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Examination of Greenhouse Conditions That Influence Scale Infestation Rates on Begonia

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in the
Department of Biology

By:
Madeline Cusick

Under the mentorship of Dr. Michele Guidone

ABSTRACT

Scale insects are common pests within greenhouse and agricultural settings. I quantified scale infestation on *Begonia* x Richmondensis, also known as the Dragon Wing begonia, within the Armstrong Campus Greenhouse from March to November of 2023. Six different treatments accounted for variations in sunlight, humidity, and temperature within the greenhouse with the goal of determining the insect's preferred environmental conditions. Morphological comparisons were also made between host plant Dragon Wing begonia and other begonias present within the greenhouse. Analysis indicated a significant difference in scale presence on replicate plants by zone and sunlight treatments, with more plants hosting at least one scale insect in the back of the greenhouse within the light rather than shade. Additionally, significant differences were detected in monthly scale density from August to October, with higher densities in the light. Data by month showed there were consistent significant differences in scale abundance between the top and bottom leaf surfaces; scale was more abundant on the leaf bottoms. In comparing the Dragon Wing to other begonia species, the Dragon Wing begonia exhibited lower tissue strength and lower trichome abundance. These results indicate that though scale infestation occurs throughout the greenhouse setting, scale insects seem to prefer high light conditions, and may secondarily select for higher humidity and temperature conditions.

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Introduction

Scale insects are a tremendous problem for global horticulture and agriculture. They are detrimental to plant health due to their active siphoning of plant sap, reducing nutrient availability for plant growth and reproduction (Mahr, 2022). All species of scale feed upon plants, but individual species of scale feed on different plant species (Mahr, 2022). In order to obtain nutrients from the plant, the scale must attach itself to a plant part (e.g. stem, leaves, branches, twigs). Once the scale attaches itself to its desired location, the insect begins to feed. All scale species feed through a tube-like structure that pierces the plant part infected by the scale; the scale then siphons the sap. Scale tends to infect perennial plants because these provide a constant availability of nutrients (Mahr, 2022). This, along with the fact that they are so easily spread by wind (Mahr, 2022), makes scale a formidable challenge. This can be devastating to those who make their livelihood off of these crops. According to Miller et al. (2014), scale insects in the U.S. alone cause billions of dollars in damages and control costs per year.

Understanding scale insect host-plant choice is important for the potential development of insect repellents, which could aid global agricultural efforts and increase revenue. Yet there has been little research into what specifically drives these insects to select their host-plants. Several theories have been presented regarding insect host-plant selection, including presence of natural enemies, ideal oviposition location, population density, and availability of high-quality hosts (Carrasco et al., 2015; Mayhew, 1997). Other theories have been presented regarding insect host-plant choice and female phenotypic plasticity, in regards to olfactory and chemosensory cues; Carrasco et al. (2015) suggested that phytophagous insects, which include scale, are somewhat selective when it comes to choosing host plants based upon plant chemical composition and olfactory signals. These olfactory and chemoreceptors may elicit memories regarding past host-plant selection and interactions, though more research is needed to further understand this phenomenon.

One of the various host plants that scale insects may be attracted to are begonias, which is an economically important ornamental angiosperm (Akintoye et al., 2013). While there has been extensive research into the scale insect itself as well as the *Begonia* genus, there has been no research published in English, to my knowledge, pertaining to begonia-scale interaction, scale-host plant selection, or scale preference for different plants of the *Begonia* genus. The purpose of this study was to understand the factors that lead to scale insect host-plant choice. First, I hypothesized that scale insects would be drawn to plants in more humid environmental conditions. Second, I hypothesized that scale insects would select host-plants based on sunlight availability; the condition most conducive to scale success being medium light, with some access to shade for predator evasion and crawler protection. Finally, I hypothesized that scale insects would select host plants in a cooler temperature environment rather than a hotter environment in order to avoid desiccation.

Methods

Study Species

Scale insects are in the order Hemiptera, family Coccoidea (McLean, 1955). Scale comes in two forms: armored and soft (Mahr, 2022). Soft scale generally grows larger than armored scale. Immature soft scale starts off as a light color, but tends to darken as they mature. Female soft scale can produce either live young or eggs, and keep their young under a soft, waxy covering until they mature. Armored scales develop a hard outer coating when mature, which differentiates them from soft scale (Mahr, 2022).

Young soft scale tends to mature around the two-week mark, and once mature, they emerge from under the waxy coating. These young, called crawlers, then spread across the plant until they find a suitable place to settle. Those that develop into males retain their wings and legs, but lack the ability to feed because of the absence of functional mouthparts. The males of the species tend to die soon after reproduction due to their inability to feed. Those that develop into females have reduced wings and legs and become immobile once they develop their covering (Mahr, 2022). Soft scale are generally found on the underside of plants, and can be found only in relatively temperate climates because of how susceptible they are to environmental changes (Useinov et. al, 2020).

Female armored scale produce either live young or eggs, which reside under the female's armored coating (Mahr, 2022). The crawlers, or young, mature around two weeks, and are then released from under the armored coating to spread across the plant to find a place to settle and feed. Once settled, female scale insects produce a sticky substance that helps them attach to the plant. They also quickly develop a waxy coating, which is a precursor to the armor exhibited by mature females. Females go through several molting phases, the first of which causes the female to lose its legs and antenna. Females then develop their armored coating and become immobile. Similar to the soft scale, male scale insects do not develop the armored coating and develop without functional mouthparts, which reduces their lifespan due to their inability to feed (Mahr, 2022). Armored scales are usually found lower on the plant, and in more varied climates than soft scales. Therefore, armored scale insects can survive better in a greater variety of environments and climates in comparison to soft scale (Miller, 2005)

Begonias are ornamental angiosperms within the order Cucurbitales, family Begoniaceae, and either genus *Hillebrandia* or *Begonia* (Ginori et al. 2020). Begonias are perennials, though they cannot withstand freezing temperatures and are therefore usually treated as annuals. These plants prefer tropical climates and shaded environments due to the fact that begonias are sensitive to high light levels, which could lead to wilting if not handled correctly. Begonias are relatively robust in high temperatures, leading them to be a popular ornamental indoor or outdoor plant choice in hotter climates (Ginori et al., 2020). If provided with the correct nutrient levels, begonias can grow to be a wide variety of colors, and produce different types and colors of flowers depending upon the species selected.

Begonia x Richmondensis was the selected study species and scale host plant for this experiment. According to Ginori et al. (2020), it is a shrub-like begonia with fibrous roots, sometimes given the common name Dragon Wing begonia. Begonias within this species have distinctly shaped leaves, and can vary from ear-shaped, to spiral-shaped, to wing shaped, which is the category that the host plant falls under. *Begonia* x Richmondensis has dark green leaf tops and red leaf bottoms and stems. It produces small, bilabiate flowers that can range from dark pink to white (Ginori et al., 2020).

Experimental Setup

In order to create the different temperature, humidity, and sunlight conditions, six treatment groups were set up within the greenhouse on the Armstrong campus. Three treatment zones were located on separate benches spaced throughout the greenhouse. One zone was located in the back of the greenhouse and had high humidity and overall low light, a second was located in the middle of the greenhouse to create a medium humidity zone and overall medium light exposure, and the third was located at the front of the greenhouse to create a low humidity zone and overall high light exposure. Within each zone there were two sunlight levels, one of which was exposed fully to light, and the other that was shaded by mesh coverings. The mesh coverings, or shades, were built from a frame of PVC piping (90 cm in length x 60 cm in width x 20 cm in heigh) with window screening stretched across the top of the frame and attached with zipties. These shades simulated the amount of sunlight exposure plants received within the lowest light zone at the back of the greenhouse. Temperature and sunlight were monitored for each of the treatments using HOBO TidbiT v2 temperature data loggers and an Apogee Instruments light meter (Model MQ-500), respectively. Humidity was monitored in each location using a BestAir brand humidity gauge.

Observational Procol

Prior to being placed into treatments, experimental plants were visually inspected and scale insects were removed to ensure that the plants were scale free before beginning the experiment. Plants were placed into treatments on March 2, 2023, were watered as necessary when the top 1 centimeter of soil became dry, and visually inspected twice a week for new insects. The number of insects found on the stems and tops and bottoms of leaves was recorded weekly to biweekly when the experiment began, but was changed to monthly beginning May 4, 2023. The same protocol was followed when measuring and recording light values. The start and final lengths of the plants themselves were also recorded.

On November 15, 2023, the photosynthetic efficiency of plants in each treatment was recorded as a function of F_v/F_m using a Handy PEA+ advanced continuous excitation chlorophyll fluorimeter. This measurement indicates if the plant was under photosynthetic stress (Joesting et al., 2020), and was important to measure in this study because the presence of scale insects on a plant can cause increased stress levels. $F_v/F_m$was taken for a single leaf from six plants in each zone-sunlight treatment combination.

Morphological Comparisons of Begonia spp.

Throughout the experiment, a lack of scale was observed on other plants within the *Begonia* genus that were grown alongside the infected host Dragon Wing begonias within the greenhouse setting. As insects did not infect any other surrounding begonia plants, additional tests were performed to determine different aspects of Dragon Wing begonia morphology in comparison to other begonia species present in the Armstrong greenhouse. These plants were *Begonia* x Erythrophylla (Beefsteak begonia), *Begonia rex* (Persian Swirl begonia-hybrid), and *Begonia* x Rhizomatous (Northern Lights begonia). The specific morphological characteristics measured were leaf tissue strength and number of trichomes present on the leaf surfaces and veins. Scale-free leaves were collected from each of the plants (10 from Dragon Wing begonias and 6 leaves from the other species). All leaves were inspected using a dissecting microscope between the magnifications of 25x and 40x, depending on what magnification yielded the best visualization results. The number of trichomes on the midrib, other veins, and in between the veins on the leaves' tops and bottoms were recorded. A second set of scale-free leaves was collected as described earlier to test tissue strength of the tops of the leaves using a penetrometer apparatus (Duffy et al., 1991).

Statistical Analysis

The presence/absence of scale on replicate plants was analyzed using a generalized linear mixed model with greenhouse zone (front, middle, back) and sunlight treatment (shade, light) as fixed factors and observation period as a random factor. Scale density for the months of August, September, and October was analyzed using a repeated measures ANOVA with the fixed factors of zone and sunlight treatment; data were fourth root transformed prior to analysis to improve normality and achieve homogeneity of variance. Due to violating the assumption of sphericity, the Greenhouse-Geisser epsilon adjusted values were utilized. Differences in scale abundance between the top and bottom surfaces of the leaves was assessed by month for August, September, and October using the Sign Test due to non-normal data.

F_v/F_m data were analyzed for zone and sunlight treatment separately. F_v/F_m data for zones were analyzed via a Kruskal Wallis test as data were not normally distributed but met the assumption of equal variance. F_v/F_m data for sunlight treatment did not meet the assumptions of normality or equal variance and were thus analyzed using the Median test.

Tissue strength data were normal with equal variance; data were analyzed using a one-way ANOVA. Midrib trichome abundance was normally distributed with heterogeneous variance; data were analyzed via Kruskal Wallis. Trichome abundance on the secondary veins and intervenous spaces was not normally distributed and lacked equal variance; these data were analyzed using the Median Test.

Photosynthetic active radiation data was fourth-root transformed in order to obtain normality and equal variance, and a two-way ANOVA was conducted for analysis. Humidity data required square-root transformation in order to achieve homogeneity of variance; data were not normally distributed, but ANOVA is robust against deviations of normality in instances of sample sizes over 30 (Underwood, 2006). In this experiment N = 78, therefore data were analyzed via two-way ANOVA. Temperature data were not normal and lacked equal variance, but the sample size (N = 1,428) was sufficiently large to proceed with a three-way ANOVA.

Results

Scale presence on replicate plants significantly differed between zone ($F_2 = 10.44$, $p < 0.0001$, Fig. 1) and sunlight treatment ($F_1 = 15.06$, $p = 0.0001$, Fig. 1), with a significant zone*sunlight interaction ($F_2 = 4.77$, $p = 0.0088$, Fig. 1). More plants in the back-light zone were infected than any other treatment combination (Fig. 1), while plants in the middle-shade zone were least infected. In contrast, plants in the front zone exhibited similar infection status across the light and shade levels (Fig. 1). Scale presence also differed by observation period (Wald $p = 0.0244$, Fig. 2); infection status dramatically increased between the months of July and August (Fig. 2).

Overall scale density generally increased from August to October ($F = 28.1698$, $p < 0.0001$), with greater density in the light ($F = 0.1027$, $p = 0.0439$), though significant interactions were present between zone and sunlight treatment ($F = 0.1946$, $p = 0.0239$) and month and zone ($F = 0.8247$, $p = 0.0127$; Fig. 3). Scale abundance across all zones and sunlight treatments was greater on the bottom surface of the leaves for all months (p for all months < 0.0001; Fig. 4).

No significant difference in F_v/F_m values was detected among plants in the three zones ($H_2 = 5.0259$, $p = 0.0810$). F_v/F_m values were significantly greater ($X^2 = 3.8889$, $p = 0.0486$) for plants in the shade (mean = 0.8166 ±0.02792 SD) than the light (mean = 0.7785 ± 0.06211 SD).

Tissue strength did not differ among the begonia species ($F_{3, 25} = 1.4862$, $p = 0.2424$). Mean tissue strength was greatest for the Beefsteak begonia (49.9771 ± 11.8171 SD) and weakest for the Dragon Wing begonia (39.8665 ± 10.6662 SD); mean tissue strength for the Northern Lights begonia (47.56 ± 10.3231) and Persian Swirl begonia (47.7908 ± 9.9070) were intermediate, but similar to the Beefsteak begonia.

Trichome abundance on the midrib was significantly greater on the Persian Swirl begonia than all other species examined ($p = 0.0002$, Steel-Dwass post-hoc test, Fig. 5). Trichome abundance on the secondary veins of the Beefsteak begonia was significantly less than the abundance observed on the other begonia species ($p = 0.0100$, Steel-Dwass post-hoc test, Fig 5). Trichome abundance in the intervenous spaces of the Dragon Wing begonia was significantly less than the other species ($p = 0.0004$, Steel-Dwass post-hoc test, Fig. 5).

Photosynthetic active radiation was significantly greater within light in comparison to shade ($p = 0.0007$) and increased moving from the back zone to the front zone ($p < 0.0001$, Fig. 6).

A significant trend in humidity data was detected between zones ($p < 0.0001$, Fig. 7); humidity was lowest at the front of the greenhouse, while no significant difference in humidity was observed between the middle and back zones (Tukey post-hoc test). Humidity was the same for light and shade ($p = 0.7907$), with no interaction between factors (zone*sunlight treatment $p = 0.9814$).

Temperature significantly differed by month ($p < 0.0001$) and zone ($p < 0.0001$), with an interaction between sunlight treatment and zone ($p = 0.0077$); all other interactions were not statistically significant (month*sunlight treatment $p = 0.9974$; month*zone $p = 0.2414$; month*sunlight treatment*zone $p = 1.000$). Temperature was highest in the front zone and lowest in the back, with peak temperatures in the months of July and August and lowest temperatures in the months of March and October (Fig. 8). Though temperature differences were present by zone, these differences were minor (1-3° F, Fig. 8).

Discussion

This study found that more Dragon Wing plants were infected with scale insects in the light, which received more PAR, than in the shade; and more plants within the back zone of the greenhouse were infected than those in the middle or front zones. There were notably more infected host plants in the light treatment of the zone located in the back of the greenhouse, and significantly less infected plants in the shade treatment in the zone located in the middle of the greenhouse. In contrast, scale density data actually reflected an increase in scale insects in the front of the greenhouse, where humidity was the lowest. These data sets indicate that scale insects prefer higher light conditions rather than shade, and that they may prefer warmer climates and lower humidity. Humidity did not differ significantly between the middle and back zones, and only minor differences in temperature were recorded within the Armstrong Greenhouse, indicating that temperature and humidity may be less important than sunlight availability in the scale-host plant selection process within a greenhouse setting. Dancewicz et al. (2017) conducted a study to determine the effect that host plant light availability had on aphid insect reproduction and fitness. This study indicated that not only did most aphids refuse to settle and reproduce on host plants in no-light and low-light conditions, but that those that did settle on plants in low-and no-light conditions had significantly shorter reproductive periods and limited population growth rates (Dancewicz et al., 2017). Just et al. (2020) observed that scale insects responded positively to warmer climates with increased abundance, survival, and egg production. Scale density appears to be, on average, greater in the front, warmest zone than other zones; this is in agreement with findings made by Meineke et. al (2013) that found scale density was thirteen times more abundant on trees in warmer climates than in cooler ones. Given this information, it can be concluded that environmental temperature may be a significant factor in scale-host plant choice, though further research is necessary to solidify these findings. This could be especially important to those who work within the greenhouse setting, in that greenhouses tend to be warmer than open-air environments.

The number of plants infected by scale insects in the months of August, September, and October was greater in comparison to all other times of the year, with the number of plants infected with scale in August being less than the number of plants infected with scale in September and October. This corresponds to when scale insects are most likely to reproduce, hatch, and disperse rapidly. Data collected from trees and shrubs by University of Minnesota showed that female scale insects lay eggs from the months of May and June, and when crawlers hatch between June and July they begin to spread and infect surrounding plants and leaves (Hahn, 2021). Other data trends were observed concerning scale density, indicating that density generally increased from August to October, with a peak in September, and was greatest in light.

Scale density trends also differed depending upon leaf surface; more scale were present on leaf bottoms in comparison to leaf tops. This trend may be attributed to the presence of a thicker cuticle on the tops of leaves, but this requires further research.

Plant health was measured using photosynthetic productivity values (F_v/F_m), where plants with F_v/F_m measurements between 0.75 and 0.83 are generally considered healthy and more productive photosynthetically, and therefore less stressed (Joesting et al., 2020). Collected fluorescence data indicated that plants in shade had higher F_v/F_m values in comparison to those in light, and were therefore less stressed; but none of the plants from either sunlight treatment deviated from within healthy F_v/F_m levels (Joesting et al., 2020). This could indicate that the specific Dragon Wing begonia host plant prefers shadier conditions over brighter conditions, but more research is needed.

Scale insect preference correlated positively with increased light conditions, and this in conjunction with F_v/F_m data indicating that plants in shade conditions are less stressed than those in light conditions, could indicate that scale insects are selecting plants in light treatments due to their compromised production of photosynthetic chemicals. Because conditions are not totally conducive for plant growth, more biological energy must be put towards the production of primary metabolites, and less towards the production of secondary metabolites, like anti-herbivory chemicals (Dancewicz et al., 2017). Dancewicz et al. (2017) also suggests that insect presence and light availability play a role in plant photosynthetic stress. Aphids actively rejected settlement on plants in no-light and low-light conditions that caused increased photosynthetic stress. In this study, while differences in F_v/F_m data trends were observed between light and shade, none of the plants within any treatment or zone exhibited unhealthy levels of photosynthetic stress, and this could be due to the reduced amount of stressors within the greenhouse setting. Factors that can affect photosynthetic productivity include water stress (Kansman et al., 2020), insect presence, and light availability (Dancewicz et al., 2017). Kansman et al. (2020) concluded that aphids are less likely to select for plants that are highly-stressed in comparison to those that are experiencing minor or no water stress.

No significant differences were observed in tissue strength among the begonia species, but data trends indicate that the Dragon Wing begonia had a weaker leaf-top tissue strength compared to the others, while the Beefsteak begonia exhibited the toughest leaf-top tissue strength. One caveat with this protocol is that the penetrometer apparatus could only measure the tissue strength of the leaf tops due to the inability to separate different leaf tissues into separate tissue samples. However, these data suggest that scale insects may select the Dragon Wing begonia as a major host plant in the greenhouse due to weaker leaf tissue. In contrast, Caldwell et al. (2016) and Peeters et al. (2007) both found that transitory insects that siphon sap for nutrients correlated positively with mechanical traits of tissue strength. This difference in results may be attributed to the scale lifecycle; scale insects remain on host plants for the duration of their lifetime, while most other siphon-feeding insects move between different host plants. Thus, scale insects must leave their host plant in relatively good condition in order to ensure the survival of their progeny. Caldwell et al. (2016) and Peeters et al. (2007) used visible damage as their indicator for insect-host plant selection, and scale insects do not produce the same type of visible plant damage during their lifecycle since their siphon is not visible to the naked eye.

Trichome data trends, along with the observed pattern of scale insects attacking and tapping veins for nutrients, may indicate that lower leaf trichome presence on veins could make the host plant more attractive for scale insect feeding in comparison to other begonias present, though previous research contradicts this idea. Nalam et al. (2021) indicated that the presence of anti-herbivory structures (like trichomes) may have a minor effect on insect-host plant selection, unless it affects phloem access.

Though sunlight availability, temperature, humidity, month, leaf surface, trichome presence, plant health, and tissue strength are all factors in insect-host plant choice, these do not encompass the totality of factors which can dictate this decision in insects, and further research is necessary to fully dissect these interactions. Future research endeavors should place a significant focus on identification of plant volatiles and anti-herbivory complexes within *Begonia* species as a basis for understanding scale insect-host plant choice, as well as for the development of environmentally safe scale insect deterrents. Production of these deterrents could increase agricultural productivity and revenue, thus bolstering economies globally. Within a greenhouse setting, conclusions can be drawn concerning optimal growth conditions for the Dragon Wing begonia, which specifically include lower light intensity. This will help to both increase the biological productivity of the Dragon Wing begonia plant, while also acting as a deterrent to scale insects, which seem to prefer plants in higher light intensity environments.

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Figures

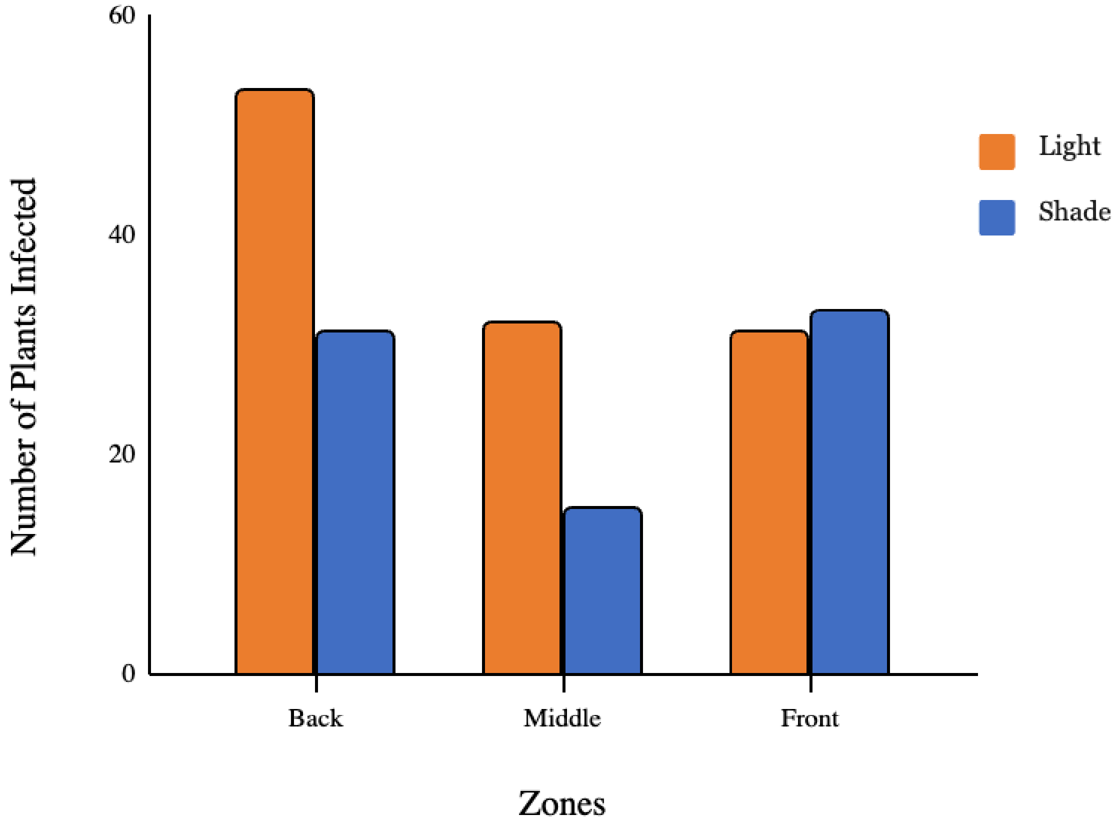

Figure 1. Number of plants infected across all dates (48 plants observed on 13 occasions) within the light and zone treatment combinations.

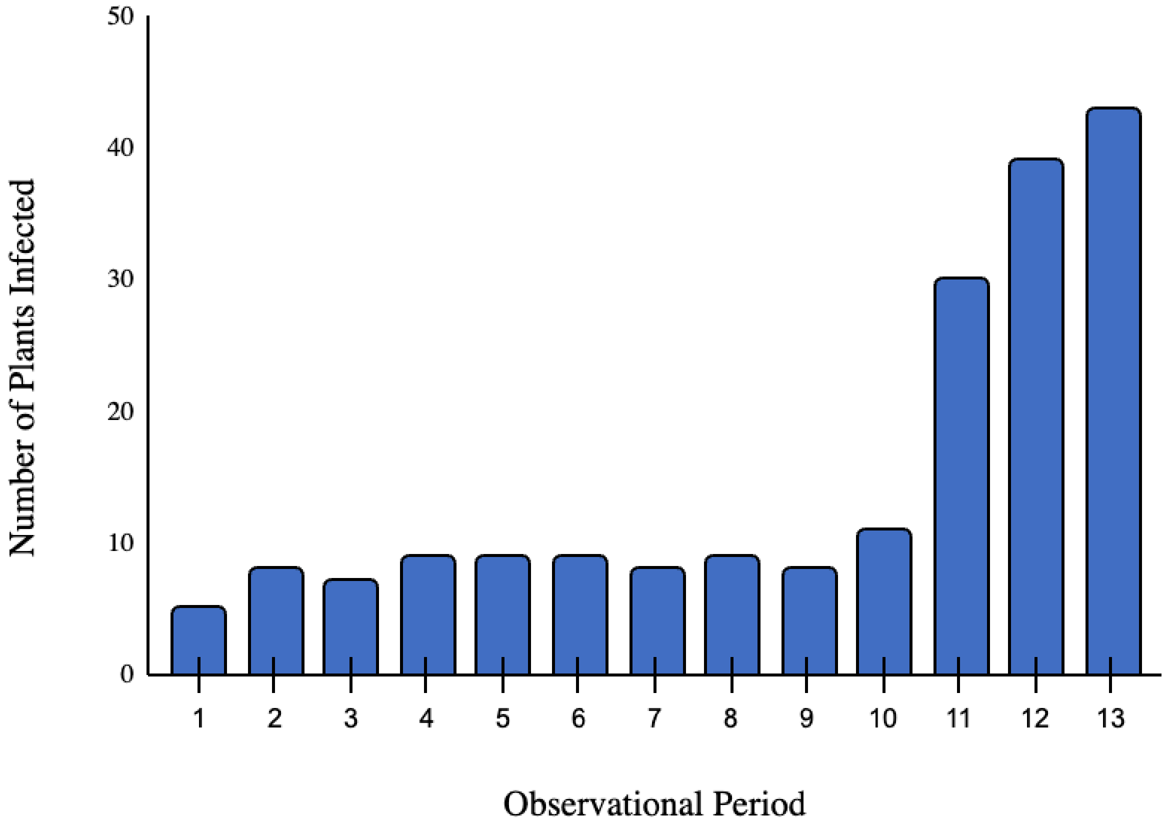

Figure 2. Number of plants infected (out of 48 total) per observational period (by date in 2023); 1) - March 9, 2) - March 23, 3) - March 30, 4) - April 6, 5) - April 13, 6) - April 20, 7) - April 27, 8) - May 4, 9) - June 2, 10) - June 30, 11) - August 16, 12) - September 13, and 13) - October 18-November 1.

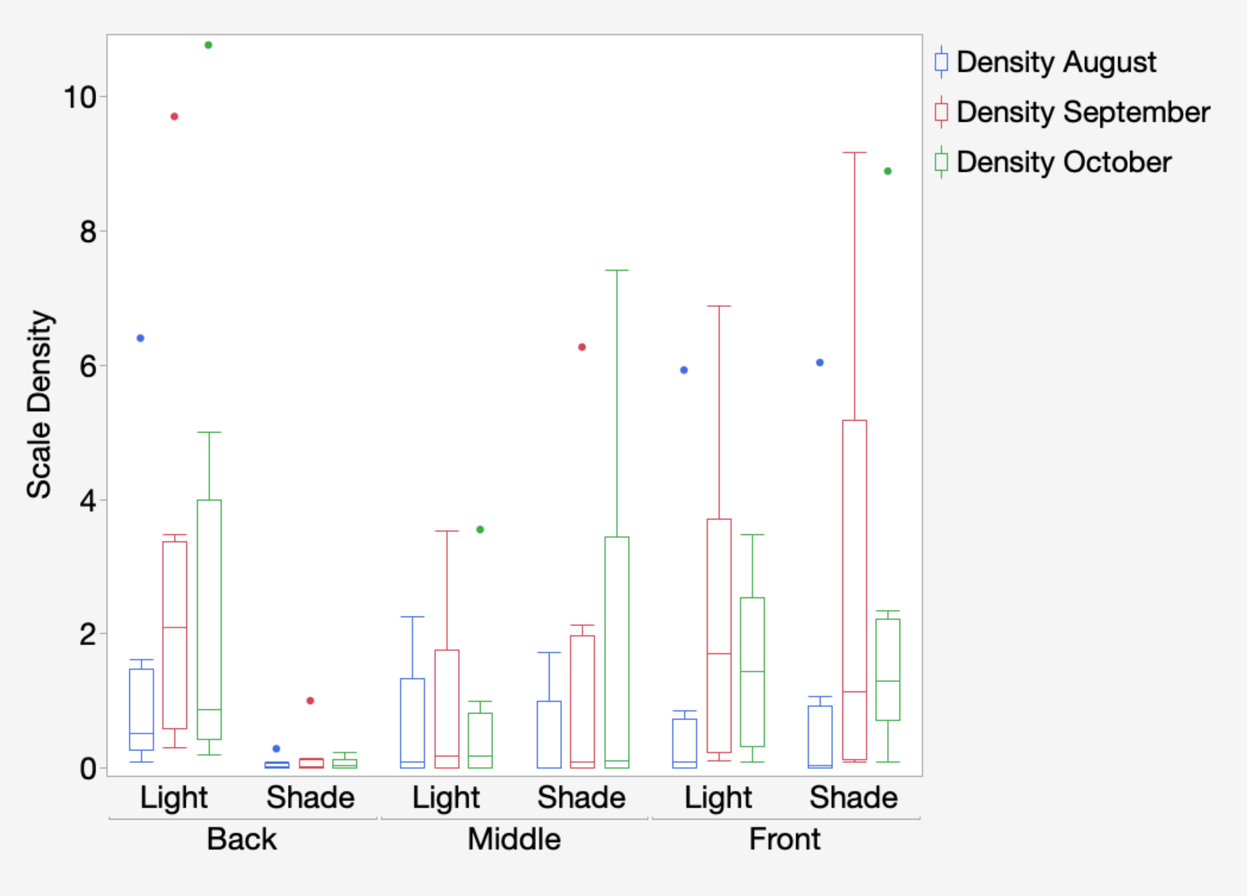

Figure 3. Scale density (mean number per leaf) by month across the light and zone treatments.

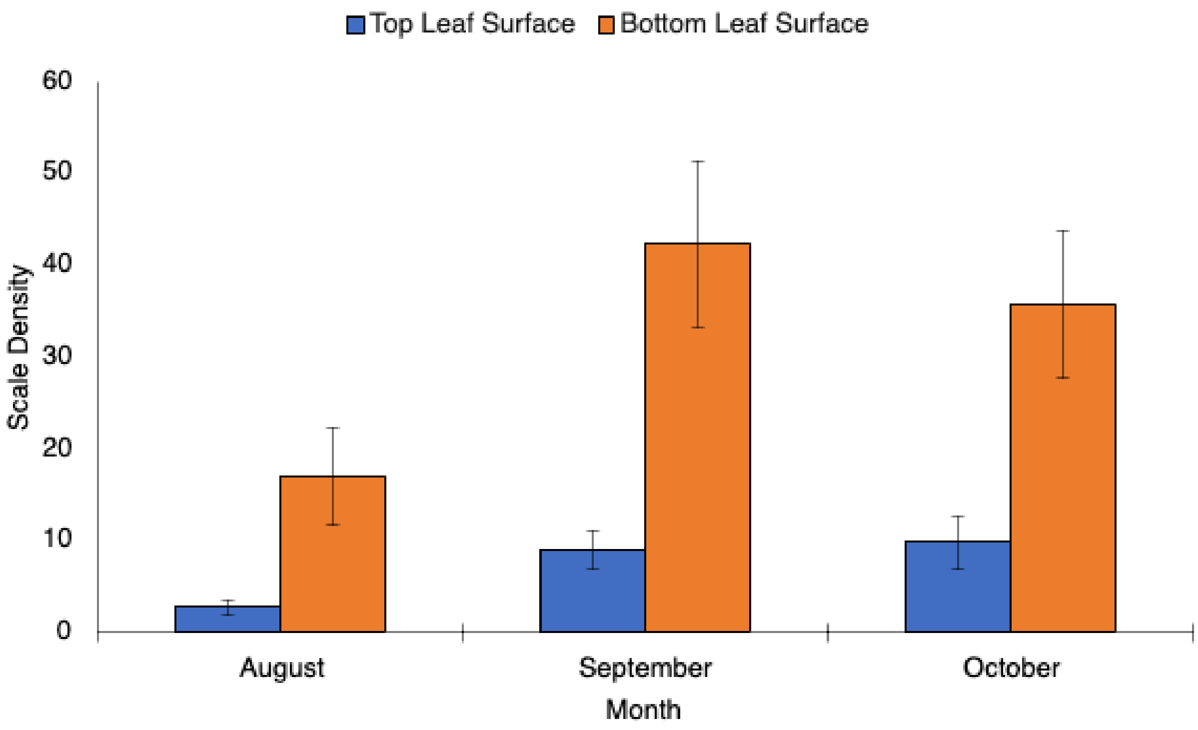

Figure 4. Scale density on top and bottom leaf surfaces across the months of August, September, and October.

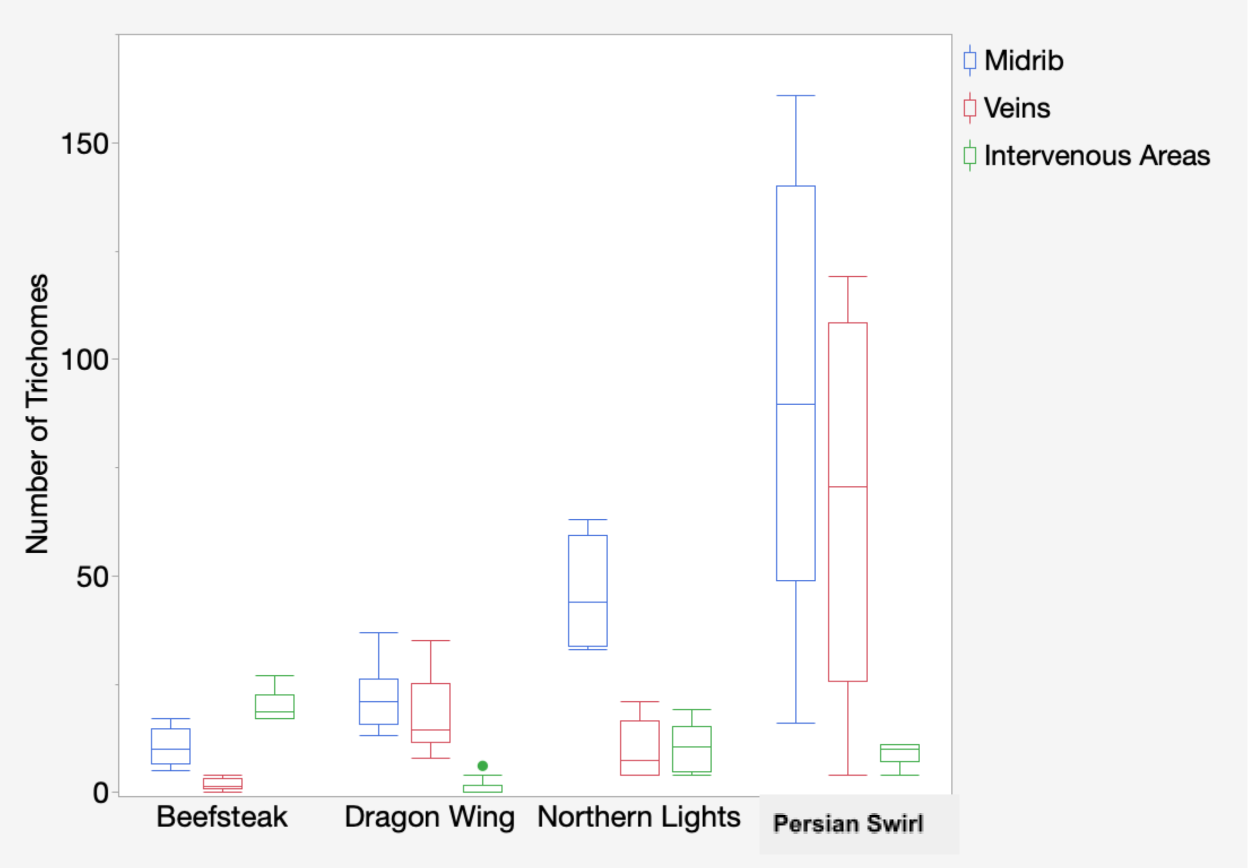

Figure 5. Trichome abundance on midrib, other veins, and intervenous leaf tissues associated with different species of begonia→Beefsteak begonia (*Begonia* x Erythrophylla), Dragon Wing begonia (*Begonia* x Richmondensis), Northern Lights begonia (*Begonia* x Rhizomatous), and Persian Swirl begonia (*Begonia rex*-hybrid).

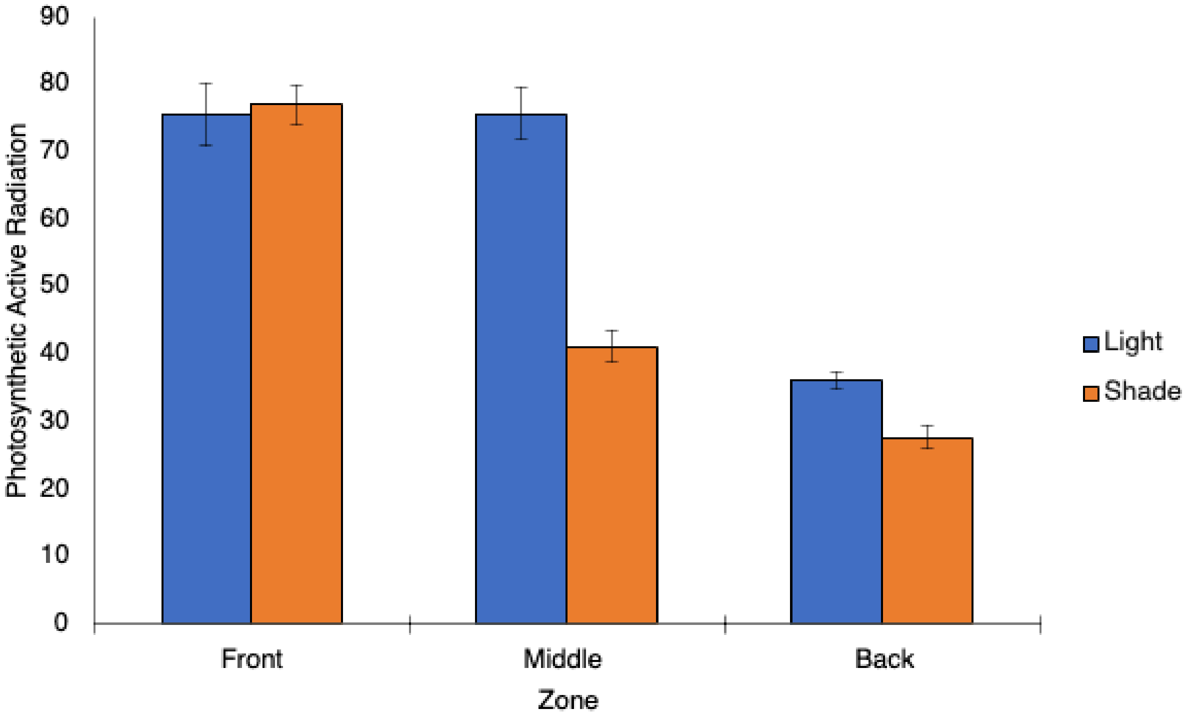

Figure 6. Mean photosynthetic active radiation levels (± 1 SD) within each light and zone treatment combination across all dates.

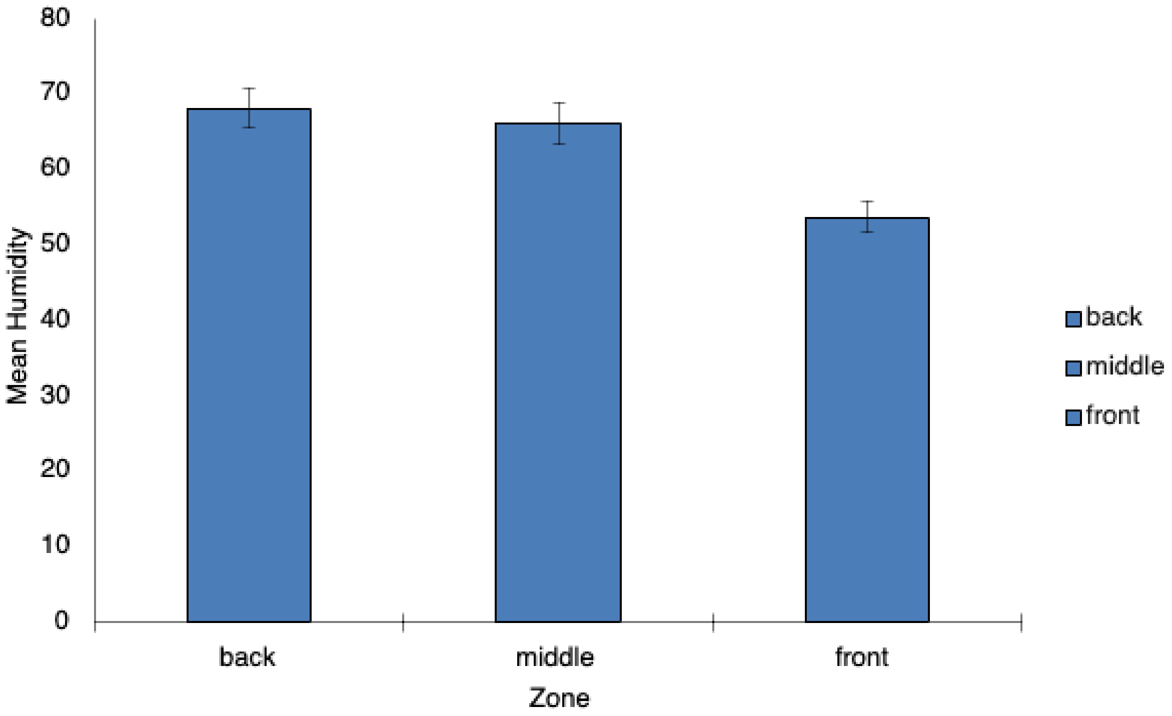

Figure 7. Mean humidity by greenhouse zone (± 1 SD) across all dates.

Figure 8. Temperature distributed by zone across all dates.