THESIS

MANIPULATION OF RESOURCE ALLOCATION TO INCREASE FECUNDITY IN *MIMULUS GEMMIPARUS*

Submitted by

Kevin Chu

Department of Biology

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Master's Committee:

Advisor: David Steingraeber

Chris Funk
Ruth Hufbauer

ABSTRACT

MANIPULATION OF RESOURCE ALLOCATION TO INCREASE FECUNDITY IN *MIMULUS GEMMIPARUS*

Mimulus gemmiparus W. A. Weber, a Colorado endemic, is a rare species that is at risk of becoming endangered. Reproduction in *M. gemmiparus* is solely by vegetative propagules (bulbils), which function analogously to seeds; sexual reproduction has not been observed in the wild. Manipulation of resource allocation in this plant may shift resources allocated for sexual reproduction to vegetative growth; the investment in increased vegetative growth should result in a direct increase in fecundity.

Two experiments were conducted to examine the effects of redistribution of resources and change in meristem fate. The first experiment used plants grown in greenhouse conditions; seemingly nonfunctional floral buds or floral buds and shoot apical meristems were removed to potentially increase bulbil production. The second experiment used plants grown indoors under growing lamps to produce plants that were similar in size to those found in their natural habitats; their shoot apical meristems were removed to potentially induce branching and thus increase vegetative growth to increase bulbil production.

Removal of floral buds or removal of both floral buds and shoot apical meristems in a greenhouse environment doubled the number of bulbils produced per plant, increased by approximately two-thirds the total bulbil biomass per plant, increased the ratio of bulbil count to stem biomass, and increased the ratio of bulbil biomass to stem biomass; however, there was a trade-off between having greater number of bulbils and individual bulbil biomass.

Solely removing the shoot apical meristem in a low-light environment had no effect on bulbil production; although branching was induced, the primary branches remained small and did not elongate. Secondary branches were formed only on plants that had their shoot apical meristem removed. The total numbers of shoot axes per plant were not significantly different between the control and treatment group; the induction of branching seemingly produces a compensatory effect, but not overcompensation for the removal of the shoot apical meristem.

In both experiments, stem biomass did not differ between the control and treatment group(s). Lighting condition in the environment the plants grew in had the greatest effect on stem development, and manipulation of meristems to form new shoots did not increase plant size. Bulbil yield may be increased in greenhouse conditions by manipulation of allocation from sexual reproduction to vegetative growth.

ACKNOWLEDGMENTS

I would like to extend thanks to all the people who made this thesis possible. First and foremost, I would like to express my deepest sense of gratitude to my academic advisor, David Steingraeber, for his mentorship and encouragement. This project could not have been completed without his guidance. I thank my committee members for their contributions to my program. I would also like to thank Colorado Parks and Wildlife for allowing collection of plant samples and also Mark Beardsley for being in the field to collect the plants used in my work. I am also appreciative to Lauren Hall, Thomas Manion, and Stephanie Szostek for their assistance in preparing this manuscript. Finally, I am grateful for the support and help from Laura Cascardi and Katherine Chu.

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CHAPTER 1 - INTRODUCTION

When hiking the trails or bushwhacking through the forests of the Colorado Front Range you may encounter the rare budding monkeyflower (*Mimulus gemmiparus* W. A. Weber), a small green plant not easily seen and at first glance a minor element when compared to the backdrop of botanical and fungal diversity. *Mimulus gemmiparus* is a plant endemic to Colorado with a particular habitat requirement and life history strategy; these plants are known from only eight general locations along the Colorado Front Range (Figure 1.1), typically along or under overhangs of south or west facing cliffs and requiring a consistent water supply usually from an aquifer (Beardsley, 1997; Steingraeber and Beardsley, 2005) (Figure 1.2). *Mimulus gemmiparus* grows in small isolated patches with few other plant species in the immediate vicinity; the known total area which this plant occupies is less than 100 m^2 (Beardsley, 1997, 2012). This plant is truly rare with its limited distribution and unassuming form.

Mimulus gemmiparus is a peculiar plant due to its unusual mode of reproduction. It is an annual species and appears to reproduce only asexually via the production of an embryo-like bulbil inside each leaf petiole. Formation of bulbils and asexual reproduction by bulbils have been observed in a variety of other plant families (e.g. Agavaceae, Brassicaceae, Liliaceae). The bulbils of other plant families, however, are not the primary mode of reproduction and the bulbils often are composed of exposed rosettes of leaves (Arizaga and Ezcurra, 1995; Moody, *et al*., 1999). Bulbils of *M. gemmiparus* are unique, however, in that they become completely surrounded and protected by another structure, i.e., the ensheathing petiole of the subtending leaf.

The general growth pattern of *M. gemmiparus* consists of two leaves and four axillary buds produced at each node, with two axillary buds initiated in the axil of each leaf. However, beginning with the third node, the proximal bud at each node becomes ensheathed by the developing petiole of the subtending leaf to form the bulbil. The distal bud at each node remains and can later develop into

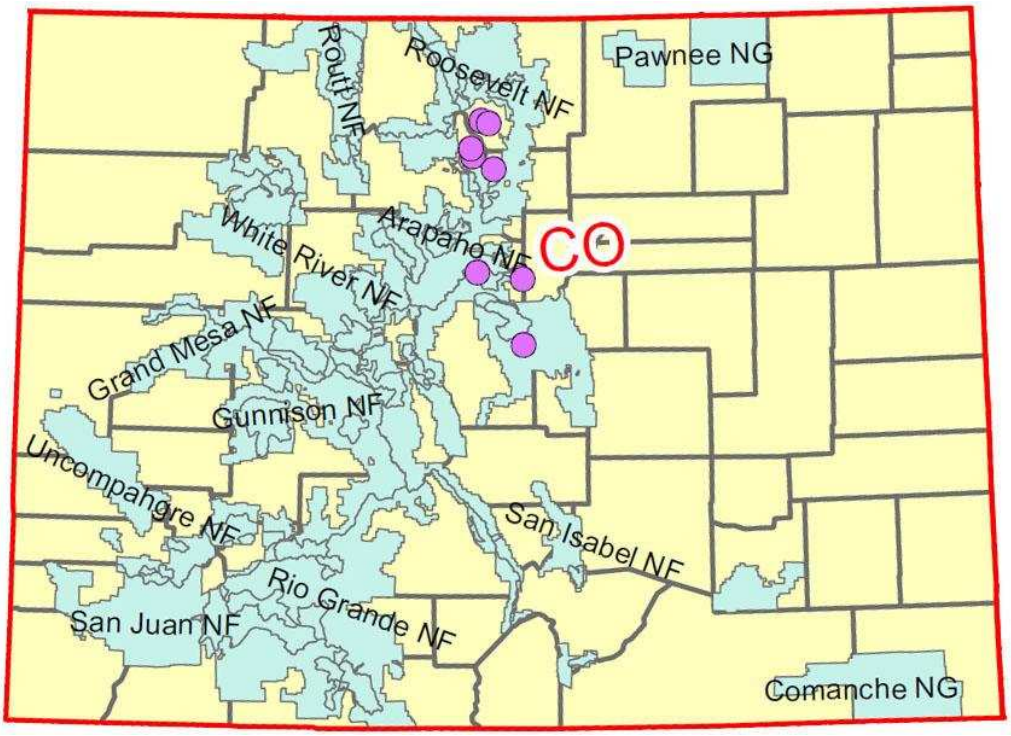

Figure 1.1. Map of *M. gemmiparus* distribution from Beatty *et al*. (2003) showing the eight locations the plants are found in Colorado and worldwide.

Figure 1.2. A patch of *M. gemmiparus* growing under a rocky outcrop (noted by the blue oval) at Staunton State Park (Steingraeber and Beardsley, 2005).

a flower or a branch (Moody *et al*., 1999). Branching usually occurs at the first few nodes and flowers may develop later from axillary buds at distal nodes, but flowers never form from a terminal bud.

The vegetative propagules form starting at the third node and branches (Figures 1.3, 1.4). The bulbils form in the petioles of the leaves and are homologous to axillary buds and analogous to seeds (Moody *et al*. 1999). The petiole likely functions analogously to a seed coat, providing protection from the elements to the bulbil. The bulbil inside the petiole is a modified vegetative shoot with a pair of cotyledon-like storage leaves and adventitious root primordia (Beardsley, 1997). The bulbils of *M. gemmiparus* are classified as brood bulbils, with storage leaves containing starch grains. Although brood tubers (in other plants) are sometimes also referred to as bulbils, brood tubers have starch stored in the stem rather than leaves (Moody *et al.*, 1999). At maturity the plant desiccates; the leaves wither away and the bulbils (with petiolar sheaths) dissociate from the stem (Weber, 1972). Bulbil dispersal can be accomplished by floating down water paths or being carried by the wind. Bulbils overwinter on the soil surface, and germination of the embryo-like plant inside the structure occurs after overwintering. Flowers may develop at distal nodes on older plants, but the flowers are seemingly nonfunctional (Weber, 1972; Beardsley 1997). Fruits and seeds have never been observed on plants in any of the populations, and reproduction is solely asexual via bulbils. Therefore, flower production and maintenance would seem to be suboptimal in terms of resource use (Figure 1.5).

Mimulus gemmiparus is a rare species and considered critically imperiled (Bunch, 2012). The persistence of a population is dependent upon the bulbils produced by the previous generation. Since reproduction of the species is asexual by bulbils, fecundity of the plant is directly linked to its vegetative growth. Protecting naturally occurring populations has involved minimizing disturbance of these areas by human activity, but in light of potential climate change and other disturbances such as fire and drought, conservation efforts have included ex-situ propagation of bulbils for planting and reintroduction in sites with suitable habitat. Can plants grown ex-situ be manipulated to increase bulbil production for use in such reintroductions? That is the central question addressed in the work that follows.

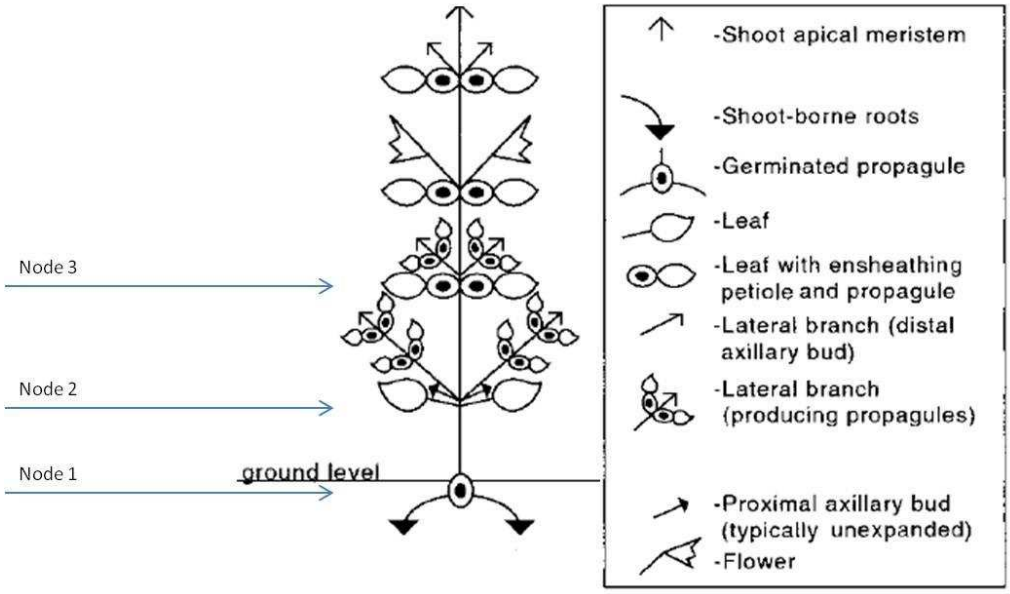

Figure 1.3. Diagram of *M. gemmiparus* from Moody *et al.* (1999) depicting the general pattern of growth. Bulbils begin to form from the third and subsequent nodes on the main stem and first and subsequent nodes of branches.

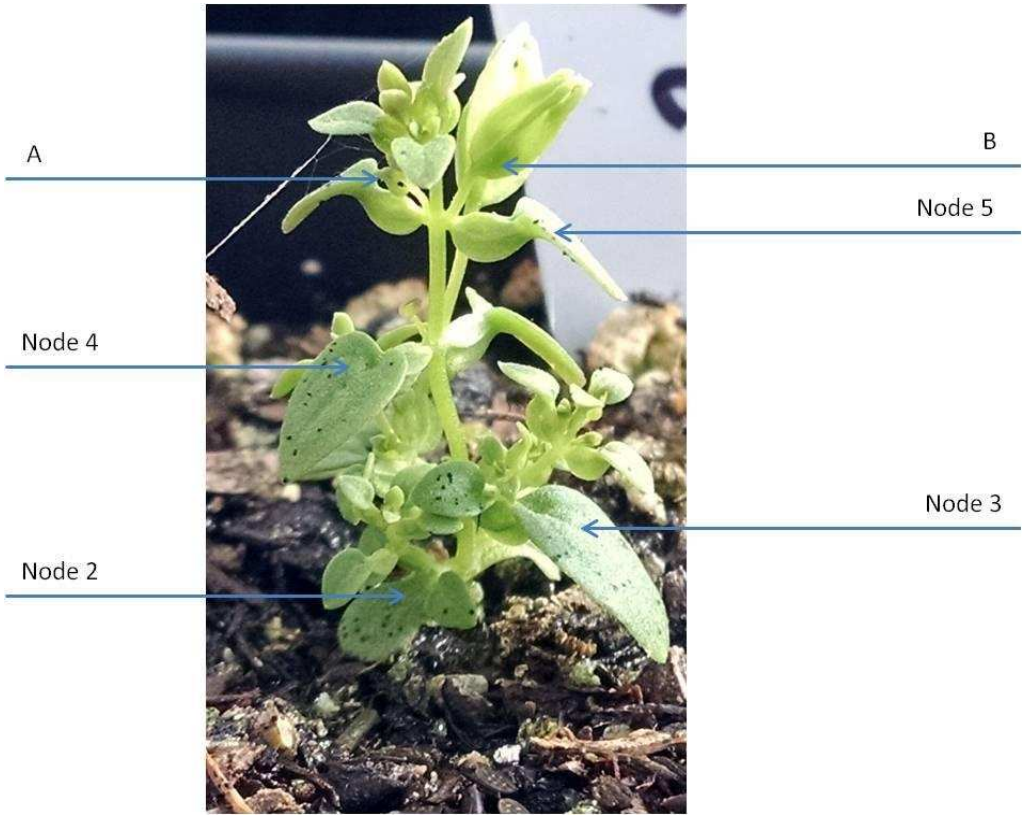

Figure 1.4. *Mimulus gemmiparus* showing above ground nodes. Branch formation can be seen on nodes 2-5, and floral buds on nodes 4 and 5. Node 5 shows axillary buds becoming a flower on the right (B) and a branch on the left (A). Node 5 shows an example of a propagule where the petiole of the leaf encases a bulbil.

Figure 1.5. Single *M. gemmiparus* plant grown in greenhouse conditions with multiple branches containing numerous flowers and floral buds.

Shoots of higher plants are composed of repeating modules of stem, leaf, and axillary bud; a plant's form is dictated by internal population dynamics and production of the modules which are arranged as units of nodes and internodes (metamers) to form the main stem and branches (Harper, 1977; Harper and Bell, 1979). The modules of a single plant can be considered to have their own birth and death rate, analogous to a species' birth and death rates in a population. Repetition of these modules forms the main stem and branches which are limited by the quantity of axillary meristems. Each meristem produced may become a vegetative shoot, reproductive shoot, or remain quiescent. If a vegetative shoot is initiated, a branch forms and increases the number of available meristems for future development. However, if a reproductive shoot is initiated, a flower forms, which prevents the formation of new meristems from that axillary bud (Harper and Bell, 1979; Watson, 1984). Branching from an axillary bud may be initiated by the loss of apical dominance where a shoot apical meristem acting as a source of auxin is removed or has grown sufficiently far away from the axillary bud that it can no longer inhibit the axillary bud from developing into a branch (Thimann and Skoog, 1933).

Throughout a plant's lifespan, it garners resources both above- and below-ground. Water and mineral nutrients are absorbed by the roots and carbon is taken up by leaves. The acquired resources and their metabolic products may be stored, spent for growth, survival, or reproduction (Boggs, 1992). Environmental factors and internal population dynamics influence the allocation of resources and generate the patterns and types of modules produced. Available resources and spacing between plants may affect the direction of growth and the amount of growth possible; conditions during growing seasons can affect the investment of resources to vegetative growth or sexual reproduction (Harper and Bell, 1979). In an optimal setting, plants may produce branches throughout the growing season and increase the number of meristems they contain before committing them to flowers for reproduction, thereby increasing fecundity (Smith, 1984; Geber, 1990). In less ideal settings, plants may allocate resources to flowering earlier to ensure reproduction and increase fecundity, creating a trade-off between reproduction and vegetative growth of the plant (Baker and Diggle, 2011). Allocating resources to sexual reproduction can reduce vegetative growth since there is an investment of resources and energy to producing flowers (Harper and Ogden, 1970).

Plants may divert resources to asexual reproduction if sexual reproduction is not a viable strategy when the environment is unfavorable. Low pollinator visitation, limited available resources, or abiotic factors may contribute to prioritizing asexual reproduction to ensure reproduction. The method for asexual reproduction varies with different species (e.g. formation of stolons, rhizomes, bulbils) and requires allocation of resources within the plant similar to allocation of resources between vegetative growth and sexual reproduction; similarly, there is a trade-off between sexual and asexual reproduction (Sutherland and Vickery, 1988; Arizaga and Ezcurra, 1995).

Beardsley (1997) has shown that there is plasticity in the development of the modules of *M. gemmiparus* based on environmental conditions. His study utilized three sets of plants, one set grown in nutrient-rich conditions, another in nutrient-deficient conditions, and the third set under light-deficient conditions. Plants responded to these conditions by allocating resources differently to stems, leaves, and bulbils, and changing their growth pattern. Metamer production, stem volume, leaf size, and bulbil size were found to be dynamic. With high resource levels (the potting soil treatment), *M. gemmiparus* allocated resources to produce more metamers and larger leaves, an investment that resulted in more bulbils produced later in life. In nutrient-deficient conditions, plants allocated resources early on to enlarging bulbils, thereby ensuring reproduction when resources were scarce; metamers were not produced as numerously and stem growth was reduced. There may not be a benefit to having these structures if they cannot be maintained. Plants growing in shaded conditions with no light available did not produce bulbils but allocated resources to stem growth where the stems elongated and widened to forage for and capture light. *Mimulus gemmiparus*' response to the different growing conditions shows that its development is influenced by the environment and suggests that while individual parts of the plant compete for resources, the allocation of resources may be directed by manipulation to specific parts.

In this thesis, I intend to present an overview of axillary bud development pathways in *M. gemmiparus* and test hypotheses regarding manipulation of *M. gemmiparus* structures. The overall goal was to manipulate plant growth and resource allocation to improve bulbil yield. The study addresses how changes in patterns of growth and resource allocation could affect meristem fate and plant

reproductive fitness. I examined how the removal of meristems and reproductive organs may influence vegetative development and production of bulbils under controlled growing conditions. Two experiments are presented, one involving the removal of floral buds and apical meristems to shift resource allocation in greenhouse-grown plants, and second involving removal of shoot apical meristems to induce branching in lab-grown plants. A discussion on the significance of the results follows each experiment. The synthesis and conclusions section integrates the two experiments together and explains the application of this research to current conservation efforts. In the appendix, I give the SAS codes for statistical tests, details regarding statistical tests, and a description of observed morphological anomalies of *M. gemmiparus* grown during the experiment.

CHAPTER 2 - EFFECTS OF REMOVAL OF FLORAL BUDS AND APICAL MERISTEMS ON PROPAGULE PRODUCTION

Experiment 1: Removal of Floral Buds and Apical Meristems in Greenhouse Environment

Introduction

Each node produced by *Mimulus gemmiparus* contains two leaves and four axillary buds; two buds become bulbils within the petioles and the other two have the normal functions of axillary buds, i.e., they can develop into a branch, develop into a flower, or remain dormant (Moody *et al.*, 1999). Leaves function to capture light and carbon, and at the same time, bulbils are produced for asexual reproduction. Thus, at each node, both a resource source and sink are produced, and some photosynthate presumably is translocated from leaf to bulbil. The other two axillary buds, if they were to develop, would either become a branch that could produce more leaves and bulbils, or become a flower. Since successful sexual reproduction has never been observed in wild *M. gemmiparus* populations, flowers are sterile and do not contribute to fitness; however, resources assimilated by the plant are used in producing and maintaining the floral structures. While branching does have an initial cost of resources for the plant, branching produces additional leaves which capture sunlight and acquire carbon for the plant, which would compensate for the investment into building a branch (Watson, 1984). In *M. gemmiparus*, the investment of resources in vegetative growth also produces a functional method of reproduction in the form of bulbils, which directly contribute to fitness.

The main goal of this study is to understand whether and how experimental manipulations of the meristem population and resource allocation affect growth patterns in the plant and ultimately, bulbil production and fitness. In *M. gemmiparus*, each leaf produces one vegetative propagule, and investment in increased vegetative growth should result in a direct increase in fecundity. Thus, by removing flowers and/or apical meristems, growth and allocation patterns may be altered in a way that increases bulbil production and fecundity.

One such manipulation is the continued removal of all flower buds as they are initiated. If resources are not allowed to be expended on sexual reproduction, then those resources could be allocated to other parts of the plant for new vegetative growth, resulting in more metamers and bulbils. Another possible result from this treatment may be increased allocation of resources to individual bulbils, likely increasing survivorship by increasing bulbil biomass. An increase in numbers of bulbils produced and/or an increase in individual bulbil mass may result from this manipulation or the plant may prioritize one over the other. An increase in the ratio of total bulbil biomass to stem biomass would be expected if the removal of floral buds results in resource reallocation to the production of more bulbils. An alternate result could be an increase in bulbil biomass but not an increase in the number of bulbils produced, which would occur if resources were reallocated to increase the biomass of existing bulbils rather than to increase the production of more bulbils. In this case, there would not be a difference in bulbil count to stem mass ratio. Since floral buds are removed they would not remain as a strong sink for resources and those resources could be reallocated to vegetative growth.

An alternative manipulation is the continued removal of the shoot apical meristems along with the flower buds as they are initiated. The treatment includes the removal of flower buds, therefore, a change in resource allocation is expected. However, a resource sink is also created by the induction of branching by removing the shoot apical meristem. Results of this manipulation may be similar to the results of the first treatment, with an increased count of bulbils and/or increased individual bulbil biomass, or it may be different from the first manipulation depending on how resources are allocated in the formation of new branches. The removal of floral buds should eliminate a strong resource sink, and continuous removal of shoot apical meristems may induce more branching, which may, in turn, increase the number of stems and number of bulbils. This treatment is expected to break apical dominance and potentially induce branching to yield more metamers and, accordingly, more bulbils.

Data collected from the experiment includes the number of bulbils produced per plant, bulbil biomass per plant, and stem biomass. The number of bulbils to stem biomass ratio should provide information on the growth form of the plant, i.e., whether there are more nodes (and bulbils) produced per unit mass of stem or if there is more elongation of stems with few nodes (Figure 2.1).

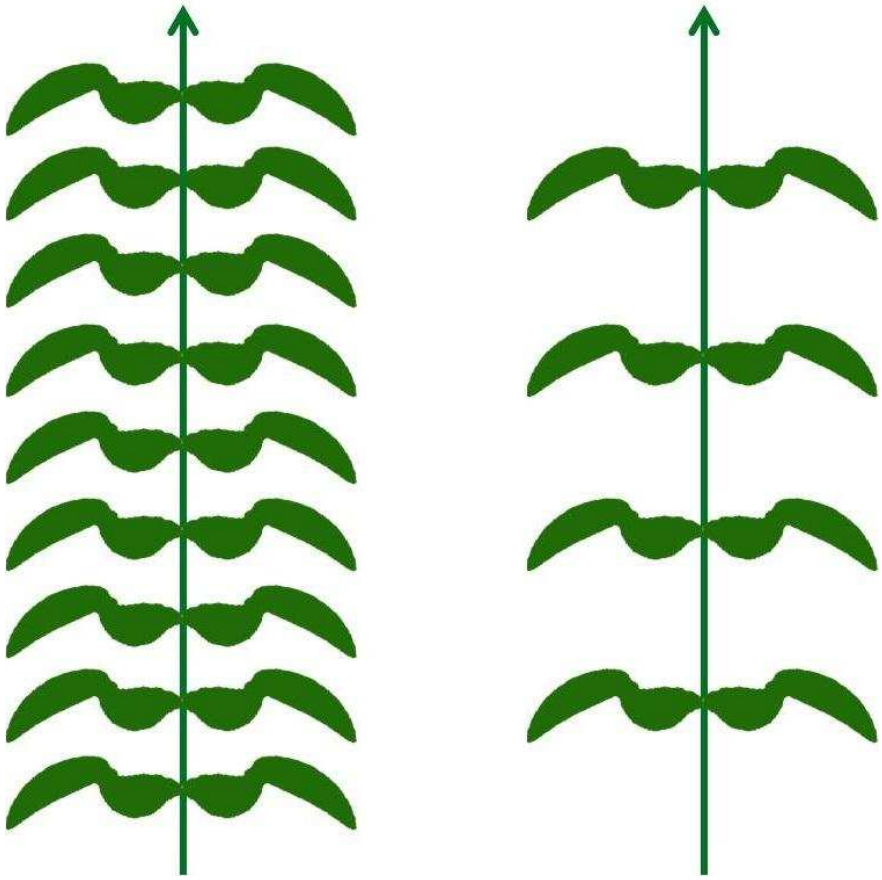

Figure 2.1. Left: diagram of *M. gemmiparus* with numerous nodes per unit mass of stem. Right: diagram of *M. gemmiparus* with sparse nodes per unit mass of stem.

The bulbil biomass to stem biomass ratio should show where resources are allocated or reallocated within the plant: high bulbil biomass to stem biomass should indicate resources being moved to the bulbils and fewer resources for growth or elongation of stems, whereas low bulbil biomass to stem biomass should indicate resources being moved to stem growth or elongation instead of bulbil production.

Materials and Methods

Mimulus gemmiparus plants were randomly collected in 2013 from the Elk Creek population in Colorado's Staunton State Park and bulbils from these plants were germinated in the Colorado State University (Fort Collins, Colorado) greenhouse in January 2014. From these plants grown in the greenhouse, bulbils were harvested to grow the plants for the experiment conducted in the summer of 2014. Using the bulbils from clones of the original plants, I conducted a greenhouse study with two treatments and one control. Each treatment group consisted of 31 replicates of cloned plants from six different original plants, with 186 plants per treatment group, and 558 plants total (Figure 2.2). The first experimental treatment was the continuous removal of floral buds as soon as buds were apparent. The second experimental treatment was the continuous removal of floral buds and shoot apical meristems, beginning when floral buds first become apparent. Removal of the shoot apical meristem by itself was not possible since it is not exposed; instead, the newest developing node was removed along with the developing floral buds from the previous node. The timing of the production of a floral bud was used to determine when to remove the shoot apical meristems because the transition to sexual reproduction is an indication of a mature plant which would be more likely to survive the stress of the treatment than a seedling (Reekie, 1997). The controls for the experiment had no removal of floral buds or shoot apical meristems (Figure 2.3). Each 3-inch pot had approximately three inch spacing between itself and its neighbors to give plants adequate room. Bulbils were germinated and grown in the greenhouse in July 2014 on Fafard 4P potting soil with high nutrient and water availability. The light regimen was sixteen hours of light and eight hours of dark; the temperature was approximately 75°F and the approximate relative humidity was 70%.

Figure 2.2. The layout of the experiment in the CSU greenhouse. Green tags representing plants with treatment one, red tags representing plants with treatment two, and white tags representing plants used as controls with no treatment applied.

Figure 2.3. Left: Plant with treatment one, with only floral buds removed. Center: Plant with treatment two, with floral buds and shoot apical meristem removed. Right: Control plant with no parts removed.

All bulbils were grouped and identified by the original source plant from which they came. Bulbils from the source plants were distributed randomly among test groups when grown and had their positions rotated multiple times per week to reduce positional and environmental effects. Two weeks after the initial potting, pots in which bulbils failed to germinate were replanted with new soil and bulbils to attempt germination again. When plants had grown to approximately two nodes, each pot was thinned to one plant per pot.

The experiment was conducted for eight weeks following thinning, with each pot containing one plant at the start of the experiment (Figures 2.4, 2.5). After eight weeks, the above-ground portion of each plant was harvested and air-dried in individual paper bags (Figure 2.6). After drying for two months, a subsample of 20 plants in each treatment group was randomly selected by random number generator for data collection. Bulbils and stems from individual plants were physically separated by hand with the aid of a dissecting probe and a Luxo 16346WT IFM Magnifier.

The number of bulbils per plant was counted by using a Sony DSC-W830 Digital Camera, Adobe Photoshop CS2, and ImageJ image-processing software. Bulbils were placed on a sheet of herbarium mounting paper and spaced apart without bulbils touching each other, and an image was taken at a distance of approximately half a meter with the camera set at 20.1 megapixels. Lighting for the photo was produced with four desk lamps with Satco Omnix LED bulbs at 830 lumens and color temperature of 3500 K. Adobe Photoshop CS2 was then used to crop each image to show only the bulbils. Cropped images were then edited by the "Spot Healing Brush Tool" to remove extraneous material that could not have been easily physically separated by hand (e.g. leaf fragments, flower petal fragments, insects, and dirt) (Figure 2.7). Bulbils that were touching each other or attached to each other at the base of the petiole and were too delicate to pull apart were also moved within the image. The background was then deleted along with any shadows along the edge of the photo, and the resulting image showed only bulbils (Figure 2.8). ImageJ (Version 1.49; Rasband, 2015) was used to count the number of bulbils on each image. The "Threshold" function converted the image to black and white to contrast the bulbils from the white background, and then the "Analyze Particles" function was configured to count the blackened bulbils with a particle size range of ten pixels to infinity (Figure 2.9). Stem and bulbil

Figure 2.4. The start of experiment with multiple bulbils germinating in each pot. Numbering on tag identifies the original source plant of the seedlings. Seedlings were later removed to contain only one plant in each pot.

Figure 2.5. *Mimulus gemmiparus* at approximately 4 weeks of growth since planting in the greenhouse.

Figure 2.6. *Mimulus gemmiparus* at the end of the experiment (approximately 8 weeks) with some plants beginning to senesce. Varied growth forms and different amount of growth observed across both treatment groups and control group.

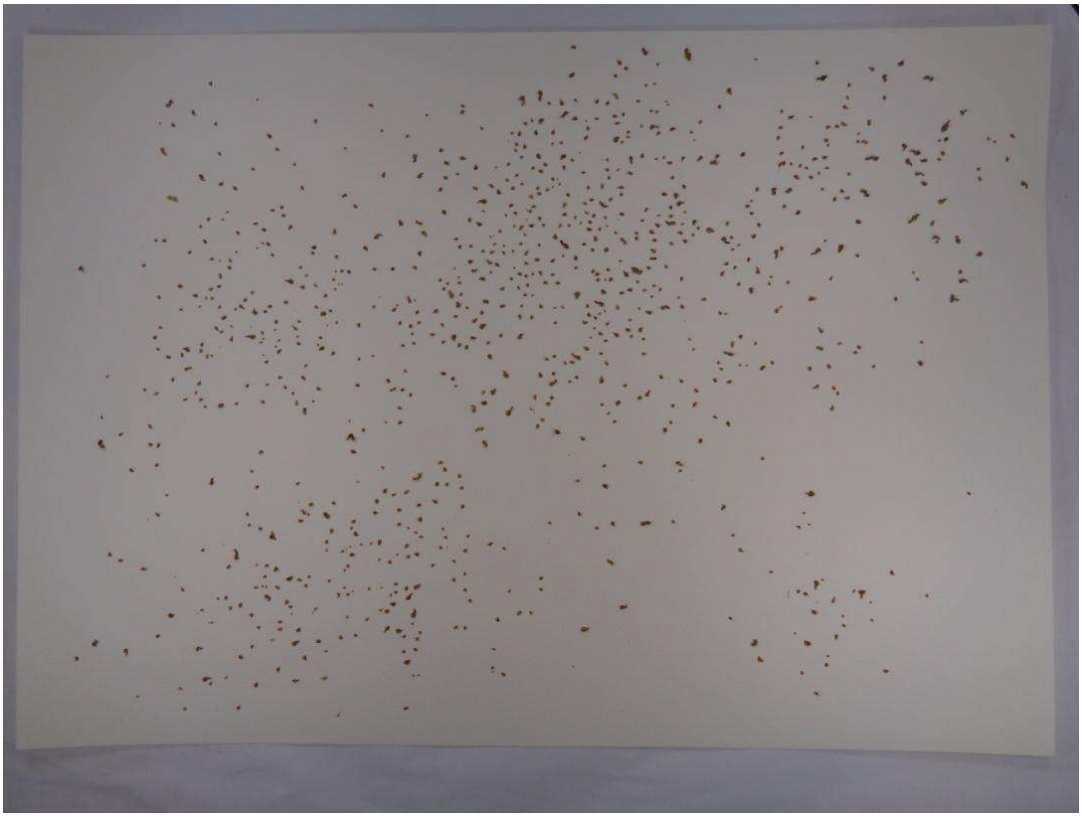

Figure 2.7. Unedited image of bulbils and other materials on a sheet of herbarium mounting paper.

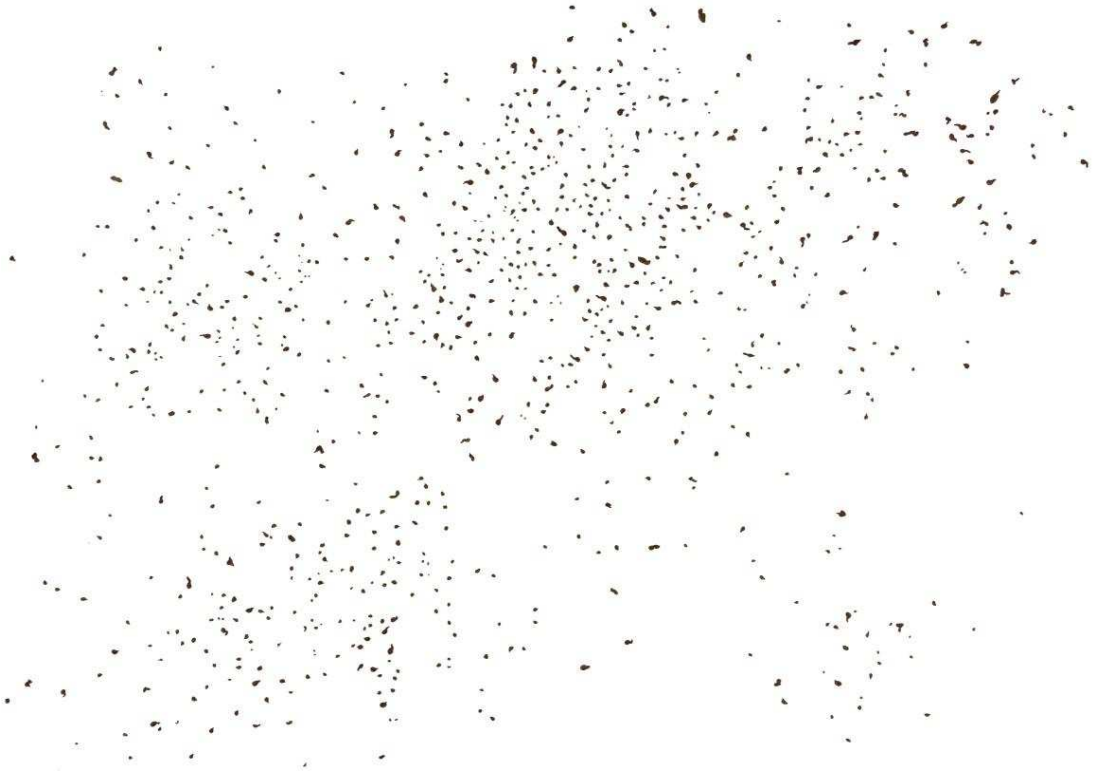

Figure 2.8. Edited image of Figure 2.7 with only bulbils showing.

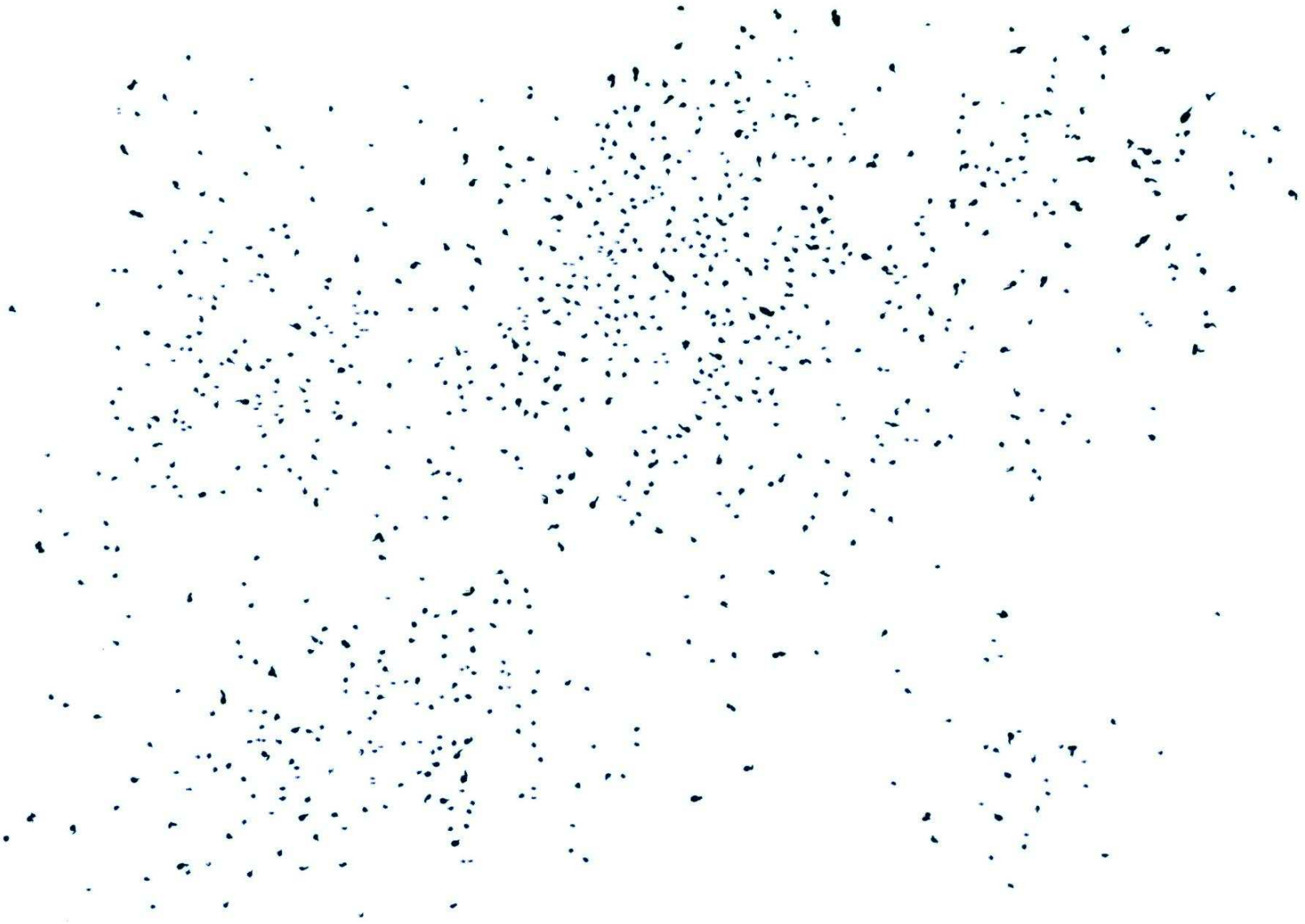

Figure 2.9. The result of analysis by ImageJ, Figure 2.8 was converted to black and white and each particle was counted. Image shows 941 bulbils, the blue color in the image are overlaid numbers identifying each individual bulbil; the actual size of this image is 4613 x 3255 pixels.

biomass for each plant was measured after the images were taken by weighing the samples on a Denver Instrument APX-100z analytical balance.

The means of the number of bulbils produced per plant, total bulbil mass (g) per plant, dry mass of the above-ground stem and branches (g), ratio of number of bulbils to stem biomass (bulbils g^{-1}), ratio of bulbil biomass to stem biomass, and average individual bulbil mass (g) per plant were compared between the control and two experimental treatments using the GLM Procedure to generate ANOVA tables ($\alpha = 0.05$) with the SAS 9.3 software package.

Results

Bulbil Count

The mean number of bulbils produced by plants with floral buds removed (trt 1) was 3770.60 ± 531.93 (95% CI). The mean number of bulbils produced by plants with floral buds and shoot apical meristems removed (trt 2) was 3260.80 ± 468.41. The mean number of bulbils produced by the control plants was 1678.40 ± 292.15. ANOVA analysis indicated that at least one group was significantly different from others ($p < 0.0001$, Figure 2.10). The least squares means analysis shows a significant difference between the control and treatment one ($p < 0.0001$) and a significant difference between the control and treatment two ($p < 0.0001$); there was no significant difference between treatment one and treatment two ($p = 0.0937$, Figure 2.11).

Total Bulbil Biomass per Plant

The mean total bulbil biomass of plants with floral buds removed (trt 1) was 0.6768 ± 0.1070 g (95% CI). The mean total bulbil biomass of plants with floral buds and shoot apical meristems removed (trt 2) was 0.5996 ± 0.2121 g. The mean total bulbil biomass of the control plants was 0.3877 ± 0.0879 g. ANOVA analysis indicated that at least one group was significantly different from others ($p = 0.0002$, Figure 2.12). The least squares means analysis shows a significant difference between the control and treatment one ($p < 0.0001$) and a significant difference between the control and treatment two ($p =$

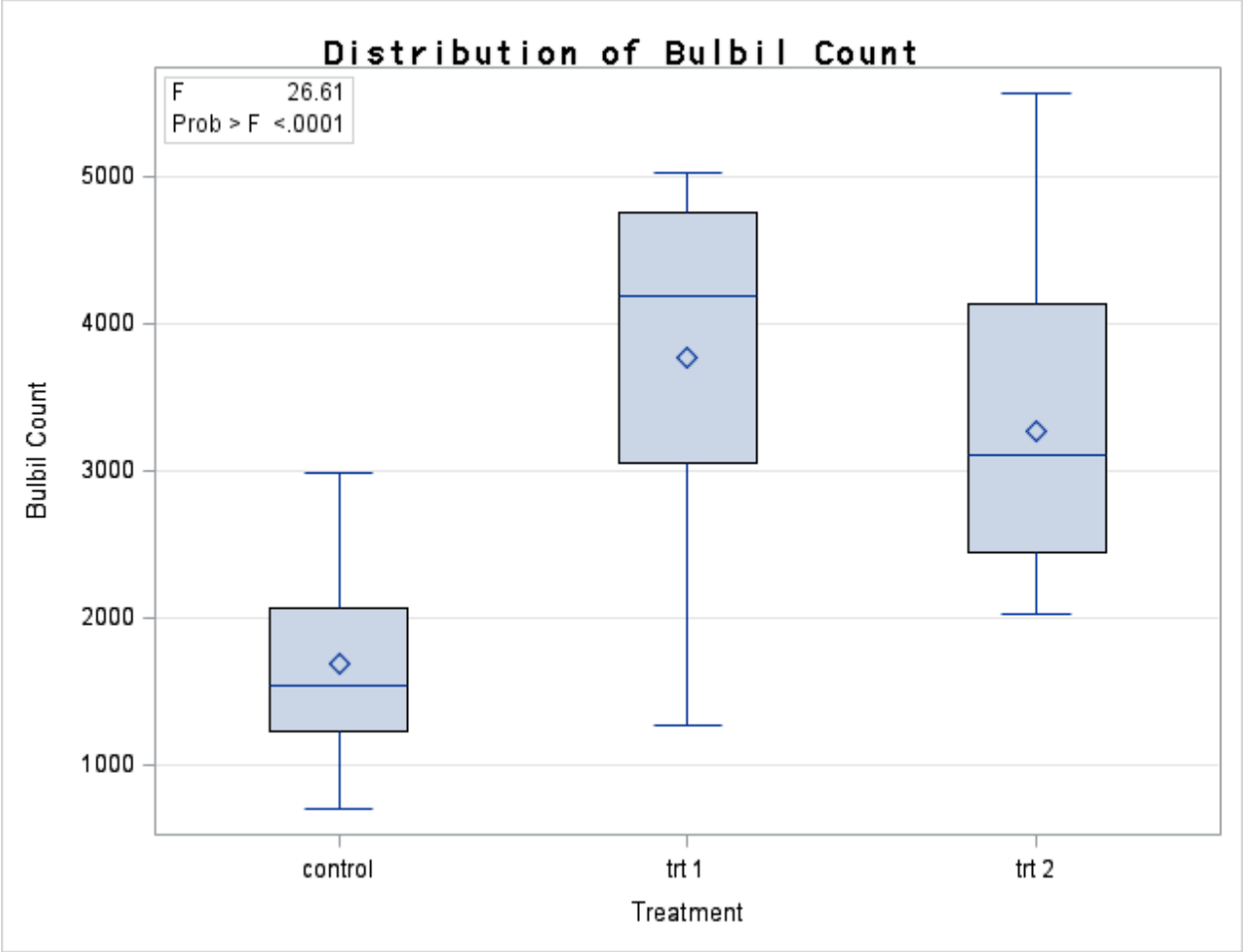

Figure 2.10. Box plots of the distribution of total bulbils produced per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Bulbil count from 20 plants sampled in each treatment group. ANOVA F-ratio = 26.61, $p < 0.0001$, $\alpha = 0.05$. Boxes represent the first quartile to the third quartile of collected data, and the extended vertical lines represent the maximum and minimum values of that data that was collected. Horizontal bars in the boxes represent the median of the data Diamond symbols in each box plot represent the mean of the treatment group.

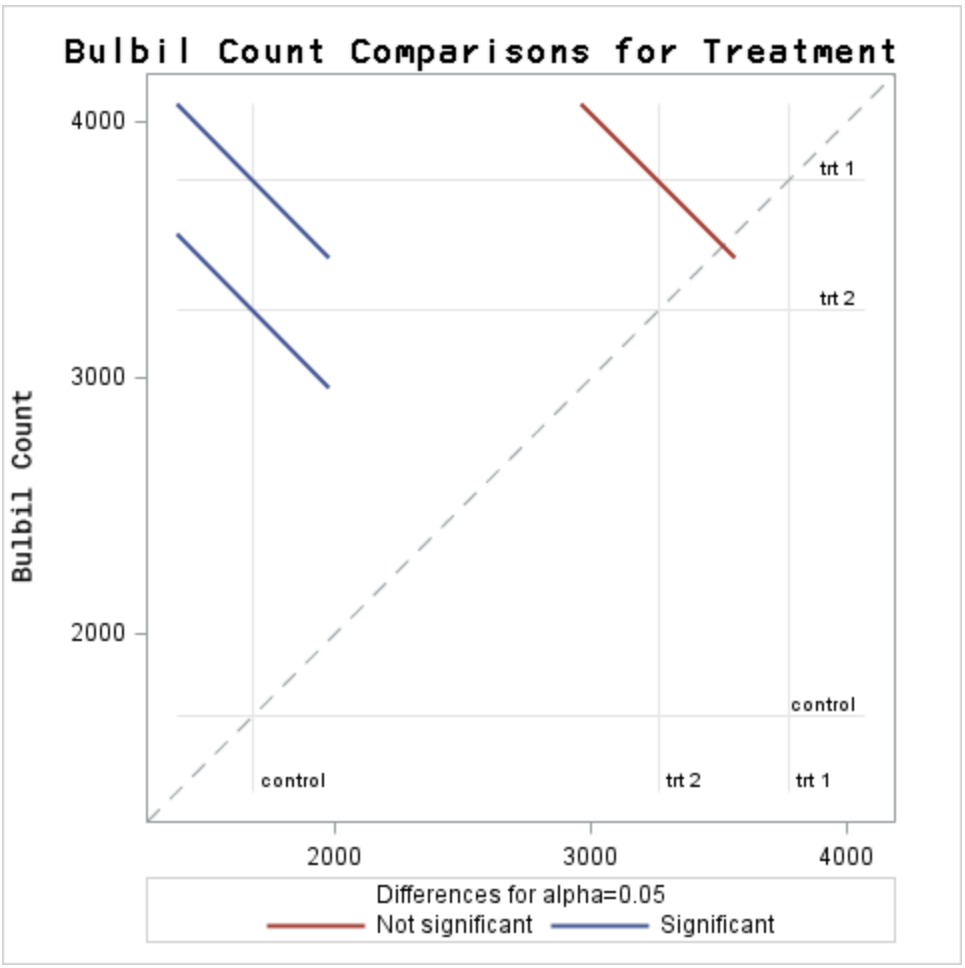

Figure 2.11. Diffogram of LS-means; comparison of means of total bulbils produced per plant by treatment groups. Horizontal and vertical axes contain the range of means. Means of treatment groups plotted on both axes, the intersections of vertical and horizontal lines represent the value of the difference in means of two groups. Dotted line represents the difference of mean = 0. Colored lines represent the 95% CI of the difference of means.

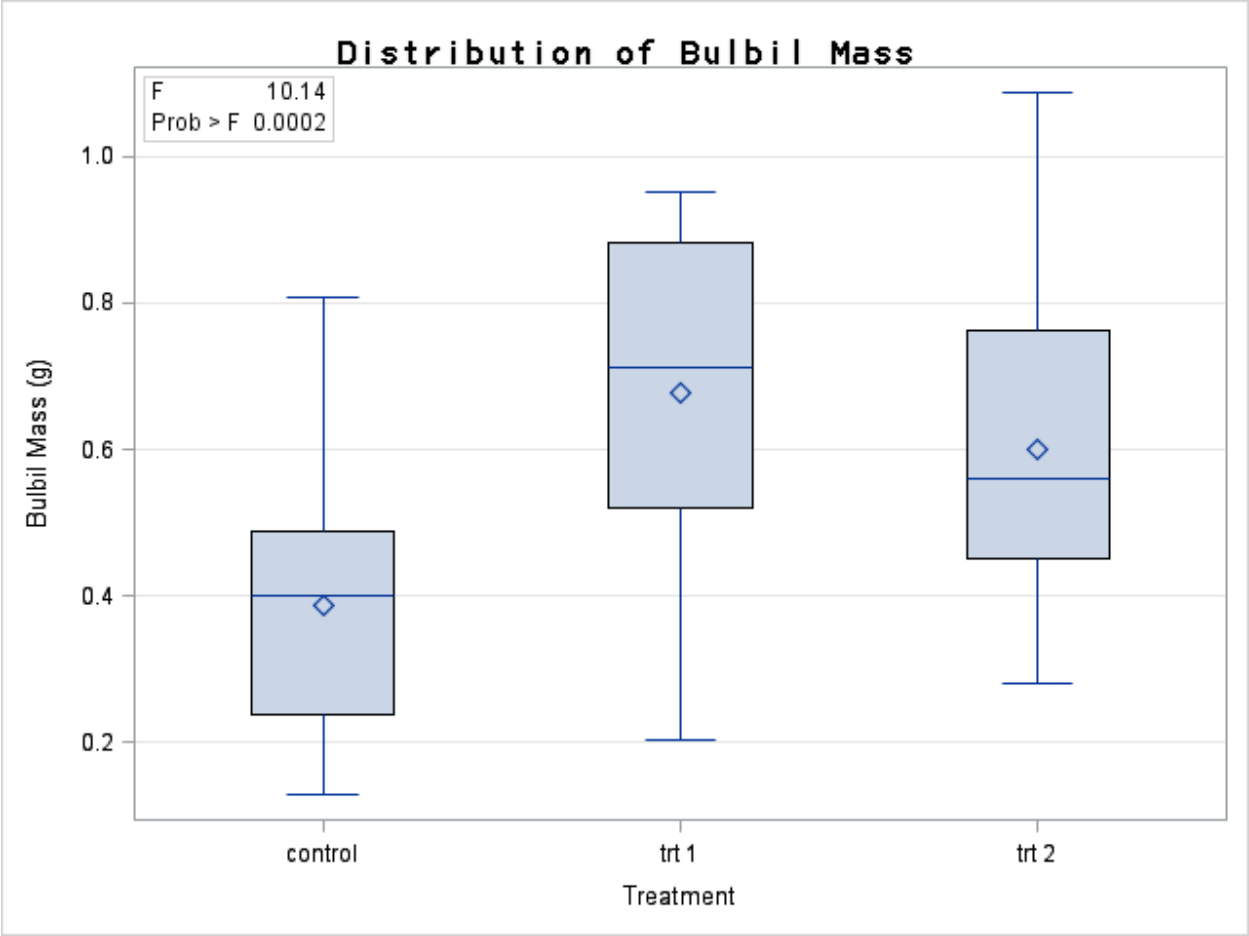

Figure 2.12. Box plots of the distribution of total bulbil biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Total bulbil biomass per plant from 20 plants sampled in each treatment group. ANOVA F-ratio = 10.14, p = 0.0002, α = 0.05. See caption on Figure 2.10 for details on box plot.

0.0023); there was no significant difference between treatment one and treatment two ($p = 0.2505$, Figure 2.13).

Average Individual Bulbil Biomass

The mean individual bulbil biomass of plants with floral buds removed (trt 1) was $1.776 \times 10^{-4} \pm 8.909 \times 10^{-6}$ g (95% CI). The mean individual bulbil biomass of plants with floral buds and shoot apical meristems removed (trt 2) was $1.816 \times 10^{-4} \pm 7.955 \times 10^{-6}$ g. The mean individual bulbil biomass of the control plants was $2.230 \times 10^{-4} \pm 2.092 \times 10^{-5}$ g. ANOVA analysis indicated that at least one group was significantly different from others ($p < 0.0001$, Figure 2.14). The least squares means analysis shows a significant difference between the control and treatment one ($p < 0.0001$) and a significant difference between the control and treatment two ($p < 0.0001$); there was no significant difference between treatment one and treatment two ($p = 0.6776$, Figure 2.15).

Stem Biomass

The mean total stem biomass of plants with floral buds removed (trt 1) was 0.2418 ± 0.0357 g (95% CI). The mean total stem biomass of plants with floral buds and shoot apical meristems removed (trt 2) was 0.2171 ± 0.0404 g. The mean total stem biomass of the control plants was 0.2045 ± 0.0453 g. There was not a significant difference between the three groups from the ANOVA analysis ($p = 0.3922$, Figure 2.16).

Ratio of Bulbil Count to Stem Biomass

The mean ratio of number of bulbils to stem biomass of plants with floral buds removed (trt 1) was 15883.02 ± 1500.06 bulbils g^{-1} (95% CI). The mean ratio of number of bulbils to stem biomass of plants with floral buds and shoot apical meristems removed (trt 2) was 15666.10 ± 1072.97 bulbils g^{-1}. The mean ratio of number of bulbils to stem biomass of the control plants was 10070.05 ± 3618.68 bulbils g^{-1}. ANOVA analysis indicated that at least one group was significantly different from others ($p = 0.0005$, Figure 2.17). The least squares means analysis shows a significant difference between the control and treatment one ($p = 0.0005$) and a significant difference between the control and treatment

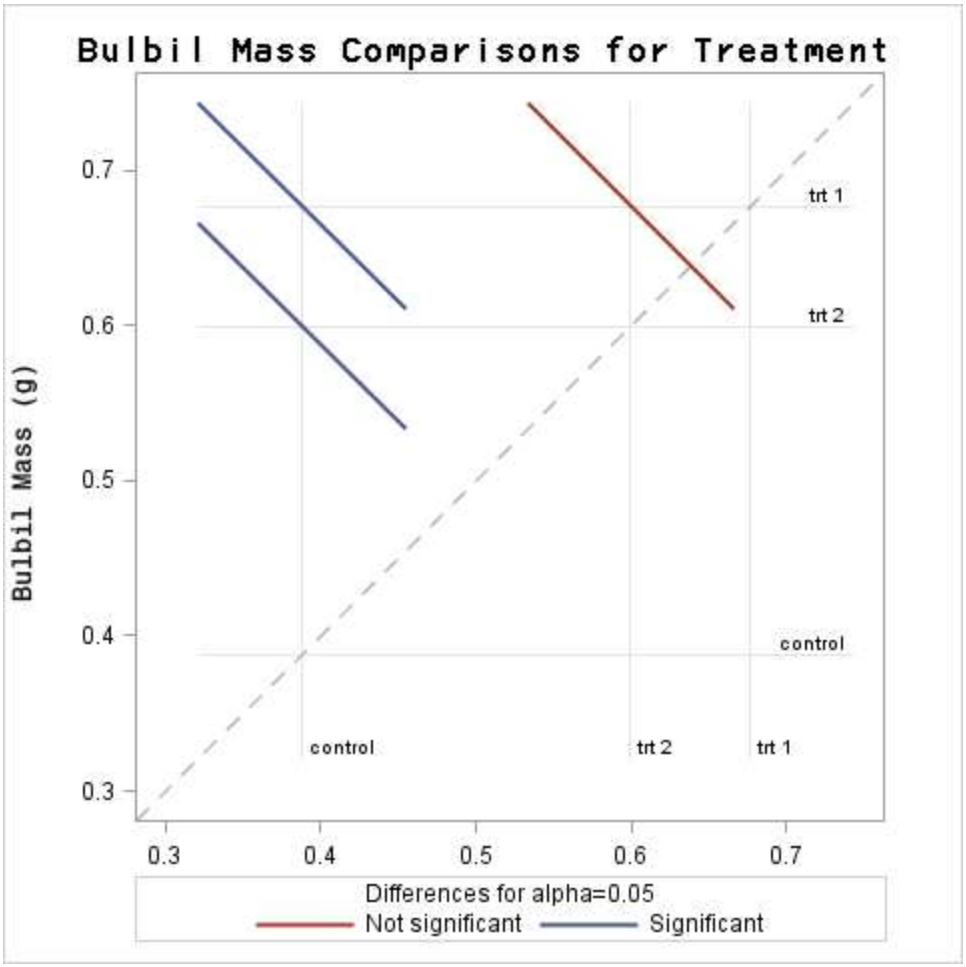

Figure 2.13. Diffogram of LS-means; comparison of means of total bulbil biomass per plant by treatment groups. See caption on Figure 2.11 for details on diffogram.

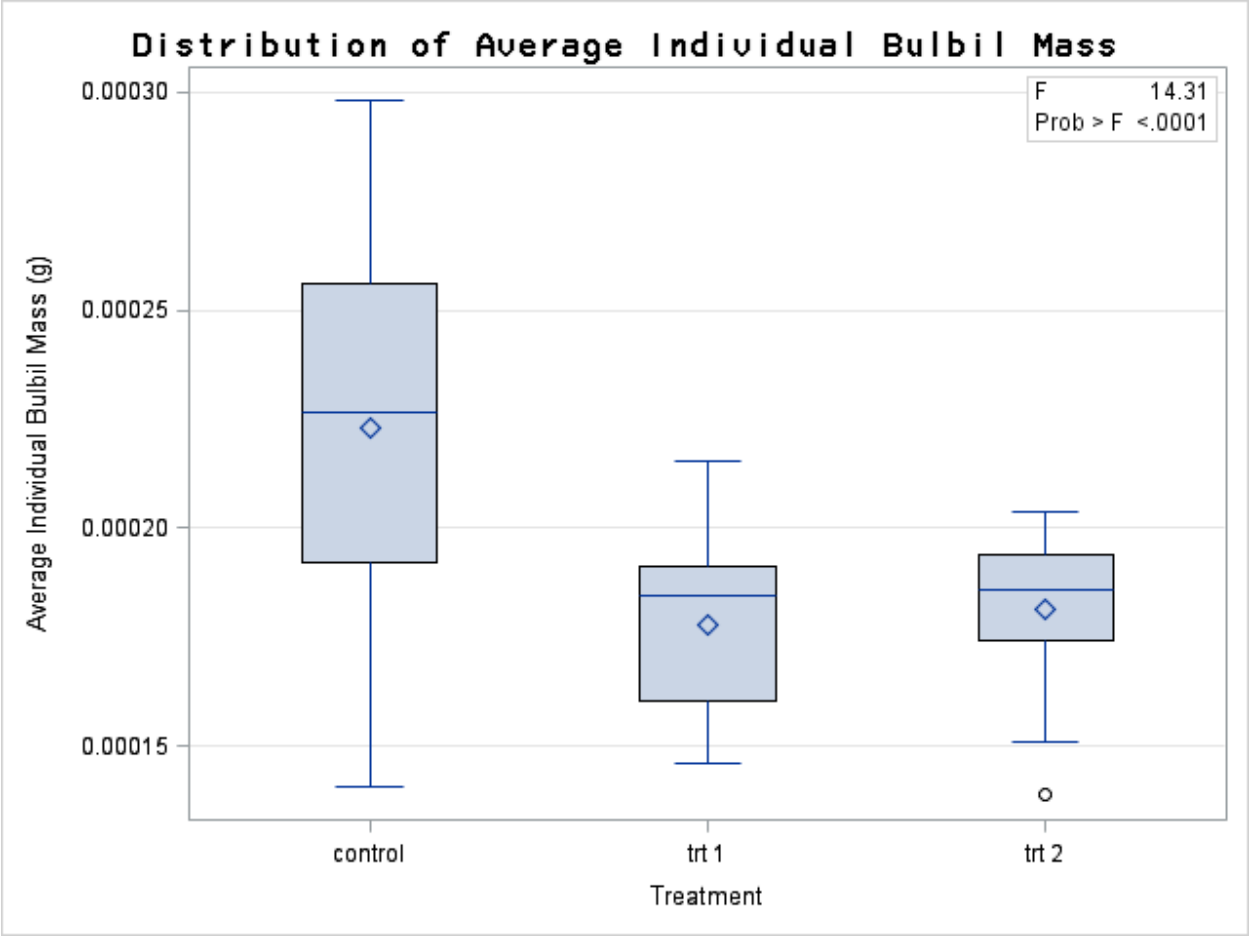

Figure 2.14. Box plots of the distribution of average individual bulbil biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Average individual bulbil biomass per plant from 20 plants sampled in each treatment group. ANOVA F-ratio = 14.31, $p < 0.0001$, $\alpha = 0.05$. See caption on Figure 2.10 for details on box plot.

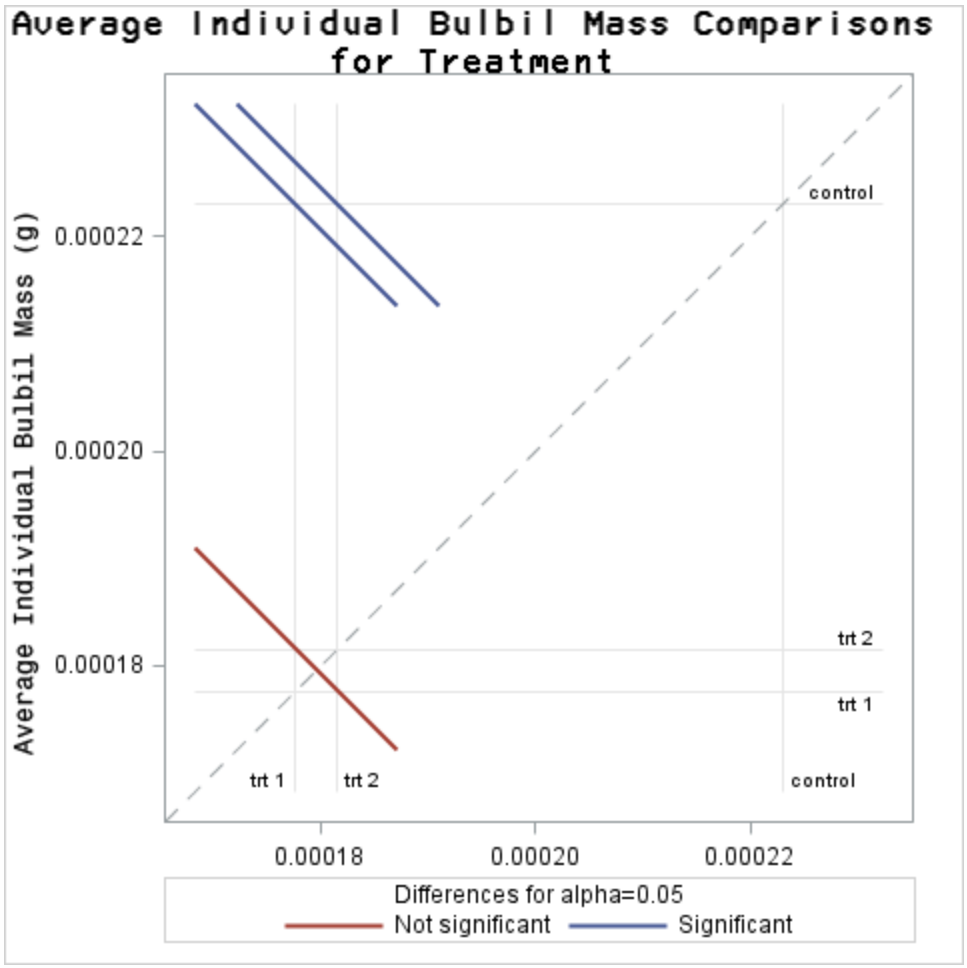

Figure 2.15. Diffogram of LS-means; comparison of means of average individual bulbil biomass per plant by treatment groups. See caption on Figure 2.11 for details on diffogram.

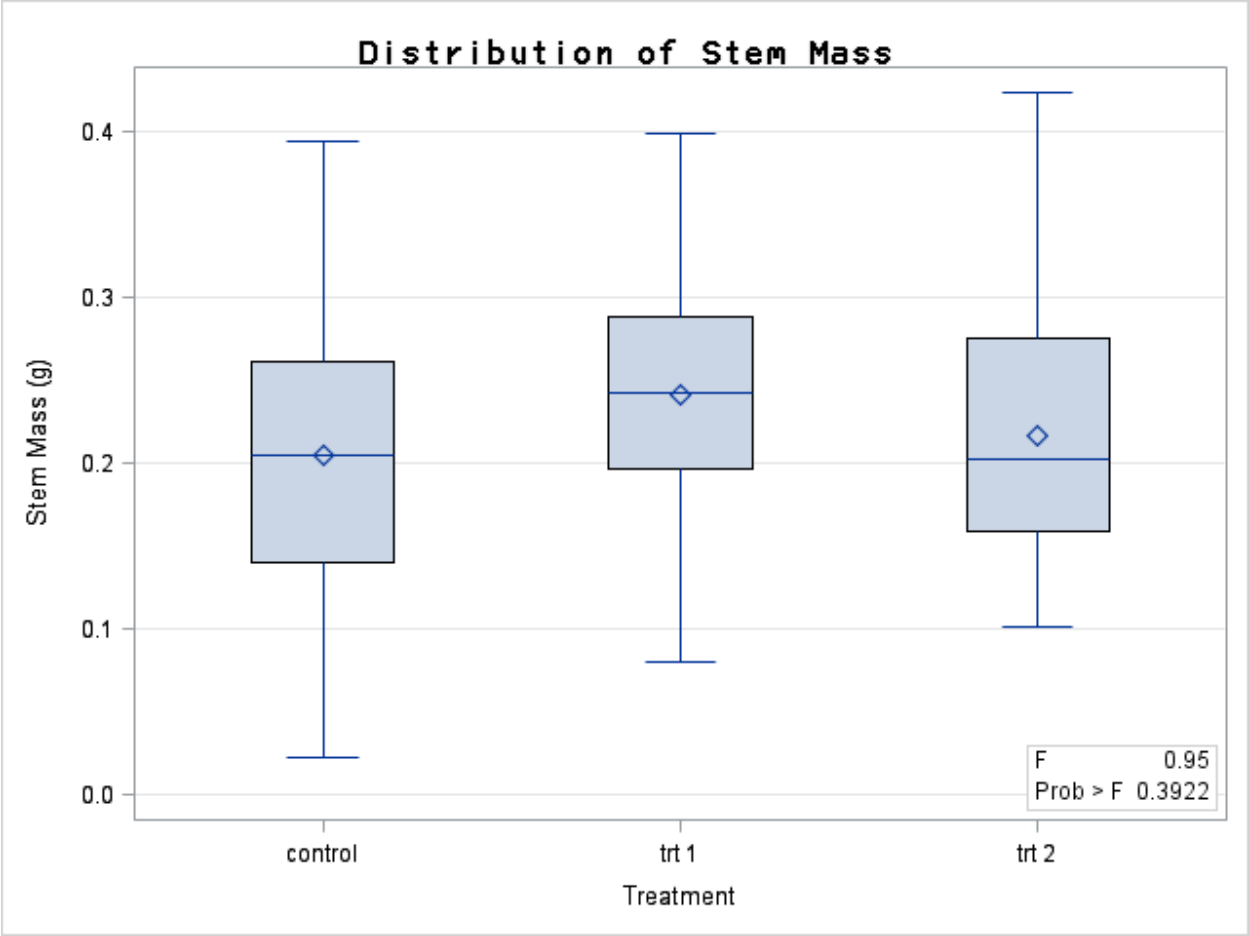

Figure 2.16. Box plots of the distribution of total stem biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Stem biomass from 20 plants sampled in each treatment group. ANOVA F-ratio = 0.95, p = 0.3922, α = 0.05. See caption on Figure 2.10 for details on box plot.

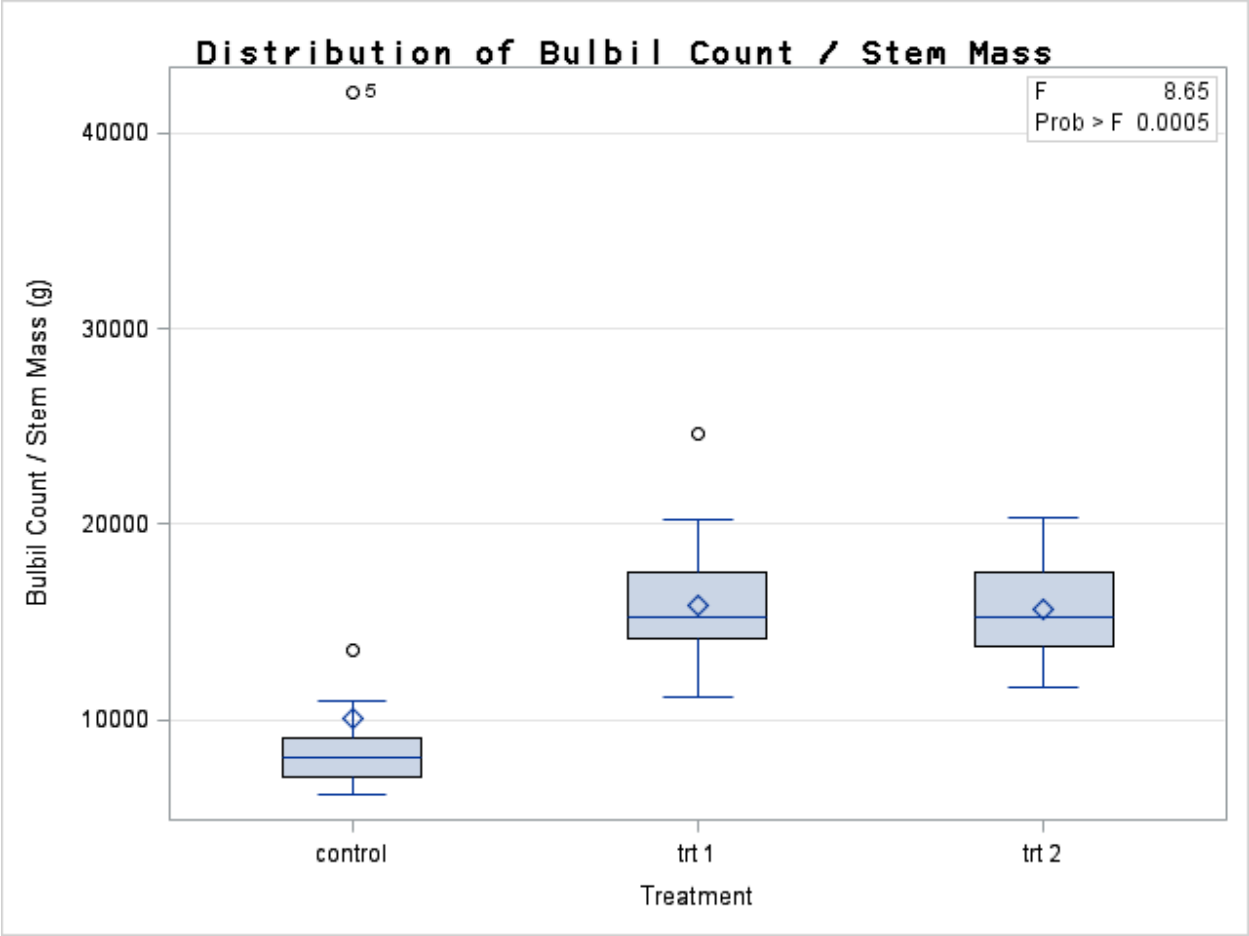

Figure 2.17. Box plots of the distribution of ratio of total bulbils produced to stem biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Ratio of total bulbils produced to stem biomass per plant from 20 plants sampled in each treatment group. ANOVA F-ratio = 8.65, p = 0.0005, α = 0.05. See caption on Figure 2.10 for details on box plot. Circle symbols represent outliers.

two ($p = 0.0008$); there was no significant difference between treatment one and treatment two ($p = 0.8916$, Figure 2.18).

A natural log transformation was used on the ratio of number of bulbils to stem biomass to normalize the data. The mean natural log transformed ratio of number of bulbils to stem biomass of plants with floral buds removed (trt 1) was 9.6548 ± 0.0908 (95% CI). The mean natural log transformed ratio of number of bulbils to stem biomass of plants with floral buds and shoot apical meristems removed (trt 2) was 9.6492 ± 0.0681. The mean natural log transformed ratio of number of bulbils to stem biomass of the control plants was 9.0972 ± 0.1920. ANOVA analysis indicated that at least one group was significantly different from others ($p < 0.0001$, Figure 2.19). The least squares means analysis shows a significant difference between the control and treatment one ($p < 0.0001$) and a significant difference between the control and treatment two ($p < 0.0001$); there was no significant difference between treatment one and treatment two ($p = 0.9491$, Figure 2.20).

Ratio of Bulbil Biomass to Stem Biomass

The mean ratio of total bulbil biomass to stem biomass of plants with floral buds removed (trt 1) was 2.8041 ± 0.2440 (95% CI). The mean ratio of bulbil biomass to stem biomass of plants with floral buds and shoot apical meristems removed (trt 2) was 2.8257 ± 0.1690. The mean ratio of bulbil biomass to stem biomass of the control plants was 2.0538 ± 0.4347. ANOVA analysis indicated that at least one group was significantly different from others ($p = 0.0004$, Figure 2.21). The least squares means analysis shows a significant difference between the control and treatment one ($p = 0.0006$) and a significant difference between the control and treatment two ($p = 0.0004$); there was no significant difference between treatment one and treatment two ($p = 0.9166$, Figure 2.22).

A natural log transformation was also used for the ratio of bulbil biomass to stem biomass to normalize the skewed data. The mean natural log transformed ratio of bulbil biomass to stem biomass of plants with floral buds removed (trt 1) was 1.0134 ± 0.0925 (95% CI). The mean natural log transformed ratio of bulbil biomass to stem biomass of plants with floral buds and shoot apical

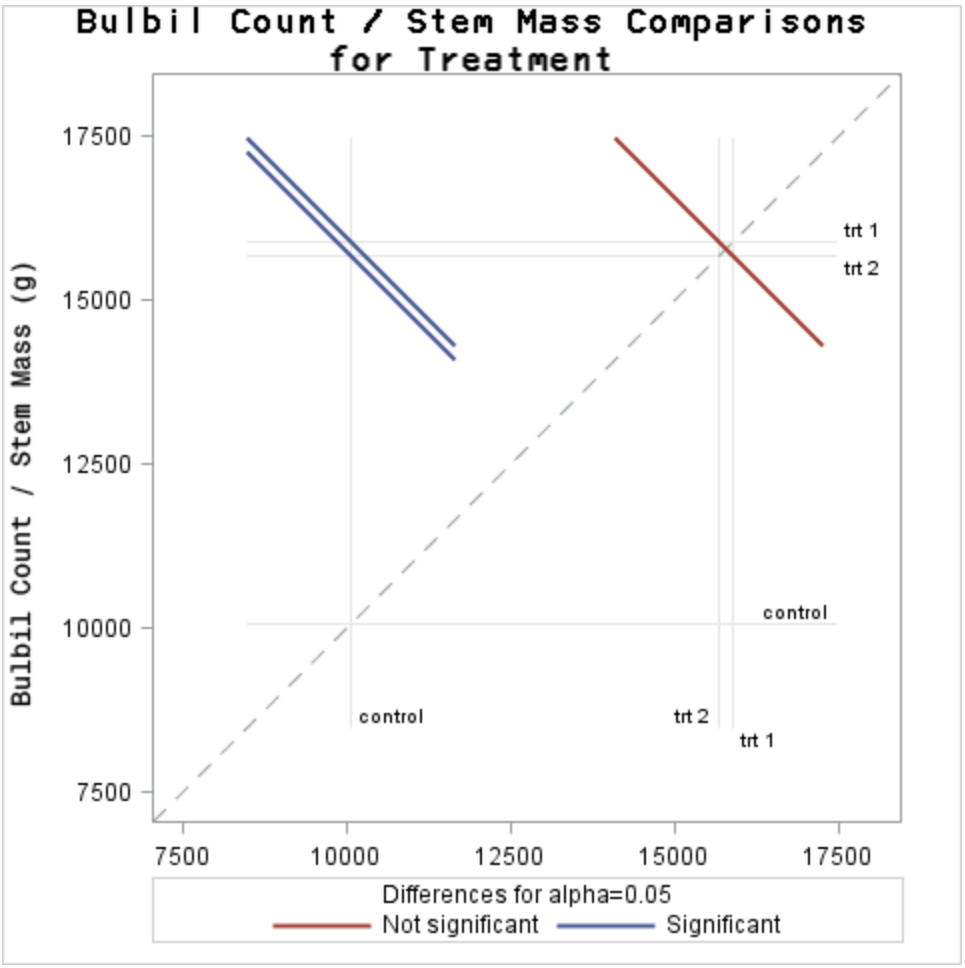

Figure 2.18. Diffogram of LS-means; comparison of means of ratio of total bulbils produced to stem biomass per plant by treatment groups. See caption on Figure 2.11 for details on diffogram.

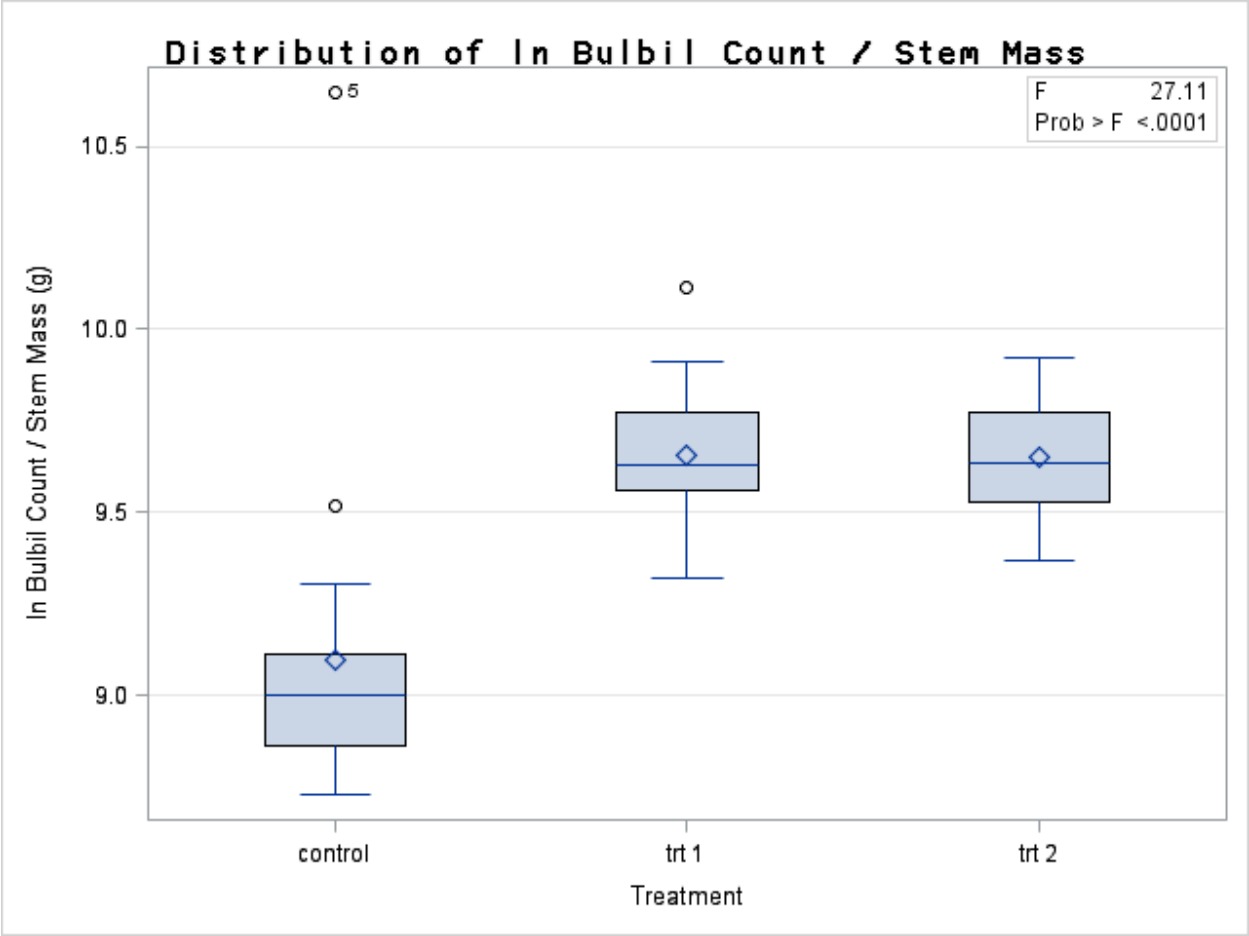

Figure 2.19. Box plots of the distribution of natural log transformed ratio of total bulbils produced to stem biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Natural log transformed ratio of total bulbils produced to stem biomass per plant from 20 plants sampled in each treatment group. ANOVA F-ratio = 27.11, $p < 0.0001$, $\alpha = 0.05$. See caption on Figure 2.10 for details on box plot. Circle symbols represent outliers.

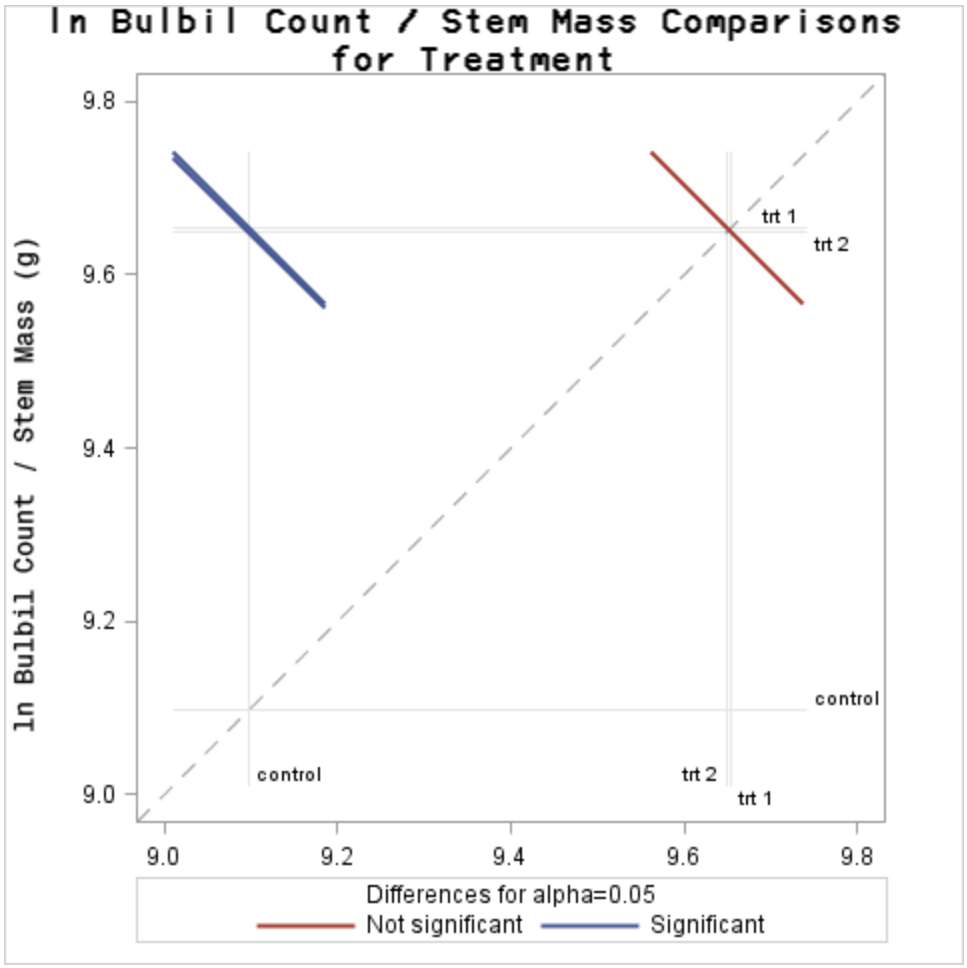

Figure 2.20. Diffogram of LS-means; comparison of means of natural log transformed ratio of total bulbils produced to stem biomass per plant by treatment groups. See caption on Figure 2.11 for details on diffogram.

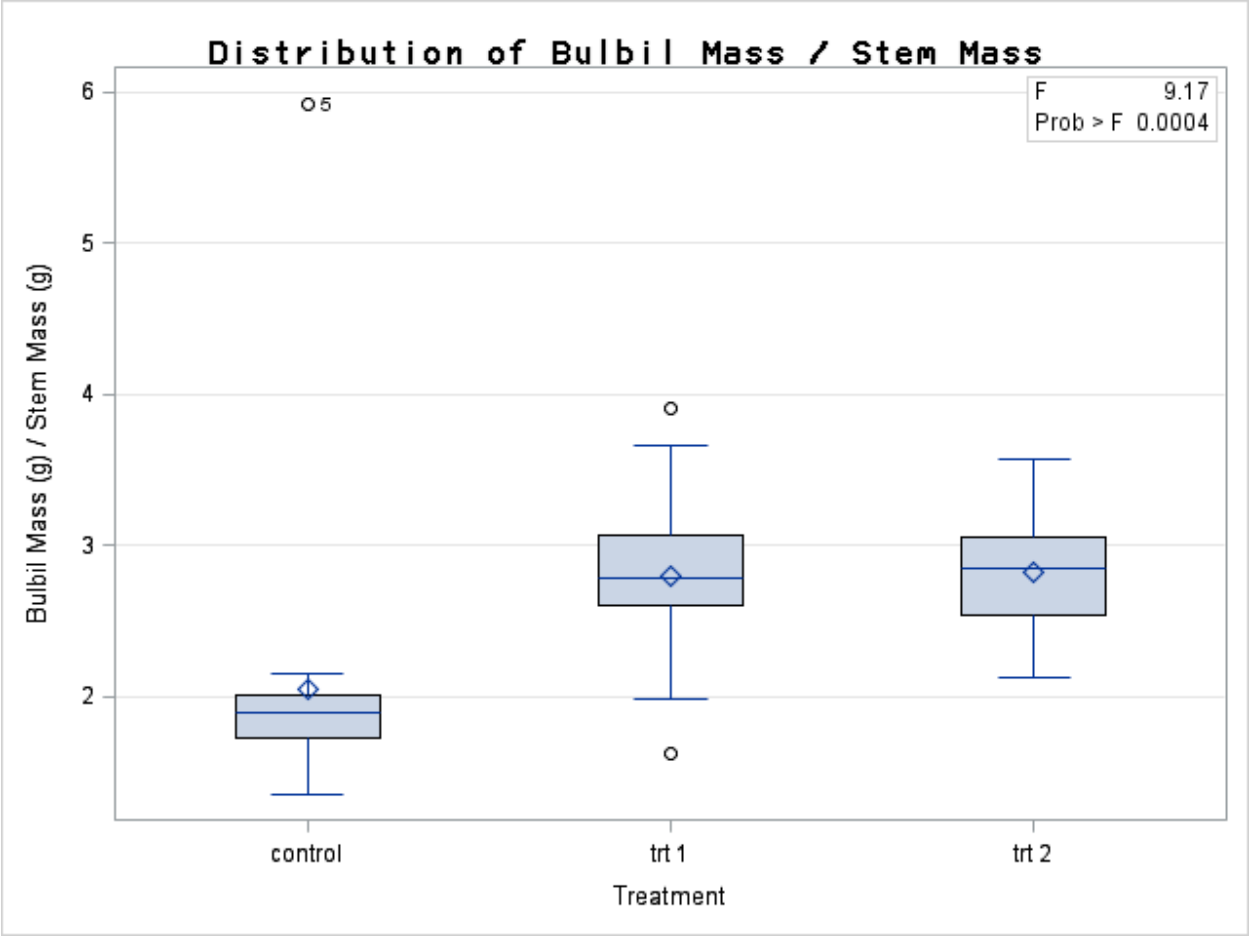

Figure 2.21. Box plots of the distribution of ratio of total bulbil biomass to stem biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Ratio of total bulbil biomass to stem biomass per plant from 20 plants sampled in each treatment group. ANOVA F-ratio = 9.17, p = 0.0004, α = 0.05. See caption on Figure 2.10 for details on box plot. Circle symbols represent outliers.

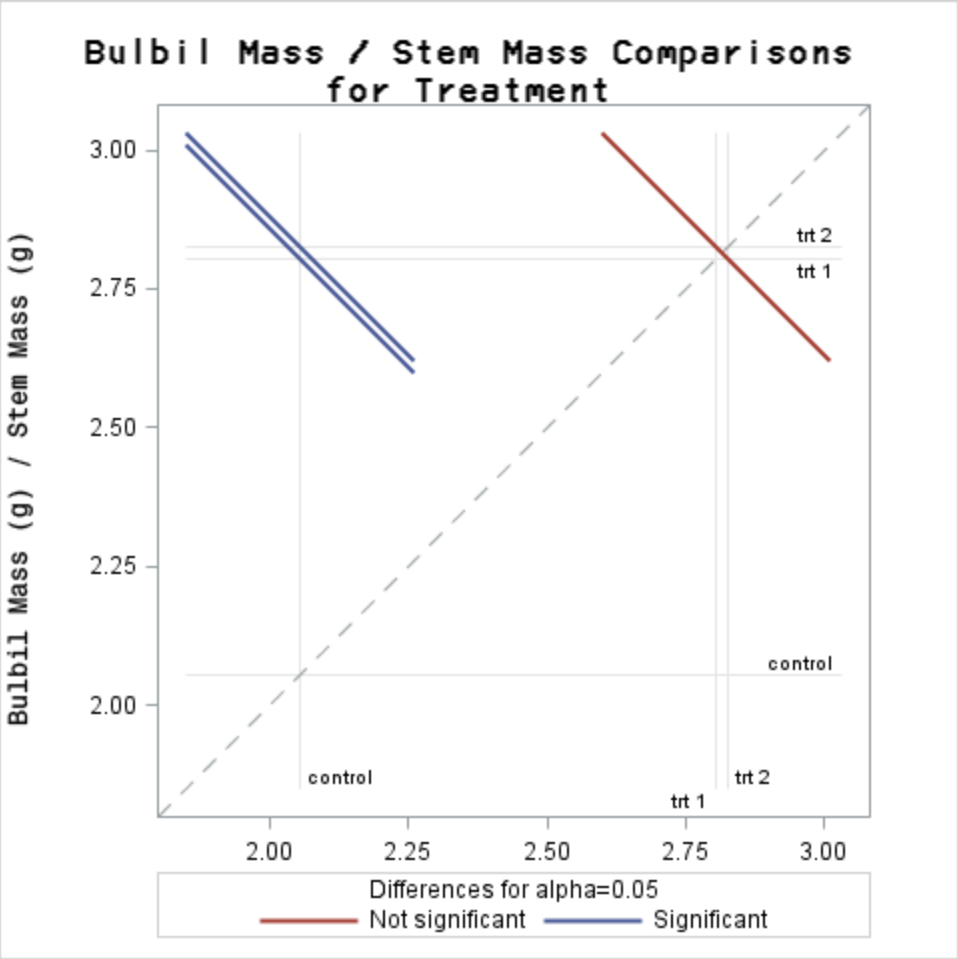

Figure 2.22. Diffogram of LS-means; comparison of means of ratio of total bulbil biomass to stem biomass per plant by treatment groups. See caption on Figure 2.11 for details on diffogram.

meristems removed (trt 2) was 1.0308 ± 0.0612. The mean natural log transformed ratio of bulbil biomass to stem biomass of the control plants was 0.6683 ± 0.1320. ANOVA analysis indicated that at least one group was significantly different from others ($p < 0.0001$, Figure 2.23). The least squares means analysis shows a significant difference between the control and treatment one ($p < 0.0001$) and a significant difference between the control and treatment two ($p < 0.0001$); there was no significant difference between treatment one and treatment two ($p = 0.7973$, Figure 2.24).

Discussion

Bulbil Count, Total Bulbil Biomass per Plant, and Stem Biomass

Significantly more bulbils were produced when *M. gemmiparus* was given one of the treatments, either removal of floral buds (treatment one), or removal of both floral buds and shoot apical meristems (treatment two), compared to the control group (Figures 2.10, 2.11). Solely removing the floral buds or removing the floral buds with the shoot apical meristems resulted in more nodes being formed, with approximately twice as many propagules compared to the control group.

Similarly, both treatments one and two resulted in greater total bulbil biomass per plant when compared to the control group, with no significant difference in bulbil biomass per plant between treatment one and treatment two (Figures 2.12, 2.13). Plants in both treatment groups appeared to shift their allocation of resources to vegetative growth, with the production of new metamers and bulbils and increasing allocation to bulbils when floral buds were removed. The induction of branching by the removal of shoot apical meristems in treatment two did not significantly affect total bulbil biomass when compared to treatment one. Branch formation was observed to be common among all plants in the experiment, including controls, and may have been caused by high nutrient, water, and sunlight availability within the greenhouse. The removal of the shoot apical meristems in treatment two may not have caused an observable effect on branch formation in a greenhouse setting.

Plants grown in the greenhouse showed variable growth among plants within treatment groups. Plants grew to different heights and widths and had different metamer densities (Figure 2.25), and this variability can be seen in the range of overlap between groups (Figures 2.10, 2.12). The data collected

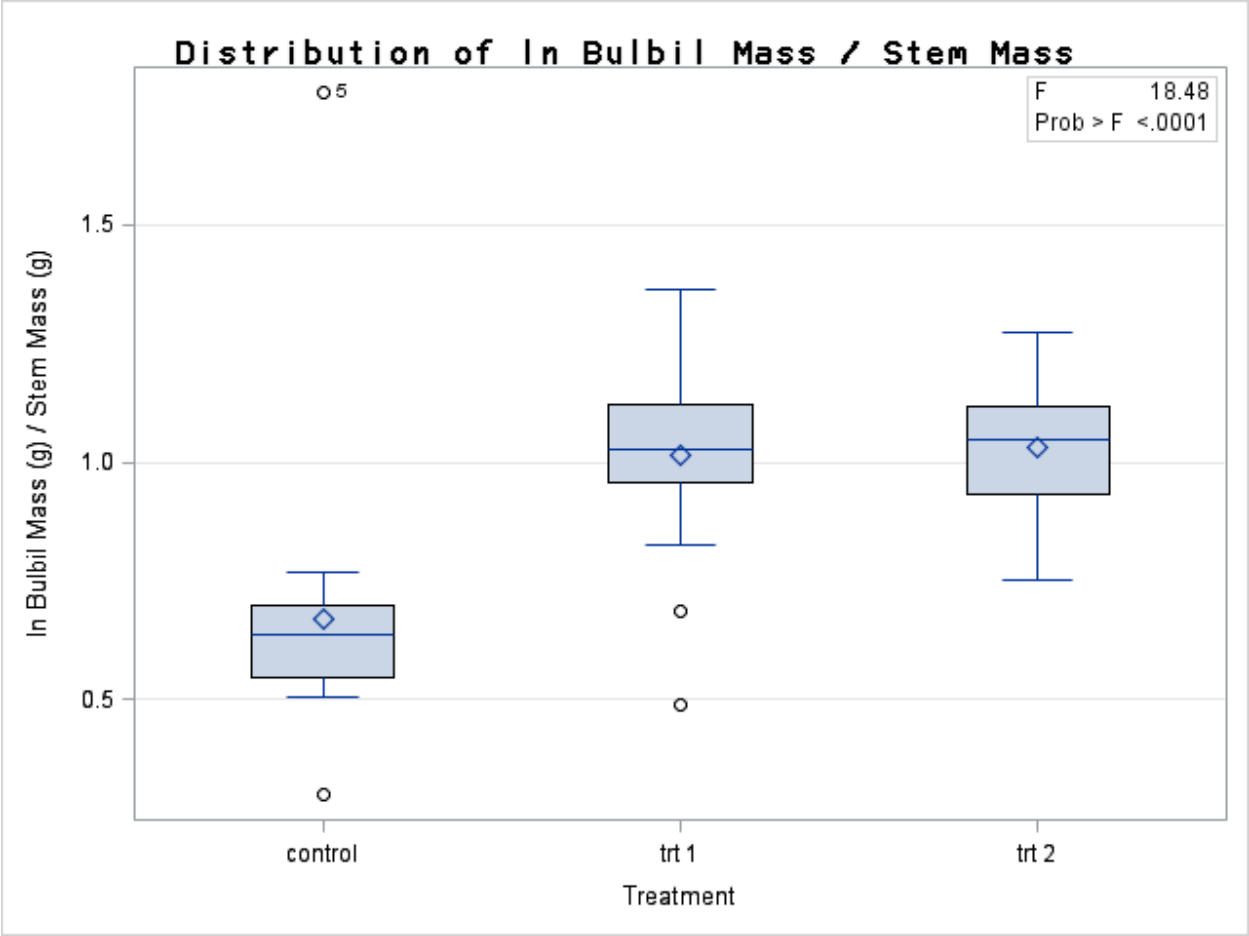

Figure 2.23. Box plots of the distribution of natural log transformed ratio of total bulbil biomass to stem biomass per plant of treatment groups: control, trt 1 (removal of floral buds), and trt 2 (removal of floral buds and shoot apical meristems). Natural log transformed ratio of total bulbil biomass to stem biomass per plant from 20 plants sampled in each treatment group. ANOVA F-ratio = 18.48, $p < 0.0001$, $\alpha = 0.05$. See caption on Figure 2.10 for details on box plot. Circle symbols represent outliers.

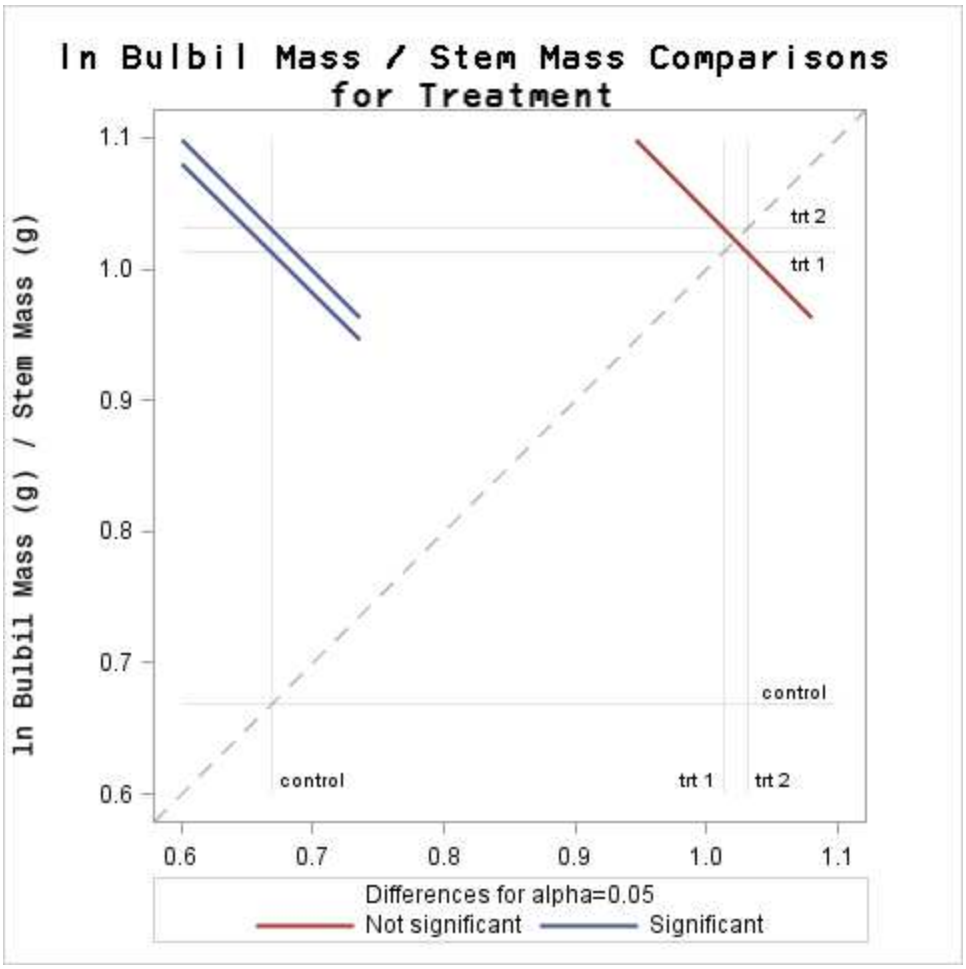

Figure 2.24. Diffogram of LS-means; comparison of means of natural log transformed ratio of total bulbil biomass to stem biomass per plant by treatment groups. See caption on Figure 2.11 for details on diffogram.

Figure 2.25. Four plants from the same lineage and from treatment two (removal of both floral buds and shoot apical meristems) displaying variation in size and internodal elongation.

for number of bulbils per plant and total bulbil biomass per plant show a significant difference between the control and both treatment one and treatment two; however an analysis of either only total bulbil count per plant or total bulbil biomass per plant does not sufficiently show whether resource allocation has shifted to produce more or larger bulbils unless the size of the plant is accounted for by including stem biomass in the analysis (Figure 2.1).

There were no significant differences between groups for total stem mass per plant (Figure 2.16). Neither treatment one (removal of floral buds) nor treatment two (removal of floral buds and shoot apical meristems) increased or decreased the total stem biomass when compared to the control group. This result suggests that manipulation of resource allocation by removal of floral buds, a strong resource sink, did not have an effect on stem growth in a greenhouse environment where growth conditions are favorable and resources are not limited. Similarly, removing shoot apical meristems to induce branching, another resource sink, did not appear to alter total stem biomass. From the data gathered, it appears the environment, rather than resource allocation, may have a stronger effect on total stem growth. Plants growing in favorable conditions, such as the greenhouse with high resource availability, would continue to accumulate stem biomass until senescence at the end of the growing season.

Average Individual Bulbil Biomass

In both treatments one and two more bulbils were produced and resources were reallocated to bulbil production; however, the average biomass of an individual bulbil was less than those in the control group (Figures 2.14, 2.15). Individual bulbils produced by the control group had greater average mass and thus potentially greater survivability. These results show there is a trade-off between producing more bulbils, and the size and presumed quality of those bulbils. Beardsley (1997) showed that germination rate is correlated with bulbil size and that smaller bulbils have a lower rate of successful germination. Although the bulbils produced with an experimental treatment are smaller, there is a greater quantity of bulbils and the decreased chance of germination may be counterbalanced by having significantly more bulbils produced per plant.

Ratio of Bulbil Count to Stem Biomass

Examination of the ratio of bulbil count to stem biomass shows both treatments had an increased number of bulbils formed per gram of stem biomass in comparison to the control group (Figures 2.17-2.20). The number of bulbils produced is standardized by the size of the plant, which is represented by the biomass of the stem tissue. The treatments did not cause the plants to increase stem biomass along with increasing bulbil production, but instead the plants produced more nodes per gram of stem, thereby producing more bulbils. The ratios indicate that plants in both treatments one and two produced more nodes close together along the stem in comparison to the control group, resulting in greater numbers of bulbils (Figures 2.26-2.28).

The growth pattern suggests a shift in reproductive allocation for plants that have undergone treatment one or treatment two. Growth conditions for *M. gemmiparus* were assumed to be favorable and plants grown in the greenhouse would be expected to allocate resources to branch production to increase the total amount of available meristems for later use in reproduction, as if it was a sexually reproducing plant, by producing flowers and increasing fecundity (Smith, 1984; Geber, 1990). However, *M. gemmiparus* does not reproduce sexually and the flowers that are formed do not increase fecundity. Plants in the control group were observed to follow this pattern by having quiescent meristems in the lower nodes sprout into branches and then form flowers later into the growing season; secondary branching and formation of adventitious roots to support a large plant were commonly observed. Plants that were given treatment one or treatment two also produced branches at the lower nodes that increased the number of available meristems, however, the floral buds were removed as they were initiated and the plants produced a greater number of nodes and thereby more available meristems and bulbils. The removal of resource sinks associated with sexual reproduction (flowers - in this case, non-functional), resulted in enhanced asexual reproduction.

Ratio of Bulbil Biomass to Stem Biomass

Both treatment groups showed a greater ratio of total bulbil biomass to stem biomass when compared to the control group (Figures 2.21-2.24). The inclusion of the stem biomass addresses the

Figure 2.26. Plant from the control group with large internodal regions.

Figure 2.27. Plant from treatment one group with short internodal regions.

Figure 2.28. Plant from treatment two group with short internodal regions, similar to plants in treatment one.

differences in plant size for an analysis of the partitioning of biomass in a plant. Plants with floral buds removed in treatment one and treatment two had a higher ratio of total bulbil biomass per gram of stem biomass; resources seemingly were shifted to production of bulbils and their storage leaves instead of maintaining floral structures. Removal of the non-functional sexual reproductive structures resulted in increased vegetative growth and production of bulbils, and thus fecundity. Some other *Mimulus* species, although they do not form bulbils, do reproduce asexually by the formation of rooted branches; sexual and asexual reproduction are negatively correlated where investment into floral structures inhibits the production of rooted branches (Sutherland and Vickery, 1988). In bulbil producing plants, such as *Agave macroacantha*, there is also a negative correlation between investment of resources to flowers and the production of bulbuls (Arizaga and Ezcurra, 1995). Similarly, *M. gemmiparus* shows the same relationship between production of floral structures and bulbils.

The life history of a plant is determined by its genotype, which dictates how it will grow and reproduce; the response to the environment produces variations in the development of the plant (Harper and Ogden, 1970). In any environment a plant may grow in, it would be limited by resource availability and the time constraint of its lifespan; resources acquired from the environment in that time are allocated or redistributed amongst competing processes within the plant (Harper and Ogden, 1970). *Mimulus gemmiparus* rarely flowers or forms branches in its natural environment, presumably due to limited resource availability or suboptimal conditions; resources are used in the growth of the plant to form bulbils as its reproductive strategy. In the greenhouse, plants presumably are not resource-limited; they are, however, constrained by their genotype. Plants grown in the greenhouse form flowers in response to a favorable environment, allowing resources to be allocated for the change in reproductive strategy due to their genotype even though the formation of flowers has no benefit to the plant. Removal of floral buds in treatment one and treatment two prevented further allocation of resources to non-functional sexual reproduction and shifted resources to growth and bulbil production. Thus, resource allocation in *M. gemmiparus* can seemingly be manipulated to increase bulbil production in a greenhouse setting.

Experiment 2: Removal of Shoot Apical Meristem in a Low-Light Environment

Introduction

Plants grown in the greenhouse for the first experiment showed pronounced growth and while conditions were assumed to be favorable, they were not the conditions found in natural environments. It seems likely that when plants remain smaller as they do in natural settings, the relative effect of meristem removal to induce branching and increase bulbil production will be more pronounced. When grown in a low light condition in the laboratory, *M. gemmiparus* grows similarly in form to the plants found in their natural habitats. The goal of this experiment is to determine whether removal of the shoot apical meristem induces branching in *M. gemmiparus* under such conditions. Removal of the shoot apical meristem may induce early branching, and possibly increase the number of shoot axes and thereby increase the number of bulbils. Data collected from the experiment includes the number of bulbils produced per plant, stem biomass, and the number of primary and secondary branches per plant. Change in the number of bulbils formed is indicative of a treatment effect on bulbil production. Change in the number of branches formed would indicate a treatment effect by breaking apical dominance and inducing branching. Similar to the first experiment, the number of bulbils to stem biomass ratio should provide information on the growth form of the plant, i.e., whether there are more nodes produced per unit mass of stem and more bulbils produced or if there is increased elongation of stems with few nodes (Figure 2.1). The number of bulbils to total stem axes ratio should show whether induction of branching increases bulbil production.

Materials and Methods

Twenty random propagules from the stock bulbils used in experiment one were grown in reduced light conditions more similar to those of the natural populations. Plants were grown in a tray with approximately two inch spacing between plants and received 16 hours of light each day at room temperature. Agrobrite High Output T5 24W 6400K were placed approximately half a meter above the trays for lighting and plants were grown on Hyponex potting soil. When plants had grown to five nodes their shoot apical meristems were removed along with the fifth node. Twenty random propagules from

the first experiment were also grown under the same conditions and used as a control group for comparison with no parts of the plant removed (Figures 2.29-2.31). Trays were rotated multiple times a week to reduce environmental effects due to their position.

Plants were harvested after approximately eight weeks of growth when the rate of growth declined and senescence began. The number of branches on each plant was counted by hand at the time of harvesting the plants. The number of bulbils produced per plant was counted using the same method as in the first experiment, by using a digital camera to image bulbils spread on a sheet of herbarium paper, editing the image with Photoshop to remove debris, and counting the bulbils with ImageJ 1.49. After the plants air dried at room temperature for a month, stem biomass was measured with a Denver Instrument APX-100z analytical balance. Means between the control and treatment group were compared with two-sample *t*-tests ($\alpha = 0.05$) by SAS 9.3 for the number of bulbils produced per plant, number of branches produced per plant, dry mass of the above-ground stem and branches (g), ratio of number of bulbils to stem biomass (bulbils g^{-1}), and ratio of number of bulbils to total stem axes.

Results

Bulbil Count

The mean number of bulbils produced by plants with the shoot apical meristem removed (trt) was 166.0 ± 44.8 (95% CI). The mean number of bulbils produced by the control plants was 126.7 ± 32.7. The mean number of bulbils produced by the control and treatment group was not statistically different ($p = 0.1464$, Figure 2.32).

Stem Biomass

The mean stem biomass of plants with the shoot apical meristem removed (trt) was 0.00542 ± 0.00143 g (95% CI). The mean stem biomass of the control plants was 0.00412 ± 0.00115 g. The mean stem biomass of the control and treatment group was not statistically different ($p = 0.1440$, Figure 2.33).

Figure 2.29. The layout of experiment with one tray containing control group and the other containing the treatment group.

Figure 2.30. The start of experiment with germinated bulbils.

Figure 2.31. Left: Control plants with no parts removed and at various ages due to variation in germination time. Center: Plant with shoot apical meristem and fifth node removed, axillary buds beginning to branch. Right: Plant with treatment after seven weeks of growth with multiple branches formed.

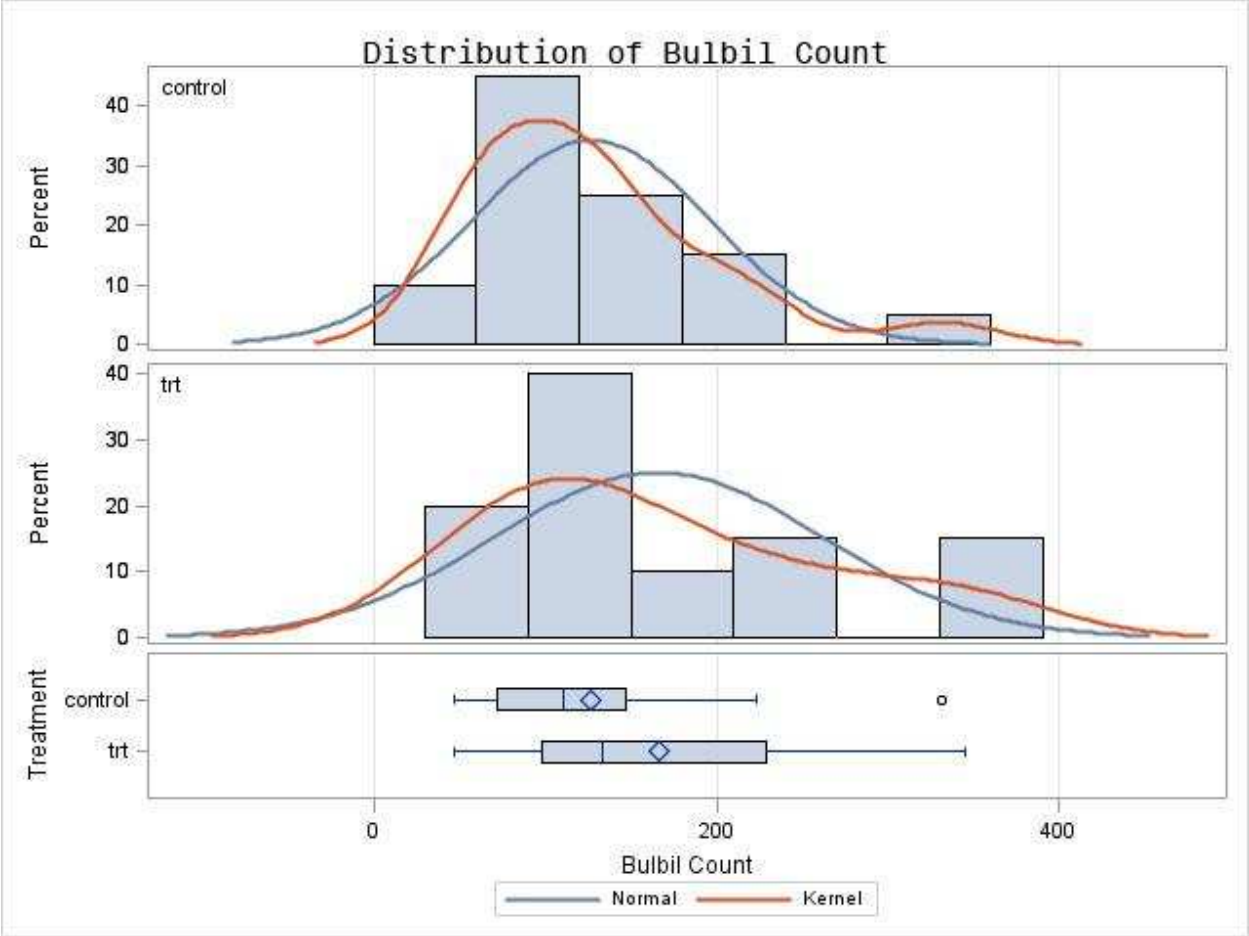

Figure 2.32. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of total bulbils produced per plant between control and treatment group (removal of the shoot apical meristem). Bulbil counts from 20 plants sampled in each group. Boxes represent the first quartile to the third quartile of collected data, and the extended horizontal lines represent the maximum and minimum values of that data that was collected. Vertical bars in the boxes represent the median of the data. Diamond symbols in each box plot represent the mean of the treatment group. Circle symbol represents an outlier.

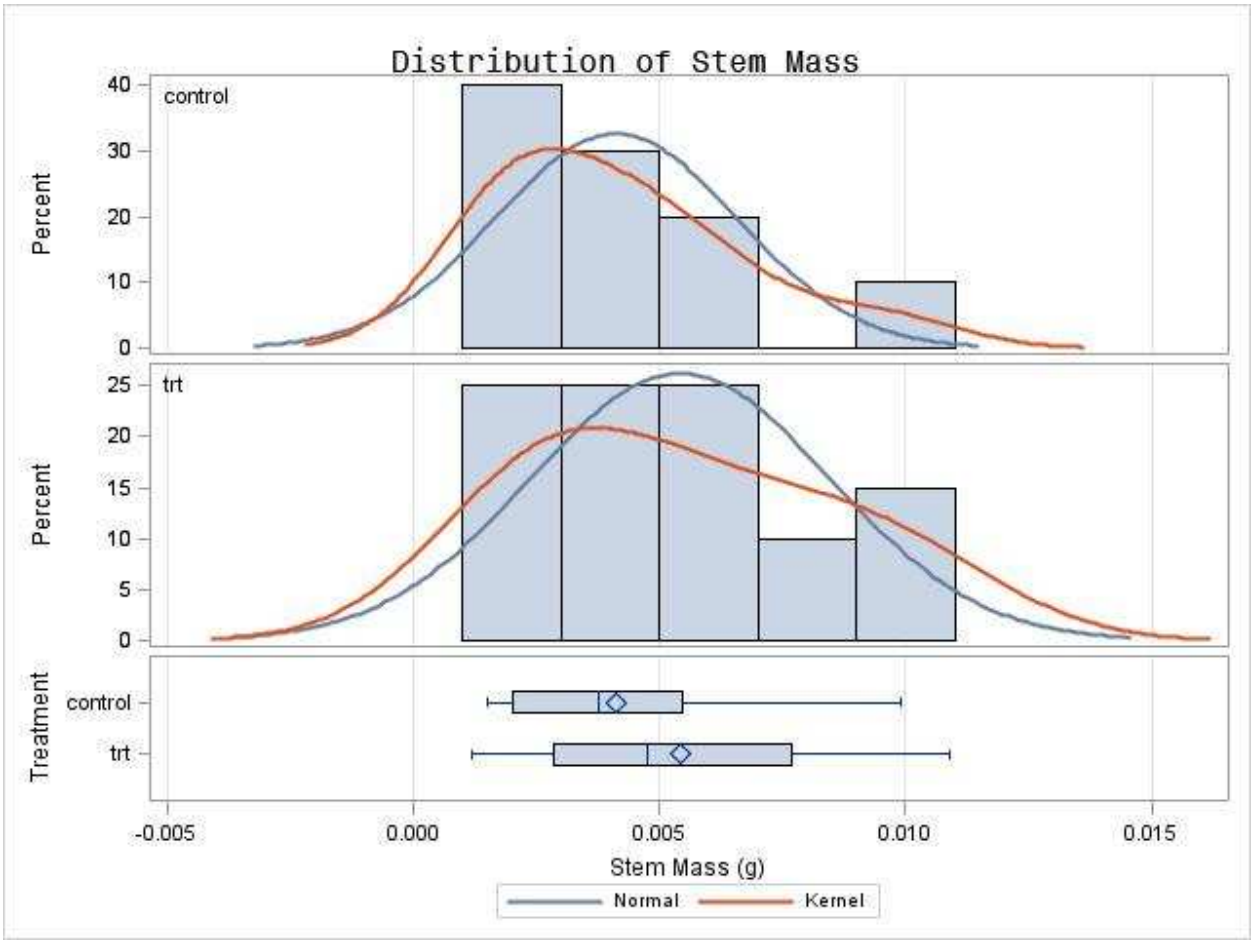

Figure 2.33. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of total stem biomass per plant between control and treatment group (removal of the shoot apical meristem). Stem biomass from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

Ratio of Bulbil Count to Stem Biomass

The mean ratio of number of bulbils to stem biomass of plants with the shoot apical meristem removed (trt) was 31679.7 ± 3046.4 g^{-1} (95% CI). The mean ratio of number of bulbils to stem biomass of the control plants was 32316.5 ± 2444.5. The mean ratio of number of bulbils to stem biomass of the control and treatment group was not statistically different (p = 0.7348, Figure 2.34).

Branch Formation and Total Stem Axes

The mean number of primary branches (shoot axis from axillary buds on the central stem) produced by plants with the shoot apical meristem removed (trt) was 6.9000 ± 0.9224 (95% CI). The mean number of primary branches produced by the control plants was 12.3500 ± 2.6487. The mean number of primary branches produced by the control and treatment group was statistically different (p = 0.0005, Figure 2.35).

The mean number of secondary branches (shoot axis from axillary buds on primary branches) produced by plants with the shoot apical meristem removed (trt) was 6.8000 ± 3.7182 (95% CI). No secondary branches were produced by plants in the control group. The mean number of secondary branches produced by the control and treatment group was statistically different (p = 0.0011, Figure 2.36).

The mean total number of branches produced by plants with the shoot apical meristem removed (trt) was 13.7000 ± 3.9189 (95% CI). The mean total number of branches produced by the control plants was 12.3500 ± 2.6487. The mean number of total branches produced by the control and treatment group was not statistically different (p = 0.5538, Figure 2.37).

The mean total number of stem axes (total number of branches plus one central stem) produced by plants with the shoot apical meristem removed (trt) was 14.7000 ± 3.9189 (95% CI). The mean total number of stem axes produced by the control plants was 13.3500 ± 2.6487. The mean number of total stem axes produced by the control and treatment group was not statistically different (p = 0.5538, Figure 2.38).

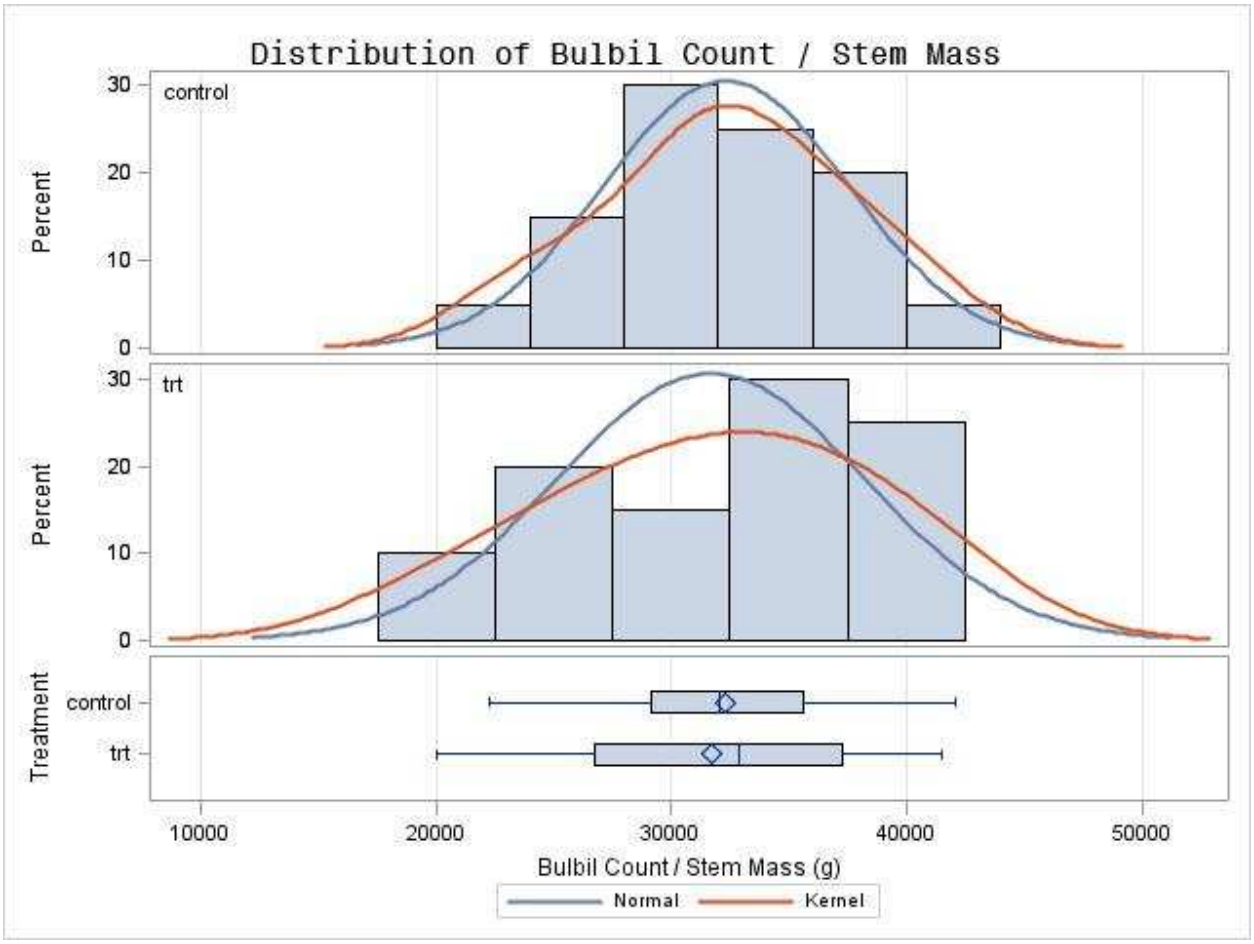

Figure 2.34. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of the ratio of total bulbils produced to total stem biomass per plant between control and treatment group (removal of the shoot apical meristem). Ratio of bulbil count to stem biomass per plant from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

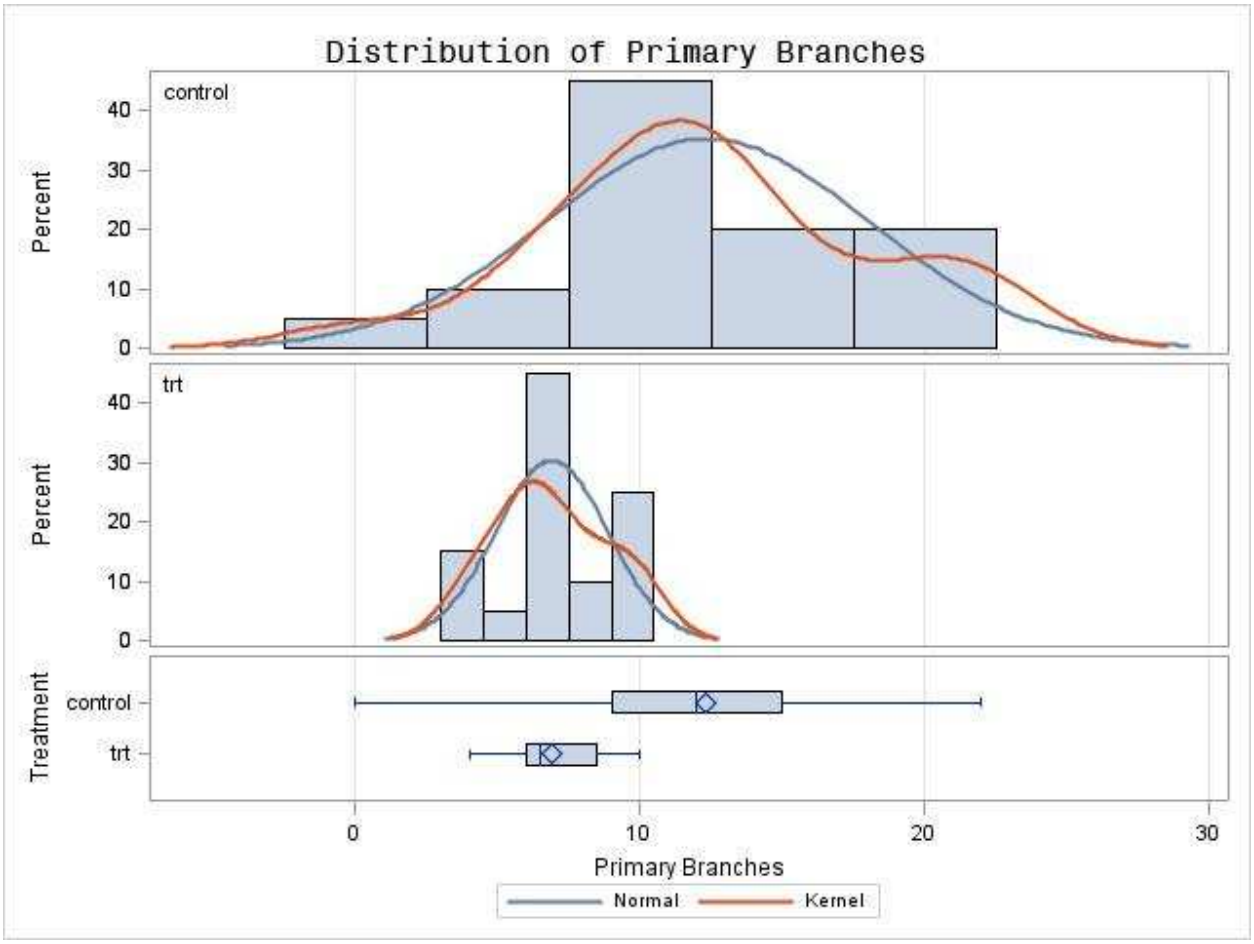

Figure 2.35. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of primary branches per plant between control and treatment group (removal of the shoot apical meristem). Count of primary branches from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

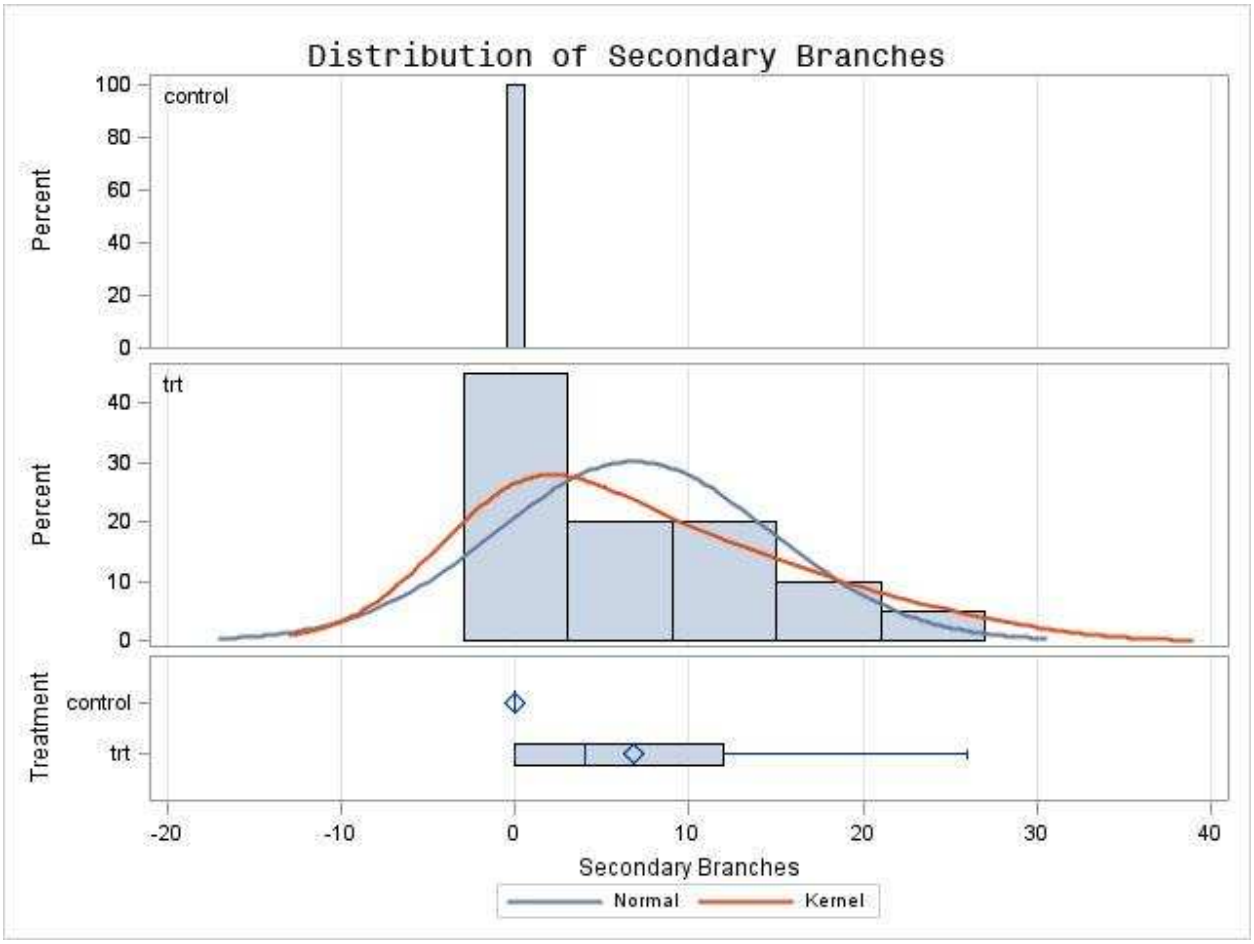

Figure 2.36. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of secondary branches per plant between control and treatment group (removal of the shoot apical meristem). Count of secondary branches from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

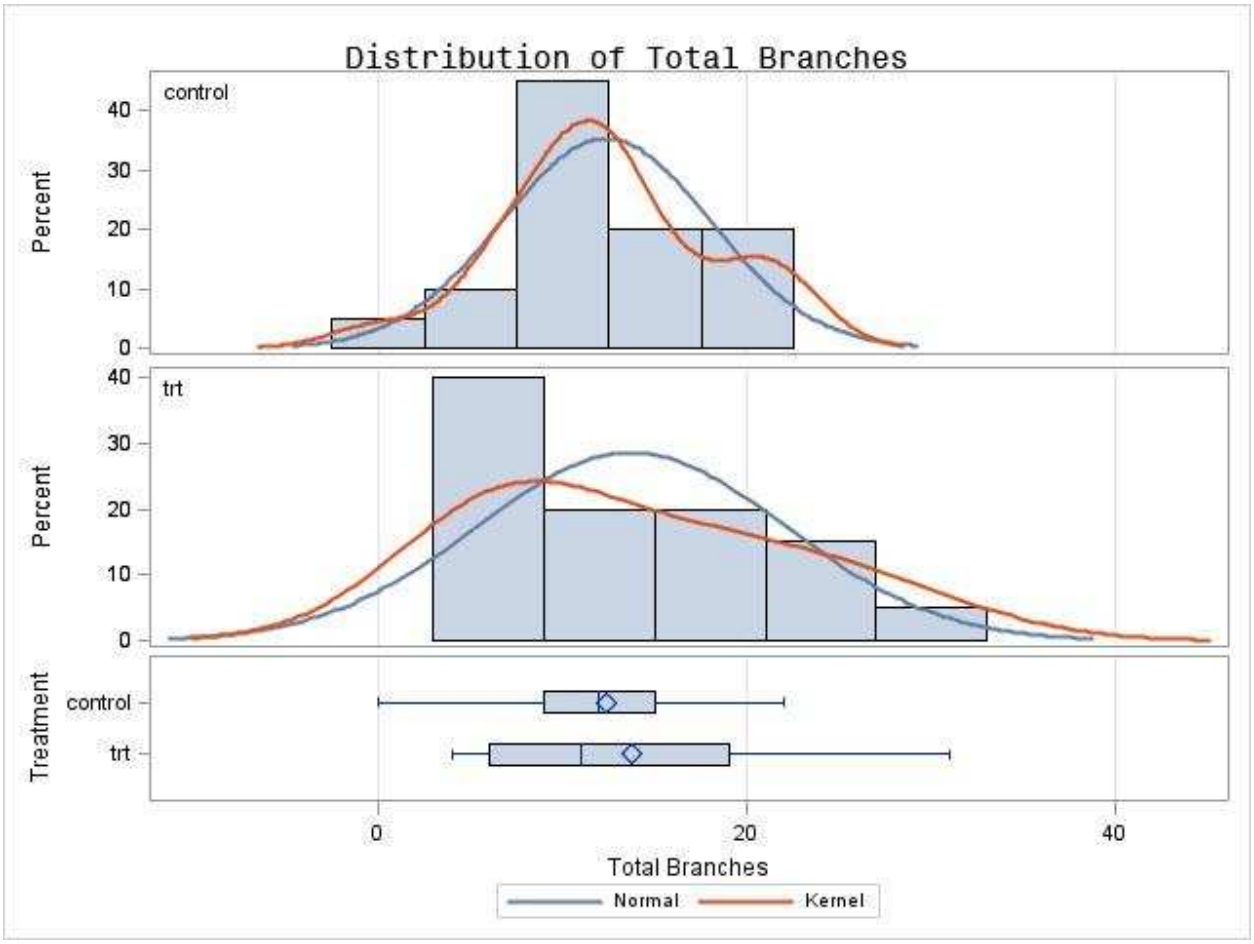

Figure 2.37. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of total branches per plant between control and treatment group (removal of the shoot apical meristem). Count of total branches from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

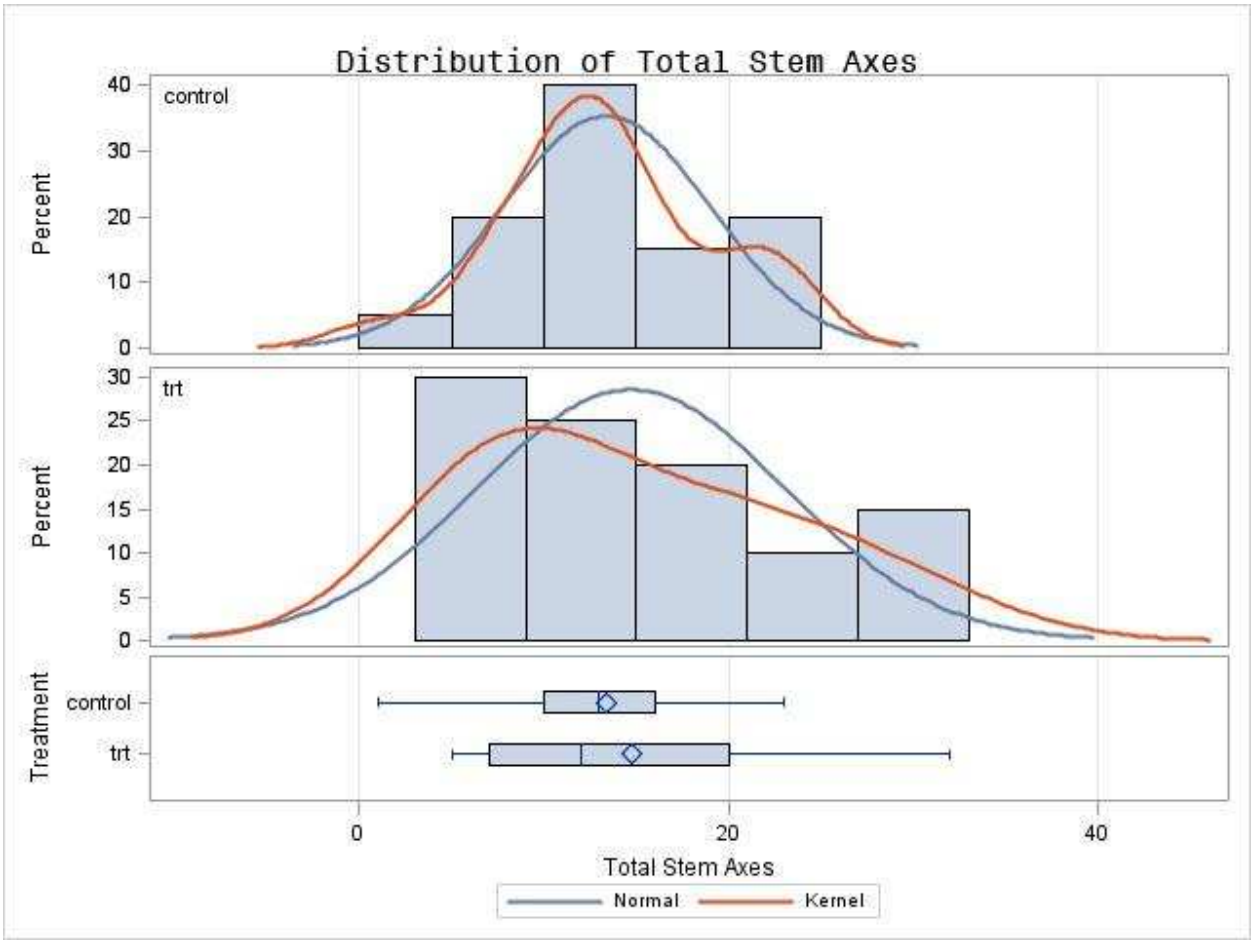

Figure 2.38. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of total stem axes per plant between control and treatment group (removal of the shoot apical meristem). Count of total stem axes from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

Ratio of Bulbil Count to Total Stem Axes

The mean ratio of number of bulbils to total stem axes of plants with the shoot apical meristem removed (trt) was 11.7643 ± 1.6744 (95% CI). The mean ratio of number of bulbils to total stem axes of the control plants was 11.7832 ± 5.7753. The mean ratio of number of bulbils to total stem axes of the control and treatment group was not statistically different ($p = 0.9948$, Figure 2.39).

A natural log transformation was used for the ratio of number of bulbils to total stem axes to normalize the skewed data. The mean natural log ratio of number of bulbils to total stem axes of plants with the shoot apical meristem removed (trt) was 2.4237 ± 0.1366 (95% CI). The mean ratio of number of bulbils to total stem axes of the control plants was 2.2633 ± 0.2487. The mean ratio of number of bulbils to total stem axes of the control and treatment group was not statistically different ($p = 0.2461$, Figure 2.40).

Discussion

Bulbil Count, Stem Biomass, and Ratio of Bulbil Count to Stem Biomass

The number of bulbils produced, stem biomass, and the ratio of bulbil count to stem biomass were not significantly different between the control and treatment groups (Figures 2.32-2.34). Under low-light conditions the removal of the shoot apical meristem did not enhance the production of bulbils, nor did it contribute to a change in the amount of stem biomass produced per plant. The ratio of bulbil count to stem biomass shows that both the treatment and control groups had a similar distribution of nodes and internodes; even though the removal of the shoot apical meristem terminated the main shoot axis in the treatment group, the total number of bulbils and total stem biomass in the branches remained the same.

Branch Formation and Total Stem Axes

The control group produced significantly more primary branches than the treatment group. However, the removal of the shoot apical meristem in the treatment group limited the number of

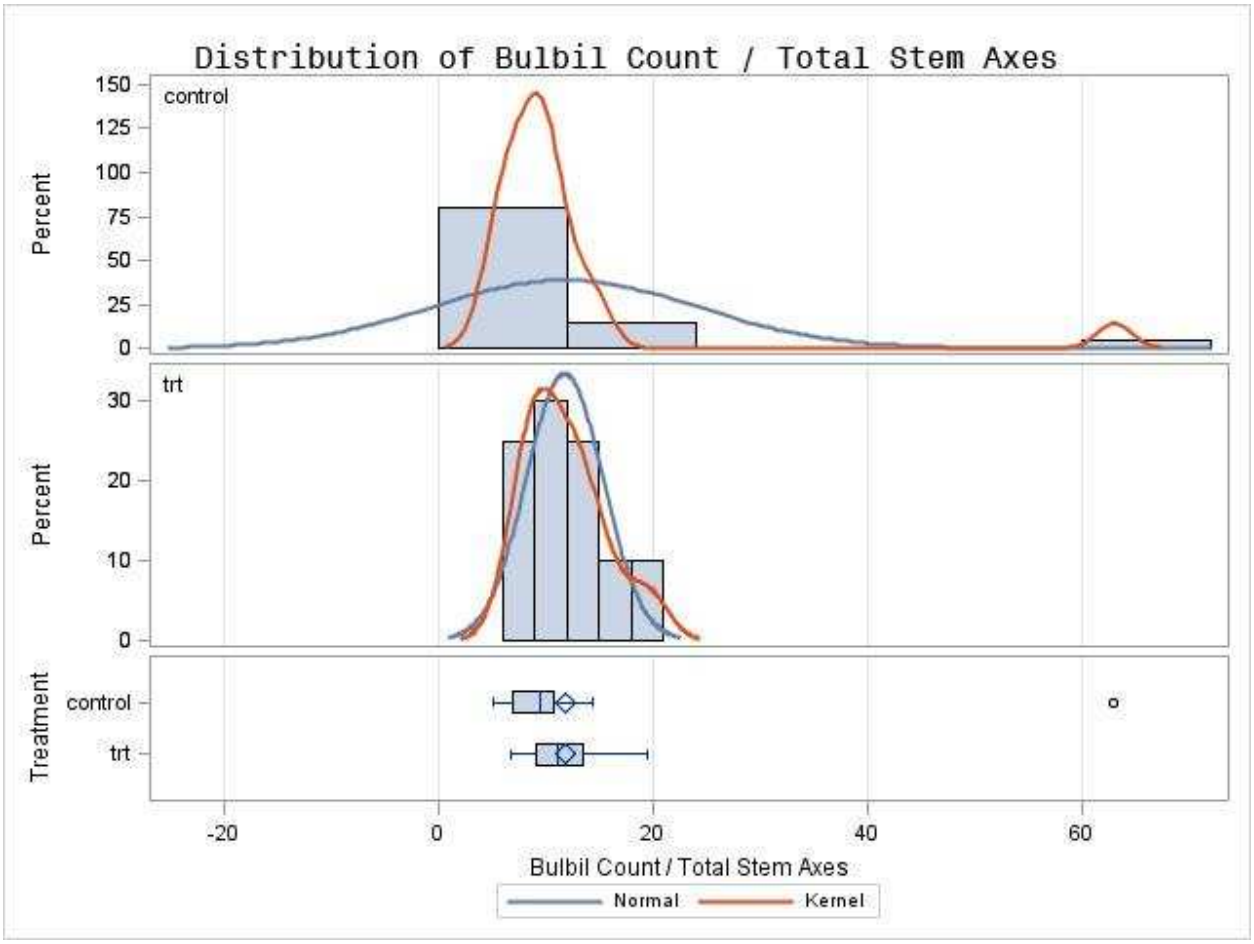

Figure 2.39. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of the ratio of total bulbils produced to total stem axes per plant between control and treatment group (removal of the shoot apical meristem). Ratio of bulbil count to total stem axes from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

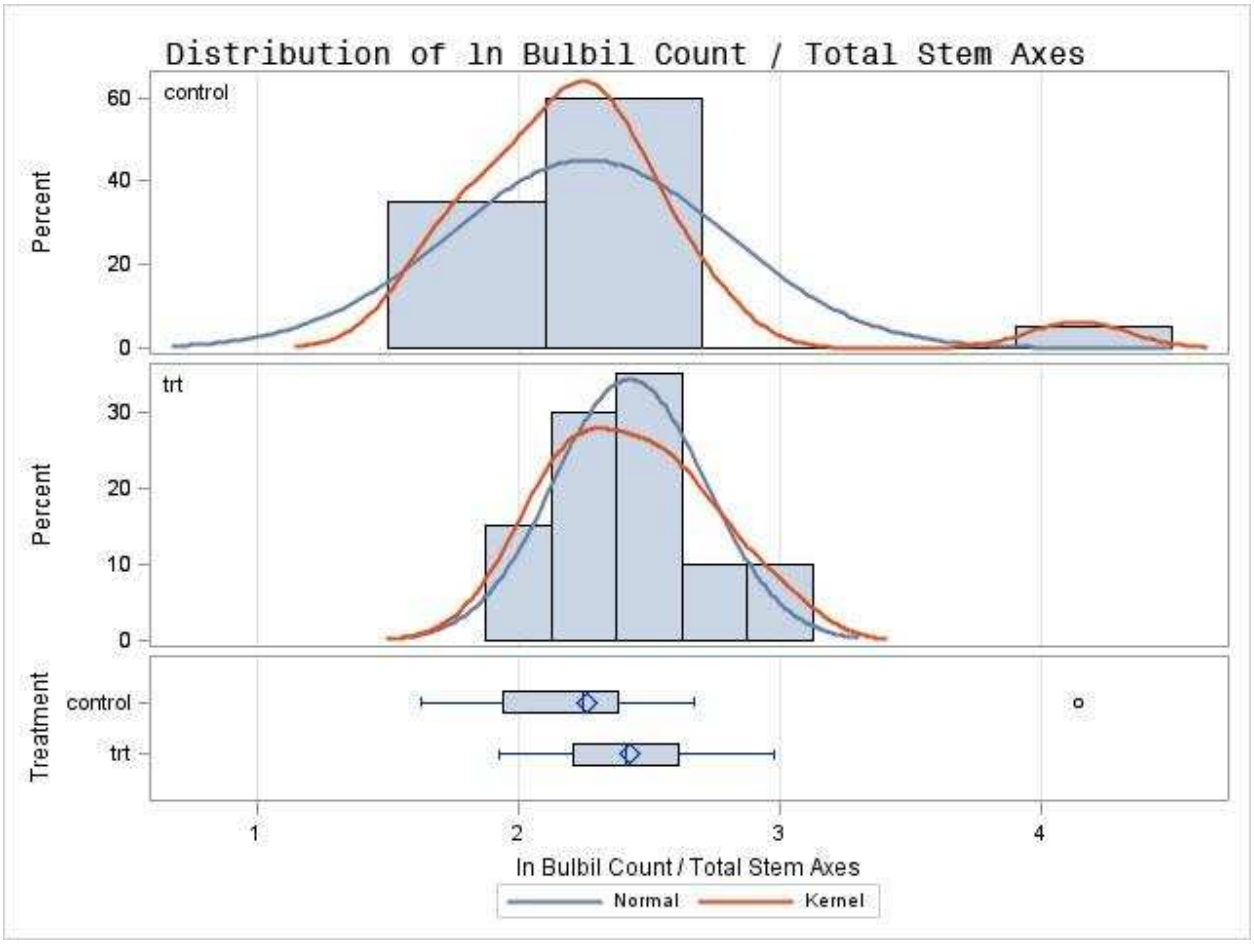

Figure 2.40. Comparative histograms, normal and kernel densities, and box plots comparing the distribution of natural log transformed ratio of total bulbils produced to total stem axes per plant between control and treatment group (removal of the shoot apical meristem). Natural log transformed ratio of bulbil count to total stem axes from 20 plants sampled in each group. See caption on Figure 2.32 for details on box plot.

primary branches a plant was able to produce since the main shoot axis contained only five nodes (10 axillary buds). Both treatment and control groups produced primary branches, but only the treatment group produced secondary branches (Figures 2.35, 2.36). There was no significant difference between the treatment and control group for total number of branches and total number of shoot axes produced (Figures 2.37, 2.38). Although both groups had similar numbers of shoot axes, the plants in the control group were composed of primary branches while the plants in the treatment group had both primary and secondary branches. The secondary branches compensated for the removal of the shoot apical meristem that resulted in the loss of potential axillary buds that may be developed on new nodes (Belsky, 1986; Paige, 1987; Baker and Diggle, 2011). In low-light conditions, the removal of the shoot apical meristem does not appear to have an effect on the production of primary branches but only on the production of secondary branches. The lack of secondary branching in the control group suggests that quiescent meristems may be viewed as a bet-hedging strategy (Baker and Diggle, 2011), with dormant meristems allowing for a rapid response to changes in the environment. Although the quiescent meristems appeared to compensate for a damaged meristem in the experiment, they also would allow for the development of floral buds or more branches if the conditions become more favorable.

Ratio of Bulbil Count to Total Stem Axes

The ratio of bulbil count to total stem axes was not significantly different between the treatment and control group (Figure 2.40). The number of bulbils and the total number of stem axes produced per plant were similar between the treatment and control group, and as such there is no difference in the ratio of the two. Although branching was induced by removal of the shoot apical meristem in the treatment group the branches remained small and did not develop more bulbils as expected.

Synthesis and Conclusions

The first experiment in manipulation of resource allocation showed no significant difference between the two treatment groups, removal of floral buds and removal of both floral buds and shoot

apical meristems, for the parameters used to assess fecundity. However, when compared to the control group, the treatment groups had higher mean bulbil count, bulbil biomass per plant, ratio of bulbil count to stem biomass, and ratio of bulbil biomass to stem biomass. Under greenhouse conditions the removal of flowers or both flowers and apical meristems doubled bulbil production, although individual bulbils were smaller. The removal of flowers or both flowers and apical meristems can be used under greenhouse conditions to increase bulbil production and thus increased fecundity. Removal of nonfunctioning flowers, a resource sink, seemingly led to reallocation of resources to produce more bulbils; however, there is a trade-off in number of bulbils to biomass of individual bulbils which could result in reduced germination rate or lower survivorship of germinating bulbils.

The second experiment (on induction of branching) showed that, contrary to expectations, there was no effect in increasing fecundity by the removal of the shoot apical meristem. In a low-light environment, the induction of branching did not increase bulbil production. Early branching was induced by the removal of the shoot apical meristem in the treatment group, however the branches remained small and did not continuously increase in length or biomass prior to senescence. Production of secondary branches was observed in only the treatment group, which seems to be caused by the removal of the shoot apical meristem; breaking apical dominance appeared to have an effect on the axillary buds that are dormant along the primary branches which, when released, developed into secondary branches. Removal of the shoot apical meristem did not change the total stem biomass of the plant, nor did it change the total number of shoot axes. A compensatory effect was observed by removing the shoot apical meristem, but there was no overcompensation to increased vegetative growth and increase bulbil production.

In both experiments, there was no significant difference in the comparison of stem biomass between the control group and the treatment group(s). The results of the second experiment give explanation for the lack of difference between the two treatment groups in the first experiment; removal of the shoot apical meristem does not increase overall stem production even though it does initiate the formation of branches. In the low-light conditions of the second experiment both the control and treatment group had the same number of shoot axes formed and stem biomass, likewise in

high-light conditions of the greenhouse the stem biomass was not different between the control group and the second treatment group with the removal of shoot apical meristems. The amount and quality of the light appears to have the greatest effect on the growth of stem tissue; and resource allocation between sexual and asexual reproduction has the greatest effect on bulbil production. In low-light conditions there might not be a method to increase bulbil production (fecundity) in individual plants, as the plants grow similarly to those found in wild populations, small and rarely producing flowers.

Mimulus gemmiparus is a habitat specialist, growing at high elevation and under overhanging cliffs (Beatty, 2003; Beardsley, 2012). In these high elevation locations there is a need for available water, adequate sunlight, and warm temperature; while these requirements may not appear to be too specific, the total number of natural sites of these plants (of which there are eight) suggests that there is a habitat preference. Plants may disperse or disappear completely from known locations. Although new populations may form in new areas following dispersal, there is a chance they may be overlooked. Climate change and human activity add to the problem of habitat availability faced by these plants. Changes in water availability during the growing season hinders a population's ability to grow and produce offspring for the following generation. Fire, either natural or due to human activity, is detrimental and has the potential to erase a population. These fragile plants should be preserved since they are extremely rare and unique.

A proposed solution to the limited habitat problem of *M. gemmiparus* is to supplement natural populations with harvested bulbils from the ex-situ propagation of bulbils in greenhouses. These plants grow much larger and more robust in the greenhouse than in their native habitats. Under greenhouse propagation, the number of bulbils produced can be manipulated by changing the plant's resource allocation. Resources that would have been used by sexual reproduction can be reallocated to vegetative growth by removing the resource sink that is the floral buds. Even though the plants produce a larger number of bulbils on their own when grown in the greenhouse, greenhouse-grown plants can be manipulated to further increase the yield of bulbils for use in reintroduction efforts.

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APPENDIX I

SAS 9.3 code used for analysis of experimental data for experiment 1: removal of floral buds and apical meristems in greenhouse environment.

```
proc glm;
        class treatment;
        model bulbil_mass__g_ = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model bulbil_count = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model stem_mass__g_ = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model Bulbil_Mass__g____Stem_Mass__g_ = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model ln_Bulbil_Mass__g____Stem_Mass__ = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model Bulbil_Count___Stem_Mass__g_ = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model ln_Bulbil_Count___Stem_Mass__g_ = treatment;
        lsmeans treatment / stderr pdiff;
proc glm;
        class treatment;
        model Average_Individual_Bulbil_Mass__ = treatment;
        lsmeans treatment / stderr pdiff;
run;
```

Table A1.1. Experimental data of control, treatment one, and treatment two for experiment 1: removal of floral buds and apical meristems in greenhouse environment.

Lineage	Treatment	Individual	Stem Mass (g)	Bulbil Mass (g)	Bulbil Count	Bulbil Mass (g) / Stem Mass (g)	ln Bulbil Mass (g) / Stem Mass (g)	Bulbil Count / Stem Mass (g)	ln Bulbil Count / Stem Mass (g)	Average Individual Bulbil Mass (g)
16	control	1	0.2830	0.4803	2259	1.6972	0.5290	7982.3322	8.9850	2.126E-04
16	control	2	0.2956	0.5796	2631	1.9608	0.6733	8900.5413	9.0939	2.203E-04
16	control	3	0.1680	0.3033	1457	1.8054	0.5908	8672.6190	9.0679	2.082E-04
16	control	4	0.2625	0.4540	1780	1.7295	0.5478	6780.9524	8.8219	2.551E-04
19	control	5	0.0220	0.1302	926	5.9182	1.7780	42090.9091	10.6476	1.406E-04
19	control	6	0.1680	0.2913	1150	1.7339	0.5504	6845.2381	8.8313	2.533E-04
19	control	7	0.2282	0.4657	1810	2.0408	0.7133	7931.6389	8.9786	2.573E-04
19	control	8	0.2597	0.4313	1604	1.6608	0.5073	6176.3573	8.7285	2.689E-04
24	control	9	0.1855	0.3685	1273	1.9865	0.6864	6862.5337	8.8338	2.895E-04
24	control	10	0.3950	0.8072	2708	2.0435	0.7147	6855.6962	8.8328	2.981E-04
24	control	11	0.3878	0.7304	2982	1.8834	0.6331	7689.5307	8.9476	2.449E-04
29	control	12	0.0786	0.1633	1070	2.0776	0.7312	13613.2316	9.5188	1.526E-04
29	control	13	0.0956	0.1291	696	1.3504	0.3004	7280.3347	8.8929	1.855E-04
33	control	14	0.1371	0.2309	1421	1.6842	0.5213	10364.6973	9.2462	1.625E-04
33	control	15	0.2250	0.4430	1890	1.9689	0.6775	8400.0000	9.0360	2.344E-04
33	control	16	0.1425	0.2464	1175	1.7291	0.5476	8245.6140	9.0174	2.097E-04
33	control	17	0.1215	0.2300	1334	1.8930	0.6382	10979.4239	9.3038	1.724E-04
33	control	18	0.2401	0.5170	2218	2.1533	0.7670	9237.8176	9.1311	2.331E-04
33	control	19	0.1443	0.2567	1289	1.7789	0.5760	8932.7789	9.0975	1.991E-04
34	control	20	0.2507	0.4964	1895	1.9801	0.6831	7558.8353	8.9305	2.620E-04
16	trt 1	1	0.2397	0.6406	4196	2.6725	0.9830	17505.2149	9.7703	1.527E-04
16	trt 1	2	0.3025	0.9033	4830	2.9861	1.0940	15966.9421	9.6783	1.870E-04
16	trt 1	3	0.3423	0.9504	4843	2.7765	1.0212	14148.4078	9.5574	1.962E-04
16	trt 1	4	0.2565	0.6500	3016	2.5341	0.9298	11758.2846	9.3723	2.155E-04
16	trt 1	5	0.3051	0.8248	4419	2.7034	0.9945	14483.7758	9.5808	1.866E-04
19	trt 1	6	0.1023	0.2031	1271	1.9853	0.6858	12424.2424	9.4274	1.598E-04
24	trt 1	7	0.2360	0.9232	4769	3.9119	1.3640	20207.6271	9.9138	1.936E-04
24	trt 1	8	0.1905	0.5291	3304	2.7774	1.0215	17343.8320	9.7610	1.601E-04
24	trt 1	9	0.1800	0.4118	2570	2.2878	0.8276	14277.7778	9.5665	1.602E-04
29	trt 1	10	0.2203	0.5996	3272	2.7217	1.0013	14852.4739	9.6059	1.833E-04
29	trt 1	11	0.2403	0.7927	4513	3.2988	1.1936	18780.6908	9.8406	1.756E-04
29	trt 1	12	0.1620	0.5146	3042	3.1765	1.1558	18777.7778	9.8404	1.692E-04
29	trt 1	13	0.2024	0.7412	4986	3.6621	1.2980	24634.3874	10.1119	1.487E-04
29	trt 1	14	0.2734	0.4454	3052	1.6291	0.4880	11163.1309	9.3204	1.459E-04
33	trt 1	15	0.2877	0.8847	4731	3.0751	1.1233	16444.2127	9.7077	1.870E-04
33	trt 1	16	0.3995	0.9350	5027	2.3404	0.8503	12583.2290	9.4401	1.860E-04
33	trt 1	17	0.2450	0.6857	3477	2.7988	1.0292	14191.8367	9.5604	1.972E-04
34	trt 1	18	0.2885	0.8806	4507	3.0523	1.1159	15622.1837	9.6564	1.954E-04
34	trt 1	19	0.2820	0.7891	4178	2.7982	1.0290	14815.6028	9.6034	1.889E-04
34	trt 1	20	0.0797	0.2306	1409	2.8934	1.0624	17678.7955	9.7801	1.637E-04
16	trt 2	1	0.1580	0.4424	2315	2.8000	1.0296	14651.8987	9.5923	1.911E-04
16	trt 2	2	0.2455	0.5884	3290	2.3967	0.8741	13401.2220	9.5031	1.788E-04
16	trt 2	3	0.2509	0.5320	3342	2.1204	0.7516	13320.0478	9.4970	1.592E-04
16	trt 2	4	0.1598	0.4597	2438	2.8767	1.0567	15256.5707	9.6328	1.886E-04
16	trt 2	5	0.2946	0.7381	4139	2.5054	0.9185	14049.5587	9.5503	1.783E-04
19	trt 2	6	0.1046	0.2805	2020	2.6816	0.9864	19311.6635	9.8685	1.389E-04
19	trt 2	7	0.1591	0.4840	2592	3.0421	1.1126	16291.6405	9.6984	1.867E-04
19	trt 2	8	0.1701	0.5443	3098	3.1999	1.1631	18212.8160	9.8099	1.757E-04
19	trt 2	9	0.1397	0.4098	2432	2.9334	1.0762	17408.7330	9.7647	1.685E-04
29	trt 2	10	0.1934	0.6913	3485	3.5745	1.2738	18019.6484	9.7992	1.984E-04
29	trt 2	11	0.2115	0.5995	2942	2.8345	1.0419	13910.1655	9.5404	2.038E-04
29	trt 2	12	0.2660	0.8679	4377	3.2628	1.1826	16454.8872	9.7084	1.983E-04
29	trt 2	13	0.1185	0.3632	2104	3.0650	1.1200	17755.2743	9.7844	1.726E-04
33	trt 2	14	0.1018	0.3129	2073	3.0737	1.1229	20363.4578	9.9215	1.509E-04
33	trt 2	15	0.2849	0.8642	4367	3.0333	1.1097	15328.1853	9.6374	1.979E-04
33	trt 2	16	0.4241	1.0875	5562	2.5643	0.9417	13114.8314	9.4815	1.955E-04
33	trt 2	17	0.3513	0.7894	4116	2.2471	0.8096	11716.4816	9.3688	1.918E-04
33	trt 2	18	0.2283	0.5767	3115	2.5261	0.9267	13644.3276	9.5211	1.851E-04
34	trt 2	19	0.3116	0.8481	4746	2.7218	1.0013	15231.0655	9.6311	1.787E-04
34	trt 2	20	0.1677	0.5122	2663	3.0543	1.1165	15879.5468	9.6728	1.923E-04

Table A1.2. ANOVA table for bulbil count.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	47607910.93	23803955.47	26.61	<.0001
Error	57	50980114.80	894387.98		
Corrected Total	59	98588025.73			

Table A1.3. ANOVA table for bulbil mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.89595491	0.44797746	10.14	0.0002
Error	57	2.51844649	0.04418327		
Corrected Total	59	3.41440140			

Table A1.4. ANOVA table for stem mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.01437013	0.00718507	0.95	0.3922
Error	57	0.43039019	0.00755071		
Corrected Total	59	0.44476033			

Table A1.5. ANOVA table for bulbil count / stem mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	434355894	217177947	8.65	0.0005
Error	57	1430972487	25104780		
Corrected Total	59	1865328380			

Table A1.6. ANOVA table for ln bulbil count / stem mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	4.10460014	2.05230007	27.11	<.0001
Error	57	4.31544979	0.07570965		
Corrected Total	59	8.42004992			

Table A1.7. ANOVA table for bulbil mass (g) / stem mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	7.72837495	3.86418748	9.17	0.0004
Error	57	24.02825471	0.42154833		
Corrected Total	59	31.75662966			

Table A1.8. ANOVA table for ln bulbil mass (g) / stem mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.67173509	0.83586754	18.48	<.0001
Error	57	2.57844978	0.04523596		
Corrected Total	59	4.25018486			

Table A1.9. ANOVA table for average individual bulbil mass (g).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2.5274961E-8	1.2637481E-8	14.31	<.0001
Error	57	5.0339018E-8	8.831407E-10		
Corrected Total	59	7.5613979E-8			

Table A1.10. Least squares means table of bulbil count for comparison of means.

Treatment	Bulbil_Count LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	1678.40000	211.46962	<.0001	1
trt 1	3770.60000	211.46962	<.0001	2
trt 2	3260.80000	211.46962	<.0001	3

Least Squares Means for effect Treatment Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Bulbil_Count			
i/j	1	2	3
1		<.0001	<.0001
2	<.0001		0.0937
3	<.0001	0.0937	

Table A1.11. Least squares means table of bulbil mass (g) for comparison of means.

Treatment	Bulbil_Mass__g_ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	0.38773000	0.04700174	<.0001	1
trt 1	0.67677500	0.04700174	<.0001	2
trt 2	0.59960500	0.04700174	<.0001	3

Least Squares Means for effect Treatment Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Bulbil_Mass__g_			
i/j	1	2	3
1		<.0001	0.0023
2	<.0001		0.2505
3	0.0023	0.2505	

Table A1.12. Least squares means table of stem mass (g) for comparison of means.

Treatment	Stem_Mass__g_ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	0.20453500	0.01943027	<.0001	1
trt 1	0.24178500	0.01943027	<.0001	2
trt 2	0.21707000	0.01943027	<.0001	3

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Stem_Mass__g_

i/j	1	2	3
1		0.1806	0.6500
2	0.1806		0.3722
3	0.6500	0.3722	

Table A1.13. Least squares means table of bulbil count / stem mass (g) for comparison of means.

Treatment	Bulbil_Count___Stem_Mass__g_ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	10070.0541	1120.3745	<.0001	1
trt 1	15883.0213	1120.3745	<.0001	2
trt 2	15666.1011	1120.3745	<.0001	3

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Bulbil_Count___Stem_Mass__g_

i/j	1	2	3
1		0.0005	0.0008
2	0.0005		0.8916
3	0.0008	0.8916	

Table A1.14. Least squares means table of ln bulbil count / stem mass (g) for comparison of means.

Treatment	ln_Bulbil_Count___Stem_Mass__g_ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	9.09715187	0.06152627	<.0001	1
trt 1	9.65475695	0.06152627	<.0001	2
trt 2	9.64918041	0.06152627	<.0001	3

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: ln_Bulbil_Count___Stem_Mass__g_

i/j	1	2	3
1		<.0001	<.0001
2	<.0001		0.9491
3	<.0001	0.9491	

Table A1.15. Least squares means table of bulbil mass (g) / stem mass (g) for comparison of means.

Treatment	Bulbil_Mass__g____Stem_Mass__g_ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	2.05377116	0.14518063	<.0001	1
trt 1	2.80407383	0.14518063	<.0001	2
trt 2	2.82567500	0.14518063	<.0001	3

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Bulbil_Mass__g____Stem_Mass__g_

i/j	1	2	3
1		0.0006	0.0004
2	0.0006		0.9166
3	0.0004	0.9166	

Table A1.16. Least squares means table of ln bulbil mass (g) / stem mass (g) for comparison of means.

Treatment	ln_Bulbil_Mass__g____Stem_Mass__ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	0.66831846	0.04755836	<.0001	1
trt 1	1.01341394	0.04755836	<.0001	2
trt 2	1.03076609	0.04755836	<.0001	3

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: ln_Bulbil_Mass__g____Stem_Mass__

i/j	1	2	3
1		<.0001	<.0001
2	<.0001		0.7973
3	<.0001	0.7973	

Table A1.17. Least squares means table of average individual bulbil mass (g) for comparison of means.

Treatment	Average_Individual_Bulbil_Mass__ LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
control	0.00022300	0.00000665	<.0001	1
trt 1	0.00017763	0.00000665	<.0001	2
trt 2	0.00018156	0.00000665	<.0001	3

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Average_Individual_Bulbil_Mass__

i/j	1	2	3
1		<.0001	<.0001
2	<.0001		0.6776
3	<.0001	0.6776	

APPENDIX II

SAS 9.3 code used for analysis of experimental data for experiment 2: removal of shoot apical meristem in low-light environment.

```
proc ttest;
        class treatment;
        var stem_mass__g_;
proc ttest;
        class treatment;
        var bulbil_count;
proc ttest;
        class treatment;
        var bulbil_count___stem_mass__g_;
proc ttest;
        class treatment;
        var primary_branches;
proc ttest;
        class treatment;
        var secondary_branches;
proc ttest;
        class treatment;
        var total_branches;
proc ttest;
        class treatment;
        var total_stem_axes;
proc ttest;
        class treatment;
        var bulbil_count___total_stem_axes;
proc ttest;
        class treatment;
        var ln_bulbil_count___total_stem_axe;
run;
```

Table A2.1. Experimental data of control and treatment for experiment 2: removal of shoot apical meristem in low-light environment.

Treatment	Individual	Stem Mass (g)	Bulbil Count	Bulbil Count / Stem Mass (g)	Primary Branches	Secondary Branches	Total Branches	Total Stem Axes	Bulbil Count / Total Stem Axes	In Bulbil Count / Total Stem Axes
control	1	0.0099	331	33434.3434	22	0	22	23	14.39	2.6666
control	2	0.0022	68	30909.0909	8	0	8	9	7.56	2.0223
control	3	0.0043	135	31395.3488	12	0	12	13	10.38	2.3403
control	4	0.0068	200	29411.7647	20	0	20	21	9.52	2.2538
control	5	0.0018	63	35000.0000	0	0	0	1	63.00	4.1431
control	6	0.0015	47	31333.3333	6	0	6	7	6.71	1.9042
control	7	0.0024	101	42083.3333	10	0	10	11	9.18	2.2172
control	8	0.0050	144	28800.0000	14	0	14	15	9.60	2.2618
control	9	0.0045	151	33555.5556	14	0	14	15	10.07	2.3092
control	10	0.0059	144	24406.7797	11	0	11	12	12.00	2.4849
control	11	0.0031	112	36129.0323	11	0	11	12	9.33	2.2336
control	12	0.0035	123	35142.8571	16	0	16	17	7.24	1.9790
control	13	0.0048	107	22291.6667	20	0	20	21	5.10	1.6283
control	14	0.0015	57	38000.0000	8	0	8	9	6.33	1.8458
control	15	0.0020	79	39500.0000	6	0	6	7	11.29	2.4235
control	16	0.0020	77	38500.0000	12	0	12	13	5.92	1.7789
control	17	0.0019	60	31578.9474	10	0	10	11	5.45	1.6964
control	18	0.0040	110	27500.0000	12	0	12	13	8.46	2.1355
control	19	0.0062	202	32580.6452	13	0	13	14	14.43	2.6692
control	20	0.0090	223	24777.7778	22	0	22	23	9.70	2.2717
trt	1	0.0100	340	34000.0000	10	14	24	25	13.60	2.6101
trt	2	0.0027	96	35555.5556	4	0	4	5	19.20	2.9549
trt	3	0.0037	100	27027.0270	9	0	9	10	10.00	2.3026
trt	4	0.0030	117	39000.0000	6	8	14	15	7.80	2.0541
trt	5	0.0035	141	40285.7143	10	0	10	11	12.82	2.5509
trt	6	0.0012	47	39166.6667	4	0	4	5	9.40	2.2407
trt	7	0.0053	106	20000.0000	6	0	6	7	15.14	2.7175
trt	8	0.0064	130	20312.5000	6	12	18	19	6.84	1.9231
trt	9	0.0025	66	26400.0000	6	0	6	7	9.43	2.2437
trt	10	0.0023	77	33478.2609	4	4	8	9	8.56	2.1466
trt	11	0.0086	241	28023.2558	7	19	26	27	8.93	2.1890
trt	12	0.0067	176	26268.6567	10	8	18	19	9.26	2.2260
trt	13	0.0033	137	41515.1515	6	0	6	7	19.57	2.9741
trt	14	0.0068	265	38970.5882	8	12	20	21	12.62	2.5352
trt	15	0.0017	58	34117.6471	6	0	6	7	8.29	2.1145
trt	16	0.0088	203	23068.1818	8	4	12	13	15.62	2.7483
trt	17	0.0109	345	31651.3761	5	26	31	32	10.78	2.3778
trt	18	0.0067	218	32537.3134	9	9	18	19	11.47	2.4401
trt	19	0.0042	122	29047.6190	7	1	8	9	13.56	2.6068
trt	20	0.0101	335	33168.3168	7	19	26	27	12.41	2.5183

Table A2.2. *T*-test statistics and test of equality of variance for bulbil count.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	-1.48	0.1464
Satterthwaite	Unequal	34.767	-1.48	0.1472

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	1.88	0.1789

Table A2.3. *T*-test statistics and test of equality of variance for stem mass (g).

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	-1.49	0.1440
Satterthwaite	Unequal	36.35	-1.49	0.1444

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	1.54	0.3537

Table A2.4. *T*-test statistics and test of equality of variance for bulbil count / stem mass (g).

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	0.34	0.7348
Satterthwaite	Unequal	36.297	0.34	0.7349

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	1.55	0.3456

Table A2.5. *T*-test statistics and test of equality of variance for primary branches.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	4.07	0.0002
Satterthwaite	Unequal	23.542	4.07	0.0005

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	8.25	<.0001

Table A2.6. *T*-test statistics and test of equality of variance for secondary branches.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	-3.83	0.0005
Satterthwaite	Unequal	19	-3.83	0.0011

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	Infty	<.0001

Table A2.7. *T*-test statistics and test of equality of variance for total branches.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	-0.60	0.5538
Satterthwaite	Unequal	33.362	-0.60	0.5543

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	2.19	0.0960

Table A2.8. *T*-test statistics and test of equality of variance for total stem axes.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	-0.60	0.5538
Satterthwaite	Unequal	33.362	-0.60	0.5543

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	2.19	0.0960

Table A2.9. *T*-test statistics and test of equality of variance for bulbil count / total stem axes.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	0.01	0.9948
Satterthwaite	Unequal	22.172	0.01	0.9948

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	11.90	<.0001

Table A2.10. *T*-test statistics and test of equality of variance for ln bulbil count / total stem axes.

Method	Variances	DF	t Value	Pr > |t|
Pooled	Equal	38	-1.18	0.2440
Satterthwaite	Unequal	29.512	-1.18	0.2461

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F	19	19	3.31	0.0122

APPENDIX III

Irregularities of *M. gemmiparus* Flowers

During experimentation in the greenhouse, there were plants that produced malformed flowers. Observed characteristics included: propagule attached to the calyx, split calyx, thin petals, the growth of propagule within the flower, branch formation within a flower, or a combination of these traits (Figures A2.1-A2.6). The cause of the abnormalities is currently unknown and this is the first observation of such disrupted flower development in *M. gemmiparus* and may be an isolated event that only appears in greenhouse settings. Neighboring plants in the greenhouse did not display abnormal flowers and only *M. gemmiparus* were affected. The malformed flowers might not be caused by an external factor such as insects or bacterial pathogens but from rapid growth due to high humidity and sunlight; the meristem within the lateral buds may not have separated correctly into the typical dormant bud and bulbil. Although flower formation was disrupted it did not appear to be harmful to the plant since the flowers are seemingly nonfunctional and the individual bulbil adjacent to the flower that may have been damaged is seemingly inconsequential to overall fecundity since the plant produced hundreds of bulbils.

Figure A2.1. Flower with a split calyx.

Figure A2.2. Branch formation within a split calyx on both flowers on the same node.

Figure A2.3. Bulbil germination on the calyx (right). Bulbil germination on calyx with the calyx being split open (left).

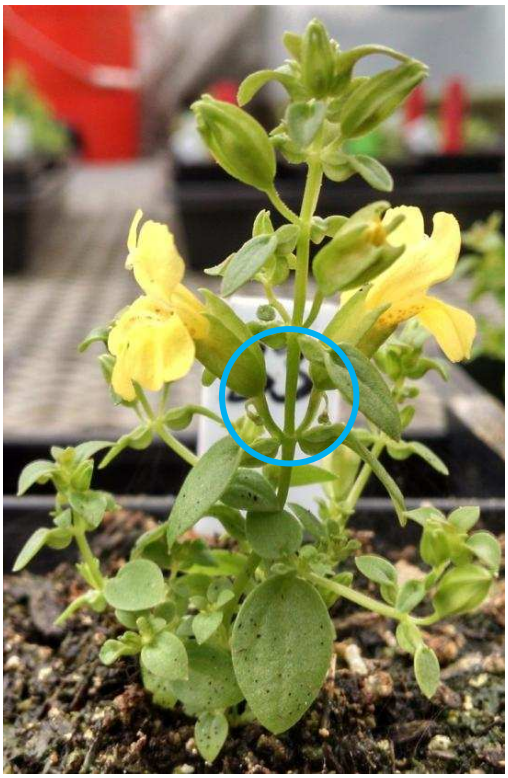

Figure A2.4. Bulbils attached to calyx of adjacent flowers, leaving empty petioles.

Figure A2.5. The development of a branch with a flower bud growing on a calyx of an older flower.

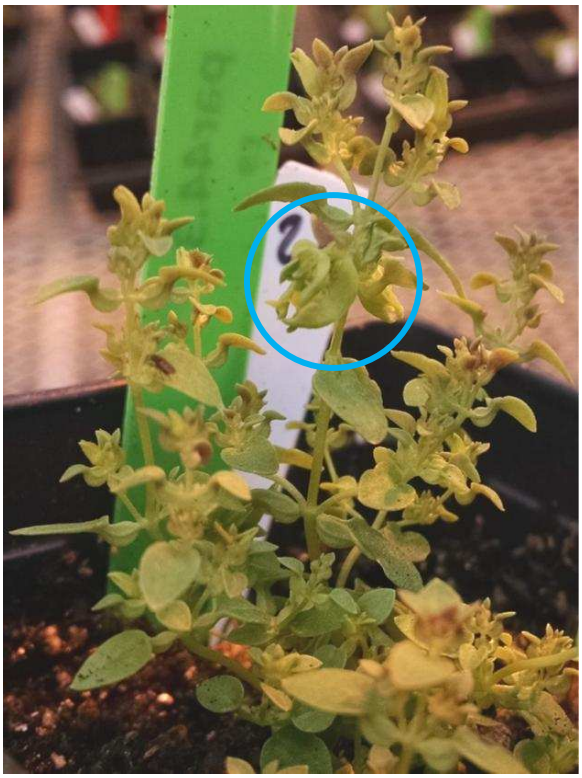

Figure A2.6. The formation of a branch through the top half of a flower and the bottom half of the flower retains three sepals and parts of the petals.