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Leaf morphology in response to changes in light intensity in *Convolvulus tricolor*

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ABSTRACT

Convolvulus tricolor, native to the Mediterranean regions of Europe, has spread across the world as an ornamental plant used in gardens and flowerpots. Multiple countries have classified this plant as invasive. Members of the *Convolvulus* family cause environmental damage due to the ability to spread and monopolize resources. To explore why this plant is so successful and invasive across different conditions, we examined the anatomical and morphological leaf characteristics across two light levels. The results showed that plants in the high light group have a greater leaf area and perimeter. Leaf shape also varied across the two light treatments, with the high light group having a greater perimeter to area ratio. While more stomata were present on the adaxial and abaxial sides of the leaves in the high light treatment, there were no differences in overall stomatal density across the treatment groups for either leaf side. One interesting trend showed a greater relative investment of low light plants into stomata on the adaxial leaf surfaces. More research is needed to demonstrate that these differences are light-dependent. These responses may contribute to this plant family's ability to thrive in a variety of environmental conditions.

Keywords: *Light intensity, morphology, stomatal density, Convolvulus tricolor*

INTRODUCTION

Climate change is directly linked to the increased rate at which invasive species establish and spread (Walther et al., 2009; Diez et al., 2012; Bellard et al., 2013). These species threaten biodiversity worldwide, as well as the economies and public health within environments that are threatened (Millennium, 2005; Early et al. 2016). The rate at which these species are spreading has increased dramatically due to disturbances of agriculture (Chytry et al., 2009; Early et al., 2016), changes in native biotic communities, (Diez et al., 2012), and adjustments in fire frequencies and regimes (Brooks et al., 2004).

There are different factors that make invasive species so successful. Alien plant species can monopolize resources making native plants struggle for survival (Brooks et al., 2004). Due to the lack of competition, invasive species can multiply and spread at a much faster rate than native species with very little opposition from the environment (MacDougall et al., 2005). One of the common approaches of controlling ongoing plant invasions is limiting light availability to alien species (Perry and Galatowitsch, 2006; MacDougall et al., 2005).

The effect of light on the performance of plants is still being studied. Responses to changes in light depend on environment, stress, seasonality, reproduction, and anatomy of different plant species (Bayat et al., 2018; Kozai, 2016). Different light wavelengths are connected to the performance of leaves and the photosynthetic properties of plants (Bayat et al. 2018). However, the way individual plants species respond to light intensity is still being researched. Invasive species and native species compete for light as a resource which ultimately affects the photosynthetic rate of the different plants and their stress levels. The invasiveness of plants is decided by a high number of adaptable traits and coordination. Invasive plants can reproduce and spread, which increases their competitiveness and fitness. This allows these plants to take over native species habitats and cause damage to the ecosystem (Osunkoya, 2014).

Invasive species have been found to respond to different levels of available light by changing leaf anatomy and cell walls (Boyne et al., 2013). This adjustment can increase or decrease the plants reproductive success. One invasive species known for its successful reproduction rate are plants in the Convolvulus genus such as Convolvulus arvensis, also known as field bindweed and Convolvulus tricolor, a type of decorative morning glory. These vining plants are successful due to their extensive root structure and asexual reproduction (Zouhar, 2004), and are highly competitive and difficult to remove. Due to the deep taproot, members of this plant genus can monopolize stored nutrients from deep within the soil, creating problems for surrounding species (Kennedy and Crafts, 1931).

The way these plants respond to light strain and stress allows us to learn more about how to control it and how it interacts with neighboring species (Liancourt et al., 2013). Studying stress tolerance helps increase the understanding of these plant's success and their control. The quantity of light affects individual leaf morphology and photosynthetic performance which leads to greater plant success

(Francis and Gilman, 2019; Dwyer et al., 2014). It is hypothesized that Convolvulus tricolor will adjust leaf morphology and performance due to different light intensities. This change has been found and studied in other invasive vines which shows anatomical differences in the shape, size, stomatal concentration, and rate of photosynthesis the individual leaves can perform (Boyne et al., 2013).

MATERIALS AND METHODS

Convolvulus tricolor were grown from seeds. A total for forty-two of them sprouted and were separated into two groups of twenty-one. Each plant was marked with notched leaves so that new, fully developed growth was tested while also accounting for the possibility of losing different specimens. These seeds were started in an Expert Gardener brand substrate mix of organic and inorganic substances. There were two different light groups placed in separate containers and placed in a greenhouse with windows facing to the south and east. The low light group was covered with a black shade cloth. The shade cloth treatment reduced light levels inside by at least 5x across different direct and indirect sunlight levels in the greenhouse (without shade, $\bar{x} = 2724\ \text{lx}, N = 4;\ \text{with shade}: \bar{x} = 504\ \text{lx}, N = 4$). The temperature and humidity levels were kept as constant as possible for both light groups. This allowed natural lighting to mimic different light environments where the plants may grow in the wild. Each plant was watered to full saturation as needed and their positions in the sections were rotated throughout the experiment.

Fully developed and undamaged leaves were harvested from each specimen from the two light groups. Following harvest, a clear nail varnish was applied to the lower (abaxial) and upper (adaxial) surfaces of the leaves. The varnish was transferred to slides and examined under a microscope to determine stomatal density (Boyne et al., 2013). Leaf shape was measured by taking a picture of the leaf and uploading it to the computer program ImageJ. This was to make a more accurate comparison between different leaf shapes, perimeter, and area. These tests were performed once using leaves after the plants were around four months old. The mean of the different measurements was calculated, and T-tests were run to determine the difference between the two groups.

RESULTS

Light had a significant effect on the mean leaf area, with higher values for the plants grown under high light conditions (T=7.465, P< .001, Table 1). Leaf shape also varied across the two light treatments, with the low light group having a greater perimeter to area ratio (U=11.0, P< .001, Table 1).

Table 1. The average leaf area, perimeter, and ratio which shows the difference in leaf size and shape for both groups.

	High Light	Low Light
Mean Leaf Area (cm^2)	13.47	5.4
Mean Leaf Perimeter (cm)	21.71	13.77
Perimeter to Area Ratio	1.61	2.55

Leaves had stomata that were generally paracytic across both groups. Stomata were found on both the adaxial and abaxial surfaces of the leaves, with more stomata found on the abaxial surfaces. The overall number of stomata were different across light treatments (U=3.00, P< .001), with a greater number of them found on the high light group. While the overall stomata numbers were different across the two groups, the stomatal density was the same (P= 0.514 P= 0.342). With each plant, the allocation of stomata on the adaxial and abaxial surfaces tended to depend on the light level they were adapted to. Plants in the high light group tended to have more abaxial than adaxial stomata. The plants in the low light tended to follow the same pattern but with less difference between the two (Figure 1). These differences in stomatal allocation between light treatments was marginally insignificant (F=2.87 P=0.098).

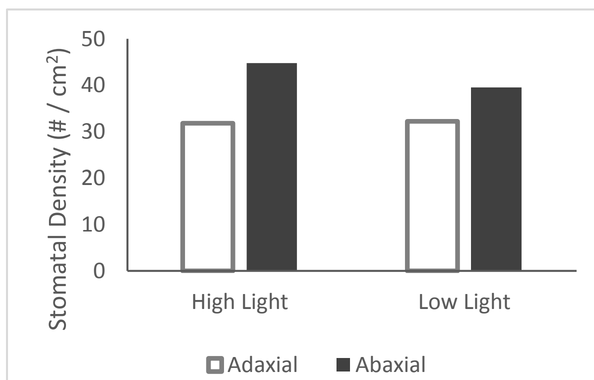

Figure 1. The stomatal density found on the adaxial and abaxial leaf surfaces for both the high light and low light groups

DISCUSSION

This study found that Convolvulus tricolor has the ability to adjust to light intensities. Other plants have been shown to respond to environmental stressors by modifying the anatomy and morphology of their leaves (Miner et al. 2005). Modifications are common in plants that grow with limited light availability (Markesteijn et al. 2007). The plants growing in lower light conditions had smaller leaves. These observations of leaf morphology and anatomy of C. tricolor align with previous research performed on other invasive vine plant species (Boyne et al., 2013). This response to light intensity is likely to be adaptive for plants that experience variable light conditions (Markensteijn et al., 2007). While this limits the investment of costly resources, it allows for the plant to function at a greater rate depending on the resources which are available. This also allows the plant to be successful in a large number of environmental conditions. This is because individual plants can adjust their photosynthetic surfaces to their surroundings.

Stomata control gas exchange, water loss, and temperature of leaves. More stomata could allow more carbon dioxide to diffuse faster into the structure of the leaf. This can increase the rate of carbon fixation where light and water are not limiting but would be inefficient where resources are scarce (Boyne et al., 2013; Osunkoya, 2014). While marginally insignificant, results showed a greater relative investment of low light plants into stomata on the adaxial leaf surfaces. For the plant to be successful in a low light environment, the greater number of stomata can allow individual leaves to function at a similar rate as the leaves with more available light. These low light plants will also lose less water as the stomata open and close which allows more stomata to be on the upper surface of the leaf due to the slower rate of evaporation.

More research is needed to demonstrate that these differences are light-dependent. More importantly, future research should focus on how these changes in leaf morphology affect overall leaf performance and photosynthetic rate. It could be that leaves from low light conditions are actually more efficient due to these adjustments in leaf shape. These responses may contribute the plant's ability to thrive in a variety of environmental conditions.

Because only one leaf per plant was examined, it cannot be determined if the light intensity effects the morphology of all the leaves on the plant or if it effects the morphology of each leaf individually. Future work should look at the plasticity in leaf shape and development when different portions of the plant experience different light levels. Understanding these adaptations may be useful when trying limit the reproduction and spread of invasive plant species.

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