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Effects of Disturbance (Mowing) on Florivory and Floral Defenses in *Solanum elaeagnifolium*, a Noxious and Worldwide Invasive Weed

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EFFECTS OF DISTURBANCE (MOWING) ON FLORIVORY AND FLORAL DEFENSES IN *SOLANUM ELAEAGNIFOLIUM*, A NOXIOUS AND WORLDWIDE INVASIVE WEED

A Thesis

by

ALEJANDRO RAFAEL VASQUEZ MARCANO

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EFFECTS OF DISTURBANCE (MOWING) ON FLORIVORY AND FLORAL DEFENSES IN *SOLANUM ELAEAGNIFOLIUM*, A NOXIOUS AND WORLDWIDE INVASIVE WEED

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ABSTRACT

Vasquez, Alejandro, Effects of Disturbance (Mowing) on Florivory and Herbivore Defenses In *Solanum elaeagnifolium*, a Noxious and Worldwide Invasive Weed Master of Science (MS), December, 2022, 97 pp, 8 figures, references, 40 titles.

Chapter 1: Literature Review- In this literature review we have examined weed management as it concerns urban and agricultural ecosystems, as well as the need for mowing assessments as it relates to floral traits and defense against herbivory.

Chapter 2: Experiments- In the experiments we examined the role of mowing on floral growth traits, floral defense traits, and their effect on Manduca sexta specialist herbivores

Chapter 3: Results- In the results we found SLN flowers to have larger diameters than unmowed flowers; however, flower mass was in line with our predictions as unmowed flowers were heavier in high mowing frequency populations. Polyphenol Oxidase (PPO) was not significantly different between mowed and unmowed and the effect of mowed and unmowed plant parts incorporated in diet was found to be pronounced in unmowed diet-fed caterpillars which were significantly heavier than their control and mowed counterparts. Physical defenses in the form of spines were also significantly higher in density in mowed plants when compared to unmowed plants.

Chapter 4: Discussion- From the results and experiments we can conclude that these findings are in line with our previous study on SLN that had not looked at floral traits but still found there to be trait induction in mowed SLN plants when compared to unmowed SLN plants.

DEDICATION

I would like to dedicate my thesis to my mother, Yosmar Marcano and father, Steven Barnhart, alongside my brother, Juan Vasquez, my grandmother Yolanda Uzcategui, and my girlfriend Jessica Ayala.

ACKNOWLEDGMENTS

I would like to give immense gratitude to Dr. Rupesh Kariyat, my thesis advisor, and undergraduate and graduate mentor for taking me into his lab 4 years ago and for continuing to mentor and support me throughout my entire academic career and achievements. I have become a researcher and educator solely because of his influence on me and he has brought out a passion in me I did not know I had. He has been such an amazing force of nature in my life and words cannot express my appreciation for him.

I would also like to thank Dr. Bradley Christoffersen and Dr. Robert Dearth for accepting to be part of my thesis committee. All the feedback provided by my committee is greatly appreciated and will help me grow as both a student and scientist.

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Lastly, I would like to thank Kariyat Lab: who have all helped, taught, and made my time as a student unforgettable.

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CHAPTER I

LITERATURE REVIEW: EFFECTS OF MOWING ON FLORAL TRAITS IN SOLANUM ELAEAGNIFOLIUM VIA PLANT AND HERBIVORE TRAITS AND FIELD DAMAGE ASSESSMENT

Introduction

In ecology, weeds are thought of through the lens of eradicating and minimizing their impact, thought of as undesirable and unwanted plants in almost every ecosystem and environment (Stefan et al. 2020). Regarding agriculture, weeds are even more scrutinized as they harm both biotic and abiotic ecosystem services because of their enhanced traits allowing them to flourish in native or introduced habitats (Chavana et al. 2021). Enhanced weed traits are numerous and encompass the ability to outcompete heterospecifics, increased fitness and defenses, as well as more vigorous germination rates. In France, Fried et al. 2020 found the most ecologically successful weed species against maize crops were those with the C4 photosynthetic pathway and summer emergence. Additionally, these weeds exhibit rapid resource acquisition through high specific leaf area (SLA) and high Ellenberg-Nitrogen (N) alongside immense colonization capacity in the form of fecundity, seed longevity, and germination. Related again to agriculture, weeds have been shown to selectively produce seeds either pre or post-crop harvest to avoid seed destruction alongside the harvest and for facilitation in seed dissemination (Clements et al. 2021). In turn, scientists and farmers possess an increasingly immense interest toward identifying and understanding weed success characteristics, with equal or more interest in how to diminish and negate these same characteristics. Multiple studies have been conducted toward the effect of chemical treatments on weeds: Misra et al. in 1974 found that the herbicides embutox plus and tok E-25 were the most effective weed killers afflicting the Japanese mint. More recent studies elaborated on these techniques and advanced toward more ethical practices, such as weed control chemicals that target enzymes of plant-specific pathways to avoid toxicological problems in mammals (Kraehmer et al. 2014). Sustainable approaches to weed management have gained tremendous interest and support, stemming from concerns including, but not limited to herbicide resistance in weed biotypes, a major concern in weed management[9]. For example, in the two Mediterranean weeds, *Diplotaxis erucoides* and *Erucaria hispanica*, a group found bipyridilium resistant biotypes with resistance to acetolactate synthase inhibtors, which alongside thousands of more studies highlighting the difficulties in managing herbicide resistant weeds (Travlos et al. 2020, Peleg and Lati 2021). Regardless of the methodology or mode of action, understanding the factors and mechanisms underlying weed success is of primary importance in their management. However, these methods of weed control and weed disturbance are chemical and do not take into account human-driven disturbances such as extensive management practices. One of the most common human-disturbance management practices practiced throughout the world is mowing, occurring in commercial, urban, and agricultural practices. While mowing is prevalent universally, it has been overlooked and does not have much research toward its effect on weeds.

A fundamental factor limiting our ability to manage weeds, is the lack of mechanistic understanding of how weed species interact with other organisms, at multiple trophic levels. In addition to traits that enhance their fitness and dispersal ability, the characteristics of weeds that either positively or negatively impact species interactions needs to be better resolved using both ecological and molecular tools (Campbell et al. 2013). For example, weeds in Greece have been found to be an important component of pest management strategies, since they can alternate hosts, act as overwintering hosts, and refuge major and minor pest species (Krigas et al. 2021; Kasper et al., 2021; Davis et al., 2020). These interactions can not only scale up to community levels but can also be examined through the angle of host location, host defenses and the factors affecting them (Portman et al., 2013**)**. And, making it more complex to resolve, genetic variation, plasticity in mating system, asexual reproduction and clonality, induced defenses through insect herbivory, disturbance or mechanical wounding can all play a significant role in these interactions (Kariyat et al. 2011, 2013; Portman et al. 2015)**.** Weeds in urban and agriculturally managed systems have been documented to have increased weed success as a result of land and soil disturbances resulting from human environmental interaction (Lozon and MacIsaac 1997, DiTomaso 2017). Clearing and draining promote erosion and damage non-weedy vegetation; however, that human effect is not pronounced on weeds (Goslee et al. 2001). A similar story was concluded by Chavana et al. 2021, finding that the noxious weed *Solanum elaeagnifolium* had been improving its fitness and defense traits as an effect of continuous mowing. When comparing unmowed and mowed *Solanum elaeagnifolium* weeds, we found that while unmowed genets produced more fruits, seeds from mowed genets were significantly heavier, and in correspondence with Fried et al. 2020, germination played a large role and in mowed plants was higher than in unmowed plants. Similarly, a study on the weed *Crepis sancta* showed germination and dispersal being again affected by human urban environments in the form of weeds growing in undisturbed areas and weeds growing on sidewalks and within the city (Cheptou et al. 2008). Putting fitness aside, plant defenses have also been studied concerning weed species. Weeds have been studied to show extraordinary adaptations to disturbance such as multiple species (common lambsquarters, field pennycress, giant foxtail, kochia) increasing their chemical defenses in an inverse relation with their disturbed or undisturbed seedbank, and vice-versa with their physical defenses (Davis et al. 2009). Although a large swath of information toward weed fitness and defense as a result of disturbance has been studied; however, most of these studies are limited and ignore floral traits affecting fitness and defenses.

Traditional plant fitness traits such as number of fruits, number of seeds, seed mass have already been studied in the Lower Rio Grande Valley (Kariyat and Chavana, 2018; Chavana et al., 2021; Kasper et al., 2021) by us, and therefore our trait identification lies elsewhere (Bigio and Yuval 2016). One study looked at the effect of herbivory on *Mimulus guttatus* and identified flower length as a key trait in growth and fitness being affected (Ivey and Carr 2005). Additionally, the effect of disturbances on herbivores requires further study because although Kariyat et al. 2012 looked at intraspecific variation in *Solanum carolinense* and found that experimental inbreeding affected the recruitment of herbivores and natural enemies in the field by selectively improving fitness when compared to inbred through better defenses, this line of research must be further expanded into anthropogenic disturbance.

The interest in using a weed species such as *S. elaeagnifolium* as the focal species- to understand disturbance through continuous mowing, stems from the knowledge we have generated using congeners and even *S. elaeagnifolium* over the past years. Previous studies have collectively found that SLN performs better under disturbance, has affinity to specific soil types, is a host for multiple herbivores that specialize on Solanaceae (also has economically important species), has a mixed mating system, but are obligate out crossers, and have wide range of physical and chemical defenses (Krigas et al. 2021, Kariyat et al., 2019; Chavana et al. 2021). Using another similar study system, *S. carolinense* (horsenettle) studies have demonstrated that genetic variation (maternal families) and breeding system (inbred vs outbred) has impacts on multi-trophic interactions (Kariyat and Stephenson, 2019) that scale up from genes involved in defense and metabolic pathways to dispersal ability of specialist herbivores (Kariyat et al 2012a,b; 2013). More specifically, outbred progenies from different maternal families have continuously outperformed their inbred counterparts in growth and fitness (Kariyat et al., 2011; 2012a), are less susceptible to herbivores in field and lab studies (Kariyat et al., 2012a,b), possess higher constitutive and induced structural defenses (Kariyat et al., 2013a), and have the ability to selectively repel herbivores, and their ovipositing adults (Kariyat et al., 2013b), while attracting their predators and natural enemies (Kariyat et al. 2013). However, these studies have mostly resorted to pairwise comparisons, and have not tested whether other common stress factors can affect these interactions, at population levels, in their native habitat. Having access to their native habitat in south Texas, SLN was the best possible candidate for these studies, incorporating the results from all the groundwork done in the past (Petanidou et al., 2018; Chavana et al., 2021; Kasper et al., 2021).

To further investigate the effect of an anthropogenic disturbance, mowing, on floral fitness and defense traits, we used a combination of field studies and lab experiments with 6 mowed and 6 unmowed sub populations of *Solanum elaeagnifolium*, Silverleaf Nightshade (SLN), to ask the following questions: (1) Does mowing influence floral fitness and defense traits, (2) How does mowing affect field herbivory on floral parts (petals and anthers) on a damage scale, (3) How do mowed and unmowed plants affect herbivore (*Manduca sexta)* mass when the plant material is added to artificial *Lepidoptera* diet, and (4) Are these effects, if any, affected by the frequency of mowing undergone by the SLN populations at different times of the year?

We hypothesized that because of continuous mowing disturbance, mowed plants will exhibit lower floral fitness traits than unmowed plants, yet their defense traits and effect on herbivores will be more pronounced as an overcompensation adaptation to consistent stress in line with how plants response to stress induced by mechanical wounding and defense signaling. To answer herbivory questions, we utilized Tobacco hornworm (*Manduca sexta*), a specialist on Solanaceae. We also hypothesized that a higher frequency of mowing would compromise fitness and defense floral traits.

CHAPTER II

EXPERIMENTS

Materials and Methods

Study Populations and Plant Materials

For the experiments explained, all Solanum *elaeagnifolium*, Silverleaf Nightshade, plant and flower material has been collected from both mowed and unmowed areas from throughout the Rio Grande Valley and Hidalgo County (Mission, McAllen, Edinburg; Texas). These populations have been monitored over 4 years by Kariyat Lab and UTRGV and the mowed populations are on a fixed mowing schedule while the unmowed populations are undisturbed. The sampled populations are spatially close being less than 30 minutes away from each other.

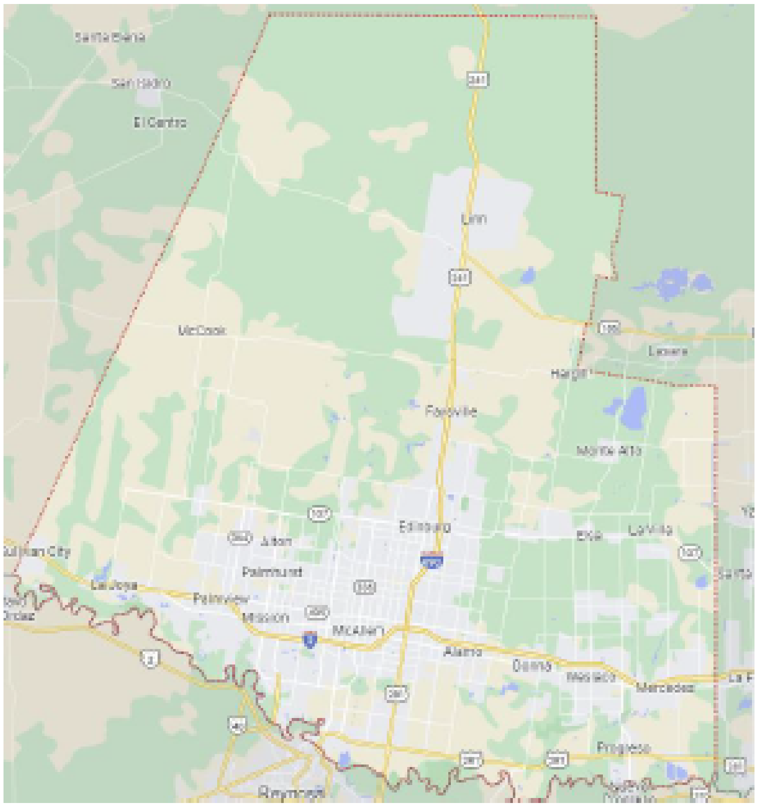

Figure 1. Hidalgo County Map (Google Maps, Hidalgo County, Texas, USA)

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Figure 2 (A) *Solanum elaeagnifolium* flowers in bloom in South Texas, (B) *S. elaeagnifolium* flower with herbivore damage on petals and poricidal anthers, (C) fifth instar *M. sexta* caterpillar, (D) Spines on *S. elaeagnifolium* pedicel.

Flower Collection

December 2021. In December 2021, flowers were collected from 6 populations (3 mowed populations: Trenton, Colorado, Duke; 3 unmowed populations: Bentsen, Red Lobster, Walmart- all populations were close to each other) from the fields previously mentioned. From mowed fields, 253 flowers were collected from 77 unique plants. From unmowed fields, 234 flowers were collected from 71 unique plants. Capable of vegetative reproduction, a form of asexual reproduction undergone by *S. elaeagnifolium* via rhizomatic roots, unique plants were considered plants more than 3 meters apart to minimize the risk of sampling cloned plants. Flowers were all collected during the same week (all sampling within 3 days) and during the same time of day
(8:00-9:00A.M.) and stored in Ziploc bags until they were left to dry in ambient laboratory conditions for 48 hours to remove excess water. Having been collected in December 2021, these SLN populations have been mowed at a high frequency.

June 2022 . In June 2022, flowers were collected again from 6 populations (3 mowed populations: Trenton, Colorado, Duke; 3 unmowed populations: Bentsen, Red Lobster, Walmart- all populations were close to each other) from the fields previously mentioned.
From mowed fields, 180 flowers were collected and from unmowed fields, 172 flowers were collected. Flowers were all collected during the same week (all sampling within 4 days) and during the same time of day (8:00-9:00A.M.) and stored in Ziploc bags until they were left to dry in ambient laboratory conditions for 48 hours to remove excess water. Having been collected in June 2022, these populations have been mowed at a low frequency.

Flower Data

Floral Diameter . All flowers were laid on a flat surface (anthers facing upwards to prevent damage to them) and their petal diameter (longest point) measured evenly and recorded in centimeters.

Floral Weight. All flowers were weighed after 48 hours of drying to obtain dry mass. For December 2021 flowers, anthers were removed and then weighed, as were petals for separate weights of each part.

Floral Anther Damage. All flowers were examined and assessed for damage on their anthers. Anther damage was assessed on a binary system, 0 for no damage and 1 for damage. Anther damage was categorized as damage on filament or anther sections of the stamen.

Floral Petal Damage. All flowers were examined and assessed for damage on their petals. Petal damage was assessed on an ordinal scale of 0-3;0 being no damage, 1 being damage is present but not major, 2 being moderate damage, and 3 being severe damage.

Manduca sexta Mass Gain Diet Experiments

High Mowing Frequency Mass Gain Diet Experiment. Artificial *Lepidoptera* diets were prepared for rearing of *Manduca sexta* caterpillars. Artificial *Lepidoptera* diet consists of a wheat-based germ diet prepared to specifications by suppliers (General Purpose Lepidoptera Diet (Product Code: F9772, Frontier Agricultural Sciences, Newark, DE,USA). 900mL of water was heated in an iron cooking pot on a hot plate with mechanical stirring until boiling, followed by the addition of 144g of General Purpose Lepidoptera Diet added in slowly to be thoroughly mixed without clumping. Once thoroughly mixed, 8g Agar powder was added into the mixture and mixed thoroughly again. The completed mixture was added to plastic Sterilite 6-Quart Storage Boxes (Walmart; Bentonville, AR, USA) and left at room temperature for 4 hours for cooling before being refrigerated. Alongside this control diet, 12 additional diets were prepared using the plant material collected previously. Diets were prepared following the above specifications; however, each diet had added plant material relating to a population. Of the 12 diets prepared, 6 diets were made from unmowed plant material, and 6 diets from mowed plant material. Of the 6 diets made from unmowed plant material, 3 were prepared from anthers and 3 were prepared from petals, and these methods were followed for mowed plant material diets as well. Anthers were removed from flowers carefully, weighed, and crushed using a mortar for 30 minutes to create a very fine powder. This fine powder was added to the *Lepidoptera* diet mixture once cool to prevent the fine powder from being broken down by heat, mixed thoroughly, and stored. The same procedure was followed for petal plant material diets.

Manduca sexta eggs were purchased (GreatLake Hornworms, MN, USA) and remained in the laboratory for 48 hours before being separated into plastic containers labeled with each diet type (12 diets, 1 control diet, N=390). 30 caterpillar eggs were placed in 2 plastic containers Sterilite 6-Quart Storage Boxes (Walmart; Bentonville, AR, USA) and fashioned with cardboard cuttings into cubicles, and labeled by caterpillar number (1-30) for data recording. Mass data was recorded every day at approximately the same time, morning, for 14 days for every caterpillar.

Plastic containers were lined with double-stacked paper towels cleaned every 2 days, and each caterpillar egg was placed on a fresh block of diet ($1cm^3$). Diet was taken out of the refrigerator 24 hours before use to diminish cold shock effects and replaced every 2 days (Methodology for diets and treatments were based on our previous studies and can be found at Watts and Kariyat, 2021; Singh and Kariyat, 2020; Tayal et al., 2020).

Low Mowing Frequency Mass Gain Diet Experiment. Artificial *Lepidoptera* diets were prepared for rearing of *Manduca sexta* caterpillars. Artificial *Lepidoptera* diet consists of a wheat-based germ diet prepared to specifications by suppliers (General Purpose Lepidoptera Diet (Product Code: F9772,Frontier Agricultural Sciences, Newark, DE,USA). Diet was prepared as previously stated for the December Diet Experiment. Only 7 diets were prepared for the June 2022 Diet Experiment (3 mowed, 3 unmowed, 1 control) and grinded plant material consisted of both anthers and petals for all treatments excluding the control treatment. Hatching rates of eggs was also recorded.

Manduca sexta eggs were purchased (GreatLake Hornworms, MN, USA) and remained in the laboratory for 48 hours before being separated into plastic containers labeled with each diet type (6 diets, 1 control diet, N=210). 30 caterpillar eggs were placed in 2 plastic containersSterilite 6-Quart Storage Boxes (Walmart; Bentonville, AR, USA) and fashioned with cardboard cuttings into cubicles, and labeled by caterpillar number (1-30) for data recording. Mass data was recorded every day at approximately the same time, morning, for 14 days for every caterpillar. Plastic containers were lined with double-stacked paper towels cleaned every 2 days, and each caterpillar egg was placed on a fresh block of diet ($1cm^3$). Diet was taken out of the refrigerator 24 hours before use to diminish cold shock effects and replaced every 2 days. Hatching rate of eggs was also recorded.

Spine Defenses

Flowers were again collected from the same 3 mowed and 3 unmowed fields sampled in June 2022. Flowers were collected randomly and in a uniform manner in which they were all cut from their plant at the first branching area to control for stem length. Flowers were taken back to the lab and spines were counted visually via hand-tally counter from the receptacle of the flower to the end of the stem. This length was then measured to generate data based on number of spines, length of stem, and spines per unit of length (Kariyat et al., 2017). Afterwards, plant materials were disposed of.

Polyphenol Oxidase (PPO) Assay

To test whether the flowers from mowed and unmowed different in chemical defenses, we also measured their polyphenol oxidase activity- commonly used as a proxy for chemical defenses[27]. We quantified PPO content (U/mg) in SLN for the low frequency of mowing populations (3 mowed, 3 unmowed) using flower tissue samples (n=8 per plant) from 3 separate genets from each field. The PPO assay performed as described in the Polyphenol Oxidase Assay Kit manual (Catalog#MBS822343; MyBioSource) with accordance with Watts and Kariyat 2022.

Quantification of PPO was performed using the equation in the Polyphenol Oxidase Assay Kit

$$PPO\ (U/g) = (ODSample - ODControl) \times VTotal / (W \times VSample / VAssay) / 0.01 / T = 233.3 \times (ODSample - ODControl) / W$$

Where OD stands for calorimetric readout of optical density at 410 nm, VTotal is the volume of sample (0.35 ml), W is weight of the sample (0.1 g of plant tissue), VSample is the volume of sample (0.05 ml), VAssay is the volume of Assay buffer (1 ml) and T is the reaction time (3 minutes).

Statistical Analyses

Except for spine density analyses, our floral traits response statistical model had three factors: population, treatment (mowed vs umowed), and mowing frequency. We pooled the populations and focused on treatment and frequency (focus of the experiment) and used Analysis of Variance for continuous variables and Ordinal logistic regression for discrete scale data. This includes both floral traits data and artificial diet data sets. Pairwise post hoc comparisons were carried our using Tukey's test. Full models with population as an additional factor for analyses, and their results are available in Supplementary data. Similarly, for caterpillar mass, early instar (day 1-5) and late instar (day 6-13) we used Anova with Tukey comparisons carried out to determine pairwise differences between control, mowed, or unmowed and low and high mowing frequencies. Although each population and treatment had separate diet made, similar to field traits, we pooled the populations to focus on treatments. Spine density was analyzed using T-Tests to determine the difference between mowed and unmowed treatments, since we only collected that data once. Polyphenol Oxdiase (PPO) was analyzed using Mann-Whitney's nonparametric test to determine difference between mowed and unmowed treatments. Full statistical models and their details are presented in Supplementary table 1.

CHAPTER III

RESULTS

Flower Data

Floral Diameter

Analyses of floral diameter between mowed (x̄=2.726cm, SEM=0.033cm) and unmowed (x̄=2.627cm, SEM=0.0283) flowers show significantly larger flowers from mowed plants when compared to flowers from unmowed plants (T-Test: P=0.0012, Fig 2A). Analyses of floral diameter between low (x̄=2.179cm, SEM=0.021) and high (x̄=3.039cm, SEM=0.026) mowing frequency plants show significantly larger flowers from high mowing frequency plants when compared to flowers from low mowing frequency (T-Test: P<0.0001, Fig 2B).

Floral Mass.

Analyses of floral mass between mowed (x̄=0.0266cm, SEM=0.0004) and unmowed (x̄=0.0316, SEM=0.0012) flowers show significantly heavier flowers from unmowed plants when compared to flowers from mowed plants (T-Test: P<0.0001, Fig 3A). Analyses of floral mass between low (x̄=0.0215, SEM=0.0003) and high (x̄=0.0344, SEM=0.001) mowing frequency plants show significantly heavier flowers from high mowing frequency plants when compared to flowers from low mowing frequency (T-Test: P=0.0308, Fig 3B)

Anther Damage.

Anthers from the flowers of unmowed plants and mowed plants had no significant difference in damage incidence (Logistic Regression; P=0.2073). Regarding mowing frequency, there was also no significant difference between low and high frequency of mowing flowers (Logistic Regression; P=0.8390).

Petal Damage

Petals from the flowers of unmowed plants had significantly more damage (0-3 scale) on petals than mowed plants (Ordinal Logistic Regression: P<0.0001, Fig 4A). There was no significant difference in petal damage between petals of high mowing frequency flowers and low mowing frequency flowers (Ordinal Logistic Regression: P=0.2817, Fig 4B)

Manduca sexta Mass Diet Experiments

Early Instar Mass

Analyses of *M. sexta* mass in early instars (Day 1-5) show significant differences between caterpillars fed on unmowed diets (x̄= 3.230mg, SEM=0.224) and caterpillars fed on mowed (x̄=2.631mg, SEM=0.183) and control (x̄=3.074mg, SEM=0.226) diets with caterpillars fed on unmowed diets being significantly heavier than both mowed and control caterpillars (ANOVA: P<0.0001, Fig 5A). With regard to frequency, early instar *M. sexta* fed on low (x̄= 6.780mg, SEM=0.355) frequency of mowing diets were significantly heavier than those fed on high frequency of mowing diets (x̄=1.308mg, SEM=0.0418) (T-Test: P<0.0001, Fig 5B).

Late Instar Mass

Analyses of *M. sexta* mass in late instars (Day 6-13) show no significant differences between caterpillars fed on unmowed (x̄543.8mg, SEM=18.23), mowed (x̄=492.6mg, SEM=20.48), and control (x̄=444.5mg, SEM=35.30) (ANOVA: P=0.6315, Fig 5C). With regard to frequency, late instar *M. sexta* fed on high (x̄= 650.6mg, SEM=15.84) frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets ($\bar{x}$=132.3mg, SEM=6.905) (ANOVA: P<0.0001, Fig 5D).

Day 1 Mass

Analyses of *M. sexta* mass on day 1 show no significant differences between caterpillars fed on unmowed, mowed, and control diets (ANOVA: P=0.0743, Fig 5E). With regard to frequency, day 1 *M. sexta* that fed on low frequency of mowing diets were significantly heavier than those fed on high mowing frequency diets (ANOVA: P<0.0001, Fig 5F).

Day 2 Mass and Mass Gain

Analyses of *M. sexta* mass on day 2 show no significant differences between caterpillars fed on unmowed, mowed, and control diets (ANOVA: P=0.7037, Fig 5G). With regard to frequency, day 2 *M. sexta* that fed on low frequency of mowing diets were significantly heavier than those fed on high mowing frequency diets (ANOVA: P<0.0001, Fig 5H). Mass gain of *M. sexta* between Day 1 and 2 was not significant for caterpillars fed on control, mowed, and unmowed diets (ANOVA: P=0.4932, Fig 6A). With regard to frequency, mass gain between day 1 and 2 was significant with caterpillars fed on low frequency of mowing diets gaining significantly more mass than high mowing frequency diets (ANOVA: P<0.0001, Fig 6B).

Day 3 Mass and Mass Gain

Analyses of *M. sexta* mass on day 3 show caterpillars fed on control diets being significantly heavier than caterpillars fed on mowed and unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets (ANOVA: P=0.0349, Fig 5I). With regard to frequency, day 3 *M. sexta* that fed on low frequency of mowing diets were significantly heavier than those fed on high mowing frequency diets (ANOVA: P<0.0001, Fig 5J). Mass gain of *M. sexta* between Day 2 and 3 was not significant for caterpillars fed on control, mowed, and unmowed diets (ANOVA: P=0.7111, Fig 6C). With regard to frequency, mass gain between day 2 and 3 was significant with caterpillars fed on high frequency of mowing diets gaining significantly more mass than low mowing frequency diets (ANOVA: P=0.0224, Fig 6D).

Day 5 Mass and Mass Gain

Analyses of *M. sexta* mass on day 5 show caterpillars fed on control diets having significantly less mass than caterpillars fed on mowed and unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets(ANOVA: P<0.0001, Fig 5K). With regard to frequency, day 5 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5L). Mass gain of *M. sexta* between Day 3 and 5 was not significant for caterpillars fed on control diets, but caterpillars fed on unmowed diets were significantly heavier than caterpillars fed on mowed diets. (ANOVA: P=0.0160, Fig 6E). With regard to frequency, mass gain between day 3 and 5 was not significant with caterpillars fed on high frequency of mowing diets and low mowing frequency diets (ANOVA: P=0.5168, Fig 6F).

Day 6 Mass and Mass Gain

Analyses of *M. sexta* mass on day 6 show caterpillars fed on control diets having significantly less mass than caterpillars fed on mowed and unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets (ANOVA: P<0.0001, Fig 5M). With regard to frequency, day 6 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5N). Mass gain of *M. sexta* between Day 5 and 6 was not significant for caterpillars fed on control and unmowed diets, yet caterpillars on mowed diets were significantly heavier than control and unmowed diet-fed caterpillars. (ANOVA: P=0.0021, Fig 6G). With regard to frequency, mass gain between day 5 and 6 was significant with caterpillars fed on high frequency of mowing diets gaining significantly more mass than low mowing frequency diets (ANOVA: P<0.0001, Fig 6H).

Day 7 Mass and Mass Gain

Analyses of *M. sexta* mass on day 7 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on mowed and unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets (ANOVA: P=0.0003, Fig 5O). With regard to frequency, day 7 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5P). Mass gain of *M. sexta* between Day 6 and 7 was not significant for caterpillars fed on control, mowed, and unmowed diets (ANOVA: P=0.9246, Fig 6I). With regard to frequency, mass gain between day 6 and 7 was significant with caterpillars fed on high frequency of mowing diets gaining significantly more mass than low mowing frequency diets (ANOVA: P=0.0110, Fig 6J).

Day 8 Mass and Mass Gain

Analyses of *M. sexta* mass on day 8 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on mowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets and control diets(ANOVA: P<0.0001, Fig 5Q). With regard to frequency, day 8 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P=0.0001, Fig 5R). Mass gain of *M. sexta* between Day 7 and 8 was not significant for caterpillars fed on control, mowed, and unmowed diets (ANOVA: P=0.0777. Fig

6K). With regard to frequency, mass gain between day 7 and 8 was significant with caterpillars fed on low frequency of mowing diets gaining significantly more mass than high mowing frequency diets (ANOVA: P=0.0004, Fig 6L).

Day 9 Mass and Mass Gain

Analyses of *M. sexta* mass on day 8 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on mowed diets and unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets (ANOVA: P<0.0001, Fig 5S). With regard to frequency, day 9 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5T). Mass gain of *M. sexta* between Day 8 and 9 was not significant for caterpillars fed on control and unmowed diets, yet caterpillars on mowed diets were significantly less heavy than control and unmowed diet-fed caterpillars. (ANOVA: P<0.0001, Fig 6M). With regard to frequency, mass gain between day 8 and 9 was significant with caterpillars fed on low frequency of mowing diets gaining significantly more mass than high mowing frequency diets (ANOVA: P<0.0001, Fig 6N).

Day 10 Mass and Mass Gain

Analyses of *M. sexta* mass on day 10 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on mowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets and control diets (ANOVA: P<0.0001, Fig 5U). With regard to frequency, day 10 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5V). Mass gain of *M. sexta* between Day 9 and 10 was not significant for caterpillars fed on control, mowed, and unmowed diets. (ANOVA: P=0.4806, Fig

6O). With regard to frequency, mass gain between day 9 and 10 was significant with caterpillars fed on low frequency of mowing diets gaining significantly more mass than high mowing frequency diets (ANOVA: P=0.0045, Fig 6P).

Day 11 Mass and Mass Gain

Analyses of *M. sexta* mass on day 11 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets(ANOVA: P<0.0001, Fig 5W). With regard to frequency, day 11 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5X). Mass gain of *M. sexta* between Day 10 and 11 was not significant for caterpillars fed on control, mowed, and unmowed diets (ANOVA: P=0.1634, Fig 6Q). With regard to frequency, mass gain between day 10 and 11 was significant with caterpillars fed on high frequency of mowing diets gaining significantly more mass than low mowing frequency diets (ANOVA: P<0.0001, Fig 6R).

Day 12 Mass and Mass Gain

Analyses of *M. sexta* mass on day 12 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets(ANOVA: P<0.0001, Fig 5Y). With regard to frequency, day 12 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5Z). Mass gain of *M. sexta* between Day 11 and 12 was not significant for caterpillars fed on control, mowed, and unmowed diets. (ANOVA: P=0.8200, Fig 6S). With regard to frequency, mass gain between day 11 and 12 was significant with caterpillars fed on high frequency of mowing diets gaining significantly more mass than low mowing frequency diets (ANOVA: P<0.0001, Fig 6T).

Day 13 Mass and Mass Gain

Analyses of *M. sexta* mass on day 13 show caterpillars fed on control diets having no significant difference in mass than caterpillars fed on unmowed diets, and caterpillars fed on unmowed diets being significantly heavier than caterpillars fed on mowed diets (ANOVA: P<0.0001, Fig 5i). With regard to frequency, day 13 *M. sexta* that fed on high frequency of mowing diets were significantly heavier than those fed on low mowing frequency diets (ANOVA: P<0.0001, Fig 5ii). Mass gain of *M. sexta* between Day 12 and 13 was not significant for caterpillars fed on control, mowed, and unmowed diets. (ANOVA: P=8981, Fig 6U). With regard to frequency, mass gain between day 12 and 13 was significant with caterpillars fed on low frequency of mowing diets gaining significantly more mass than high mowing frequency diets (ANOVA: P=0.0029, Fig 6V).

Spine Defenses

Number of Spines

Analyses of number of spines from receptacle to stem-end of mowed ($\bar{x}$=25.6989, SEM=1.1762) and unmowed ($\bar{x}$=13.955, SEM=1.2161) flowers show that mowed flowers have significantly more spines than unmowed flowers (ANOVA: P<0.0001, Fig 7A).

Length

Analyses of pedicel length of mowed ($\bar{x}$=1.57097, SEM=0.04622) and unmowed ($\bar{x}$=1.50115, SEM=0.04779) flowers show no difference in length between mowed and unmowed flowers (ANOVA: P=0.2950, Fig 7B).

Number of Spines per Unit Length

Analyses of number of spines per unit length of mowed ($\bar{x}=16.5693$, SEM=0.67189) and unmowed ($\bar{x}=8.8072$, SEM=0.69468) flowers show that mowed flowers have significantly more spines per unit length than unmowed flowers (ANOVA: $P<0.0001$, Fig 7C).

Polyphenol Oxidase (PPO)

Analyses of Polyphenol Oxidase shows that PPO activity between mowed and unmowed flowers from low mowing frequency SLN was not significantly different (Mann Whitney: $P=0.5039$, Fig 8).

Table 1. Statistical details for the effect of mowed, unmowed, high, and low frequency of mowing on *S. elaeagnifolium* floral diameter, floral mass, *M. sexta* mass, and spine density. The two factors of interest were treatment and frequency. *P* values <0.05 are in boldface.

Trait	***Source of Variation***	***df***	***SS***	***F***	***P***
Floral Mass	Treatment (Mowed/Unmowed)	1	0.01645	67.1497	**<0.0001**
	Frequency (High/Low)	1	0.001146	4.6777	**0.0308**
Floral Diameter	Treatment (Mowed/Unmowed)	1	1.58445	10.5446	**0.0012**
	Frequency (High/Low)	1	12.7806	85.0557	**<0.0001**
Early Instar Mass Day 1-5	Treatment (Mowed/Unmowed/Control)	2	713.363	23.5448	**<0.0001**

Table 1, cont.

	Frequency (High/Low)	1	5679.631	374.9166	<**0.0001**
Late Instar Mass Day 6-13	Treatment (Mowed/Unmowed/Control)	2	277439	0.4598	0.6315
	Frequency (High/Low)	1	99100097	328.4680	<**0.0001**
Spine Density	Treatment (Mowed/Unmowed)	1	2708.3071	64.5082	<**0.0001**
			L-R ChiSquare		
Petal Damage	Treatment (Mowed/Unmowed)	1	14.44474		<**0.0001**
	Frequency (High/Low)	1	1.158853		0.2817

Table 1, cont.

Anther Damage	Treatment (Mowed/Unmowed)	1	1.589976		0.0273
	Frequency (High/Low)	1	0.04125		0.8390

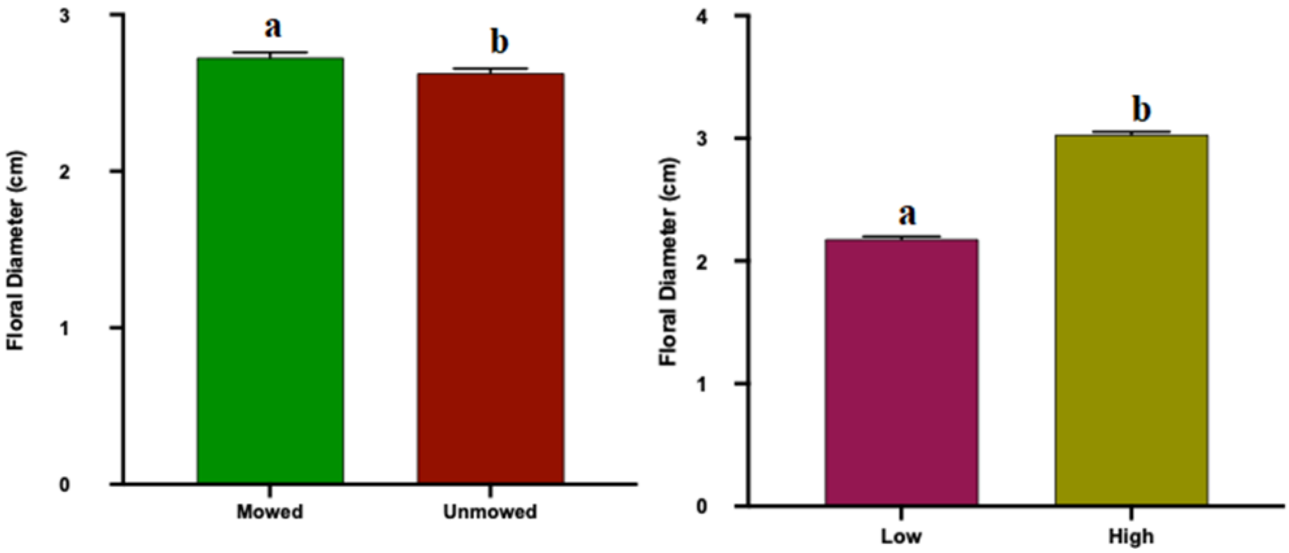

Figure **3.** Mean diameter of flowers for (A) mowed and unmowed treatments on field collected *Solanum elaeagnifolium* (**B)**. low mowing frequency and high mowing frequency treatments. Different letters indicate significant differences among mowed and unmowed treatments or low and high mowing frequency determined by post hoc analyses using Tukey's test (P<0.05).

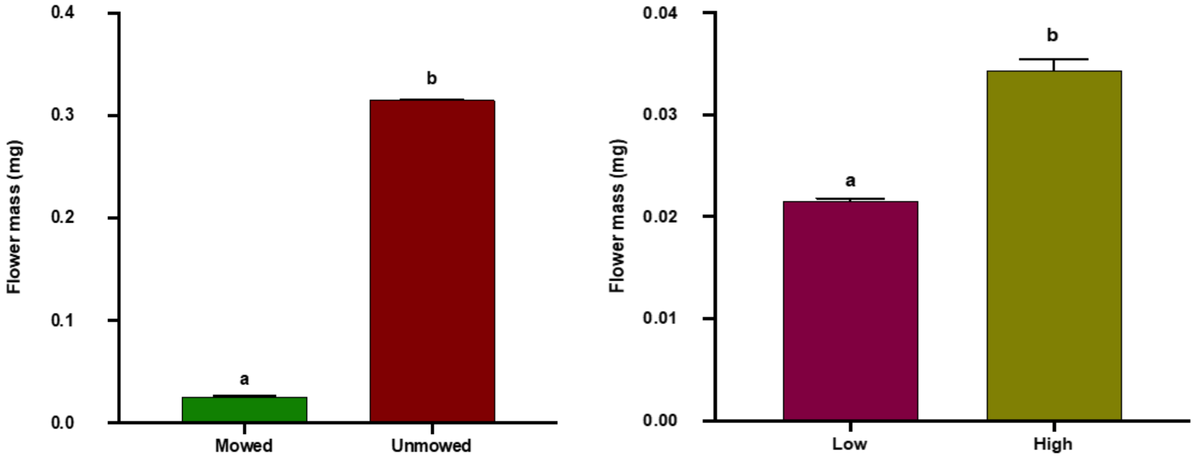

Figure **4**. Mean mass of flowers from (A) mowed and unmowed treatments on field collected *Solanum elaeagnifolium.* (B) Mean mass of flowers from low and high mowing frequency treatments on field collected *Solanum elaeagnifolium.* Different letters indicate significant differences among mowed and unmowed treatments or low and high mowing frequency determined by post hoc analyses using Tukey's test (P<0.05).

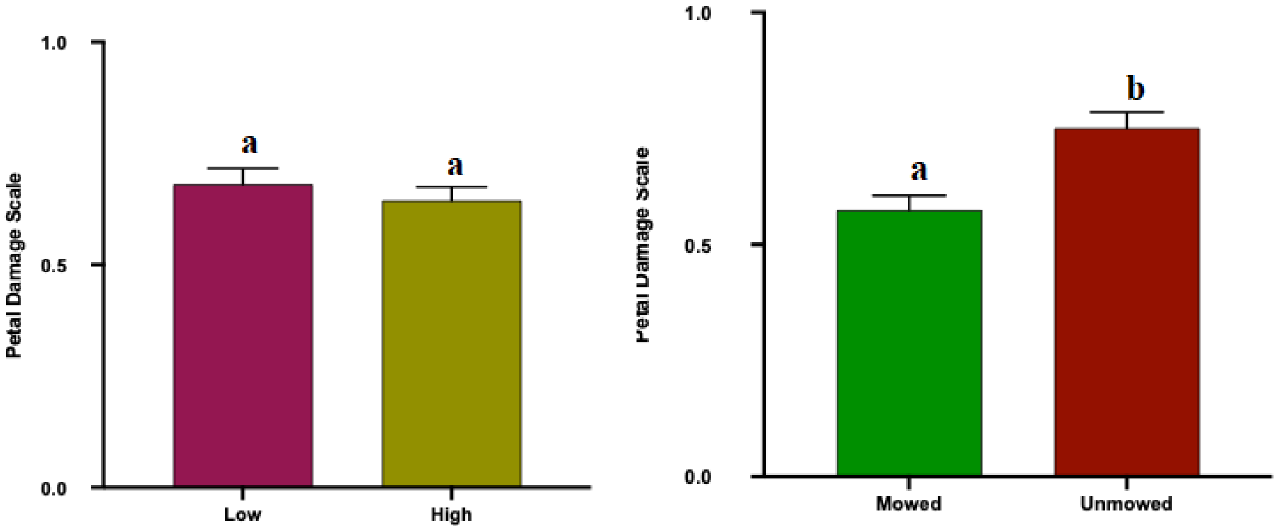

Figure **5**. Mean damage on 0-3 scale of (A) flowers from mowed and unmowed treatments on field collected *Solanum elaeagnifolium.* (B**)** flowers from low and high mowing frequency treatments on field collected *Solanum elaeagnifolium*. Different letters indicate significant differences among mowed and unmowed treatments or low and high mowing frequency determined by post hoc analyses using Tukey’s test (P<0.05).

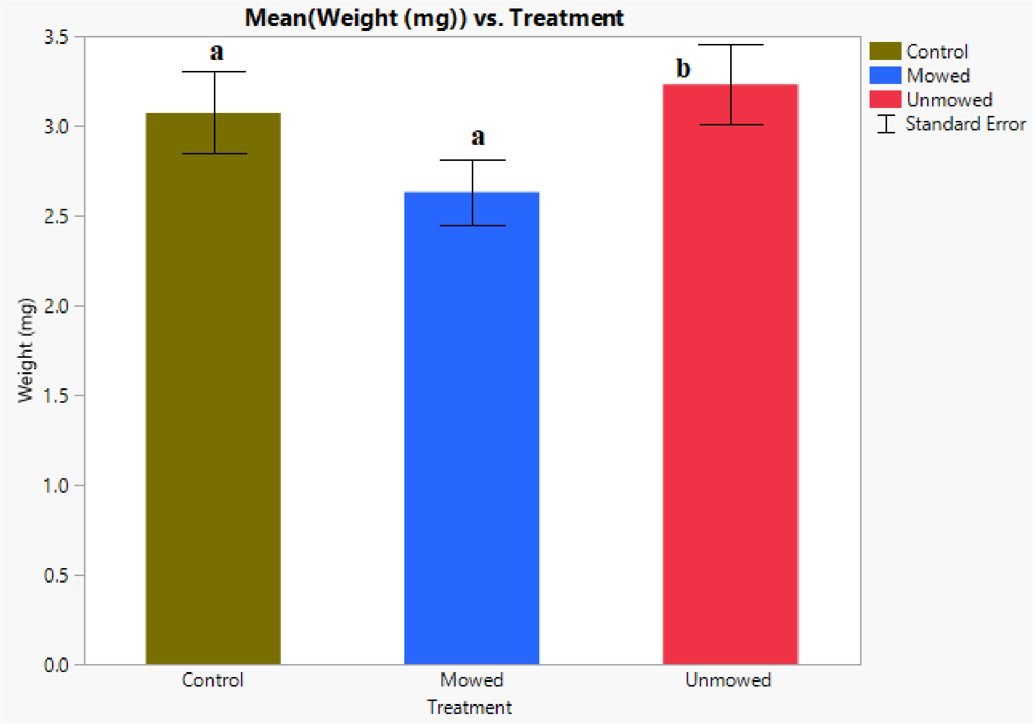

Figure 6A. Caterpillar Experiment- Mass of Early Instar Caterpillars based on Diet in Control vs Mowed vs Unmowed diets. (P<0.0001)

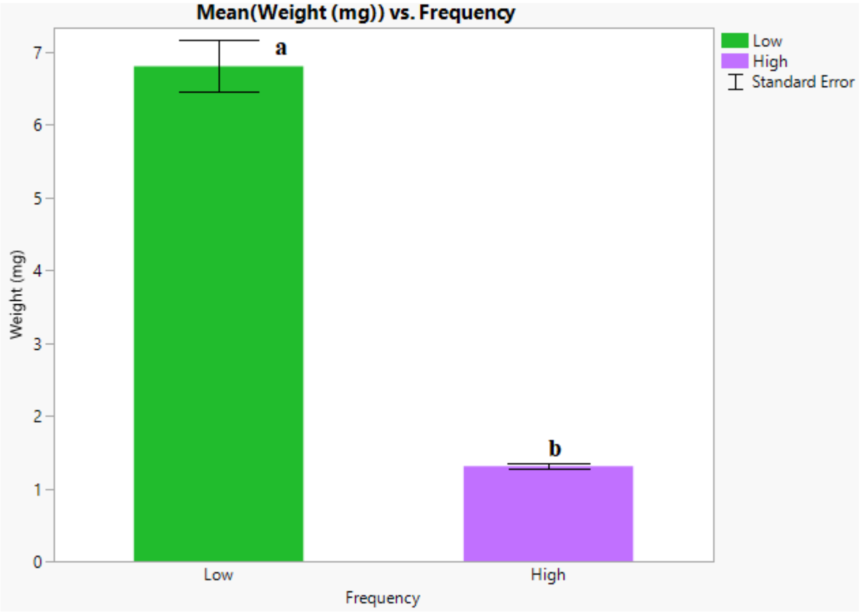

Figure 6B. Caterpillar Experiment- Mass of Early Instar Caterpillars based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001)

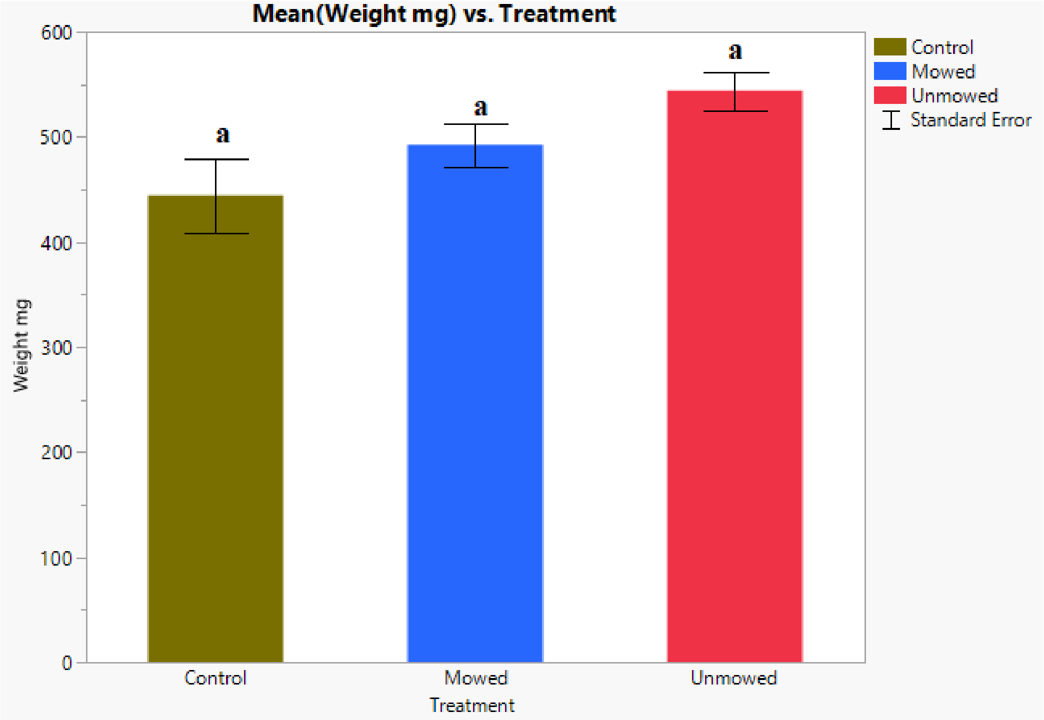

Figure 6C. Caterpillar Experiment- Mass of Late Instar Caterpillars based on Diet in Control vs Mowed vs Unmowed Diets (P=0.6315)

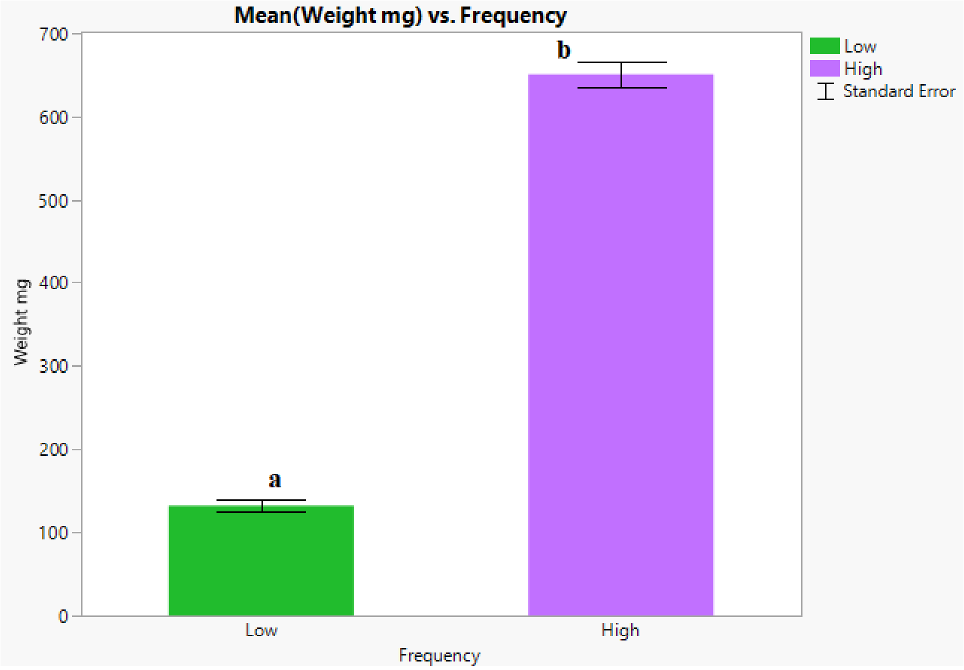

Figure 6D. Caterpillar Experiment- Mass of Early Instar Caterpillars based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001)

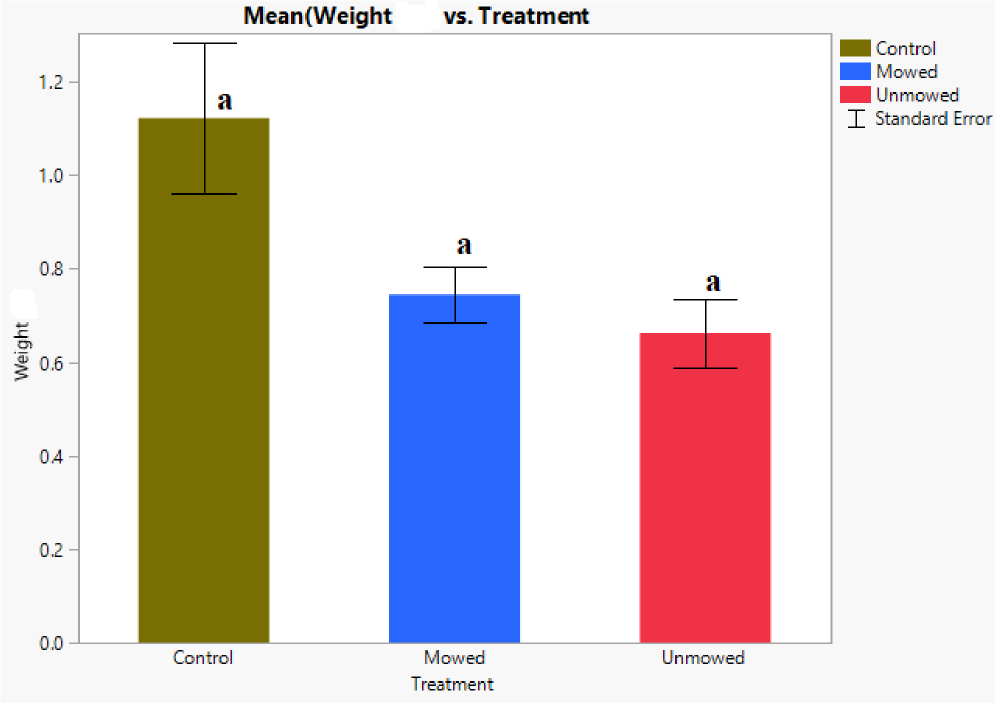

Figure 6E. Caterpillar Experiment- Mass on Day 1 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0743)

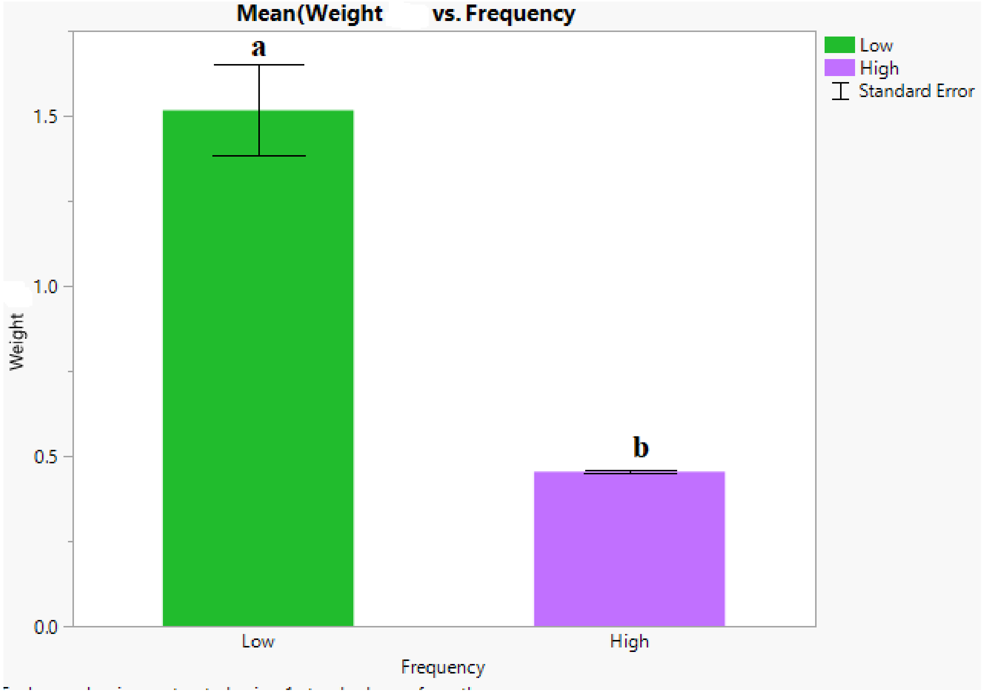

Figure 6F. Caterpillar Experiment Mass on Day 1 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

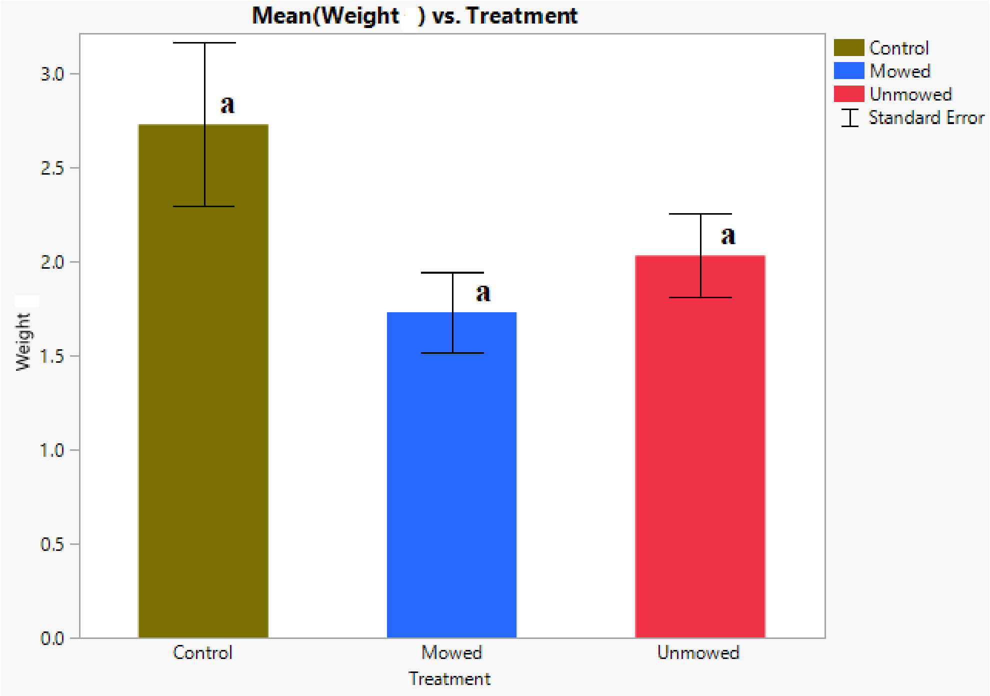

Figure 6G. Caterpillar Experiment- Mass on Day 2 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.7037)

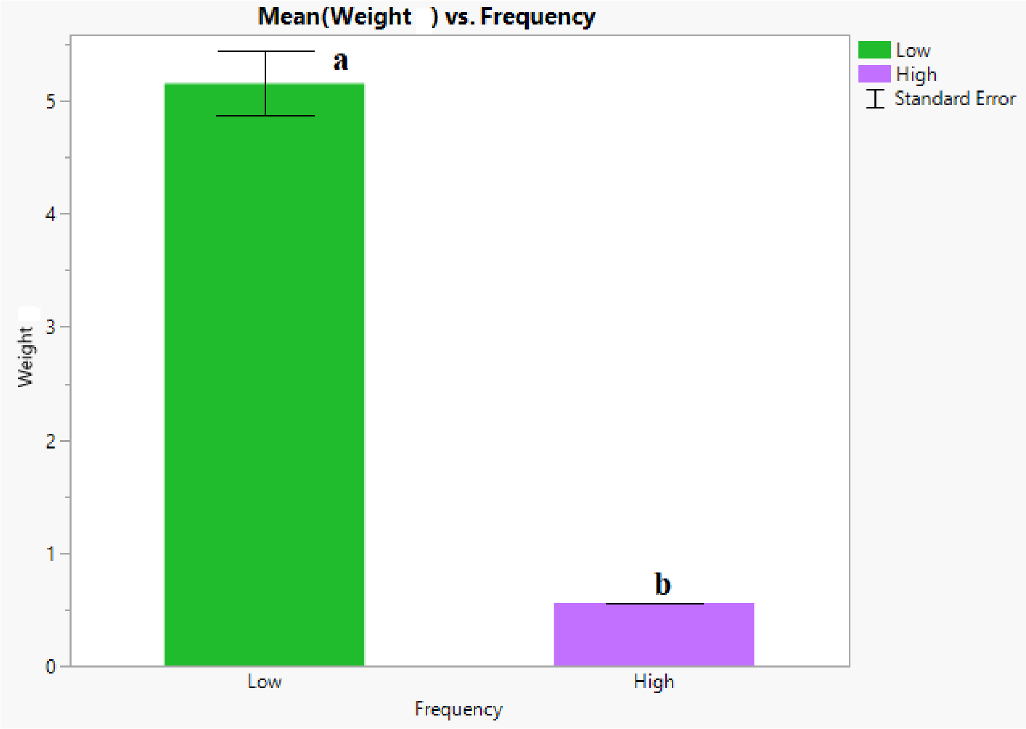

Figure 6H. Caterpillar Experiment Mass on Day 2 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

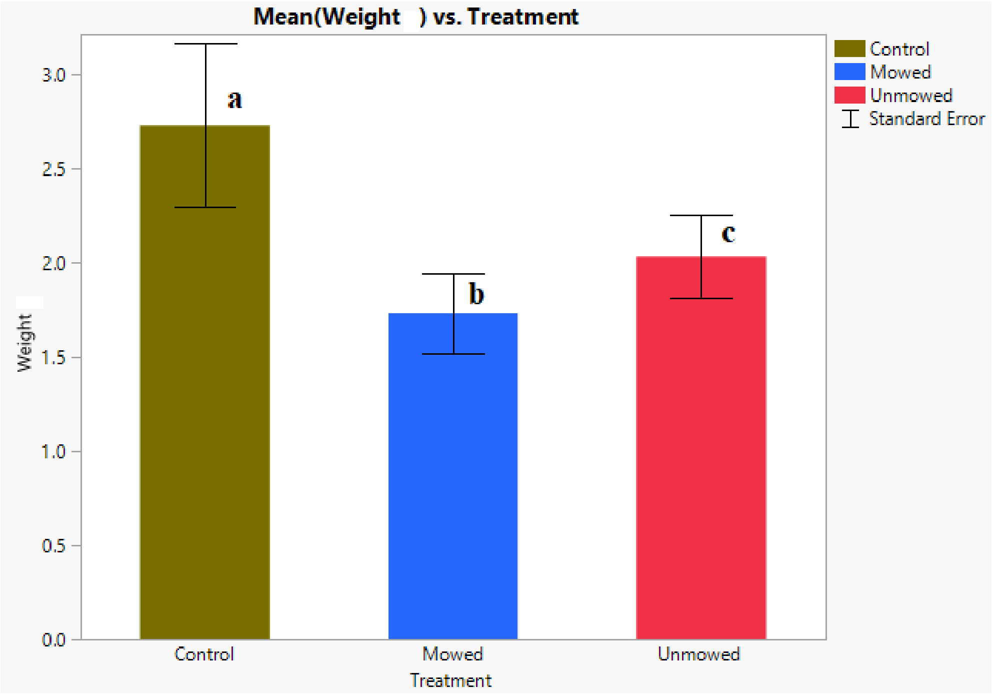

Figure 6I. Caterpillar Experiment- Mass on Day 3 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0349)

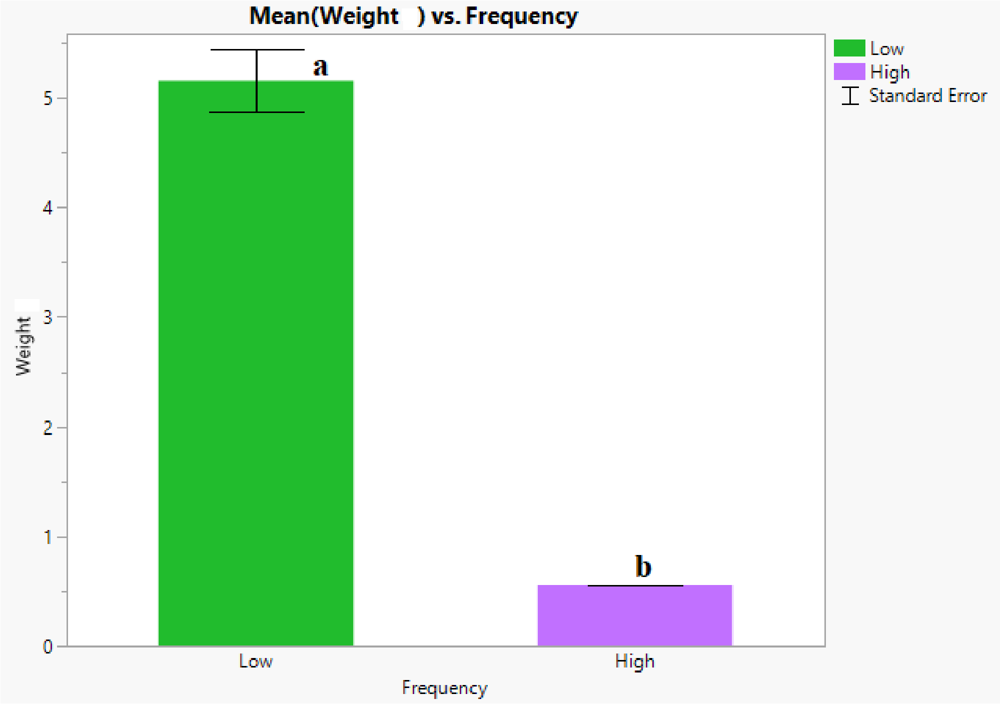

Figure 6J. Caterpillar Experiment Mass on Day 3 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

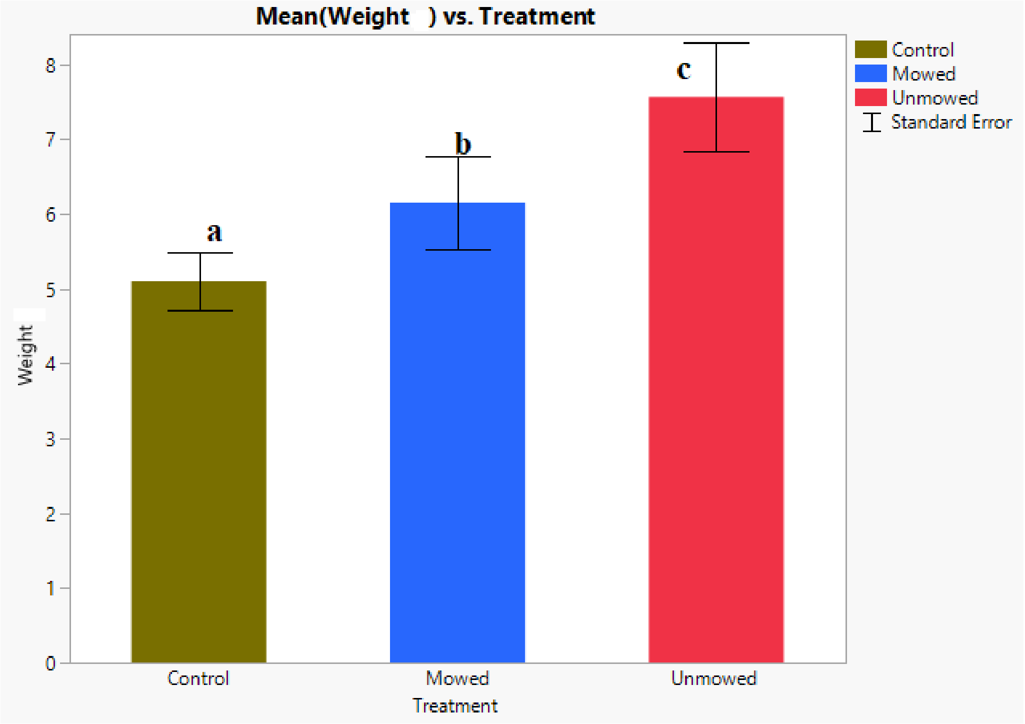

Figure 6K. Caterpillar Experiment- Mass on Day 5 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

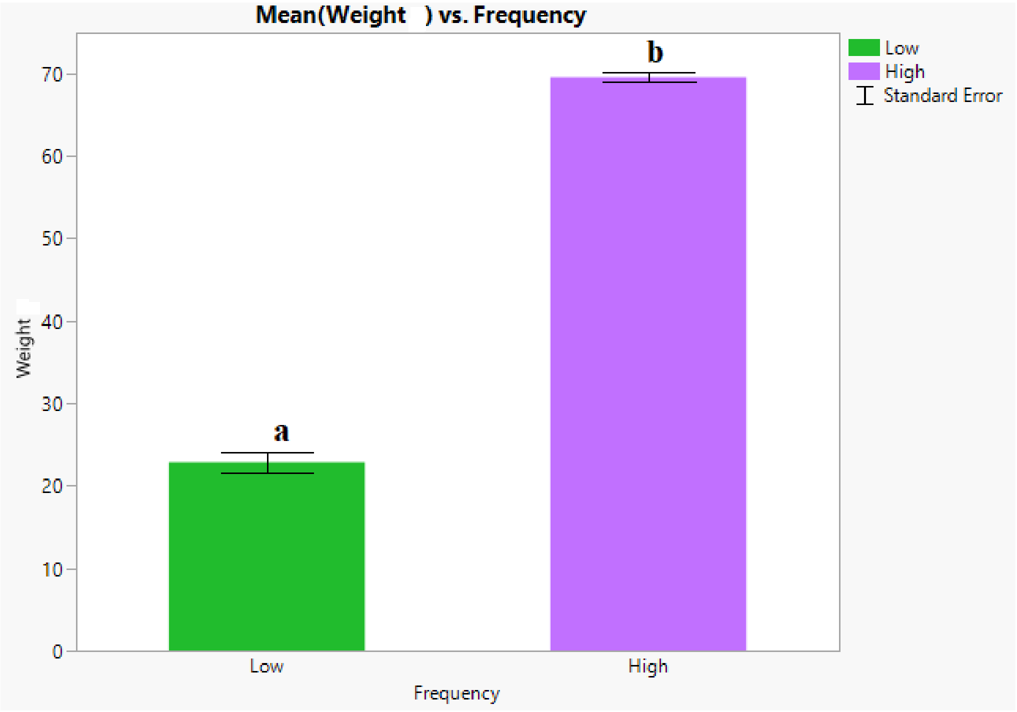

Figure 6L. Caterpillar Experiment Mass on Day 5 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

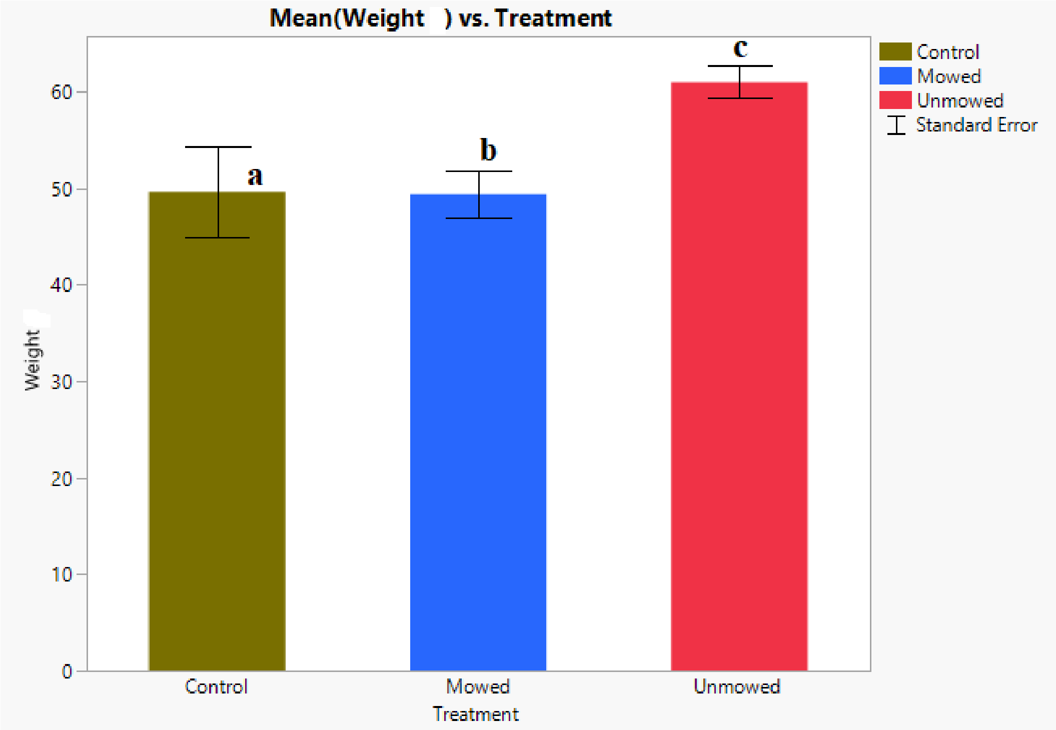

Figure 6M. Caterpillar Experiment- Mass on Day 6 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

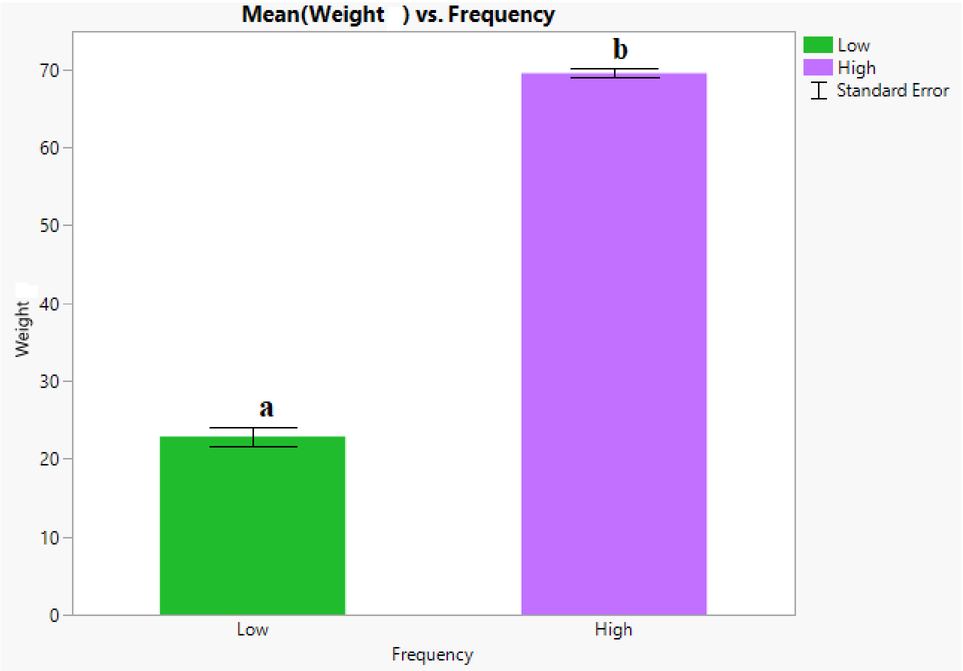

Figure 6N. Caterpillar Experiment Mass on Day 6 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

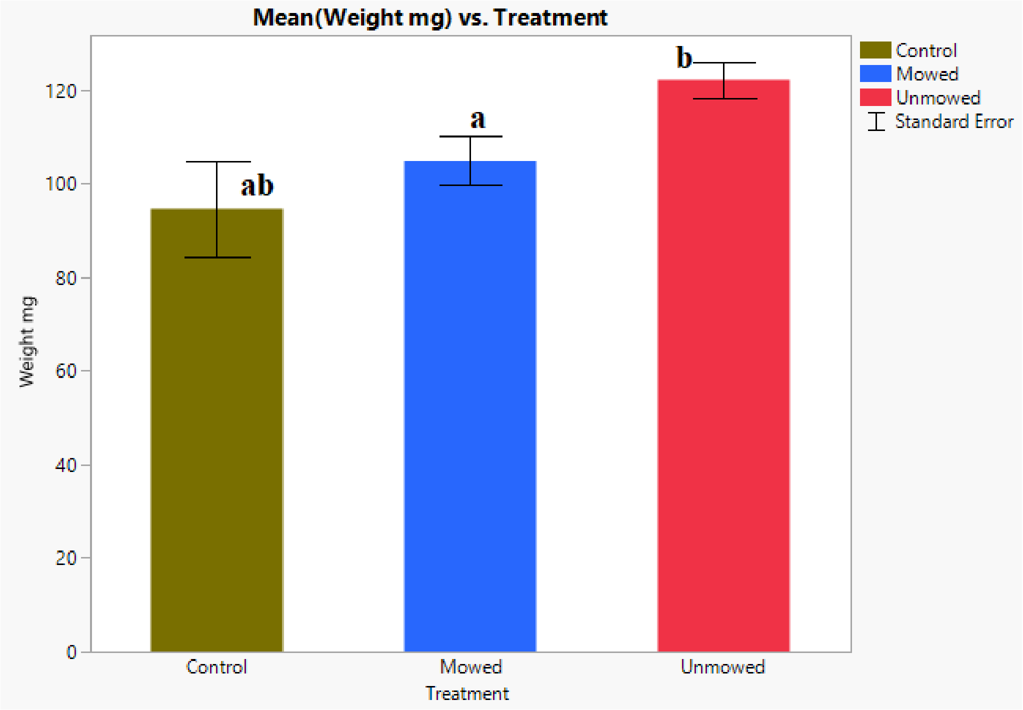

Figure 6O. Caterpillar Experiment- Mass on Day 7 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0003)

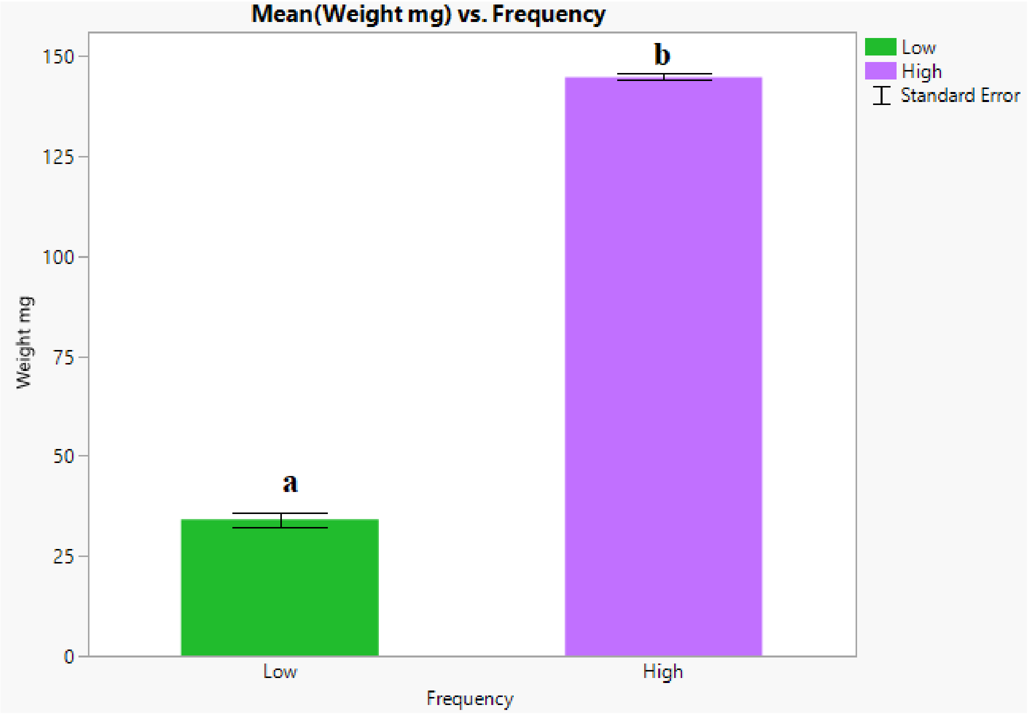

Figure 6P. Caterpillar Experiment Mass on Day 7 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

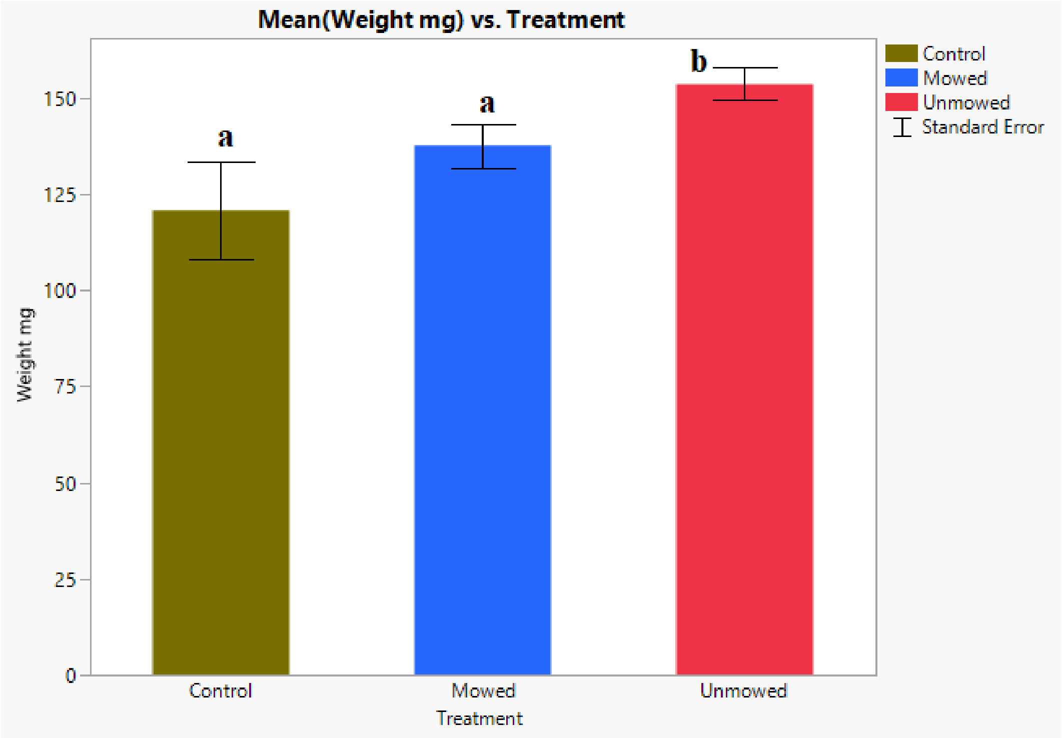

Figure 6Q. Caterpillar Experiment- Mass on Day 8 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0001)

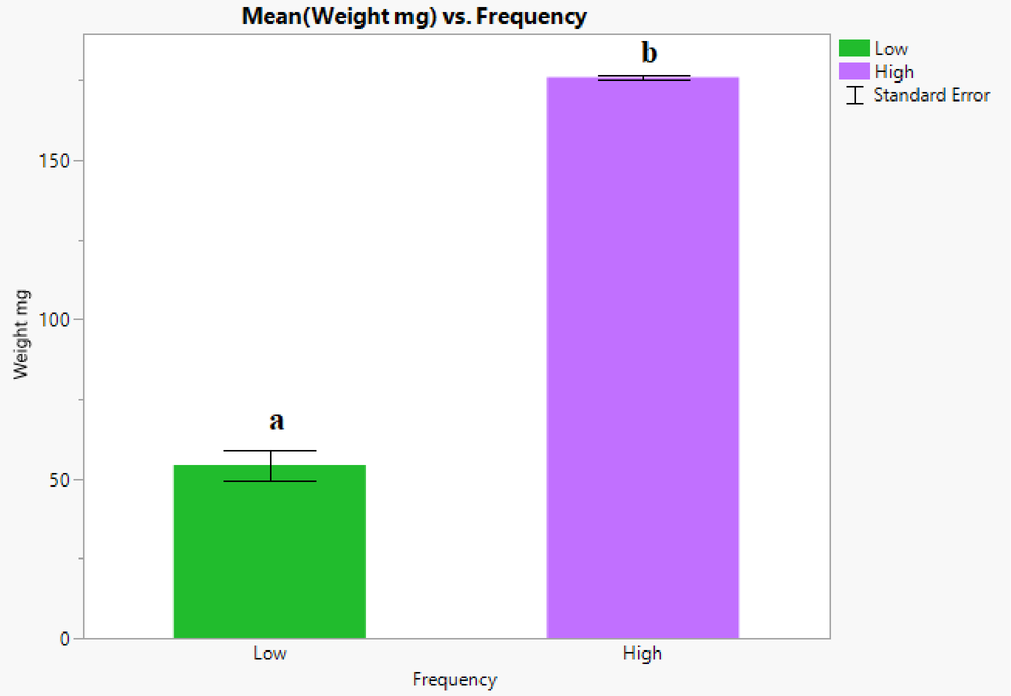

Figure 6R. Caterpillar Experiment Mass on Day 8 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

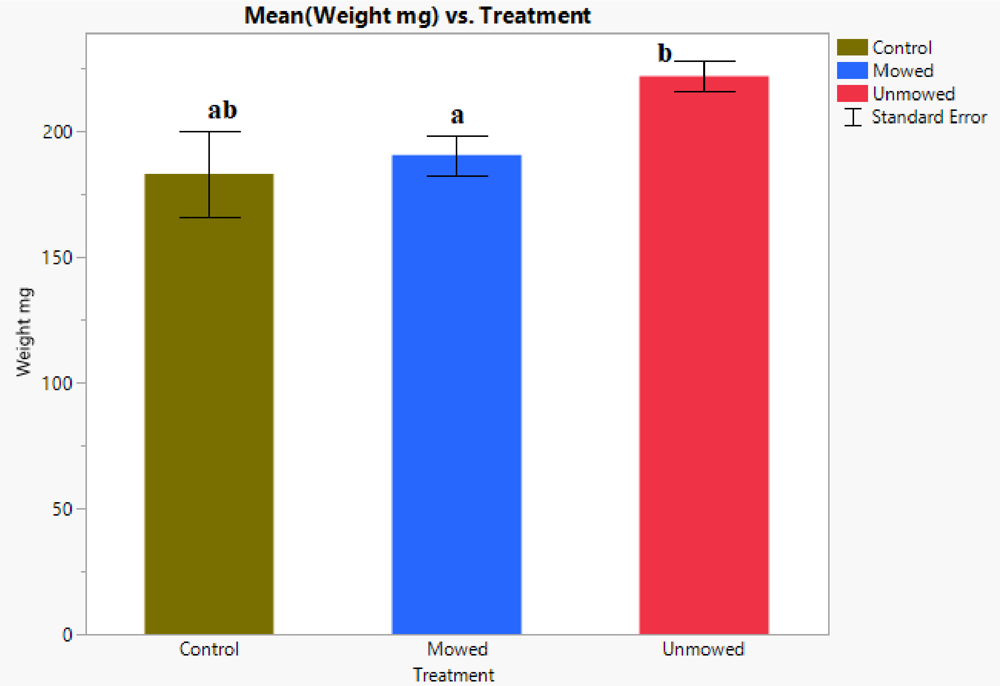

Figure 6S. Caterpillar Experiment- Mass on Day 9 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

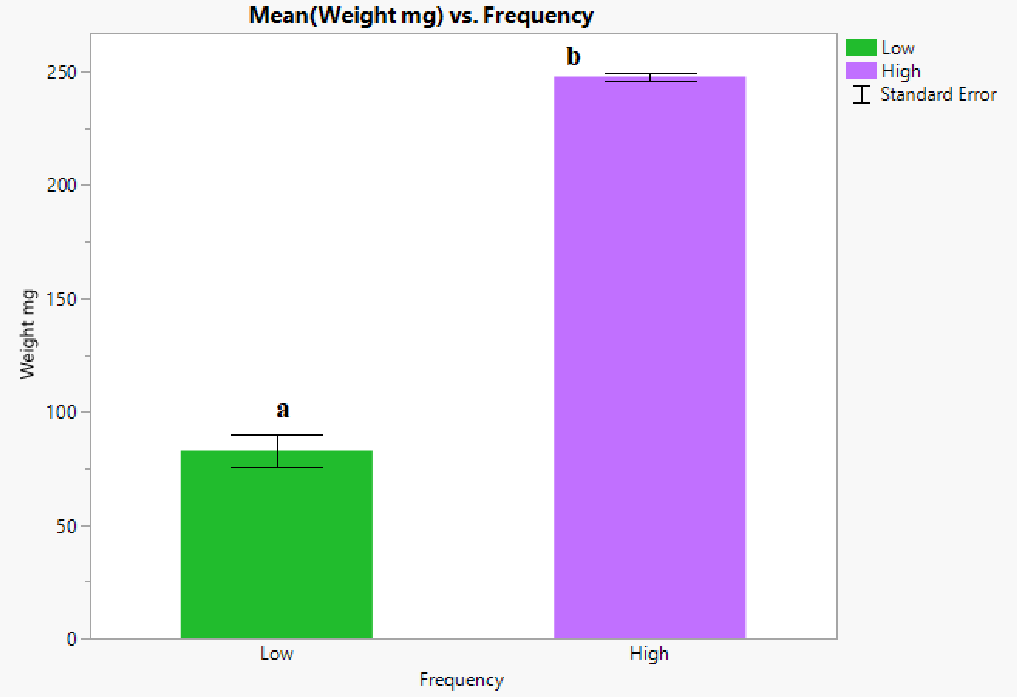

Figure 6T. Caterpillar Experiment Mass on Day 9 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

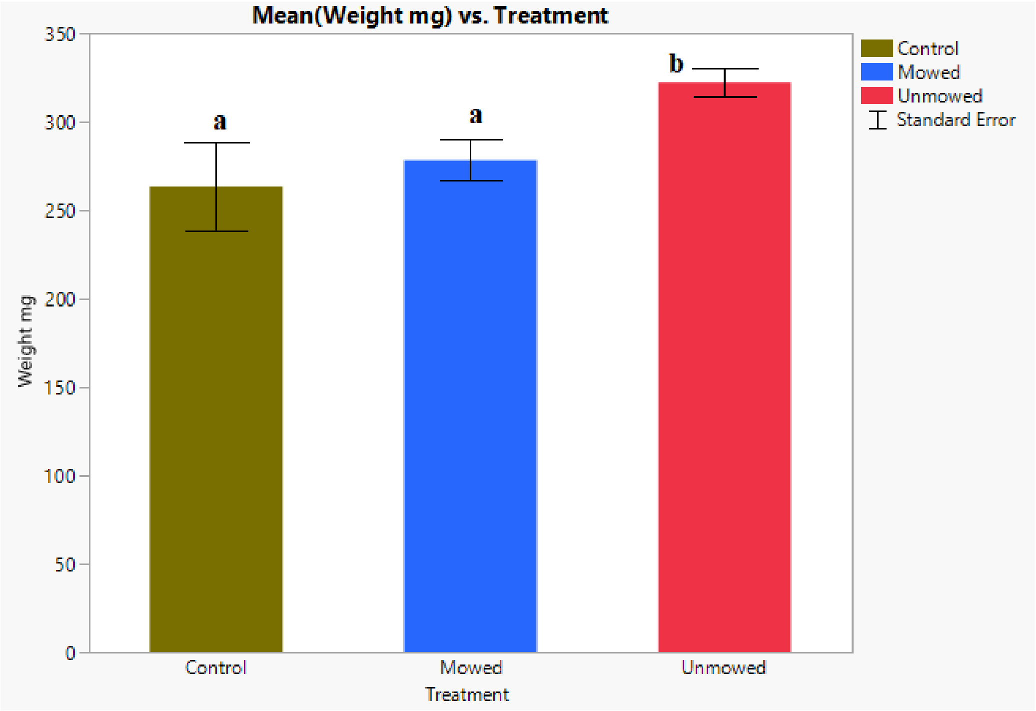

Figure 6U. Caterpillar Experiment- Mass on Day 10 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

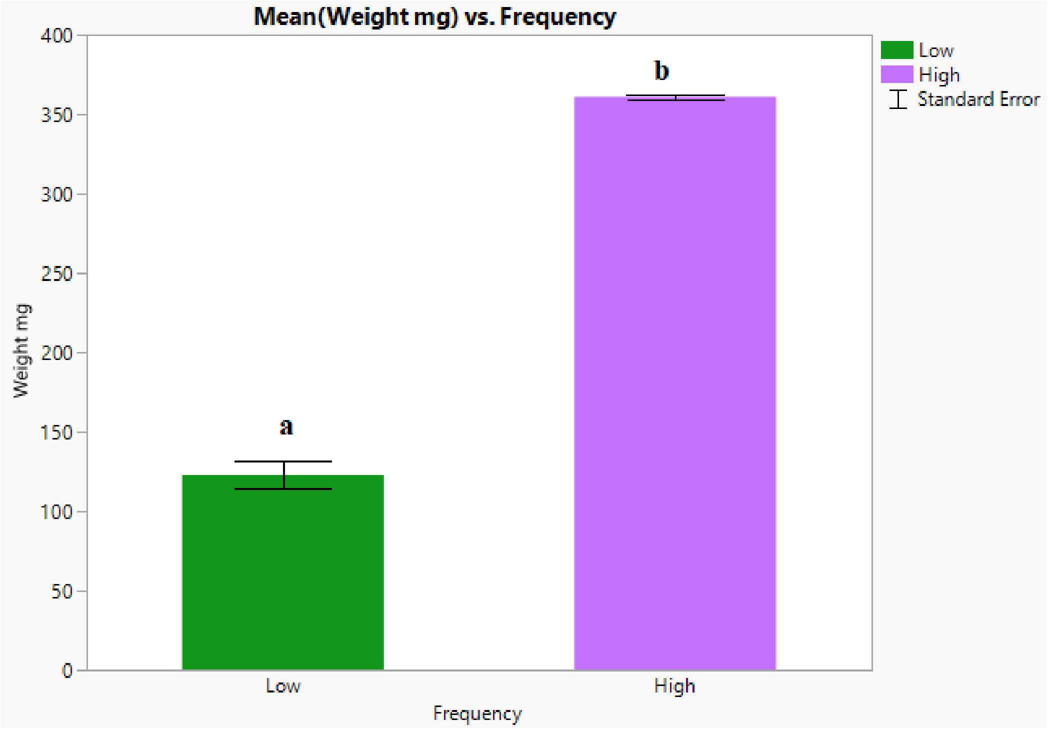

Figure 6V. Caterpillar Experiment Mass on Day 10 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

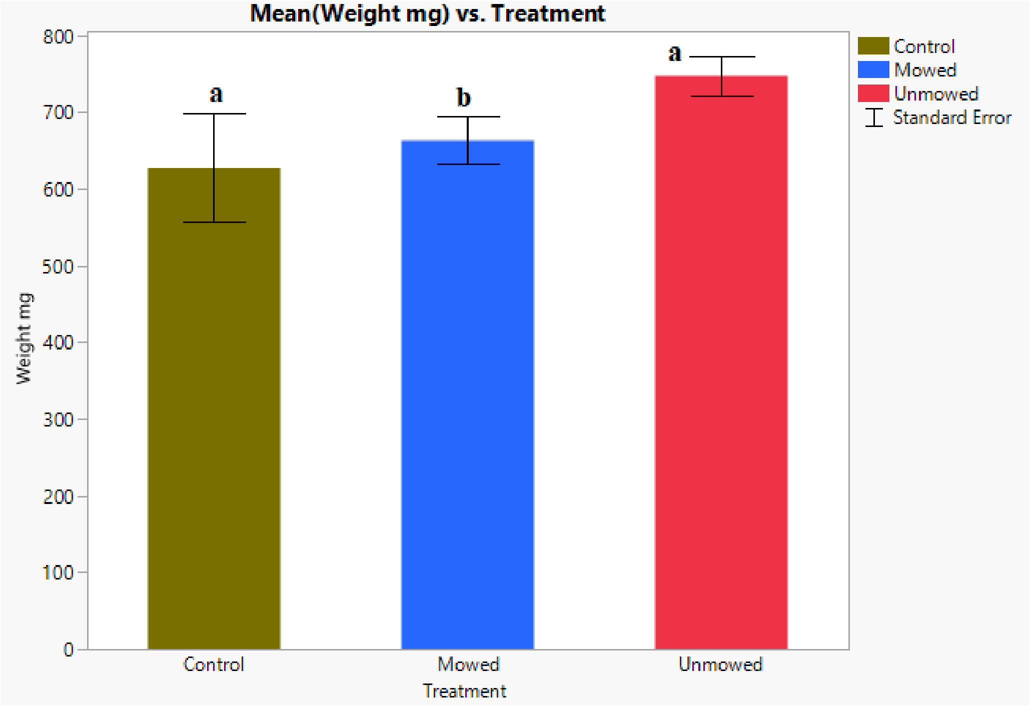

Figure 6W. Caterpillar Experiment- Mass on Day 11 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

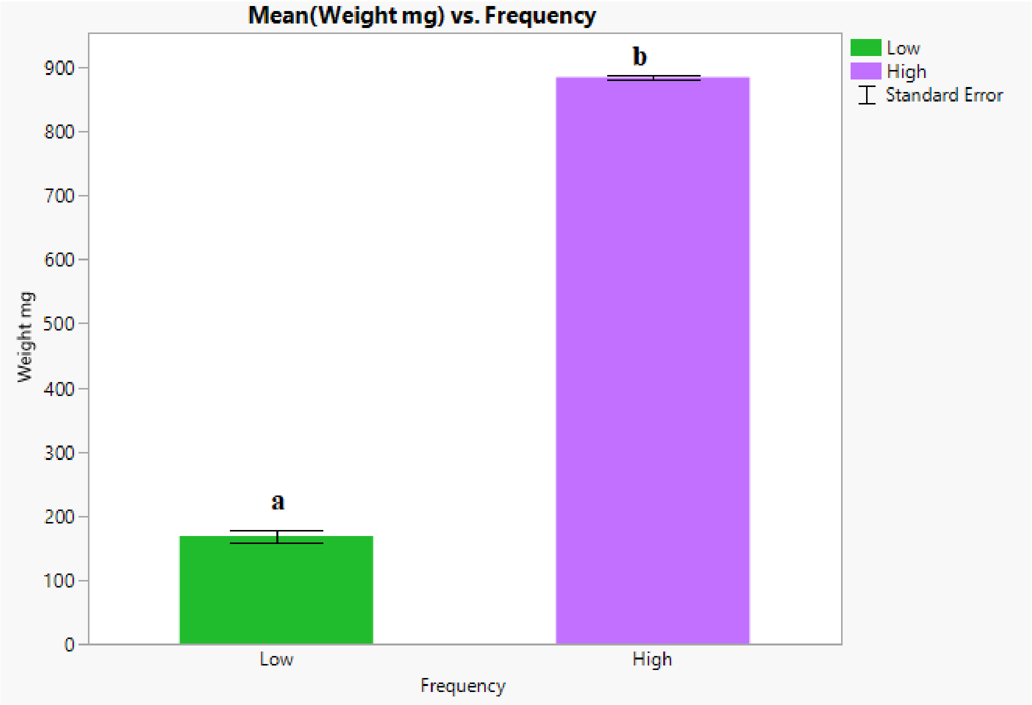

Figure 6X. Caterpillar Experiment Mass on Day 11 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

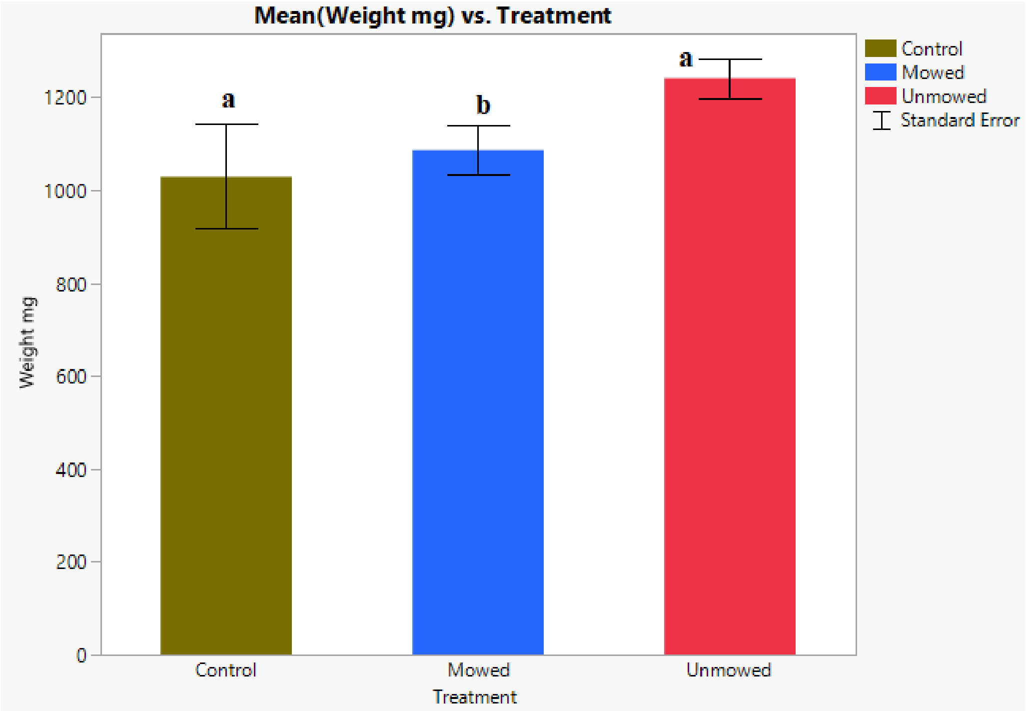

Figure 6Y. Caterpillar Experiment- Mass on Day 12 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

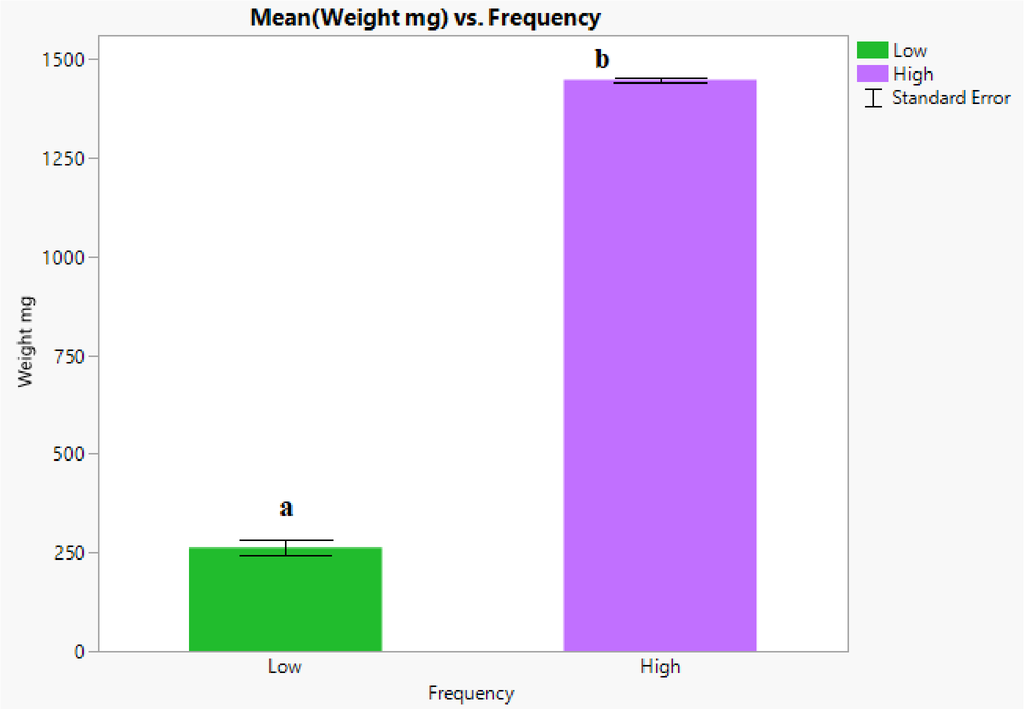

Figure 6Z. Caterpillar Experiment Mass on Day 12 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

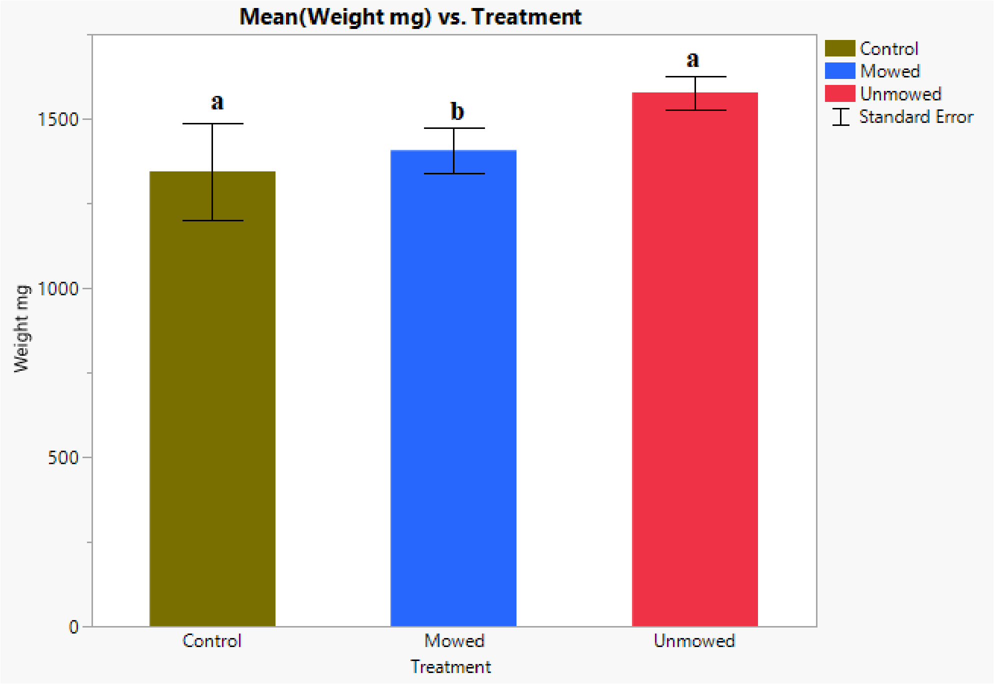

Figure 6i. Caterpillar Experiment- Mass on Day 13 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001)

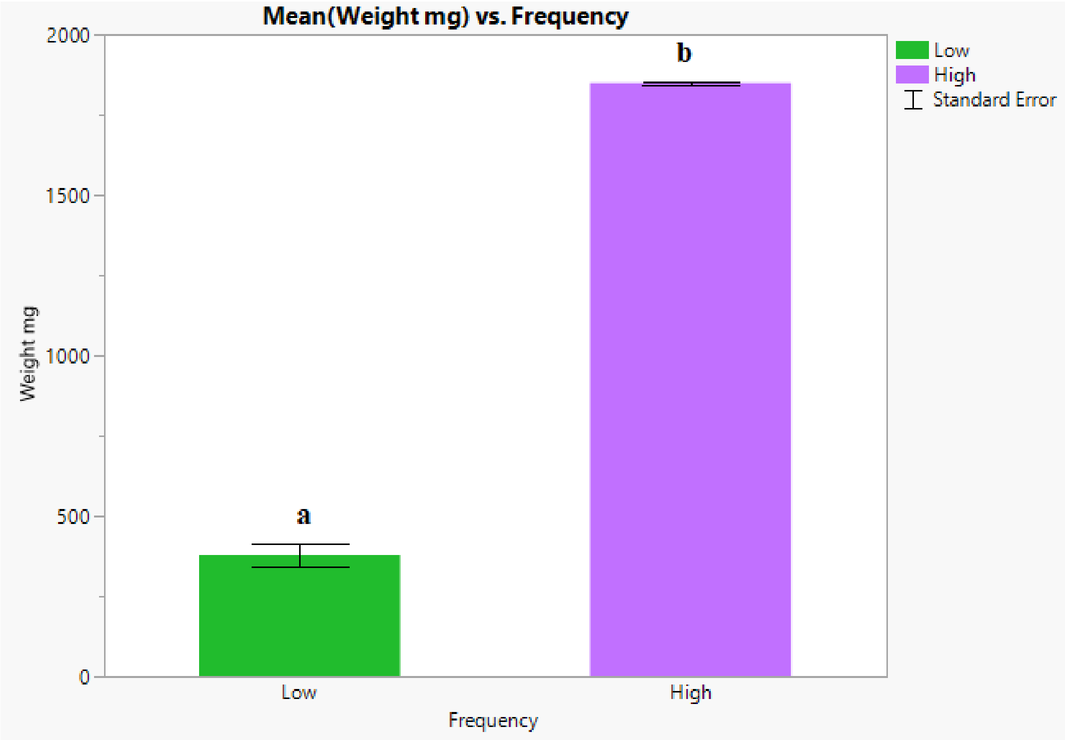

Figure 6ii. Caterpillar Experiment Mass on Day 13 based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

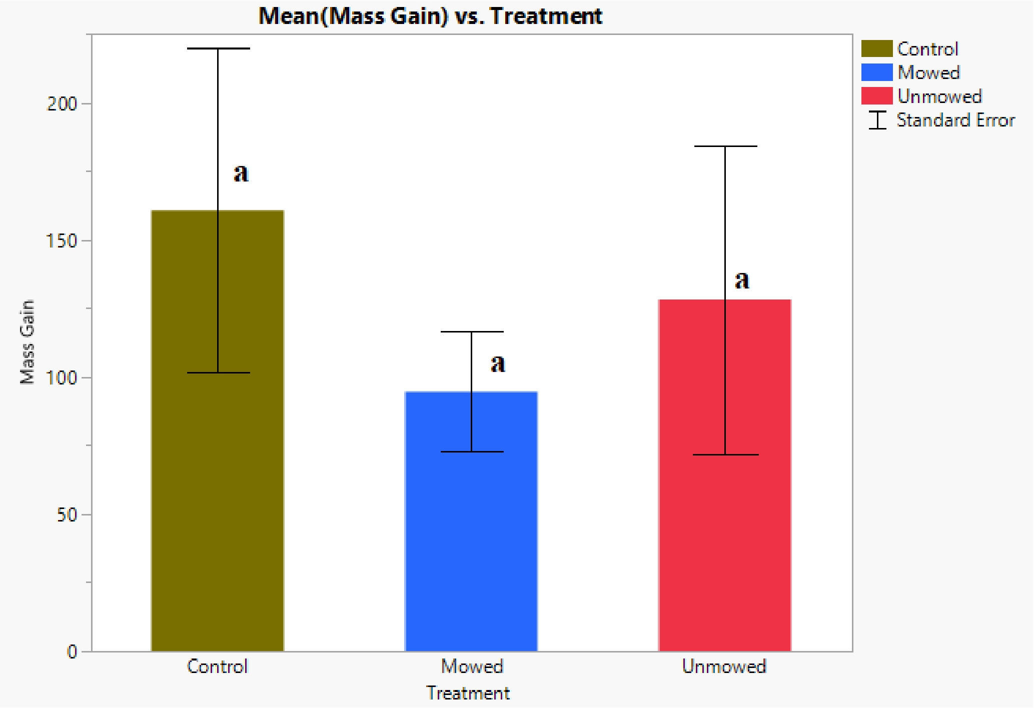

Figure 7A. Caterpillar Experiment Mass Gain From Day 1 to 2 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.4932).

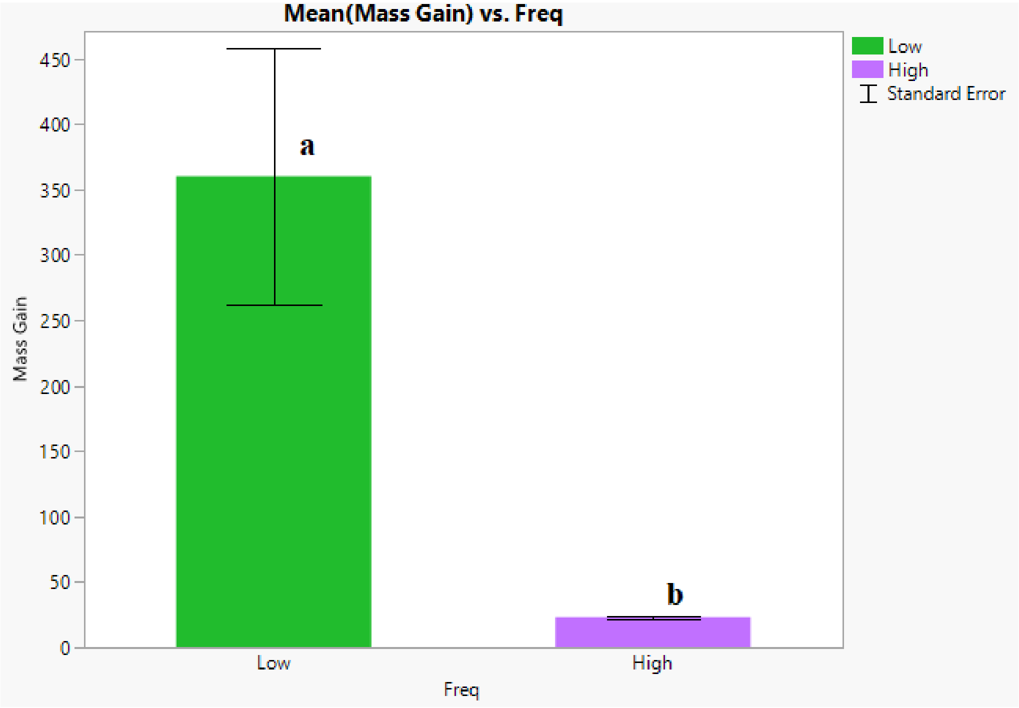

Figure 7B. Caterpillar Experiment Mass Gain From Day 1 to 2 Based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

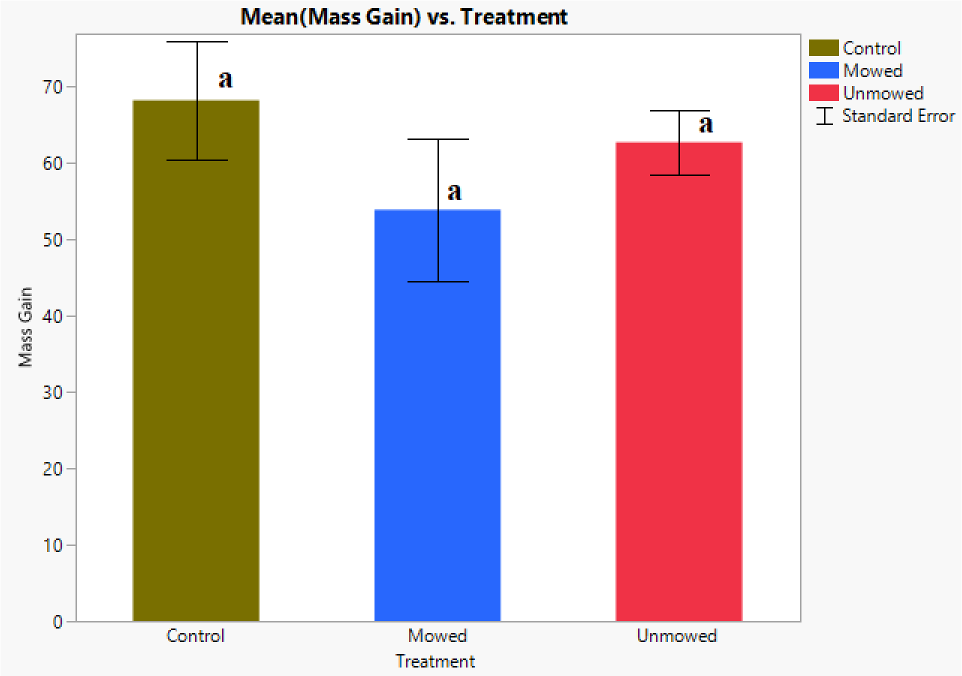

Figure 7C. Caterpillar Experiment Mass Gain From Day 2 to 3 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.7111).

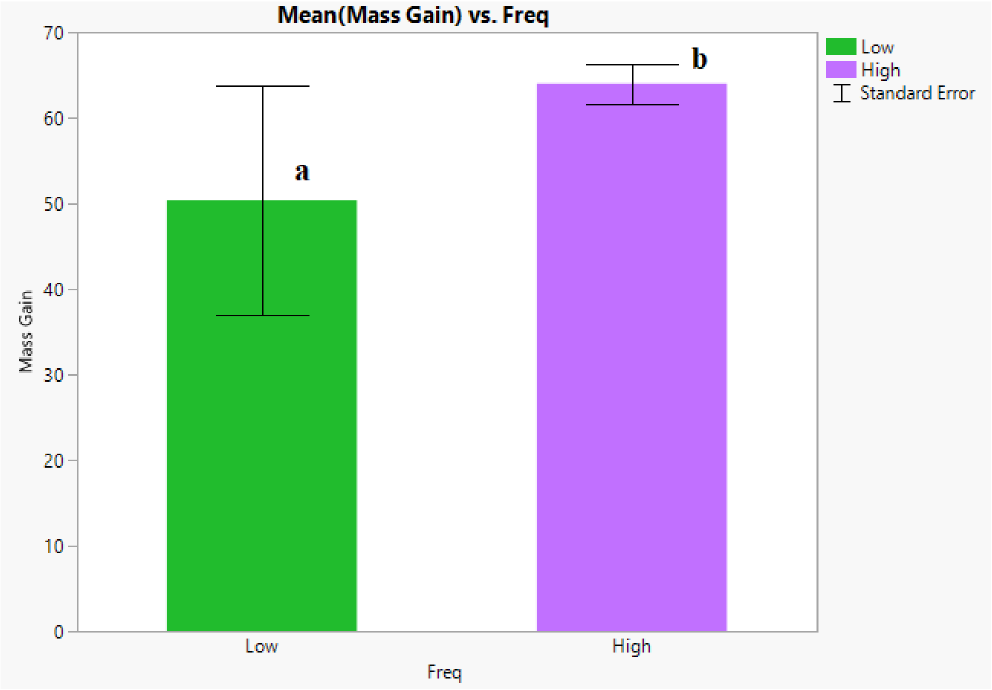

Figure 7D. Caterpillar Experiment Mass Gain From Day 2 to 3 Based on Diet in Low vs High Frequency of Mowing Diets (P=0.0224).

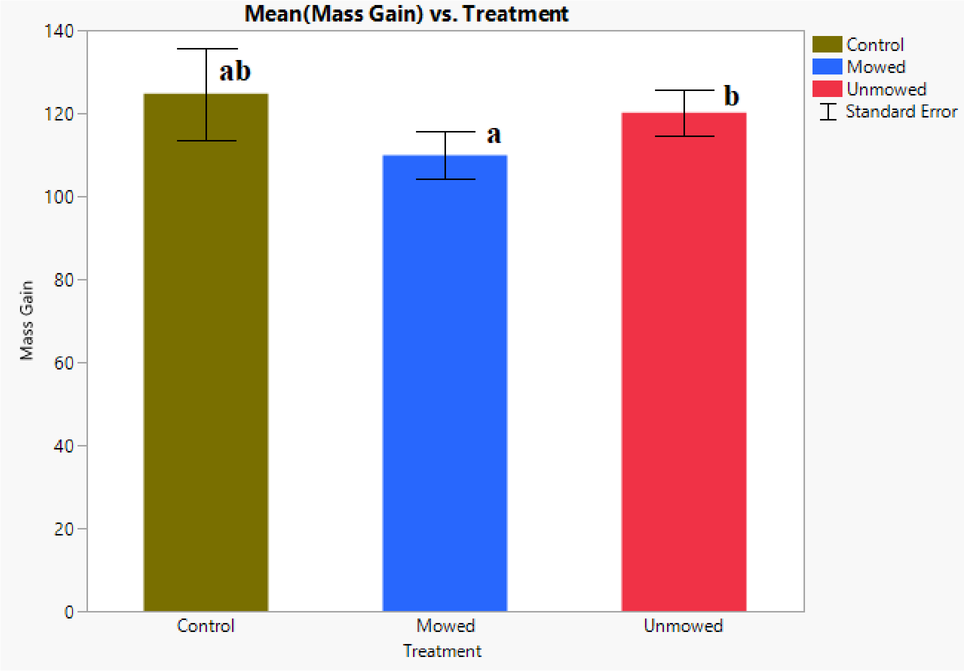

Figure 7E. Caterpillar Experiment Mass Gain From Day 3 to 5 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0160).

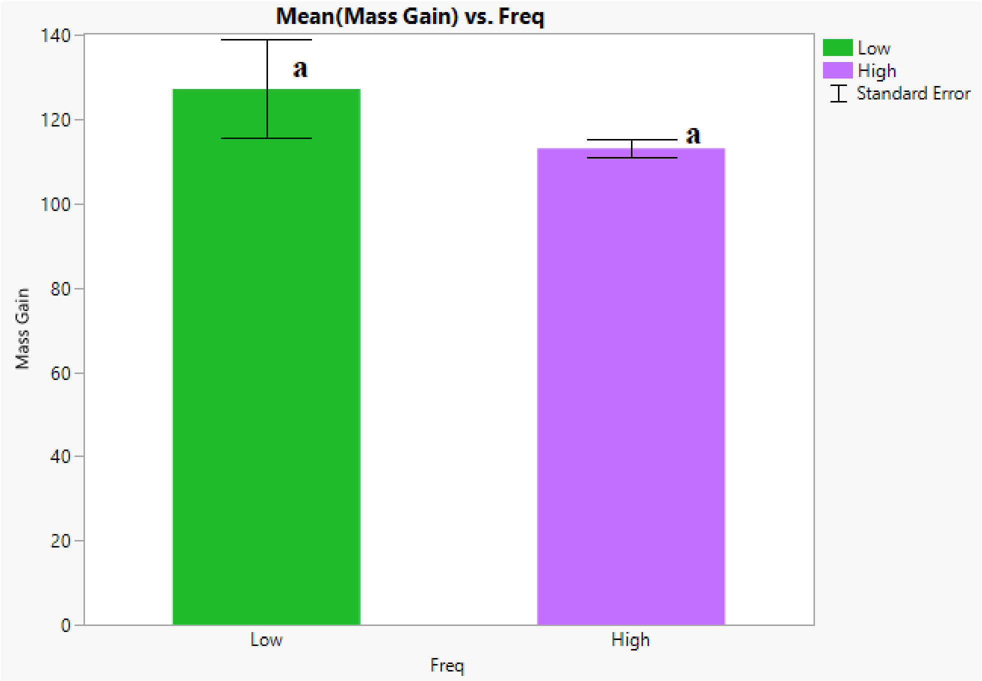

Figure 7F. Caterpillar Experiment Mass Gain From Day 3 to 5 Based on Diet in Low vs High Frequency of Mowing Diets (P=0.5168).

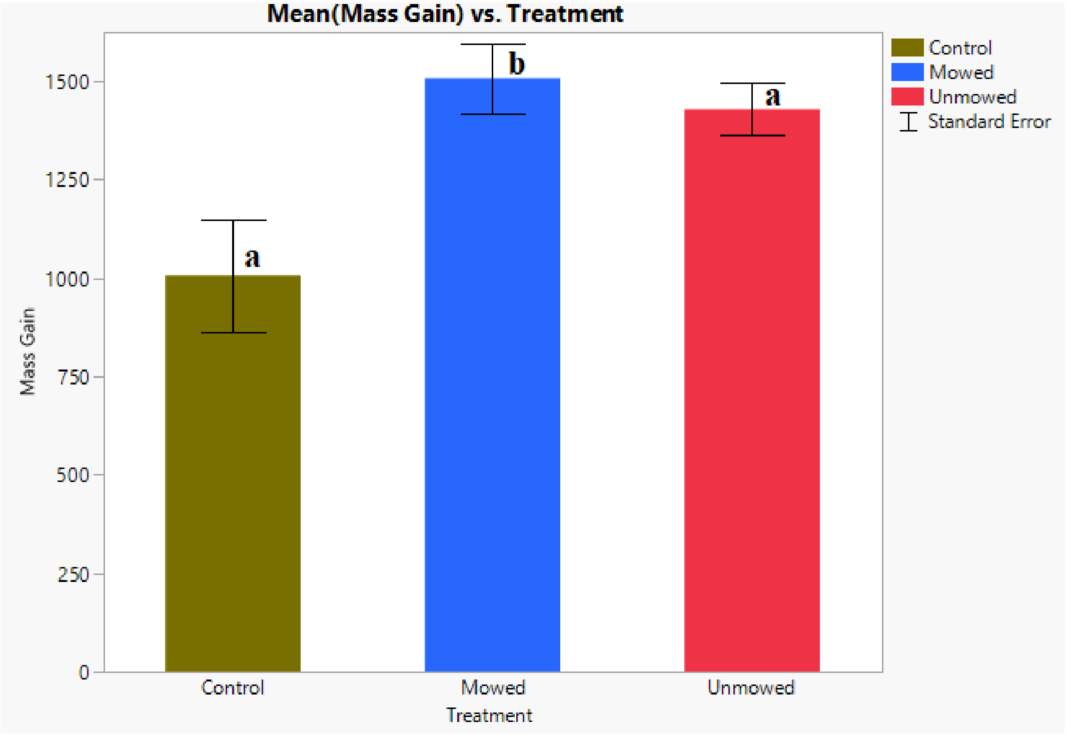

Figure 7G. Caterpillar Experiment Mass Gain From Day 5 to 6 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0021).

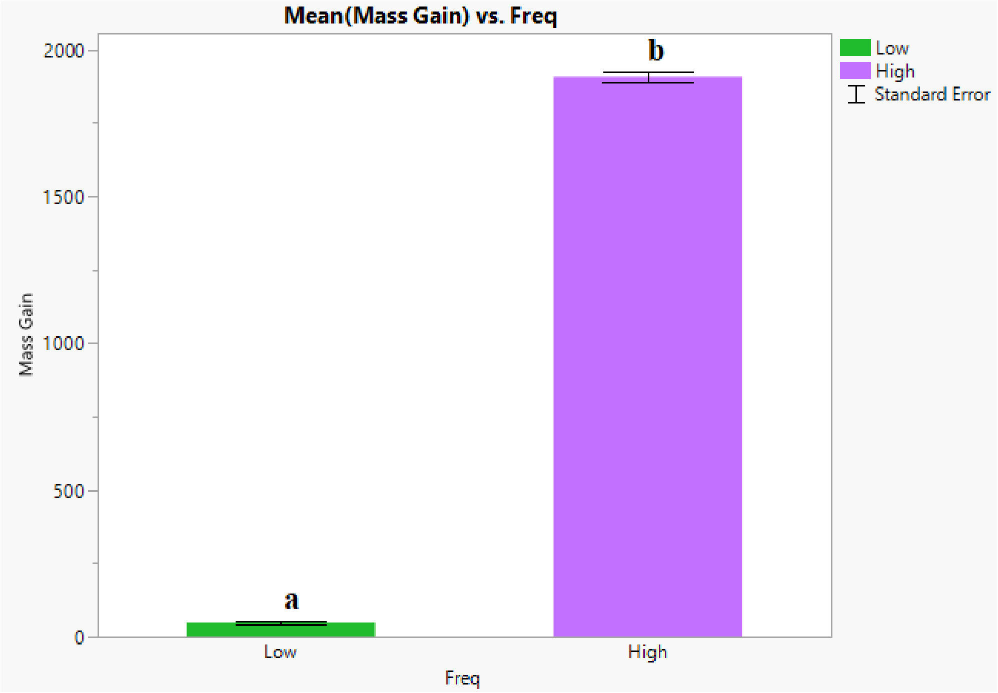

Figure 7H. Caterpillar Experiment Mass Gain From Day 5 to 6 Based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

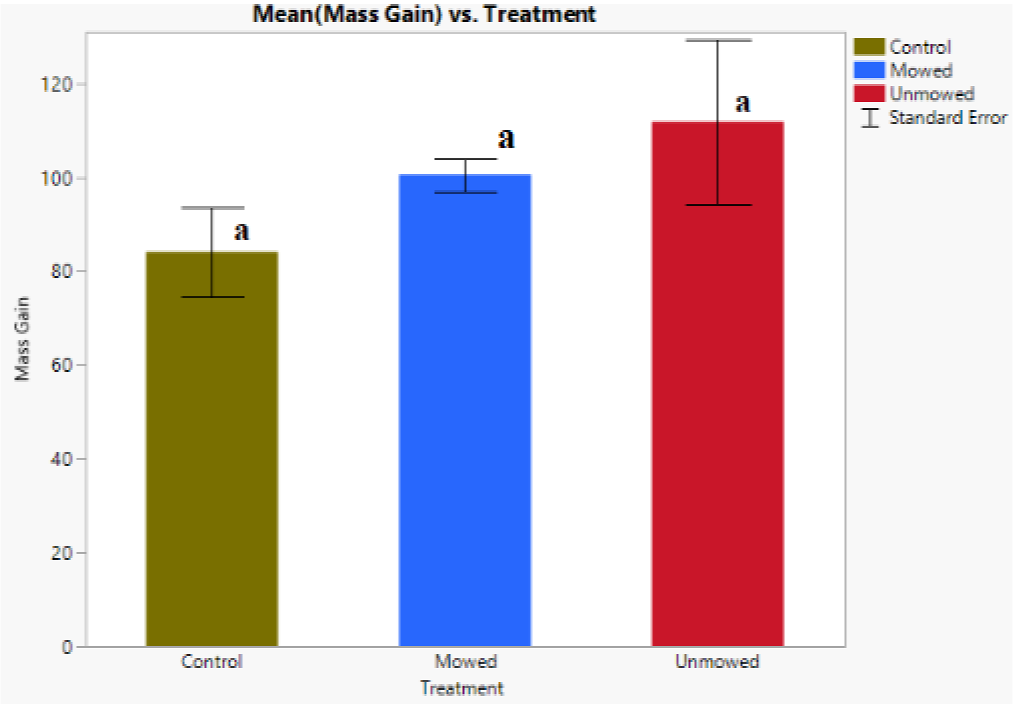

Figure 7I. Caterpillar Experiment Mass Gain From Day 6 to 7 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.9246).

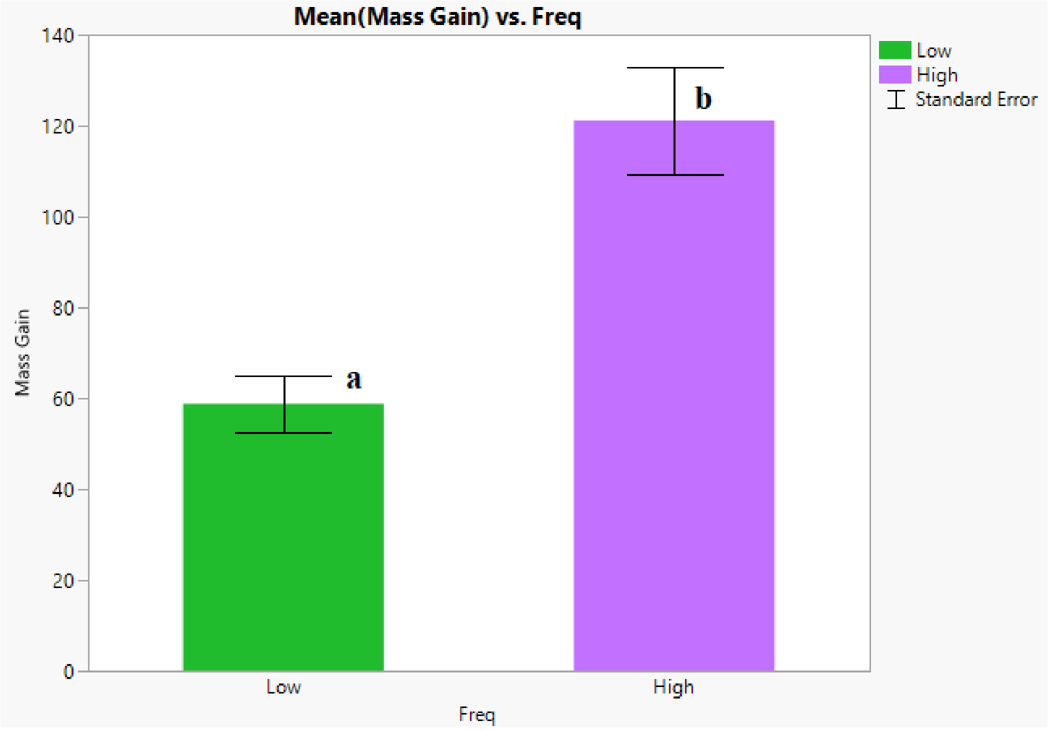

Figure 7J. Caterpillar Experiment Mass Gain From Day 6 to 7 Based on Diet in Low vs High Frequency of Mowing Diets (P=0.0110).

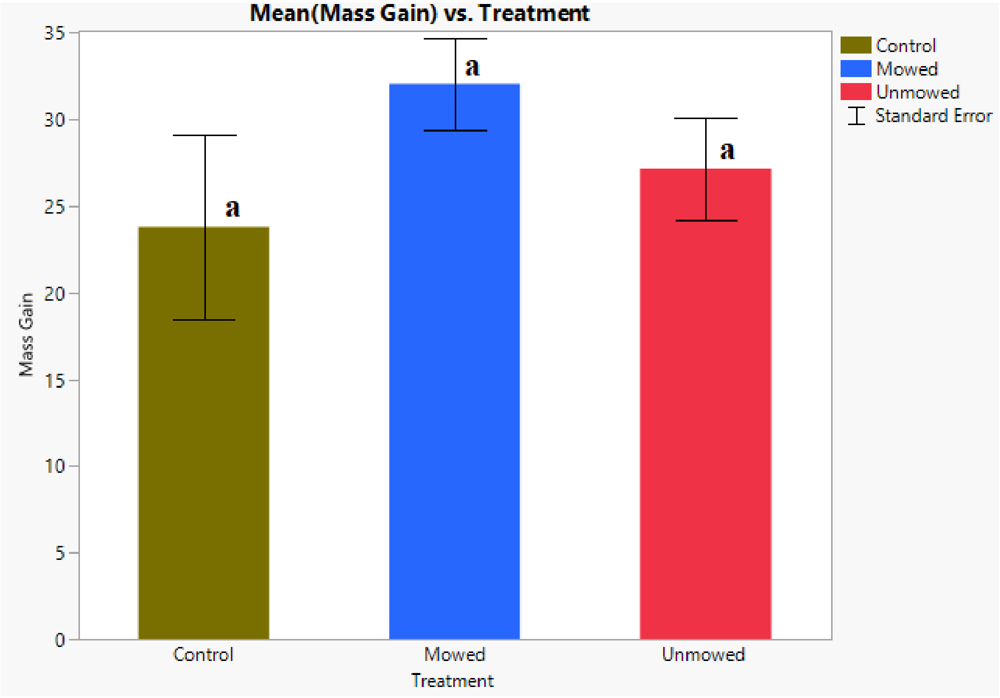

Figure 7K. Caterpillar Experiment Mass Gain From Day 7 to 8 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.0777).

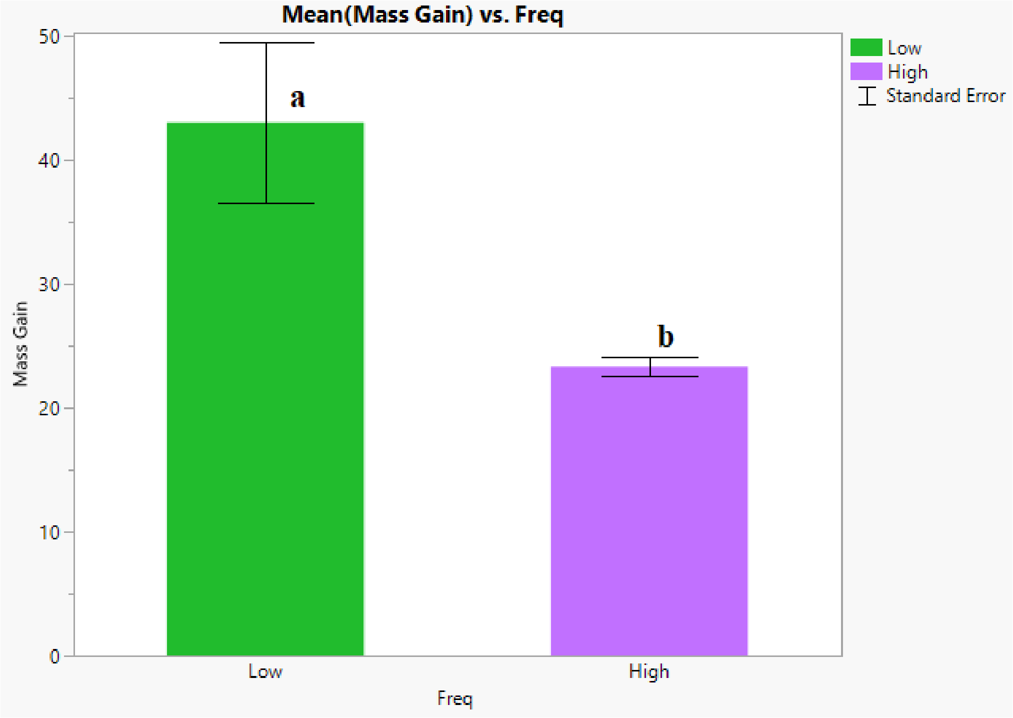

Figure 7L. Caterpillar Experiment Mass Gain From Day 7 to 8 Based on Diet in Low vs High Frequency of Mowing Diets (P=0.0004).

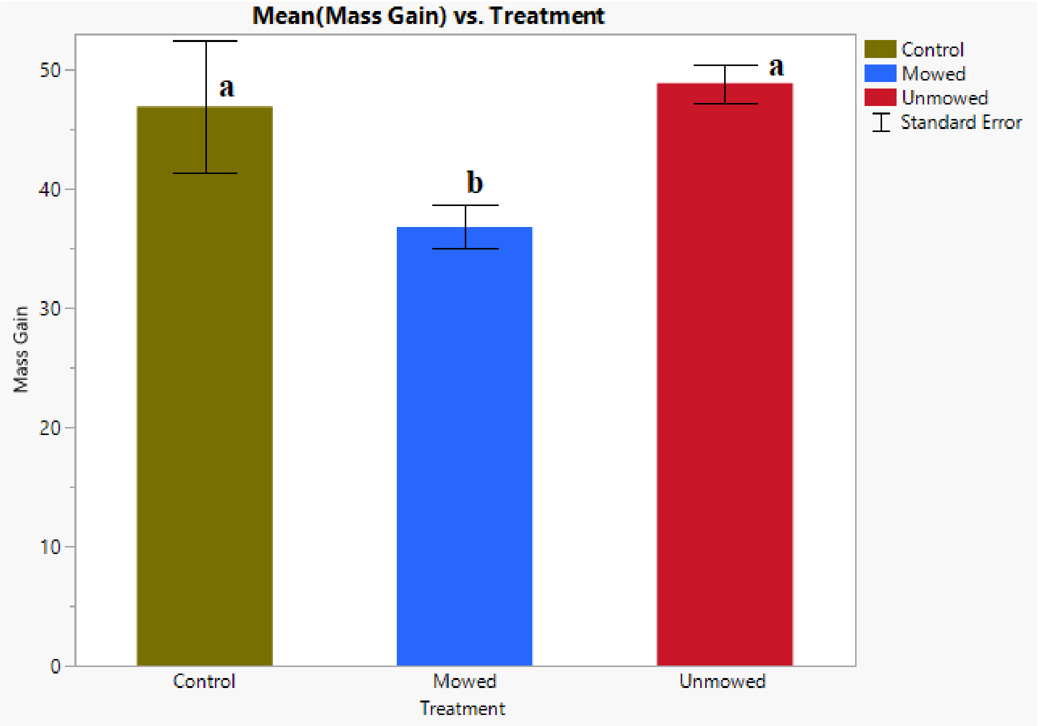

Figure 7M. Caterpillar Experiment Mass Gain From Day 8 to 9 Based on Diet in Control vs Mowed vs Unmowed Diets (P<0.0001).

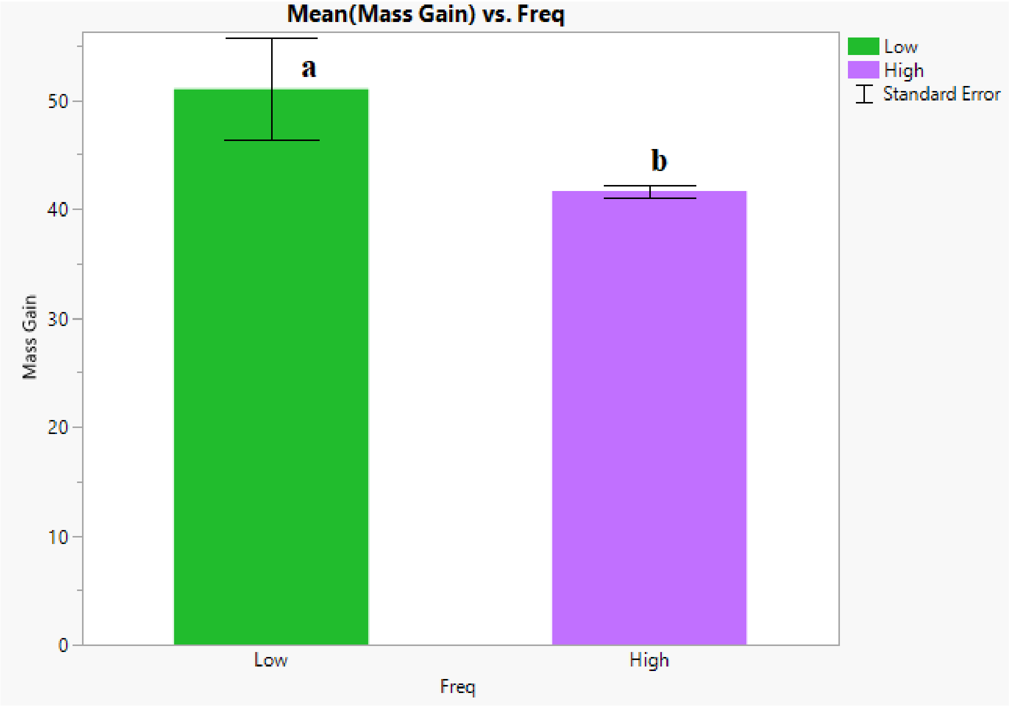

Figure 7N. Caterpillar Experiment Mass Gain From Day 8 to 9 Based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

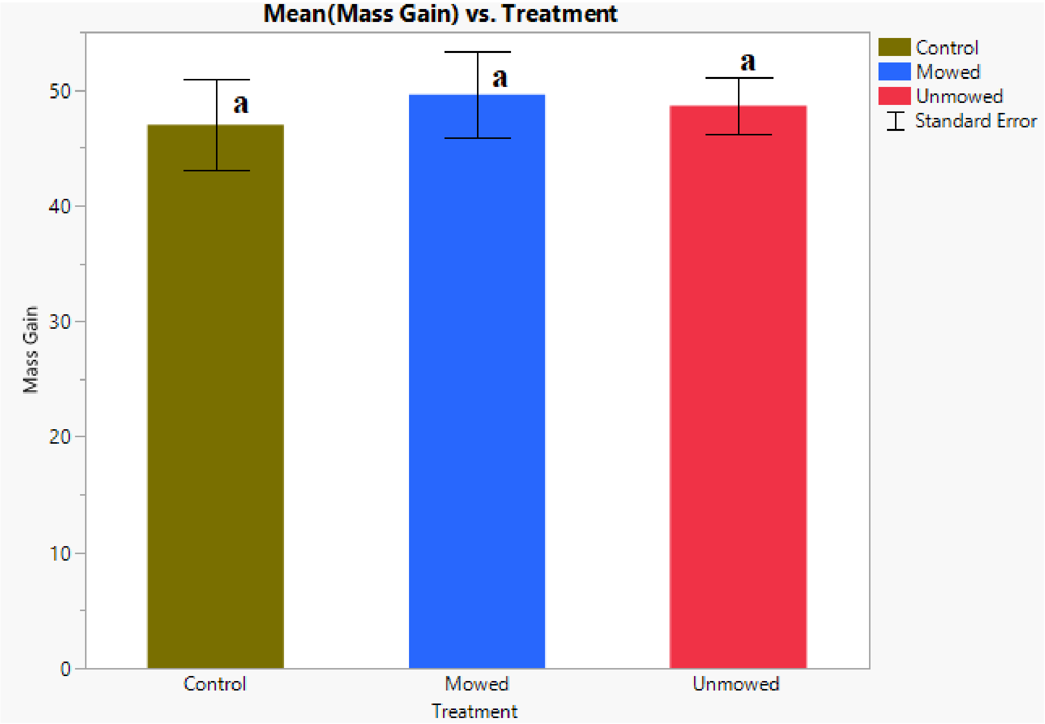

Figure 7O. Caterpillar Experiment Mass Gain From Day 9 to 10 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.4806).

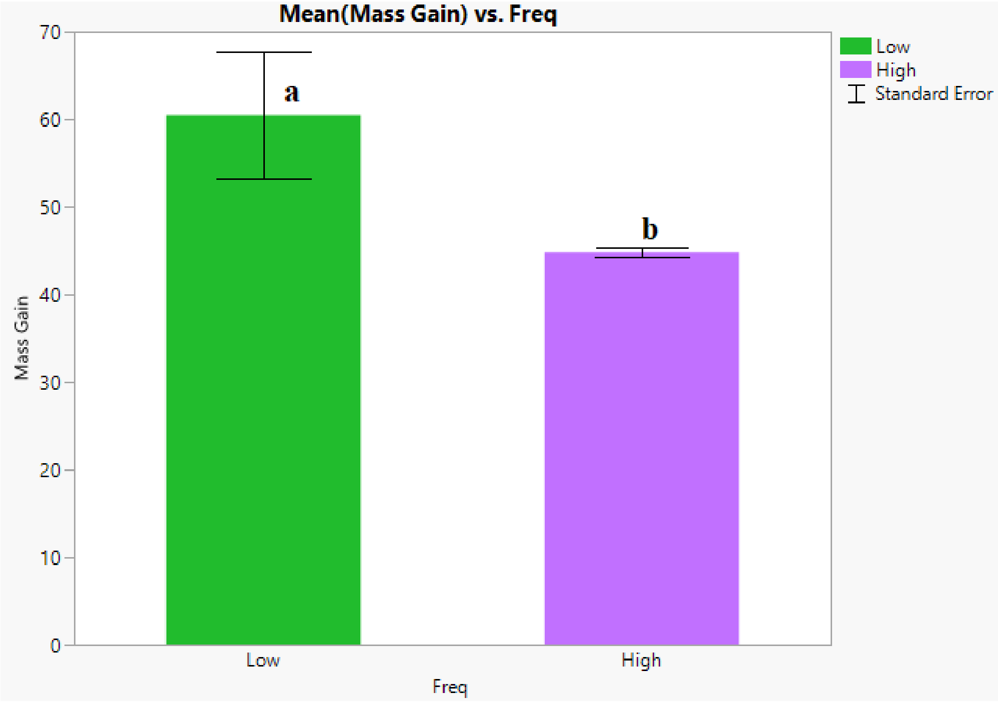

Figure 7P. Caterpillar Experiment Mass Gain From Day 9 to 10 Based on Diet in Low vs High Frequency of Mowing Diets (P=0.0045).

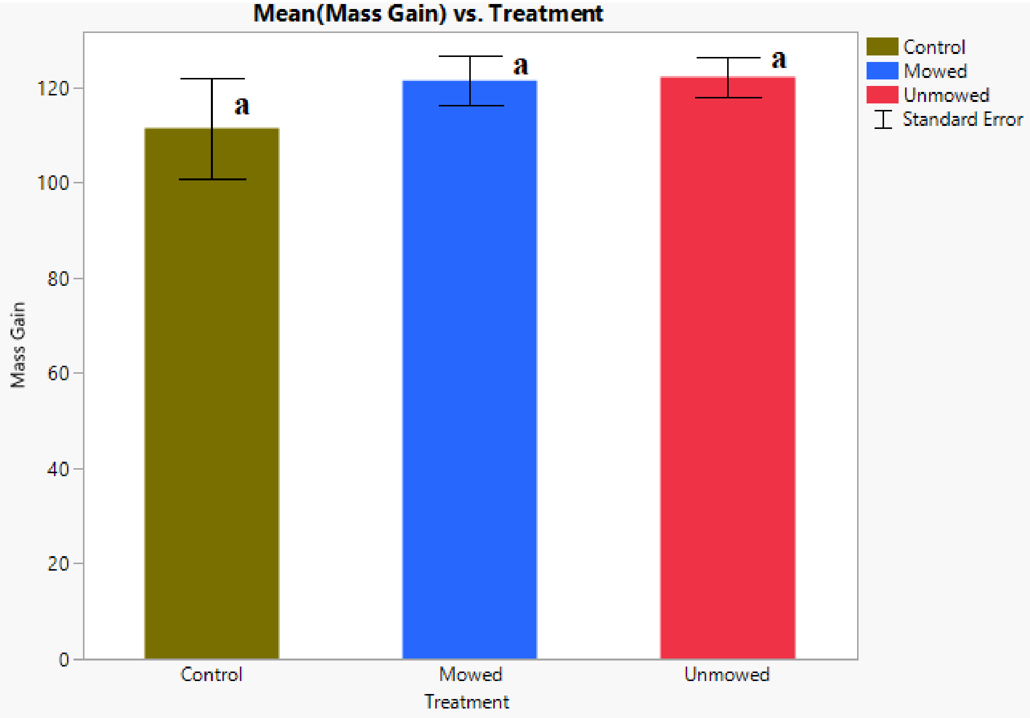

Figure 7Q. Caterpillar Experiment Mass Gain From Day 10 to 11 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.1634).

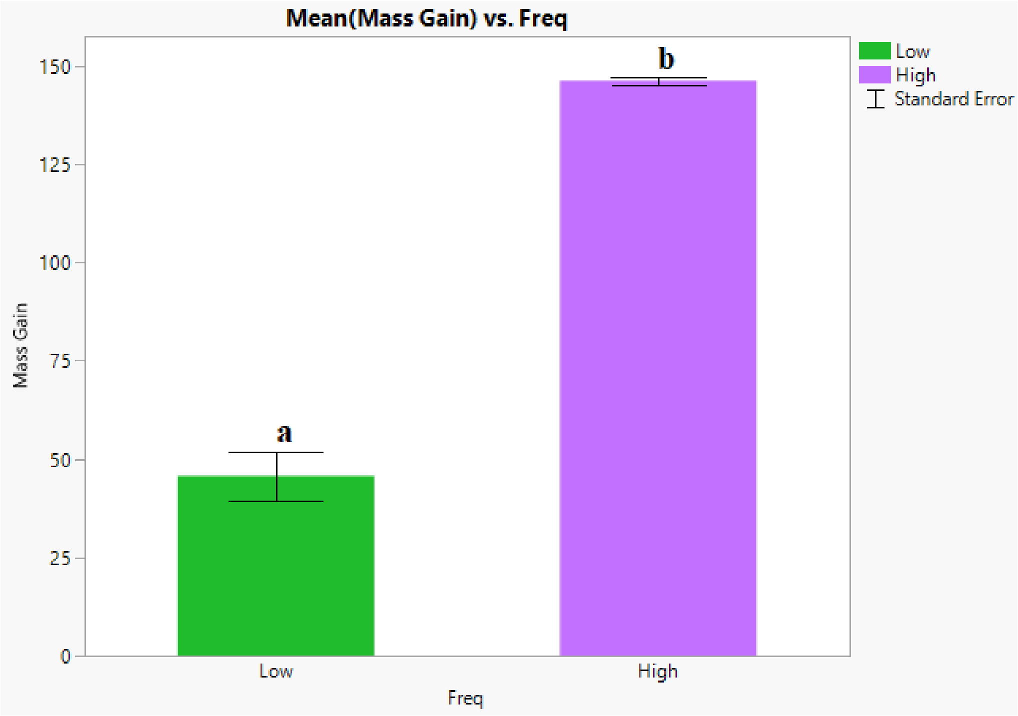

Figure 7R. Caterpillar Experiment Mass Gain From Day 10 to 11 Based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

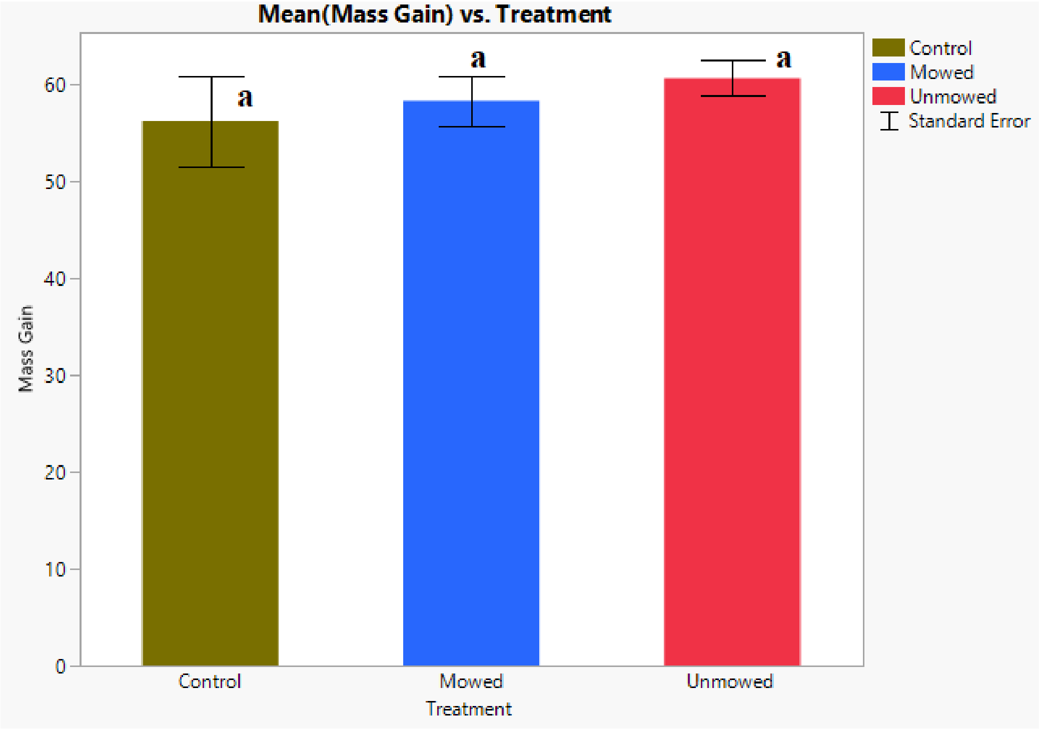

Figure 7S. Caterpillar Experiment Mass Gain From Day 11 to 12 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.8200).

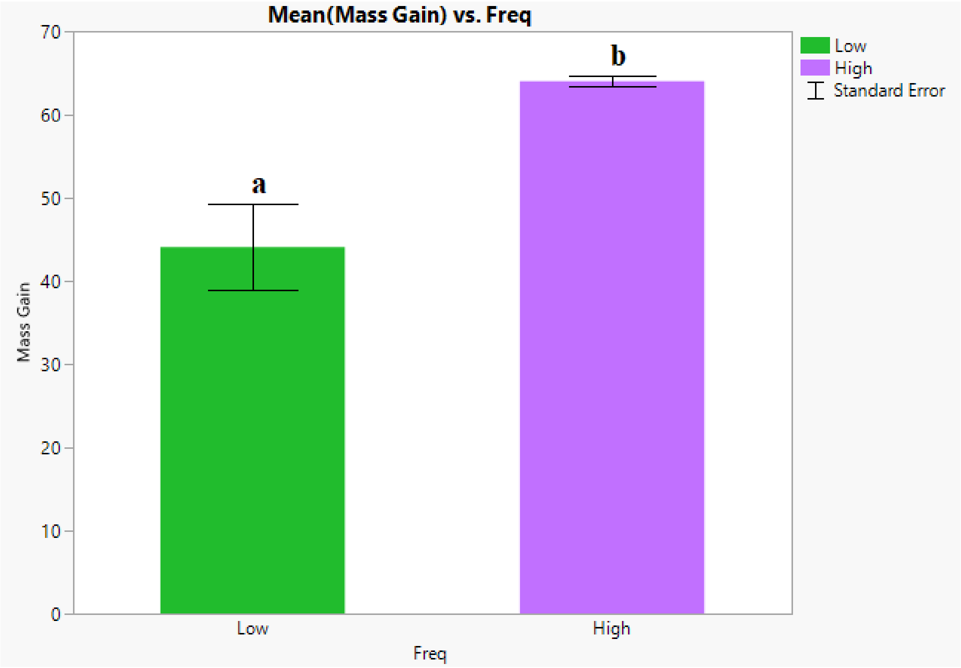

Figure 7T. Caterpillar Experiment Mass Gain From Day 11 to 12 Based on Diet in Low vs High Frequency of Mowing Diets (P<0.0001).

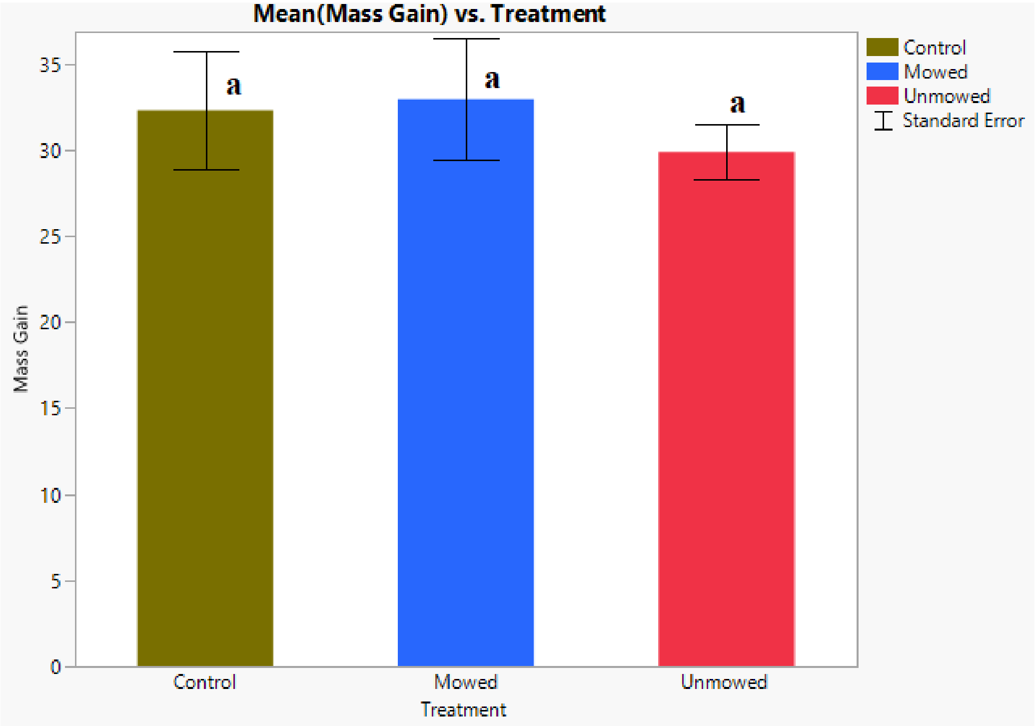

Figure 7U. Caterpillar Experiment Mass Gain From Day 12 to 13 Based on Diet in Control vs Mowed vs Unmowed Diets (P=0.8981).

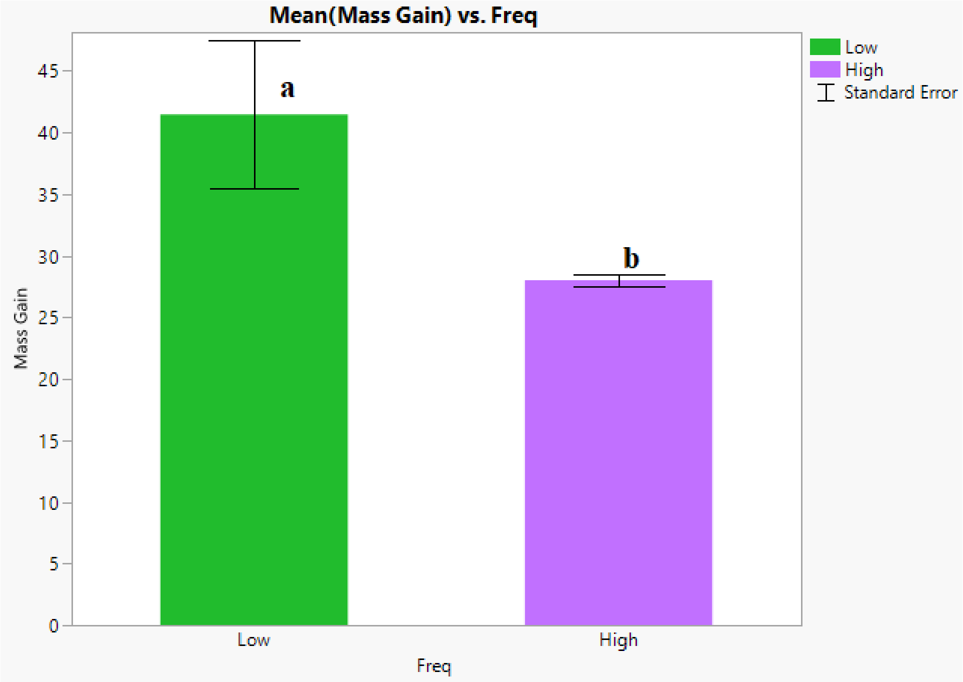

Figure 7V. Caterpillar Experiment Mass Gain From Day 12 to 13 Based on Diet in Low vs High Frequency of Mowing Diets (P=0.0029).

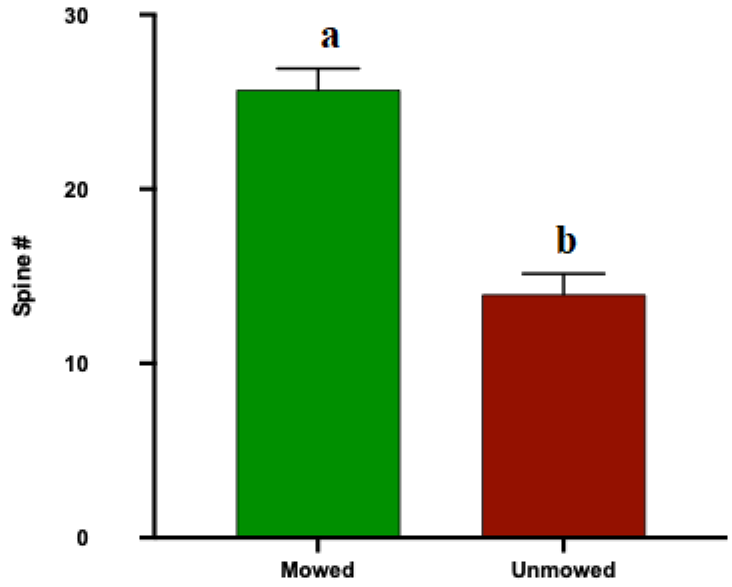

Figure 8A. Mean spine number of *Solanum elaeagnifolium* flower from mowed and unmowed treatments. Different letters indicate significant differences among mowed and unmowed treatments determined by post hoc analyses using Student's T-Test (P<0.05).

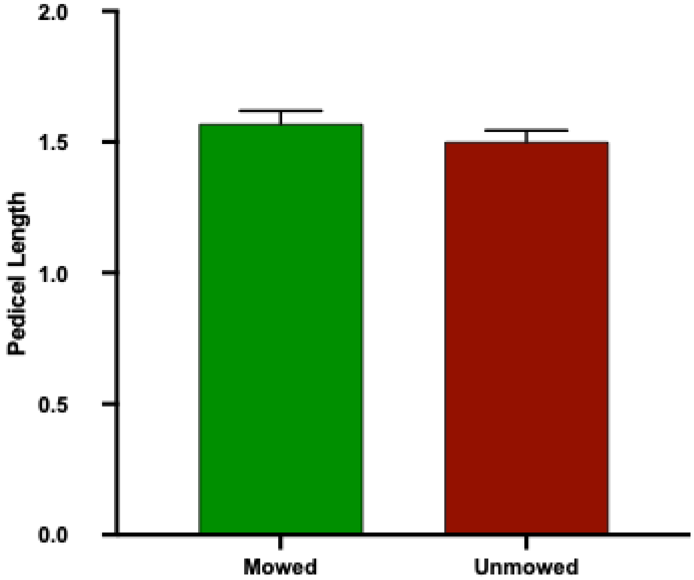

Figure 8B. Mean length of *Solanum elaeagnifolium* pedicels from mowed and unmowed treatments. Different letters indicate significant differences among mowed and unmowed treatments determined by post hoc analyses using Student's T-Test ($P<0.05$).

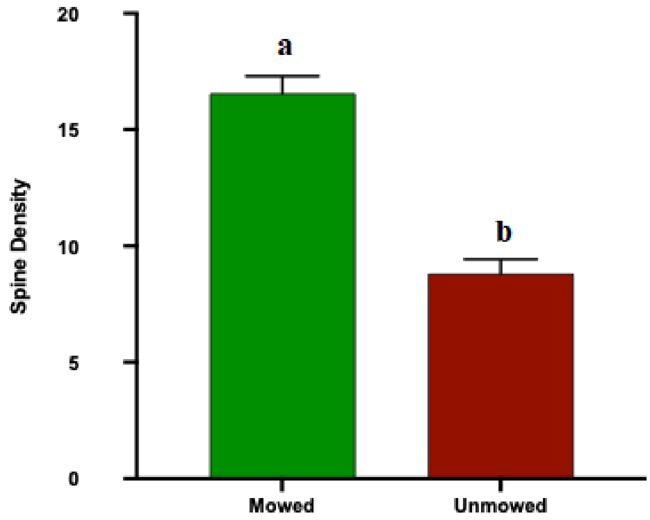

Figure 8C. Mean spine density of *Solanum elaeagnifolium* flower pedicels from mowed and unmowed treatments. Different letters indicate significant differences among mowed and unmowed treatments determined by post hoc analyses using Student's T-Test (P<0.05).

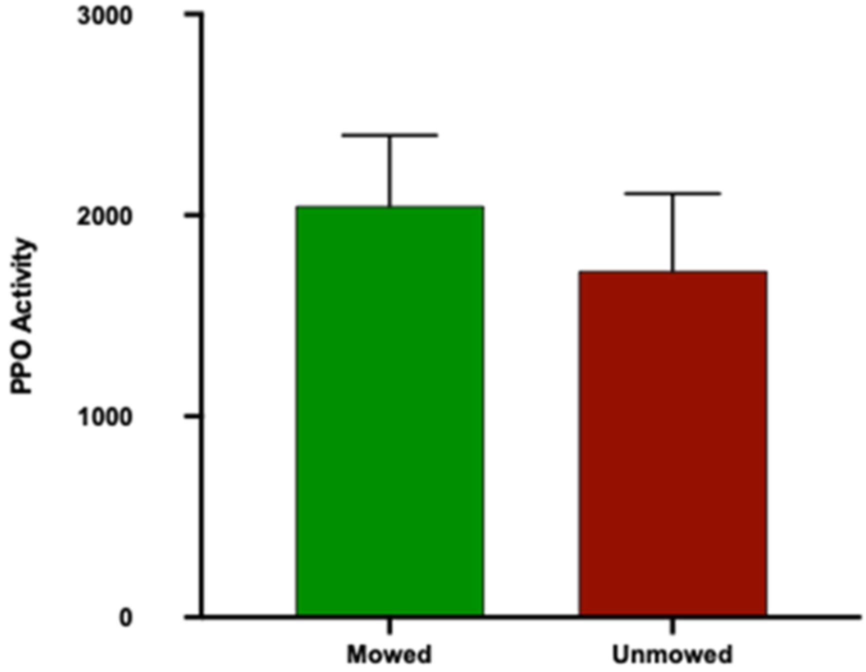

Figure 9. Mean Polyphenol Activity of *Solanum elaeagnifolium* flowers from mowed and unmowed treatments. Mann-Whitney analysis shows that the treatments did not statistically differ at $P<0.05$

CHAPTER IV

DISCUSSION

In this study, we examined how disturbance (mowing) and frequency of mowing affects floral traits, plant defenses, and herbivory on SLN. Interestingly, we found that mowed flowers had larger diameters than unmowed flowers, but flower mass was in line with our hypothesis as unmowed flowers were heavier in high mowing frequency populations. Polyphenol Oxidase (PPO) content was found to not be significantly different between mowed and unmowed treatments, which indicates perhaps lower concentration of the plant defense compound in floral parts as opposed to leaves where it is known to deter herbivores. (Ludlum et al. 1991, Tayal et al. 2020). We also found that mowed flowers had significantly less damage on petals, indicating that the stress due to continuous mowing led to an increase in the induction of defense traits, although there was no effect of frequency on petal and anther damage. To compound these effects, we also show that mowed flowers had higher spine density, a major anti-herbivore defense in Solanaceae (Kariyat 2013a; Kariyat 2017). Taken together, we show that mowing increased floral defense traits, and for a species like SLN that produce over ~100 flowers per plant, has tremendous consequences for spread, dispersal, and invasion success (Krigas et al. 2021, Petanidou et al 2018; Chavana et al.,2021; Kasper et al., 2021).

While examining the effect of mowed and unmowed plant parts incorporated into artificial diet, we found similar results regarding herbivore deterrence. In early *M. sexta* instars, we found no significant difference between larvae fed on control and mowed diets (although mowed diet-fed individuals were smaller than all treatments); however, unmowed diet-fed caterpillars were significantly heavier than their control and mowed counterparts. This again echoes the findings of petal and anther damage: that the continuous mowing stress has an effect on the plants and flowers that can be traced even through diet experiments. In late instars, there was no significant difference in mass between any of the three treatments, which may indicate that the effect of mowing as a disturbance is more pronounced on early instar *M. sexta*. This indication is supported by previous research that has shown food quality effects being more pronounced in early instars of holometabolous insects when manipulating food sources (Holmes et al. 2020; Tayal et al., 2020a,b; Singh and Kariyat 2020; Kariyat et al., 2019). With regard to mowing frequency, which has less previous data to draw conclusions upon, early instar mass was larger within low mowing frequency individuals and the opposite effect was found in late instar larvae as high mowing frequency individuals were significantly heavier. Haghkerdar et al. 2019 found detrimental effects as a result of repeat disturbances on multiple invertebrate taxa including insects, which aligns itself with the lower mass of high mowing frequency larvae observed during the early instars. This, however, contradicts the late instar masses as they were higher in high frequency of mowing, yet this can be attributed to stress-induced vigorous growth responses. Recalling back to our previous study, Chavana et al. 2021 documented the effect of mowing as a disturbance on the generalist *Leptinotarsa texana*, as well as a different feeding guild through *Aphis craccivora.* This stated, these must also be reproduced to gain a similar understanding of disturbance on floral characteristics as we have done with *M. sexta.* Alongside our findings there is clear evidence that mowing as a disturbance has detrimental effects on the Solanacea specialist, *Manduca sexta*.

Additionally, to strengthen the study future experiments would involve rearing of *M. sexta* on lab-grown *S. elaeagnifolium* to understand if similar effects observed in diet experiments can be recreated on the adult plants. In the same vein, an expansion of characteristics and traits to be measured would also strengthen the study such as evaluating the effect of mowing as a disturbance not only on herbivores mentioned previously, but also on pollinators and pollination visits in the field. Various studies have examined the effect of disturbances on pollinator communities, showing the negative impacts of anthropogenic disturbance on pollinators in American grasslands; however, bees have been found to disproportionately thrive in medium-disturbance environments in African savannas (Hanberry et al. 2021, Stein et al. 2018). Consequently, our data supports the notion that increased defenses as a result of disturbance have allowed SLN to mitigate herbivore damage and outcompete non-weedy plants in South Texas. These results coincide with our previous work on SLN which found similar results with regard to SLN defense against herbivores as a result of mowing, showing *M. sexta* gaining significantly less mass on mowed plants (Chavana et al. 2021). Additionally, we reiterate the importance of showing the increased germination rates from mowed SLN plants as a result of continuous mowing because it enhances the ability of SLN to not only diminish herbivore stress but also benefit from anthropogenic stress, and that the methods meant to control these weeds actually leads to them returning more rapidly to the environment. Following this, SLN growing in urban soils have been observed to have reduced herbivory as well, supporting their ability to thrive in disturbed, urban environments where other plants cannot. (Kasper et al 2021).

With regard to mowing frequency, which has less previous data to draw conclusions upon, early instar mass was larger within low mowing frequency individuals and the opposite effect was found in late instar larvae as high mowing frequency individuals were significantly heavier. Haghkerdar et al. 2019 found detrimental effects because of repeat disturbances on multiple invertebrate taxa including insects, which aligns itself with the lower mass of high mowing frequency larvae observed during the early instars. This, however, contradicts the late instar masses as they were higher in high frequency of mowing, yet this can be attributed to stress-induced vigorous growth responses. Although we could not disentangle the effects of mowing frequency, our findings show clearly that mowing as a disturbance has detrimental effects on the Solanaceae specialist herbivore, *M. sexta*.

Taken together, our data supports that premise that mowing has significant impact on floral traits, affecting both their fitness traits but also their ability to defend themselves against herbivore insects. Mowing as a disturbance has strong indications of being an important environmental anthropogenic disturbance and needs to be better understood. For example., while our data from field and lab showed defense trait induction, our experiments did not have enough resolution to examine spatial and temporal variation in defenses, including secondary metabolites, and gene expression (Kariyat et al., 2012a; 2012b). We also did not examine trophic consequences for moving, including the attraction of pollinators and predators (Tayal et al. 2021) And finally, additional experiments should also examine how mowing affects floral scent with possible consequences for fitness, as most buzz pollinating species, use multi modal host selection (flower size, color, flower density, and scent in Solanum genus (Tayal et al., 2021, Kariyat et al., 2021).

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