter being added each week.

Height was recorded every other day through the day at which no new buds were formed. Flowers and buds were recorded on the last day of growth. Plants were then cut off at their base. Above ground, wet mass was recorded before being placed into a 65° C drying oven for two days. Above ground, dry mass was then recorded. Root length was also recorded, but the collection method proved to be unreliable in the hydroponic growth method used, so the data was not used.

The study found that day 16 height had a high correlation with dry mass (0.67) as well as dry mass having a high correlation with flowers plus buds (0.8). This, along with day 16 being at the beginning of the flowering stage led to day 16 height being the key predictor for plant fitness when selecting for the most tolerant individuals.

APPENDIX B: NUTRIENT SOLUTION RECIPE

Table B. 1. Nutrient recipe used to create nutrients stock solutions. Both were solutions were utilized throughout this study.

	Stock (g/L)	**Control (mL/20L)**	**Low Calcium (mL/20L)**
KNO_3	101.1	30	30
$Ca(NO_3)_2$ X $4H_2O$	236.2	20	0.22
$NH_4H_2PO_4$	115.08	10	10
MgSO4 X $7H_2O$	246.5	5	5
FeDDHA	1	25	25
H_3BO_3	1.55	5	5
$MnCl_2$ X $4H_2O$	0.4	5	5
$MgNO_3$ X $6H_2O$	256.4	0	19.1
Micro (Together)	5	5	5
KCl	3.73	NA	NA
$ZnCl_2$	0.27	NA	NA
$CuCl_2$	0.07	NA	NA
Na_2MoO_4	0.12	NA	NA

APPENDIX C: DATA

Data collected over the course of the selection experiment from all generations and treatments.

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
1.1	Low Calcium	G0	17	11.2	10.6
1.2	Low Calcium	G0	NA	NA	NA
1.3	Low Calcium	G0	17	11	9.8
1.4	Low Calcium	G0	17	20.9	19.1
1.5	Low Calcium	G0	16	16.5	16.5
1.6	Low Calcium	G0	17	12.3	11.6
1.7	Low Calcium	G0	17	14	11.2
1.8	Low Calcium	G0	15	14.3	17.7
1.9	Low Calcium	G0	16	15.8	15.8
1.10	Low Calcium	G0	16	11.3	11.3
1.11	Low Calcium	G0	15	7.7	11.6
1.12	Low Calcium	G0	15	9.1	12.4
1.13	Low Calcium	G0	17	18.8	15.9
1.14	Low Calcium	G0	16	14	14
1.15	Low Calcium	G0	17	17.9	17
1.16	Low Calcium	G0	15	7.9	10.8
1.17	Low Calcium	G0	16	10.6	10.6
1.18	Low Calcium	G0	16	13.3	13.3
1.19	Low Calcium	G0	18	17.4	12.9
1.20	Low Calcium	G0	15	8.3	10.1
1.21	Low Calcium	G0	17	10.6	9.8

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
1.22	Low Calcium	G0	16	9.2	9.2
1.23	Low Calcium	G0	16	11.1	11.1
1.24	Low Calcium	G0	15	7.4	9
2.1	Low Calcium	G0	18	13.1	7.9
2.2	Low Calcium	G0	16	8.5	8.5
2.3	Low Calcium	G0	NA	NA	NA
2.4	Low Calcium	G0	16	17.8	17.8
2.5	Low Calcium	G0	18	8.8	6.4
2.6	Low Calcium	G0	15	4.7	5
2.7	Low Calcium	G0	16	11.3	11.3
2.8	Low Calcium	G0	15	13.7	18.2
2.9	Low Calcium	G0	17	11.5	10.1
2.10	Low Calcium	G0	16	7	7
2.11	Low Calcium	G0	16	10.1	10.1
2.12	Low Calcium	G0	17	4.9	5.5
2.13	Low Calcium	G0	18	6.8	5.6
2.14	Low Calcium	G0	15	10.2	12.3
2.15	Low Calcium	G0	17	10.1	8.3
2.16	Low Calcium	G0	16	13.6	13.6
2.17	Low Calcium	G0	14	4.4	9.1
2.18	Low Calcium	G0	16	11.2	11.2
2.19	Low Calcium	G0	17	10.2	8.5
2.20	Low Calcium	G0	17	6.3	6.1
2.21	Low Calcium	G0	14	6.1	8.2
2.22	Low Calcium	G0	16	7.7	7.7

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
2.23	Low Calcium	G0	15	14.3	15.8
2.24	Low Calcium	G0	15	7.9	9.1
3.1	Low Calcium	G0	16	9.2	9.2
3.2	Low Calcium	G0	17	13.1	11.3
3.3	Low Calcium	G0	17	9.1	8.5
3.4	Low Calcium	G0	17	14	11.9
3.5	Low Calcium	G0	18	7.8	6.5
3.6	Low Calcium	G0	18	14.5	8.4
3.7	Low Calcium	G0	NA	NA	NA
3.8	Low Calcium	G0	15	8.6	10.2
3.9	Low Calcium	G0	17	9.4	7.6
3.10	Low Calcium	G0	17	11.7	9.9
3.11	Low Calcium	G0	16	11.4	11.4
3.12	Low Calcium	G0	17	21.8	18.6
3.13	Low Calcium	G0	14	7.3	12.3
3.14	Low Calcium	G0	14	5.7	9.8
3.15	Low Calcium	G0	15	9.2	11
3.16	Low Calcium	G0	16	5.3	5.3
3.17	Low Calcium	G0	16	8.6	8.6
3.18	Low Calcium	G0	NA	NA	NA
3.19	Low Calcium	G0	16	8.1	8.1
3.20	Low Calcium	G0	17	8.3	6.9
3.21	Low Calcium	G0	17	12.5	11
3.22	Low Calcium	G0	NA	NA	NA
3.23	Low Calcium	G0	16	7.8	7.8

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
3.24	Low Calcium	G0	18	11.2	8.4
4.1	Low Calcium	G0	18	23.3	12.8
4.2	Low Calcium	G0	NA	NA	NA
4.3	Low Calcium	G0	18	13.4	8.5
4.4	Low Calcium	G0	17	8.6	8.2
4.5	Low Calcium	G0	NA	NA	NA
4.6	Low Calcium	G0	16	8.8	8.8
4.7	Low Calcium	G0	19	13.5	8.9
4.8	Low Calcium	G0	16	6.7	6.7
4.9	Low Calcium	G0	17	13.3	12.9
4.10	Low Calcium	G0	NA	NA	NA
4.11	Low Calcium	G0	NA	NA	NA
4.12	Low Calcium	G0	17	12.4	9.6
4.13	Low Calcium	G0	NA	NA	NA
4.14	Low Calcium	G0	18	16.7	11.8
4.15	Low Calcium	G0	17	8.6	6.8
4.16	Low Calcium	G0	16	10.7	10.7
4.17	Low Calcium	G0	15	15.9	19.8
4.18	Low Calcium	G0	NA	NA	7.4
4.19	Low Calcium	G0	16	10.9	10.9
4.20	Low Calcium	G0	15	9.6	12.2
4.21	Low Calcium	G0	17	13.3	6.5
4.22	Low Calcium	G0	16	5.8	5.8
4.23	Low Calcium	G0	17	11.1	6.2
4.24	Low Calcium	G0	17	8.2	6.4

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
5.1	Low Calcium	G0	NA	NA	NA
5.2	Low Calcium	G0	16	8	8
5.3	Low Calcium	G0	16	18.9	18.9
5.4	Low Calcium	G0	NA	NA	NA
1.1	Low Calcium	G1	17	16.9	15.4
1.2	Low Calcium	G1	16	17.4	17.4
1.3	Low Calcium	G1	18	18.1	17.9
1.4	Low Calcium	G1	16	14.2	14.2
1.5	Low Calcium	G1	16	17	17
1.6	Low Calcium	G1	17	14.7	13.2
1.7	Low Calcium	G1	18	18	15.1
1.8	Low Calcium	G1	18	12.5	10.8
1.9	Low Calcium	G1	17	19.2	16.9
1.10	Low Calcium	G1	NA	NA	NA
1.11	Low Calcium	G1	18	15.1	14.8
1.12	Low Calcium	G1	17	15	14.1
1.13	Low Calcium	G1	18	14.9	12.6
1.14	Low Calcium	G1	18	15.9	15.6
1.15	Low Calcium	G1	NA	NA	NA
1.16	Low Calcium	G1	18	16.7	15.8
1.17	Low Calcium	G1	19	8.8	5.7
1.18	Low Calcium	G1	18	13.3	12.1
1.19	Low Calcium	G1	18	14.4	14.3
1.20	Low Calcium	G1	18	13.6	11.8
1.21	Low Calcium	G1	NA	NA	NA

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
1.22	Low Calcium	G1	17	14.8	14.8
1.23	Low Calcium	G1	17	14.2	14.2
1.24	Low Calcium	G1	NA	NA	NA
2.1	Low Calcium	G1	NA	NA	NA
2.2	Low Calcium	G1	16	15.1	13.4
2.3	Low Calcium	G1	21	29.2	19.7
2.4	Low Calcium	G1	18	15.8	15.6
2.5	Low Calcium	G1	16	13	13
2.6	Low Calcium	G1	17	15.9	14.1
2.7	Low Calcium	G1	17	14.4	13.1
2.8	Low Calcium	G1	18	12.7	11.2
2.9	Low Calcium	G1	18	20.8	19.2
2.10	Low Calcium	G1	16	12.9	12.9
2.11	Low Calcium	G1	22	26.3	17.6
2.12	Low Calcium	G1	18	19.3	17.8
2.13	Low Calcium	G1	22	23.9	13.9
2.14	Low Calcium	G1	16	13.1	13.1
2.15	Low Calcium	G1	16	12.9	12.9
2.16	Low Calcium	G1	22	20.4	10.7
2.17	Low Calcium	G1	16	13.2	13.2
2.18	Low Calcium	G1	18	17.8	16.3
2.19	Low Calcium	G1	17	17.3	16.9
2.20	Low Calcium	G1	NA	NA	NA
2.21	Low Calcium	G1	26	21	16.2
2.22	Low Calcium	G1	17	14.9	12.8

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
2.23	Low Calcium	G1	18	17.2	16.5
2.24	Low Calcium	G1	18	15.8	14.5
3.1	Low Calcium	G1	16	15.9	15.9
3.2	Low Calcium	G1	NA	NA	NA
3.3	Low Calcium	G1	NA	NA	NA
3.4	Low Calcium	G1	16	15.4	15.4
3.5	Low Calcium	G1	18	15.6	14.2
3.6	Low Calcium	G1	17	17.5	16.7
3.7	Low Calcium	G1	16	19.1	19.1
3.8	Low Calcium	G1	18	16	14.8
3.9	Low Calcium	G1	17	16.7	15.2
3.10	Low Calcium	G1	18	16.1	14.3
3.11	Low Calcium	G1	18	17.4	16.4
3.12	Low Calcium	G1	16	19.8	19.8
3.13	Low Calcium	G1	18	18.4	17.5
3.14	Low Calcium	G1	19	17.5	17.3
3.15	Low Calcium	G1	19	17.7	17.1
3.16	Low Calcium	G1	16	12	12
3.17	Low Calcium	G1	16	18.5	18.5
3.18	Low Calcium	G1	17	14.2	13
3.19	Low Calcium	G1	NA	NA	NA
3.20	Low Calcium	G1	16	14.1	14.1
3.21	Low Calcium	G1	18	14.2	14
3.22	Low Calcium	G1	NA	NA	NA
3.23	Low Calcium	G1	17	14	12.6

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
3.24	Low Calcium	G1	NA	NA	NA
1.1	Low Calcium	G2	17	9.6	9.2
1.2	Low Calcium	G2	16	9.5	9.5
1.3	Low Calcium	G2	18	14.3	13
1.4	Low Calcium	G2	18	10.8	10.1
1.5	Low Calcium	G2	NA	NA	NA
1.6	Low Calcium	G2	16	13.9	13.9
1.7	Low Calcium	G2	NA	NA	NA
1.8	Low Calcium	G2	16	12.7	12.7
1.9	Low Calcium	G2	17	9.4	8.2
1.10	Low Calcium	G2	16	12.4	12.4
1.11	Low Calcium	G2	16	10.1	10.1
1.12	Low Calcium	G2	17	10.6	9.1
1.13	Low Calcium	G2	NA	NA	NA
1.14	Low Calcium	G2	17	13.1	12.9
1.15	Low Calcium	G2	17	13.4	13.3
1.16	Low Calcium	G2	NA	NA	NA
1.17	Low Calcium	G2	17	9.7	9.1
1.18	Low Calcium	G2	16	10.3	10.3
1.19	Low Calcium	G2	16	8.9	8.9
1.20	Low Calcium	G2	16	10.4	10.4
1.21	Low Calcium	G2	NA	NA	8.4
1.22	Low Calcium	G2	19	10.3	9.6
1.23	Low Calcium	G2	NA	NA	4.8
1.24	Low Calcium	G2	18	9.1	6

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
2.1	Low Calcium	G2	19	11	8.4
2.2	Low Calcium	G2	18	13.3	12.7
2.3	Low Calcium	G2	18	10.5	9.8
2.4	Low Calcium	G2	NA	NA	NA
2.5	Low Calcium	G2	NA	NA	7.9
2.6	Low Calcium	G2	16	9.4	9.4
2.7	Low Calcium	G2	16	10.6	10.6
2.8	Low Calcium	G2	19	7.5	6.9
2.9	Low Calcium	G2	18	11.2	10.5
2.10	Low Calcium	G2	18	8	8
2.11	Low Calcium	G2	16	11.4	11.4
2.12	Low Calcium	G2	NA	NA	4.8
2.13	Low Calcium	G2	18	12.3	10.5
2.14	Low Calcium	G2	18	10.7	7.3
2.15	Low Calcium	G2	16	11.8	11.8
2.16	Low Calcium	G2	16	10.9	10.9
2.17	Low Calcium	G2	16	10.6	10.6
2.18	Low Calcium	G2	17	7.9	7.7
2.19	Low Calcium	G2	NA	NA	NA
2.20	Low Calcium	G2	17	11.5	10.4
2.21	Low Calcium	G2	17	13.9	12.6
2.22	Low Calcium	G2	19	10.3	10.2
2.23	Low Calcium	G2	16	10.7	10.7
2.24	Low Calcium	G2	16	9.8	9.8
1.2	Control	G0	15	NA	14.4
1.7	Control	G0	16	NA	15.1

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
1.12	Control	G0	13	NA	19.6
1.16	Control	G0	14	NA	12.9
1.24	Control	G0	15	NA	16.8
2.4	Control	G0	15	NA	17
2.9	Control	G0	13	NA	22.9
2.19	Control	G0	15	NA	13.1
2.22	Control	G0	14	NA	28.2
2.23	Control	G0	14	NA	12.5
3.3	Control	G0	15	NA	19.7
3.6	Control	G0	13	NA	24.3
3.17	Control	G0	15	NA	18.9
3.18	Control	G0	14	NA	18.3
3.24	Control	G0	15	NA	17.1
4.1	Control	G0	15	NA	17.1
4.6	Control	G0	16	NA	27.6
4.12	Control	G0	14	NA	25.4
4.18	Control	G0	15	NA	16.2
4.23	Control	G0	15	NA	27.5
1.1	Control	G1	21	14.3	8.2
1.2	Control	G1	19	13.8	9.9
1.3	Control	G1	18	14.8	12.7
1.4	Control	G1	18	15.7	14.6
1.5	Control	G1	19	13.6	9.8
1.6	Control	G1	20	17	12.3
1.7	Control	G1	20	13.4	9.4
1.8	Control	G1	20	18.6	13.3
1.9	Control	G1	20	15.3	11
1.10	Control	G1	19	14.3	11.1
1.11	Control	G1	18	17.6	16.2
1.12	Control	G1	18	13.8	11.3
1.13	Control	G1	19	13.6	9.9
1.14	Control	G1	19	11.3	8.9
1.15	Control	G1	17	18.3	18
1.16	Control	G1	19	18.9	15.3
1.17	Control	G1	18	14.9	13.4
1.18	Control	G1	19	22.8	16.6
1.19	Control	G1	19	14.6	11.2
1.20	Control	G1	19	19.3	15.1

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
1.21	Control	G1	19	13.5	10
1.22	Control	G1	18	16.2	14.7
1.23	Control	G1	19	13.9	10.7
1.24	Control	G1	17	16.4	16
2.1	Control	G1	19	19.2	14.8
2.2	Control	G1	18	18.2	15.3
2.3	Control	G1	19	17.7	5.1
2.4	Control	G1	21	10.3	7.6
2.5	Control	G1	19	12.3	9.7
2.6	Control	G1	20	19.1	13.8
2.7	Control	G1	19	15.6	9.9
2.8	Control	G1	19	22.6	18.6
2.9	Control	G1	21	14.8	8.7
2.10	Control	G1	20	15.8	12.3
2.11	Control	G1	20	11.9	5.8
2.12	Control	G1	19	13.2	10.1
2.13	Control	G1	NA	NA	NA
2.14	Control	G1	17	15.7	15.6
2.15	Control	G1	NA	NA	NA
2.16	Control	G1	NA	NA	NA
2.17	Control	G1	19	18.8	15.3
2.18	Control	G1	19	13.9	12
2.19	Control	G1	20	19.2	12.1
2.21	Control	G1	20	12.2	7.3
2.22	Control	G1	19	11.7	8.8
2.23	Control	G1	18	13.5	11.8
2.24	Control	G1	18	19.9	18
3.1	Control	G1	17	15.4	14.9
3.2	Control	G1	20	19.1	14.2
3.3	Control	G1	19	14.4	11.1
3.4	Control	G1	18	21.1	18.5
3.5	Control	G1	19	20.5	15.9
3.6	Control	G1	21	22.3	10.8
3.7	Control	G1	18	15.1	12.9
3.8	Control	G1	20	15.9	10.8
3.9	Control	G1	20	21.2	13.7
3.10	Control	G1	20	13.7	8.3
3.11	Control	G1	19	15.9	12.9

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
3.12	Control	G1	22	15.9	5.4
3.13	Control	G1	22	8.8	2.8
3.14	Control	G1	20	13	10.2
3.15	Control	G1	20	15.9	11.7
3.16	Control	G1	NA	NA	NA
3.17	Control	G1	23	10.7	2.4
3.18	Control	G1	NA	NA	NA
3.19	Control	G1	20	15.9	10.3
3.20	Control	G1	18	16.8	15.2
3.21	Control	G1	19	12.5	8.9
3.22	Control	G1	20	15.8	9.5
3.23	Control	G1	20	15.7	9.7
3.24	Control	G1	19	21	17.8
1.1	Control	G2	16	20.5	20.5
1.2	Control	G2	17	23.2	20.4
1.3	Control	G2	14	16.8	21.6
1.4	Control	G2	15	17.9	23.2
1.5	Control	G2	16	19.8	19.8
1.6	Control	G2	14	23.3	26.3
1.7	Control	G2	15	16.1	19.6
1.8	Control	G2	16	19.9	19.9
1.9	Control	G2	14	16.7	23
1.10	Control	G2	15	19.4	23.9
1.11	Control	G2	17	28.4	25.8
1.12	Control	G2	15	20.8	26.8
1.13	Control	G2	16	20.1	20.1
1.14	Control	G2	14	14.2	21.6
1.15	Control	G2	15	16.9	20.7
1.16	Control	G2	16	15.4	15.4
1.17	Control	G2	15	16.6	21.7
1.18	Control	G2	14	13.4	26.4
1.19	Control	G2	14	23.5	30.8
1.20	Control	G2	15	19.5	26.1
1.21	Control	G2	14	17.6	22.7
1.22	Control	G2	14	19.9	26
1.23	Control	G2	16	21.3	21.3
1.24	Control	G2	15	22.3	26.3
2.1	Control	G2	15	18	20.8

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
2.2	Control	G2	18	12.8	5.6
2.3	Control	G2	13	14.8	22.6
2.4	Control	G2	13	23.1	32.5
2.5	Control	G2	15	20.3	24.3
2.6	Control	G2	15	23.8	29.8
2.7	Control	G2	NA	NA	NA
2.8	Control	G2	14	17.8	22.9
2.9	Control	G2	15	20.6	23.6
2.10	Control	G2	14	18	23.5
2.11	Control	G2	NA	NA	NA
2.12	Control	G2	15	17.7	21.8
2.13	Control	G2	14	22.2	27.7
2.14	Control	G2	16	13.6	13.6
2.15	Control	G2	14	23.8	27.4
2.16	Control	G2	14	15.3	17.7
2.17	Control	G2	15	19.5	21.5
2.18	Control	G2	17	13.4	13.4
2.19	Control	G2	14	12	12.6
2.20	Control	G2	15	19.2	21.6
2.21	Control	G2	14	18.9	25.2
2.22	Control	G2	14	20.7	26.4
2.23	Control	G2	15	19.3	21.6
2.24	Control	G2	14	13.8	16.8
3.1	Control	G2	NA	NA	NA
3.2	Control	G2	15	19.7	21.9
3.3	Control	G2	14	19.6	26.1
3.4	Control	G2	14	16.5	23.2
3.5	Control	G2	20	10.8	6
3.6	Control	G2	15	18.4	20.3
3.7	Control	G2	16	15	15
3.8	Control	G2	15	23.7	25.3
3.9	Control	G2	15	18.8	19.8
3.10	Control	G2	15	14.9	16.3
3.11	Control	G2	16	25.1	25.1
3.12	Control	G2	14	19.5	23.6
3.13	Control	G2	15	19.2	22.1
3.14	Control	G2	17	23.2	21.7
3.15	Control	G2	15	19.1	21.9

Table C.1. Raw data collected for G0, G1, and G2 in control and low Calcium solution (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flower Height	D16 Height
3.16	Control	G2	15	15.8	19.8
3.17	Control	G2	13	18.4	25
3.18	Control	G2	13	19.9	24.5
3.19	Control	G2	15	17.3	18.4
3.20	Control	G2	15	19.8	22.1
3.21	Control	G2	18	20.6	14.8
3.22	Control	G2	13	20	25.9
3.23	Control	G2	15	22	23.4
3.24	Control	G2	15	16.4	18.2

Table C.2. Raw data collected for top 20 plants from G0, G1, and G2 in control and low Calcium solutions

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flow Height	D16 Height	F+B	D24H
1.4	Low Calcium	G0	17	20.9	19.1	24	29.6
1.5	Low Calcium	G0	16	16.5	16.5	17	23.2
1.8	Low Calcium	G0	15	14.3	17.7	25	27.1
1.9	Low Calcium	G0	16	15.8	15.8	32	31.8
1.12	Low Calcium	G0	15	9.1	12.4	17	19.6
1.13	Low Calcium	G0	17	18.8	15.9	31	38.8
1.14	Low Calcium	G0	16	14	14	5	14.7
1.15	Low Calcium	G0	17	17.9	17	13	24.8
1.19	Low Calcium	G0	18	17.4	12.9	34	27.9
1.18	Low Calcium	G0	16	13.3	13.3	19	28.1
2.4	Low Calcium	G0	16	17.8	17.8	23	25.2
2.8	Low Calcium	G0	15	13.7	18.2	35	27.5
2.14	Low Calcium	G0	15	10.2	12.3	16	23.3
2.16	Low Calcium	G0	16	13.6	13.6	26	26.3
2.23	Low Calcium	G0	15	14.3	15.8	28	26.4

Table C.2. Raw data collected for top 20 plants from G0, G1, and G2 in control and low Calcium solutions (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flow Height	D16 Height	F+B	D24H
3.12	Low Calcium	G0	17	21.8	18.6	31	33.9
4.1	Low Calcium	G0	18	23.3	12.8	16	26.2
4.9	Low Calcium	G0	17	13.3	12.9	6	15
4.17	Low Calcium	G0	15	15.9	19.8	46	36.7
5.3	Low Calcium	G0	16	18.9	18.9	29	32.6
1.2	Low Calcium	G1	16	17.4	17.4	22	28.3
1.3	Low Calcium	G1	18	18.1	17.9	26	28.9
1.5	Low Calcium	G1	16	17	17	28	32.1
1.9	Low Calcium	G1	17	19.2	16.9	21	25.8
2.3	Low Calcium	G1	21	29.2	19.7	23	33.3
2.9	Low Calcium	G1	18	20.8	19.2	26	29.6
2.11	Low Calcium	G1	26.3	22	17.6	27	23.4
2.12	Low Calcium	G1	18	19.3	17.8	19	22.9
2.18	Low Calcium	G1	18	17.8	16.3	24	25.6
2.19	Low Calcium	G1	17	17.3	16.9	21	23.1
2.21	Low Calcium	G1	26	21	16.2	20	24
2.23	Low Calcium	G1	18	17.2	16.5	18	28.6
3.6	Low Calcium	G1	17	17.5	16.7	28	27.3
3.7	Low Calcium	G1	16	19.1	19.1	17	29.8
3.11	Low Calcium	G1	18	17.4	16.4	16	27.5
3.12	Low Calcium	G1	16	19.8	19.8	26	31
3.13	Low Calcium	G1	18	18.4	17.5	33	23.6
3.14	Low Calcium	G1	19	17.5	17.3	27	25.2
3.15	Low Calcium	G1	19	17.7	17.1	31	26.8

Table C.2. Raw data collected for top 20 plants from G0, G1, and G2 in control and low Calcium solutions (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flow Height	D16 Height	F+B	D24H
3.17	Low Calcium	G1	16	18.5	18.5	25	26.3
1.2	Low Calcium	G2	16	9.5	9.5	31	12.2
1.3	Low Calcium	G2	18	14.3	13	12	14.4
1.6	Low Calcium	G2	16	13.9	13.9	17	15.6
1.8	Low Calcium	G2	16	12.7	12.7	21	20.3
1.10	Low Calcium	G2	16	12.4	12.4	13	10.1
1.18	Low Calcium	G2	16	10.3	10.3	30	16.5
1.19	Low Calcium	G2	16	8.9	8.9	22	16.1
1.20	Low Calcium	G2	16	10.4	10.4	23	14.7
2.2	Low Calcium	G2	18	13.3	12.7	7	12.4
2.7	Low Calcium	G2	16	10.6	10.6	24	23.3
2.9	Low Calcium	G2	18	11.2	10.5	4	10.9
2.11	Low Calcium	G2	16	11.4	11.4	23	7
2.13	Low Calcium	G2	18	12.3	10.5	6	11.3
2.15	Low Calcium	G2	16	11.8	11.8	3	12.1
2.16	Low Calcium	G2	16	10.9	10.9	5	11.2
2.20	Low Calcium	G2	17	11.5	10.4	8	13.8
2.21	Low Calcium	G2	17	13.9	12.6	34	14.9
2.22	Low Calcium	G2	19	10.3	10.2	17	10.6
2.23	Low Calcium	G2	16	10.7	10.7	13	22.8
2.24	Low Calcium	G2	16	9.8	9.8	24	12
1.2	Control	G0	15	NA	14.4	NA	NA
1.7	Control	G0	16	NA	15.1	NA	NA
1.12	Control	G0	13	NA	19.6	NA	NA
1.16	Control	G0	14	NA	12.9	NA	NA
1.24	Control	G0	15	NA	16.8	NA	NA
2.4	Control	G0	15	NA	17	NA	NA

Table C.2. Raw data collected for top 20 plants from G0, G1, and G2 in control and low Calcium solutions (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flow Height	D16 Height	F+B	D24H
2.9	Control	G0	13	NA	22.9	NA	NA
2.19	Control	G0	15	NA	13.1	NA	NA
2.22	Control	G0	14	NA	28.2	NA	NA
2.23	Control	G0	14	NA	12.5	NA	NA
3.3	Control	G0	15	NA	19.7	NA	NA
3.6	Control	G0	13	NA	24.3	NA	NA
3.17	Control	G0	15	NA	18.9	NA	NA
3.18	Control	G0	14	NA	18.3	NA	NA
3.24	Control	G0	15	NA	17.1	NA	NA
4.1	Control	G0	15	NA	17.1	NA	NA
4.6	Control	G0	16	NA	27.6	NA	NA
4.12	Control	G0	14	NA	25.4	NA	NA
4.18	Control	G0	15	NA	16.2	NA	NA
4.23	Control	G0	15	NA	27.5	NA	NA
1.6	Control	G1	20	17	12.3	25	27.4
1.8	Control	G1	20	18.6	13.3	11	20.9
1.17	Control	G1	18	14.9	13.4	13	16.9
1.19	Control	G1	19	14.6	11.2	17	26.6
1.24	Control	G1	17	16.4	16	24	25.3
2.1	Control	G1	19	19.2	14.8	17	21.8
2.3	Control	G1	19	17.7	5.1	15	10.9
2.7	Control	G1	19	15.6	9.9	30	28.4
2.14	Control	G1	17	15.7	15.6	14	19.9
2.17	Control	G1	19	18.8	15.3	18	20.7
2.19	Control	G1	20	19.2	12.1	16	22.4
3.3	Control	G1	19	14.4	11.1	16	21.5
3.2	Control	G1	20	19.1	14.2	27	28.2
3.7	Control	G1	18	15.1	12.9	22	26.1
3.9	Control	G1	20	21.2	13.7	18	25.8
3.14	Control	G1	20	13	10.2	12	19.3
3.15	Control	G1	20	15.9	11.7	12	20.2
3.21	Control	G1	19	12.5	8.9	14	19.4
3.23	Control	G1	20	15.7	9.7	19	24.9
3.24	Control	G1	19	21	17.8	10	23.6
1.3	Control	G2	14	16.8	21.6	15	22.3
1.6	Control	G2	14	23.3	26.3	12	26.2
1.10	Control	G2	15	19.4	23.9	21	31.8

Table C.2. Raw data collected for top 20 plants from G0, G1, and G2 in control and low Calcium solutions (continued)

Plant ID	Treatment	Generation	Days to 1st Flower	1st Flow Height	D16 Height	F+B	D24H
1.13	Control	G2	16	20.1	20.1	24	26.2
1.14	Control	G2	14	14.2	21.6	21	24.4
1.17	Control	G2	15	16.6	21.7	18	25.9
1.24	Control	G2	15	22.3	26.3	25	27.2
2.3	Control	G2	13	14.8	22.6	25	24.2
2.4	Control	G2	13	23.1	32.5	45	39.8
2.5	Control	G2	15	20.3	24.3	31	30.7
2.6	Control	G2	15	23.8	29.8	30	38.3
2.14	Control	G2	16	13.6	13.6	22	21.2
2.20	Control	G2	15	19.2	21.6	27	25.6
3.3	Control	G2	14	19.6	26.1	26	34.6
3.4	Control	G2	14	16.5	23.2	29	35.8
3.13	Control	G2	15	19.2	22.1	28	27.3
3.14	Control	G2	17	23.2	21.7	25	36.1
3.16	Control	G2	15	15.8	19.8	28	33
3.17	Control	G2	13	18.4	25	34	28.2
3.24	Control	G2	15	16.4	18.2	16	21

Table C.3. Raw data for G2 tolerance control and low Calcium populations in low Calcium solution

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16 Height
1.1	Control	16	16.8	16.8
1.2	Control	NA	NA	NA
1.3	Control	16	18.9	18.9
1.4	Control	15	17.2	18.3
1.5	Control	16	17.8	17.8
1.6	Control	16	19.1	19.1
1.7	Control	16	17.2	17.2
1.8	Control	15	21.8	22.8
1.9	Control	NA	NA	NA
1.10	Control	16	21.3	21.3
1.11	Control	16	23.4	23.4
1.12	Control	15	11.8	13.5
1.13	Control	17	12.4	11.5
1.14	Control	17	18.9	17.8

Table C.3. Raw data for G2 tolerance control and low Calcium populations in low Calcium solution (continued)

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16 Height
1.15	Control	17	18.1	16.7
1.16	Control	17	14.2	12.9
1.17	Control	NA	NA	NA
1.18	Control	16	16.8	16.8
1.19	Control	NA	NA	NA
1.20	Control	17	18.1	16.6
1.21	Control	16	17.8	17.8
1.22	Control	15	18.3	21.3
1.23	Control	16	16.7	16.7
1.24	Control	17	22.4	20.7
2.1	Control	16	17.6	17.6
2.2	Control	16	18.4	18.4
2.3	Control	15	17.7	19.8
2.4	Control	16	21.2	21.2
2.5	Control	15	13.5	14.7
2.6	Control	16	17.3	17.3
2.7	Control	16	17	17
2.8	Control	17	18.1	16.1
2.9	Control	16	11.7	11.7
2.10	Control	17	11.3	14.8
2.11	Control	NA	NA	NA
2.12	Control	16	18.9	18.9
2.13	Control	15	15.9	16.8
2.14	Control	15	19.8	21.7
2.15	Control	17	22.9	19.7
2.16	Control	16	16	19.8
2.17	Control	17	30.2	27.6
2.18	Control	16	20.5	20.5
2.19	Control	16	14.9	14.9
2.20	Control	17	17.9	15
2.21	Control	15	15.5	17.4
2.22	Control	16	18.3	18.3
2.23	Control	16	20.6	20.6
2.24	Control	16	17.9	17.9
3.1	Low Calcium	17	15.1	4.6
3.2	Low Calcium	17	21.1	11.1
3.3	Low Calcium	15	19.8	21.7

Table C.3. Raw data for G2 tolerance control and low Calcium populations in low Calcium solution (continued)

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16 Height
3.4	Low Calcium	17	16.2	16
3.5	Low Calcium	16	17.5	17.5
3.6	Low Calcium	16	15.2	15.2
3.7	Low Calcium	16	9.4	9.4
3.8	Low Calcium	14	11.6	14.9
3.9	Low Calcium	16	18	18
3.10	Low Calcium	15	20.3	22.2
3.11	Low Calcium	14	16	18.7
3.12	Low Calcium	14	17.7	21.6
3.13	Low Calcium	NA	NA	NA
3.14	Low Calcium	16	17.9	17.9
3.15	Low Calcium	17	19	5.7
3.16	Low Calcium	17	18.6	13.9
3.17	Low Calcium	17	19.8	8.8
3.18	Low Calcium	NA	NA	NA
3.19	Low Calcium	15	13.6	15.5
3.20	Low Calcium	15	16.2	18.1
3.21	Low Calcium	15	16.4	18.3
3.22	Low Calcium	15	16.9	17.8
3.23	Low Calcium	15	19.5	18.3
3.24	Low Calcium	14	19.2	23.4
4.1	Low Calcium	16	13.2	13.2
4.2	Low Calcium	15	16.9	18.9
4.3	Low Calcium	15	16.7	19
4.4	Low Calcium	15	12.8	14.1
4.5	Low Calcium	15	21	23.2
4.6	Low Calcium	15	18.5	21.1
4.7	Low Calcium	17	11.2	20.8
4.8	Low Calcium	17	15.9	5.9
4.9	Low Calcium	17	19.7	11.1
4.10	Low Calcium	NA	NA	NA
4.11	Low Calcium	15	18.4	19.9
4.12	Low Calcium	16	12.2	12.2
4.13	Low Calcium	15	17.6	18.8
4.14	Low Calcium	15	14.9	16.7
4.15	Low Calcium	16	16.9	16.9
4.16	Low Calcium	15	14.3	16.7

Table C.3. Raw data for G2 tolerance control and low Calcium populations in low Calcium solution (continued)

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16 Height
4.17	Low Calcium	14	13.3	17.8
4.18	Low Calcium	16	16	16
4.19	Low Calcium	16	19.7	19.7
4.20	Low Calcium	16	15.2	15.2
4.21	Low Calcium	15	15.8	18.7
4.22	Low Calcium	14	18.2	23.1
4.23	Low Calcium	15	16.1	18.6
4.24	Low Calcium	15	17.3	19.4

Table C.4. Raw data for G3 tolerance control and low Calcium populations in low Calcium solution

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16H	D24H	F+B	Dry Mass
1.1	Control	14	12.1	11.8	11.9	18	0.103
1.2	Control	NA	NA	1.8	3	0	0.009
1.3	Control	15	10.9	11.7	12	20	0.122
1.4	Control	15	8.4	9.8	9.8	19	0.12
1.5	Control	18	9.8	8.5	9.9	15	0.093
1.6	Control	16	11.6	11.6	10.9	24	0.104
1.7	Control	14	10.8	11.5	12	11	0.093
1.8	Control	14	13.2	14.1	14.3	25	0.087
1.9	Control	15	16.9	7.5	8.5	17	0.111
1.10	Control	18	12.8	9.3	16.5	6	0.047
1.11	Control	17	6.7	6.2	10.6	22	0.044
1.12	Control	17	8.1	8	8.4	24	0.097
1.13	Control	16	8.6	8.6	14.1	17	0.065
1.14	Control	17	9.9	9.4	10.8	17	0.088
1.15	Control	17	8.3	8.1	8.7	18	0.099
1.16	Control	18	8.7	9	9	20	0.098
1.17	Control	16	13.7	13.7	17.4	17	0.102
1.18	Control	17	11.4	9.9	13.9	22	0.092
1.19	Control	NA	NA	NA	NA	NA	NA
1.20	Control	16	13.5	13.5	14.5	15	0.07
1.21	Control	17	11.6	7.9	8.2	15	0.071
1.22	Control	16	11.4	11.4	11.9	20	0.112
1.23	Control	18	10	9.8	10.3	24	0.047

Table C.4. Raw data for G3 tolerance control and low Calcium populations in low Calcium solution (continued)

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16H	D24H	F+B	Dry Mass
1.24	Control	15	11.2	11.5	20.4	27	0.085
2.1	Control	18	9.4	9.3	9.8	20	0.095
2.2	Control	18	11.9	7.2	13	19	0.091
2.3	Control	16	8.2	8.2	9.4	12	0.156
2.4	Control	16	2.5	12.5	12.9	24	0.121
2.5	Control	17	11.8	10.1	16.2	15	0.069
2.6	Control	16	9.7	9.7	11.1	9	0.073
2.7	Control	15	9	9.6	10.2	29	0.079
2.8	Control	16	10.7	10.7	11.9	17	0.072
2.9	Control	19	8.3	6.5	9.4	22	0.03
2.10	Control	NA	NA	NA	NA	NA	NA
2.11	Control	16	10.6	10.6	12	21	0.071
2.12	Control	18	9.2	5.4	12.8	19	0.037
2.13	Low Calcium	17	7.5	7.2	7.8	18	0.142
2.14	Low Calcium	17	8.4	7	8.6	20	0.148
2.15	Low Calcium	19	13.5	12.4	15	21	0.113
2.16	Low Calcium	17	9	8.5	9.2	21	0.073
2.17	Low Calcium	16	10.1	10.1	12.5	18	0.066
2.18	Low Calcium	20	7.5	6.2	7.6	13	0.061
2.19	Low Calcium	19	7.8	7.7	7	6	0.137
2.20	Low Calcium	18	9.9	9.8	10.3	15	0.086
2.21	Low Calcium	16	11.4	11.4	15.8	29	0.076
2.22	Low Calcium	16	13.2	13.2	15.5	31	0.089
2.23	Low Calcium	17	12.9	10.1	16.1	18	0.093
2.24	Low Calcium	17	13.3	14	17.2	21	0.089
3.1	Low Calcium	17	11.7	10	14.3	13	0.094
3.2	Low Calcium	15	14.7	16.6	17.5	28	0.097
3.3	Low Calcium	15	12.3	12.7	13	24	0.151
3.4	Low Calcium	16	9.5	9.5	10.4	16	0.136
3.6	Low Calcium	16	8.5	8.5	9.2	23	0.135
3.7	Low Calcium	17	8.9	8.8	9.1	20	0.107
3.8	Low Calcium	15	9.5	10.4	11.5	10	0.101
3.9	Low Calcium	16	9.5	9.5	6.1	20	0.064
3.10	Low Calcium	16	7.4	7.4	7.6	17	0.107
3.11	Low Calcium	NA	NA	NA	NA	NA	NA
3.12	Low Calcium	15	10.2	10.6	11	23	0.129
3.13	Low Calcium	16	11	11	14.2	21	0.106
3.14	Low Calcium	18	6.8	6.5	6.9	18	0.101

Table C.4. Raw data for G3 tolerance control and low Calcium populations in low Calcium solution (continued)

Plant ID	Treatment	Days to 1st Flower	1st Flower Height	D16H	D24H	F+B	Dry Mass
3.15	Low Calcium	NA	NA	4	7.3	0	0.068
3.16	Low Calcium	20	8.3	5.1	9.1	22	0.045
3.17	Low Calcium	16	7.3	7.3	13	22	0.146
3.18	Low Calcium	18	8.8	8.2	9.1	25	0.068
3.19	Low Calcium	18	9.2	7.9	9.6	17	0.093
3.20	Low Calcium	17	19	17.8	20	22	0.131
3.21	Low Calcium	NA	NA	4.1	4.8	0	0.088
3.22	Low Calcium	17	9.4	9.6	9.9	14	0.107
3.23	Low Calcium	16	10.3	10.3	10.8	16	0.099
3.24	Low Calcium	NA	NA	NA	NA	NA	NA