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Influence Of Shaded Conditions On Development Of Asteraceae Species Native to Kansas

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INFLUENCE OF SHADED CONDITIONS ON DEVELOPMENT OF ASTERACEAE SPECIES NATIVE TO KANSAS

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by

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the Master of Science Degree

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ABSTRACT

Due to the heterogeneity of habitats, all plants are exposed to at least some degree of shade during their lifetime. Reduced light intensity, drops in R:FR ratio, and limited blue light are cues for plants to perceive competition; the shade avoidance syndrome (SAS) is common for grassland species while shade tolerance (ST) is common for forest species when competition is perceived. SAS is characterized by elongation of stems and petioles, reduced branches, decreased leaf area, decreased shoot biomass, and increased number of ramets. ST is characterized by little elongation of stems and petioles, high chlorophyll content and high chlorophyll *a/b* ratio in leaves, low root-shoot ratio, and thinner leaves. In this study, germination of six native Asteraceae species was tested against 10%, 50%, and 100% of natural light in a greenhouse. Measurements of growth and reproduction were made in two species under the same light conditions. Shaded conditions decreased germinabilities of seeds in all species that were tested. Increased light conditions resulted in increased growth for both species. When exposed to shaded conditions, both species displayed several traits that are related with shade tolerance modifications such as little elongation of stems and petioles, higher specific leaf area, higher chlorophyll *a/b* ratio, reduced leaf area and root/shoot biomass, and fewer and thinner leaves. In summary, plants displayed unexpected strategies and a high resilience to grow and develop under shaded conditions.

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PREFACE

This thesis follows the style of *Environmental and Experimental Botany*.

INTRODUCTION

Environmental factors, such as local weather, climate, seasons, or position of the plant in the community, directly alter quality and intensity of light that is available for plants, strongly influencing their entire life cycle (Kami et al., 2010; Bian et al., 2014, Patel et al., 2017). Therefore, due to the heterogeneity of habitats, all plants are exposed to at least some degree of shade during their lifetime (Valladares and Niinemets, 2008).

The light signal is perceived by three classes of specialized information-transducing plant photoreceptors: red (R) and far-red (FR) light-absorbing phytochromes, the blue/UV-A light-absorbing cryptochromes, and phototropins (Franklin, 2008). Plant photoreceptors continuously sense and respond to fluctuating light conditions and modulate plant growth and development accordingly (Fiorucci and Fankhauser, 2017). Interactions among the different classes of photoreceptors and their downstream signaling pathways mediate both adaptive responses, such as phototropism, and developmental transitions, such as germination and flowering (Kami et al., 2010; Dierck et al., 2017).

Plants usually grow and develop in dynamic environments, competing with surrounding neighbors over limited resources such as light, water, and nutrients (Keuskamp et al., 2010). Reduced light intensity, drops in R:FR ratio, and limited blue light are cues for plants to perceive competition and display two contrasting mechanisms of response, the Shade avoidance syndrome and shade tolerance (Gommers et al., 2013). Additionally, while shade avoidance responses are also induced by cues that indicate neighbor proximity, such mechanical stimulation, and presence of plant volatile substances, shade-tolerance

responses are known to be elicited in plants mainly via decreases in light intensities (Gruntman et al., 2017).

Shade avoidance syndrome and its metabolic pathways are well described in the literature for model species such as *Arabidopsis thaliana* (Ciolfi et al., 2013) and crop species (Carriedo et al., 2016). Shade avoidance syndrome is a group of responses such as enhanced growth of the hypocotyl and petioles, more erect position of the leaves, and reduced branching, causing substantial changes in plant body form and function (Casal, 2012; Gommers, et al., 2013; Ballaré and Pierik, 2017). Also, responses such as acceleration of flowering, reduced resources for storage and reproduction associated with reduced seed set, and truncated fruit development are also common if the shaded condition is prolonged (Morelli and Ruberti, 2002). These responses are often accompanied by reductions in leaf area, shoot biomass and the size of harvestable organs, a likely consequence of the reallocation of resources towards reproductive structures (Franklin, 2008).

Shade avoidance responses result in optimizing the deployment of leaves into light gaps, balancing resource allocation between shoots and roots, optimizing leaf gas exchange, nutrient uptake as a function of the degree of shading, and adaptively regulating interactions with herbivores, pathogens, and microorganisms (Ballaré and Pierik, 2017). Furthermore, some plants may respond to light-competition cues by displaying traits that could improve light interception and minimize competitive interactions such as growing

away from neighbors, increasing internode length of stolons and rhizomes, and actively positioning new ramets in less crowded patches of the habitat (Gruntman et al., 2017).

Shade tolerance is a concept that refers to a multifaceted property of plants to tolerate low light levels that is achieved by different suites of traits in different species (Valladares and Niinemets, 2008). When exposed to shade, tolerant species display several physiological, anatomical, and systemic adjustments that promote plant performance under limited light conditions and minimize losses (Gommers et al., 2013). Some plant features associated with shade tolerance include high specific leaf area, high chlorophyll content in leaves, high chlorophyll *a/b* ratio in leaves, high concentration of anti-herbivory metabolites in leaves, low root/shoot biomass ratio, high fractional investment of plant mass in leaves, and high carbohydrate storage (Valladares and Niinemets, 2008). Furthermore, typical shade-tolerant species suppress shade-avoidance traits, displaying little or absent elongation responses in stems and petioles (Valladares and Niinemets, 2008).

Shade avoidance responses are more common for grassland species because of their characteristics of all individuals in the community to usually have similar heights, and overcoming the canopy to reach light is possible (Gommers et al., 2013). Conversely, shade tolerance responses are more common for species that occur in understory forests that are constantly exposed to shaded conditions and tend to increase carbon gain instead of avoiding shade (Gommers et al., 2013).

Light is also an important environmental signal for plants during germination, as the ability of plants to detect variations of light intensity, quality, or periodicity provides the seed with valuable information about its environment (Fenner and Thompson, 2005). This helps to determine where and when germination takes place, which is an essential mechanism for seed survival (Chanyenga et al., 2012).

Seeds are commonly classified as positive photoblastic, negative photoblastic, or non-photoblastic according to their light requirements for germination. Positive photoblastic seeds require light presence to germinate, negative photoblastic seeds require the absence of light to germinate, and non-photoblastic seeds germinate regardless of the light condition (Vazquez-Yanes and Orozco-Segovia, 1993).

For instance, the chances of successful seed establishment may be determined by whether the germinating seed is buried in the soil or laid down on its surface. If it is buried, its depth is crucial for emergence; if it is on the surface, then the degree of shade may be decisive for seedling establishment. Large-seeded seedlings may emerge successfully from a much greater depth than light can penetrate, whereas small-seeded seedlings usually may not; consequently, it is more likely for small-seeded species to have light as a requirement for germination than for large-seeded species (Milberg et al., 2000). However, certain species of Fabaceae and Poaceae tend to germinate readily in the dark regardless of seed size, while seeds of Cyperaceae and Asteraceae are mostly light-requiring (Fenner and Thompson, 2005).

Several mathematical expressions have been proposed to quantify germination; the most common are germinability, mean germination time, mean germination rate, and synchronization index (Ranal and Santana, 2006). These characteristics are essential to describe commercial, physiological, and ecological features of the germination process, predicting the degree of success of a species based on their capacity to spread germination through time (Ranal and Santana, 2006). Also, knowledge of seed biology is fundamental for understanding establishment, succession, and regeneration processes that occur in plant communities (Vazquez-Yanes and Orozco-Segovia, 1993). Because light requirements for seed germination are different among different species (Bewley et al., 2013), they are often assumed to be adaptations to the habitat where the species occur (Meyer et al., 1990).

Germinability is a measurement of the germination capacity; it is defined as the percentage of seeds that had the emergence of a living embryo by the end of the experiment (Ranal and Santana, 2006). The mean germination time measures the speed of germination at a specific condition, and it is calculated as the mean of the number of seeds that germinated at each moment during the experimental condition (Ranal and Santana, 2006).

The germination process under shaded conditions has been studied extensively for tropical forest tree species (Pereira de Souza and Valio, 2001; McLaren and McDonald, 2003; Godoi and Takaki, 2004; Torres-Torres et al., 2018), temperate trees (Figueroa and Lusk, 2001), and herbaceous (Jankowska-Blaszczuk and Daws, 2007). In summary, evergreen rainforest pioneer species require light to germinate, climax species tolerate

shade, and under seasonal rainforest environments, most of the pioneer species tend to germinate equally well in bright light or shaded conditions (Thusithana et al., 2018).

For North American grassland species, most studies are about grasses and little is known about forbs. Grasses vary in their light requirements for germination; some have an obligate requirement of light for germination, others have their germination increased in various degrees under higher light intensities, and still others that do not require light for germination (Khan and Gulzar, 2003).

The tallgrass prairie of North America is temperate, mesic grasslands characterized by the abundance of grasses that can attain heights of more than 2 m (Ladd and Oberle, 1995; Knapp et al., 1998). Forbs are an integral part of the prairie and contribute the most to species diversity, they are also good indicators to evaluate the conditions of a grassland (Hickman and Hartnett, 2002). Most of the forbs that occur in Kansas are Asteraceae and Fabaceae species (Haddock, 2005) and they are adapted and flexible to meet the constantly changing conditions that naturally occur in their environment (Küchler, 1974). However, there are no studies investigating how Kansas native forbs grow and develop under shaded conditions.

Therefore, the main question that permeated every section of this study was how Kansas native Asteraceae species respond to shaded conditions during each stage of their development. The primary objective was to characterize and compare the response patterns of Kansas native Asteraceae species to shaded conditions during their germination, vegetative development and growth, and reproduction.

The hypotheses associated with this project were that Asteraceae species that are native to Kansas and that inhabit the Tallgrass Prairie are expected to:

1. Have similar responses to shaded conditions due to their evolutionary relatedness;
2. Have a great diversity of mechanisms to cope with shaded conditions due to the high heterogeneity that naturally occur in their environment;
3. Produce positive photoblastic seeds because it is a common requirement in this family and because of the relatively small sizes of their seeds;
4. Display shade avoidance syndrome when exposed to shaded conditions, a common mechanism for grassland species;
5. Have their reproduction accelerated by shaded conditions, a common strategy of shade avoiders.

MATERIALS AND METHODS

The project consisted of three sets of measures on plants: germination, vegetative development, and reproduction. All experiments were conducted in greenhouse conditions during the spring and autumn of 2018. The time of each experiment, photosynthetic active radiation (PAR), and temperatures are shown in Table 1.

Table 1: Greenhouse conditions during times of germination, vegetative development, and reproduction experiments. Values are expressed as means of hourly measures from 8:00 am to 6:00 pm for six days randomly picked during each experiment ± standard errors.

			PAR (µmol m^{-2} s^{-1})		
Experiment	**Temperature (°C)**	**Duration of light exposure (hours)**	**10% of light Treatment**	**50% of light Treatment**	**100% of light Treatment**
Germination	23.2±0.3	8	27.99±3.9	172.69±24.6	392.22±60.8
Vegetative development	23.4±0.1	8	22.31±2.8	112.51±11.3	182.6±19.5
Reproduction	23.2±0.3	8	27.99±3.9	172.69±24.6	392.22±60.8

I. Plant material and growth conditions

Achenes (seeds) of *Ageratina altissima* (L.) R.M. King & H. Rob. var. *altissima* (White snakeroot), *Aster drummondii* Lindl. (Drummond's Aster), *Eutrochium purpureum* (L.) E.E. Lamont (Sweet Joe-Pye Weed), *Rudbeckia laciniata* L. (Cutleaf coneflower), and *Solidago ulmifolia* Muhl. ex Willd. (Elmleaf Goldenrod) were purchased from the Kansas Native Plant Society online store, and exposed to a 90 d cold stratification at -15 °C. Some seeds were used on the germination experiment and some of the seeds were sown and

grown in 10 cm wide and 12.5 cm tall pots containing MiracleGro Potting Mix (0.21% N, 0.07% P, 0.14% K; Scotts Company, Marysville, Ohio, USA) for approximately two months under the conditions of light, temperature and humidity of the Fort Hays State University Greenhouse (Hays, Kansas, USA; 38° 57'N, 99°23'W).

II. Shade treatments

Plants were exposed to three light levels: full sunlight conditions or 100% of natural light; approximately 50% of light, and approximately 10% of light (Table 1).

The 10% and 50% of light treatments were provided by 91.4 x 91.4 x 66 cm boxes (Fig. 1).

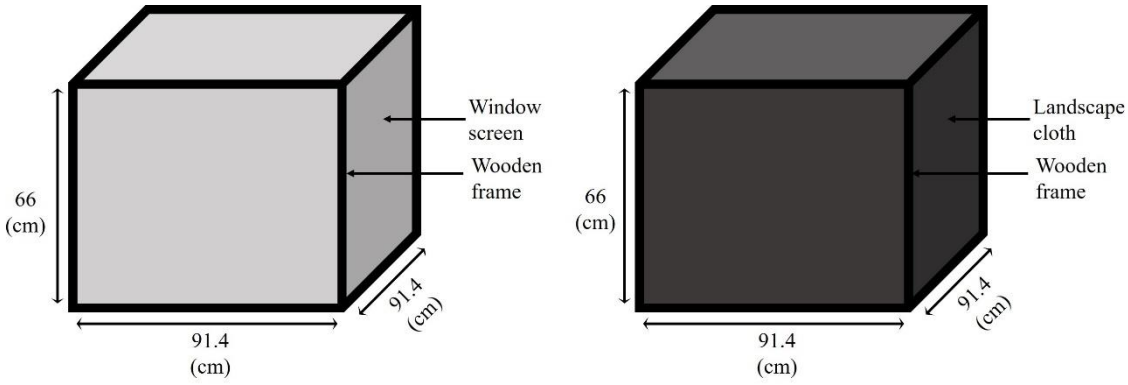

Figure 1: Representative figures of the boxes used to provide 50% of light (left) and 10% of light (right) during the germination, vegetative development, and reproduction experiments.

The boxes were made of a wooden frame and aluminum screen (Phifer, Tuscaloosa, Alabama, USA) for the 50% light treatment. Similarly, the 10% light treatment was provided by a box made of a wooden frame and a polyethylene weed block landscape fabric (Vigoro, Sylacauga, Alabama, USA). Measurements of light intensities within each treatment were made hourly with the LI-190R quantum sensor (LI-COR, Lincoln, Nebraska, USA) between 8 am and 6 pm for six days randomly assigned during each experiment. Black shade nets, as well as white ones, reduce the incident radiation over the plants without influencing the quality of light spectrum (Costa et al., 2018).

III. Germination experiment

The germination experiment consisted of exposing seeds in petri dishes containing filter paper moistened with 5 ml of distilled water to 0%, 10%, 50%, and 100% of light. For the 0% of light treatment, petri dishes were wrapped in two layers of aluminum foil. For all treatments, each petri dish was sealed with parafilm to avoid evaporation of water. Petri dishes were examined daily for 20 days and the number of seeds that had the emergence of the primary root was recorded. For each species, 20 seeds were assigned to each petri dish, and five petri dishes were assigned to each of the four light treatments (Fig. 2).

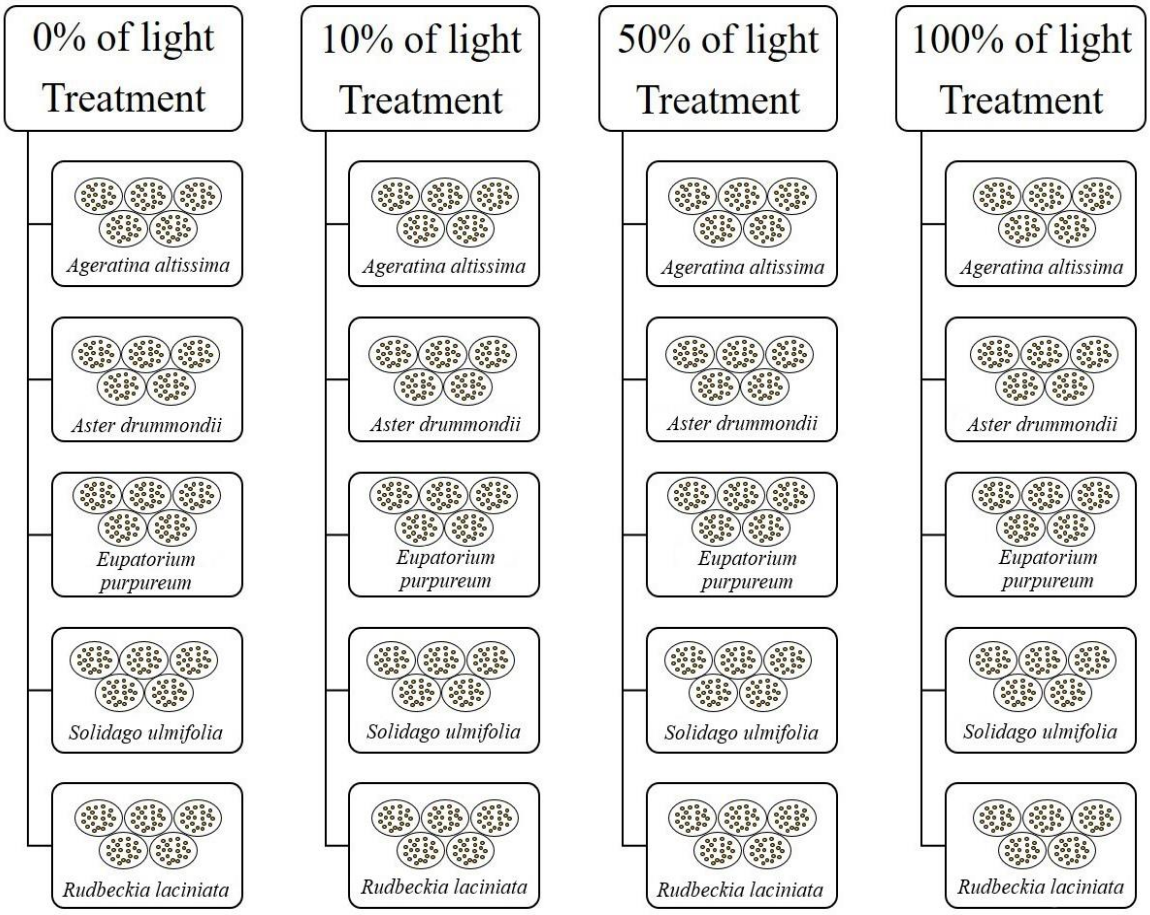

Figure 2: Germination experimental design. Seeds of four Asteraceae species were exposed to 0%, 10%, 50%, and 100% of light, and germination was checked daily. There were 20 seeds per petri dish per species, with five replicate petri dishes per treatment

Germinability measurement was the proportion, in percentage, of seeds that germinated within each treatment (Ranal and Santana, 2006). Mean germination time is the weighted mean of the germination time, in time units, and the number of germinated

seeds at the intervals established for the data collection was used as weight (Ranal and Santana, 2006).

Mean germination time can be used as an evaluation index of the speed of occupation for some species, seeds can be classified as fast (mean time <5 days); intermediate (mean time 5 to 10 days) and slow (mean time > 10 days) (Ferreira et al., 2001).

IV. Vegetative development

The vegetative development experiment consisted of measurements of growth and physiology. Approximately two-months-old *Ageratina altissima and Rudbeckia laciniata* plants were transplanted to 10 cm wide and 12.5 cm tall pots with fresh Miracle-Gro Potting Mix and exposed to 10%, 50%, and 100% of light for four weeks. Sample sizes were 11 plants for *Ageratina altissima* and 10 plants for *Rudbeckia laciniata* (Fig. 3).

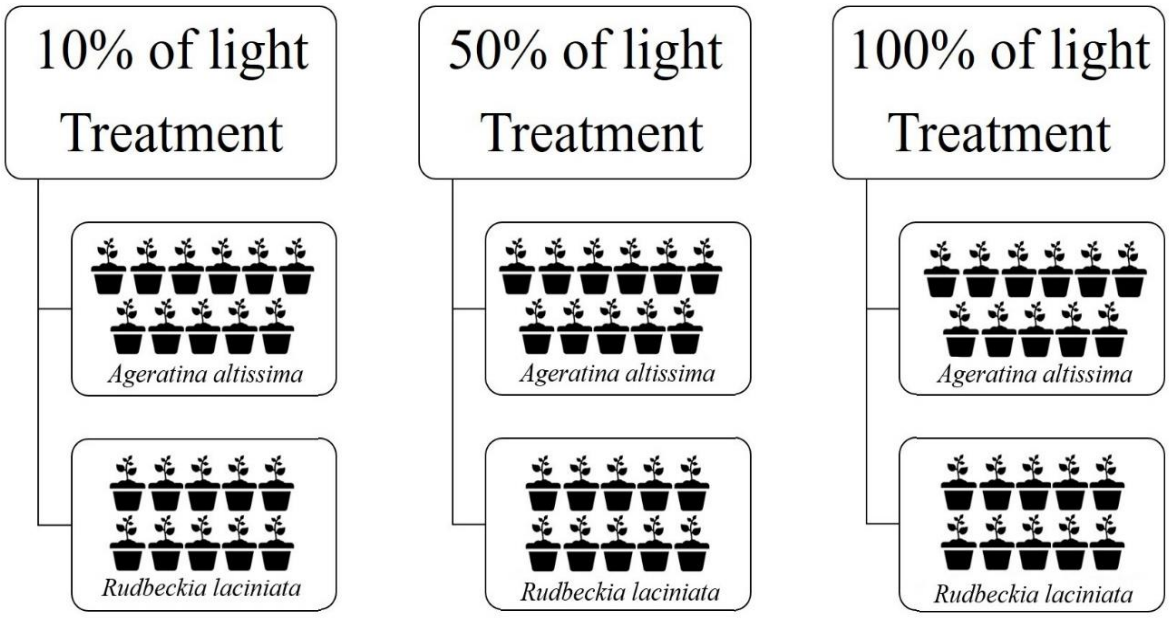

Figure 3: Vegetative development experimental set up. Two-months-old *Ageratina altissima* and *Rudbeckia laciniata* plants were exposed to 10%, 50%, and 100% of light for four weeks. Sample sizes were 11 plants for *Ageratina altissima* and 10 plants for *Rudbeckia laciniata*.

Each pot was consistently watered as needed, and the positions of the pots were randomized weekly within each treatment. Heights of *Ageratina altissima* stems were measured once per week from the visible bottom part of the stem to its apex. Due to its rosette growth form, petiole lengths of *Rudbeckia laciniata* were recorded rather than stem height. Additionally, the number of leaves, nodes, branches, and ramets were also recorded

once per week for both species. At the end of the experiment, plants were harvested for physiological measures, where chlorophyll concentrations in leaves where quantified.

After measuring the fresh weight of leaves without petioles, leaf area was measured by scanning leaves with a flatbed scanner and analyzing images with ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Pixels of leaves were counted and compared with pixels produced by the image of an object of known area. Leaves were dried at 60°C for 48 h for dry biomass measurement. The dry leaf biomass divided by the fresh leaf biomass is the leaf dry matter content (LDMC), the specific leaf area (SLA) is the leaf area divided by leaf dry mass, and leaf thickness (LT) is the $(SLA \times LDMC)^{-1}$ (Vile et al., 2005). Also, shoots and roots were dried at 60°C for 48 h for biomass measurement.

Chlorophylls *a* and *b* were extracted with a buffer solution composed of 5 mM Tris-HCl, 0.5 mM MgCl, 0.2 mM cysteine hydrochloride, and 0.2% w/v PVP-40 (Maricle, 2010). First, a 27 mm^2 leaf disc was obtained from fresh young leaves and its weight was measured, 100 µl buffer solution was added, leaf disc was ground with a chilled mortar and pestle. Then, 40 µl of the grindate was collected, mixed with 960 ml of 100% ethanol, and absorbances were quantified with a spectrophotometer according to Lichtenthaler and Wellburn (1983), which chlorophyll *a* (µg of chlorophyll/ml of solution)=$(13.95A_{665}-6.88A_{649})$, chlorophyll *b* (µg of chlorophyll/ml of solution) = $(24.96A_{653}-7.32A_{665})$, and total chlorophyll content (mg chl/g leaf) = chl concentration (µg/ml)x(1mg/1,000µg)x(0.04 ml/0.004g).

V. Reproduction experiment

Approximately one-month-old *Ageratina altissima* plants were transplanted to 10 cm wide and 12.5 cm tall pots containing Miracle Gro Potting Mix (0.21% N, 0.07% P, 0.14% K; Scotts Company, Marysville, Ohio, USA) and exposed to three light conditions, 10%, 50%, and 100% of light (Table 1). Each pot was consistently watered as needed, and the positions of the pots were randomized weekly within each treatment. The time for the first flower bud to emerge, as well as the number of plants that had flowers within each treatment, were recorded after eight weeks of treatment.

VI. Data Analysis

All data analyses were performed with R-Project Software Version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Germination data were normally distributed and had equal variances, so two Two-way analysis of variances (ANOVA) were performed, and the significance level considered was 0.03333 after B-Y correction (Narum, 2006).

Vegetative development data were normally distributed and had equal variances, so multiple Two-way ANOVAs were performed, and the significance level considered was 0.01656 after B-Y correction (Narum, 2006). Post-hoc comparisons for significant p-values were performed using HSD Tukey test, and the significance level considered was 0.05.

For measurements of the number of ramets, and reproduction measurements, multiple Chi-Square tests of independence were performed, with the significance level of 0.05.

RESULTS

I. Germination

Germinability means were as low as 8% in 0% light, but were typically much higher in the light treatments, up to 100% in some cases (Fig. 4). Conditions of 0% and 10% of light decreased germinabilities of seeds in all species that were tested ($p<0.001$).

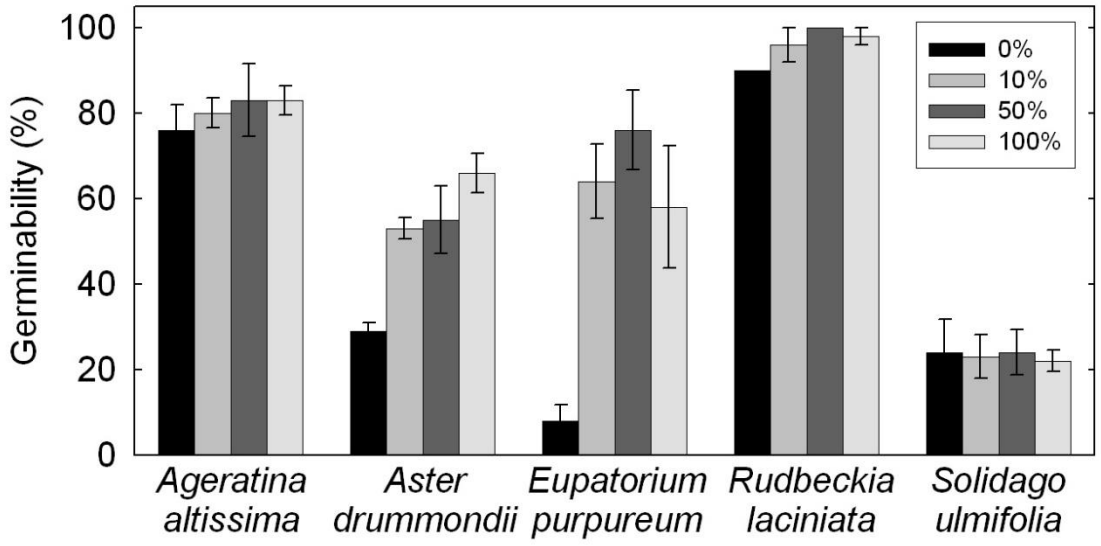

Figure 4: Germinabilities (%) of plant species across light treatments. Bars represent means of five replicates ± standard errors.

Mean germination times ranged from 2.12 to 10.7 days across species and treatments (Fig. 5). Mean germination times were usually reduced in low light treatments ($p=0.012$) and across species ($p<0.001$).

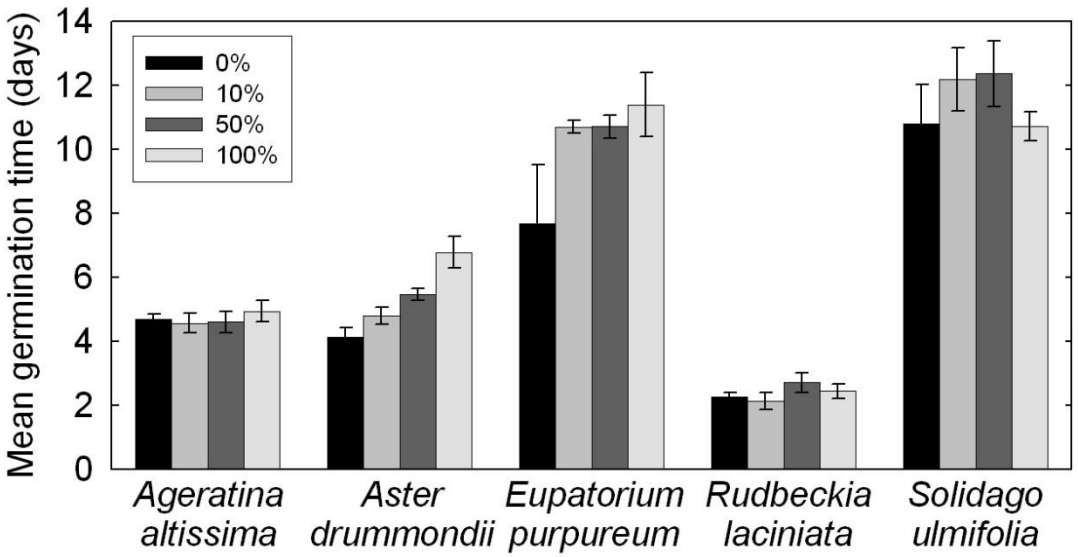

Figure 5: Mean germination time (days) of plant species across light treatments. Bars represent means of five replicates ± standard errors.

II. Vegetative development and growth

Mean stem heights in *Ageratina altissima* ranged from 9.76 cm to 18.09 cm by the conclusion of the experiment (Fig. 6) and shaded conditions resulted in reduced stem heights ($p=0.007$).

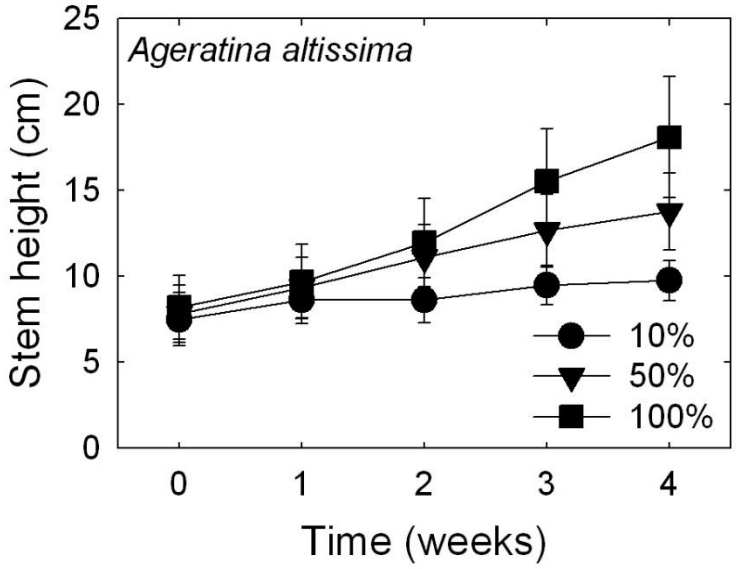

Figure 6: Stem height (cm) of *Ageratina altissima* plants over time (weeks) in different light treatment. Points represent means of 10 plants ± standard errors.

Mean petiole length in *Rudbeckia laciniata* ranged from 2.9 to 4.13 mm and were not significantly different across treatments (p=0.247) (Fig. 7).

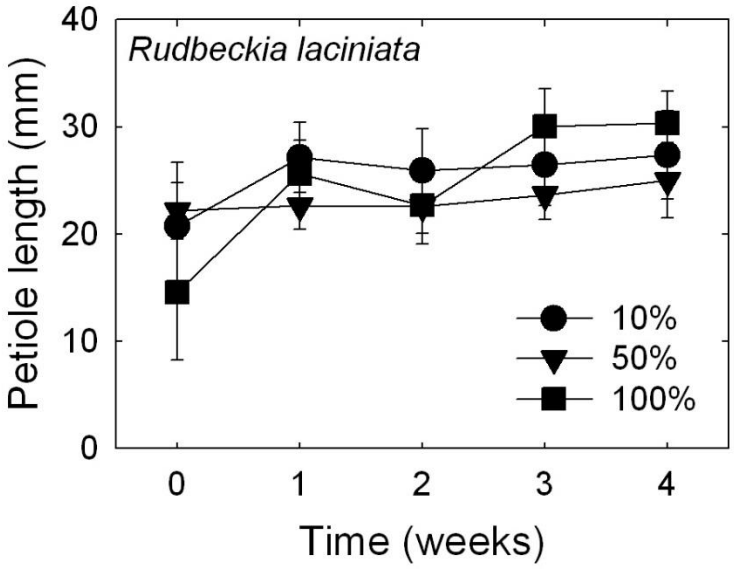

Figure 7: Petiole length (mm) of *Rudbeckia laciniata* leaves over time (weeks) in different light treatments. Points represent means of 11 replicate plants ± standard deviations.

Mean leaf number per plant ranged from 3.43 to 48.89 across species and treatments (Fig. 8). Plants of both species had fewer leaves when exposed to 10% of light ($p<0.001$) and a consistent number of leaves when exposed to 50% and 100% of light.

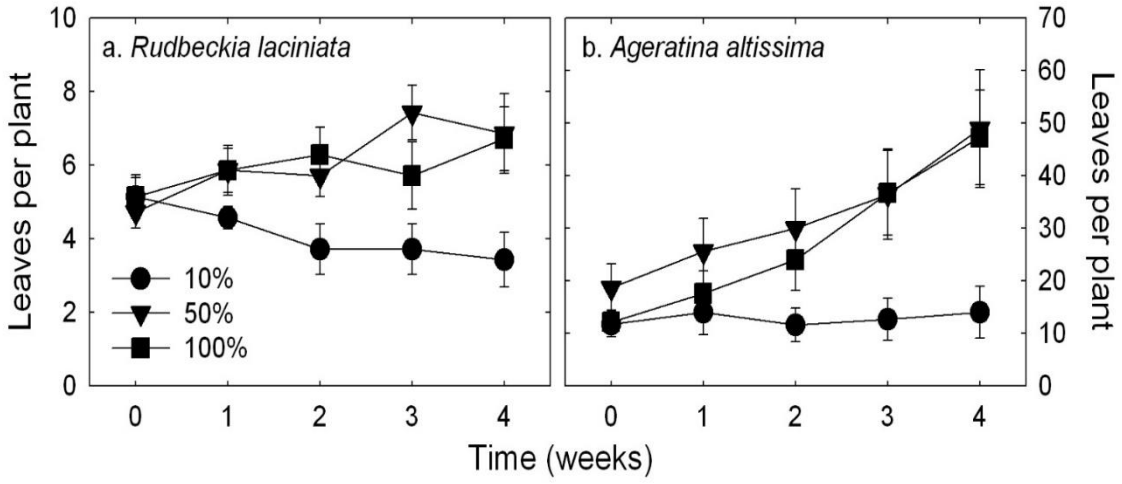

Figure 8: Number of leaves produced per plant over time in different light treatments. Points represent means of 10 plants ± standard error.

Higher light intensity treatments resulted in increased branching in *Ageratina altissima* ($p=0.005$), but not in *Rudbeckia laciniata*, that did not produce branches during the experiment. *A. altissima* plants exposed to 10% of light did not develop branches, plants under 50% of light had a mean number of 1.33 branches per plant, and plants under 100% of light had a mean of 2.55 branches (Fig. 9).

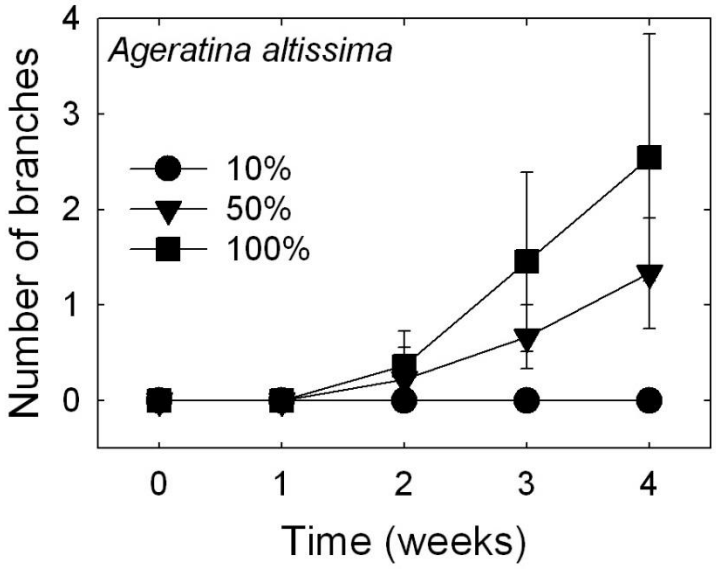

Figure 9: Number of branches that *Ageratina altissima* produced over time (weeks) in different light treatments. Points represent means of 10 plants ± standard error.

Shaded conditions resulted in reduced plant growth and a decreased root/shoot ratio for both species. The mean shoot biomass (Fig. 10), root biomass (Fig. 11), and root/shoot ratio (Fig. 12) of *A. altissima* and *R. laciniata* were reduced for plants exposed to 10% of light ($p<0.004$), except for *A. altissima* plants that had a root/shoot biomass of 1.01 under 50% of light.

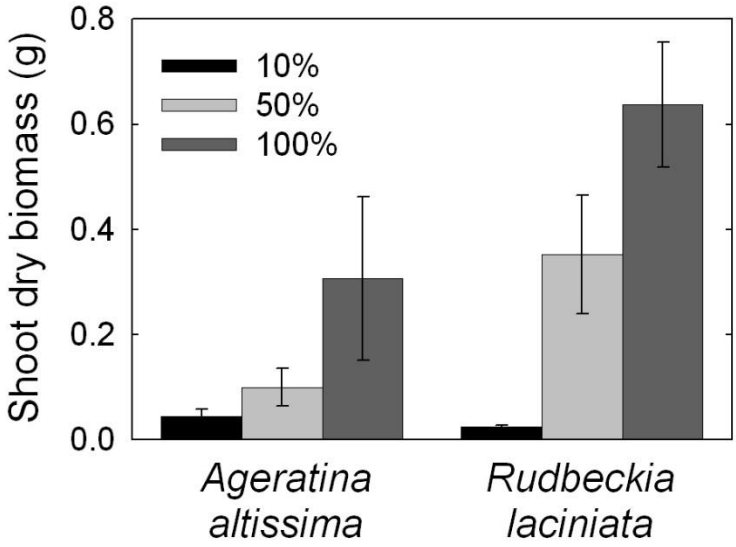

Figure 10: Shoot dry biomass (g) after four weeks of growth in different light treatments. Bars represent means of 10 plants ± standard errors.

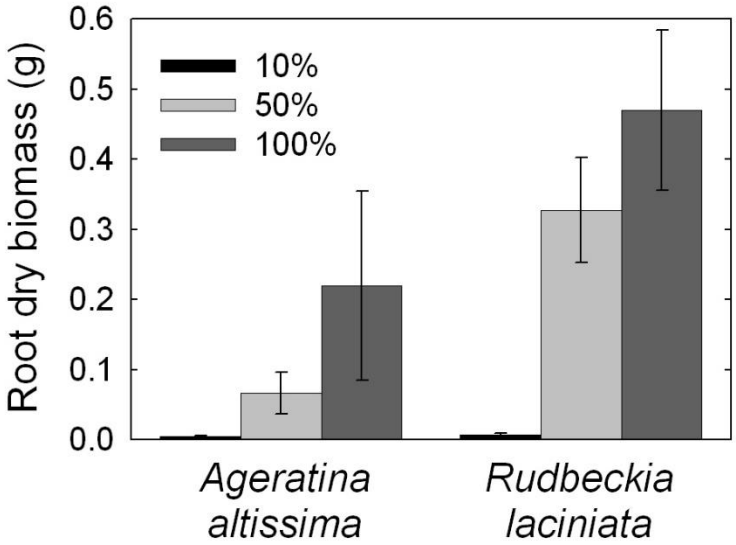

Figure 11: Root dry biomass (g) after four weeks of growth in different light treatments. Bars represent means of 10 plants ± standard errors.

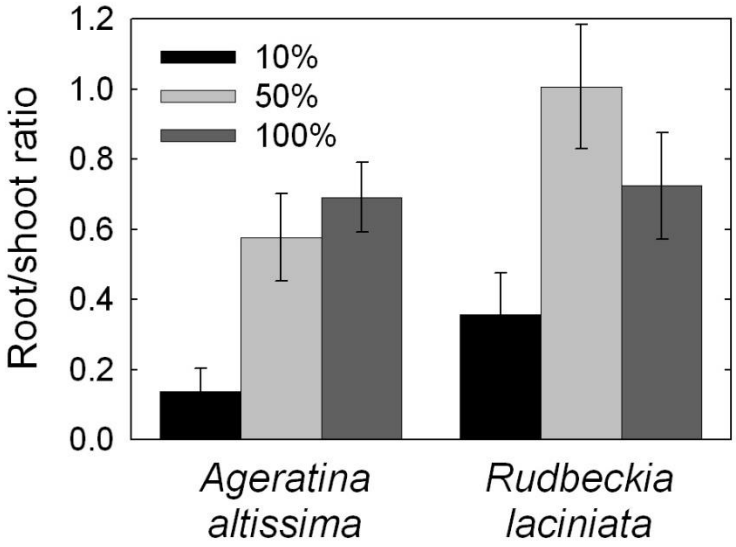

Figure 12: Root/shoot dry biomass ratio after four weeks of growth in different light treatments. Bars represent means of 10 replicate plants ± standard errors.

Vegetative reproduction of *Ageratina altissima* was also influenced by light in the experiment (p=0.012). *Ageratina altissima* had 0.39 ramets per plant under 50% of light, and 0.26 ramets per plant under 100% of light (Fig. 13). Conversely, *Rudbekia laciniata* plants did not have any ramets.

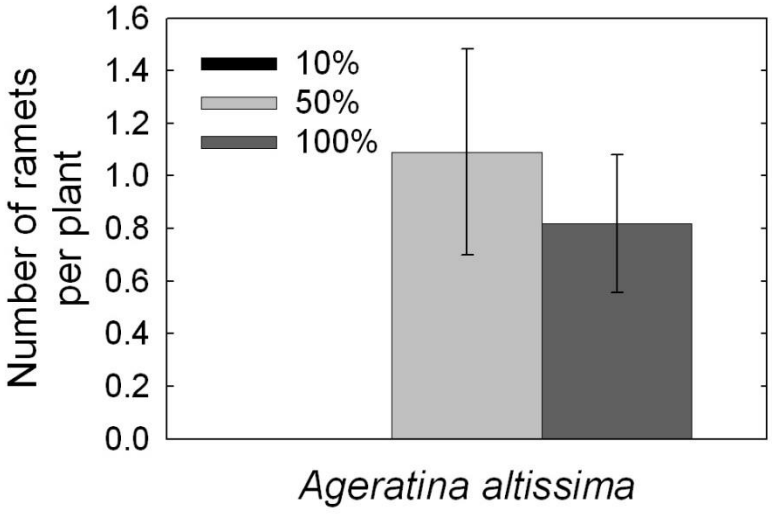

Figure 13: Number of ramets produced by plants after four weeks of growth in different light treatments. Plants exposed to the 10% of light treatment produced no ramets. Bars represent means of 10 replicate plants ± standard errors.

Mean leaf areas of plants ranged from 1.61 to 9.06 cm^2 (Fig. 14), with an increased leaf area associated with increased light for both species ($p=0.014$).

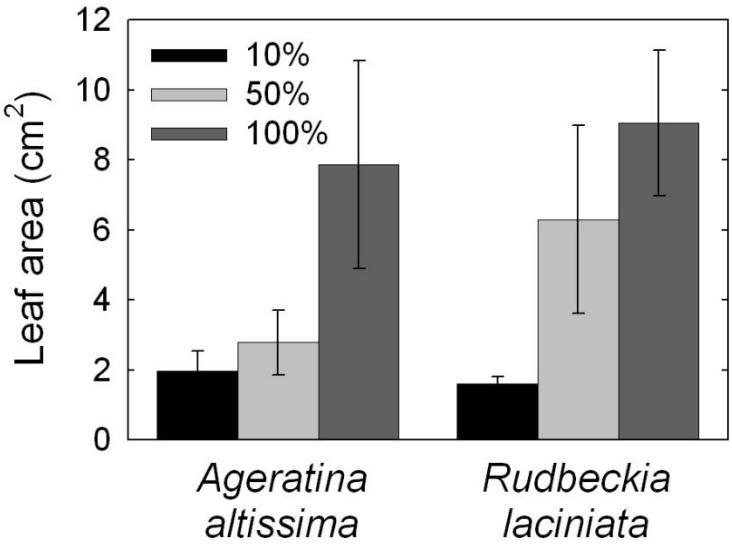

Figure 14: Leaf area (cm^2) of *Ageratina altissima* and *Rudbeckia laciniata* across light treatments after four weeks of growth. Bars represent means of 10 replicate plants ± standard errors.

Plants exposed to 10% of light had thinner leaves than plants in 50% or 100% of light ($p=0.002$). Mean leaf thickness of plants ranged from 0.008 to 0.024 cm, with a positive relationship with light in both species (Fig. 15).

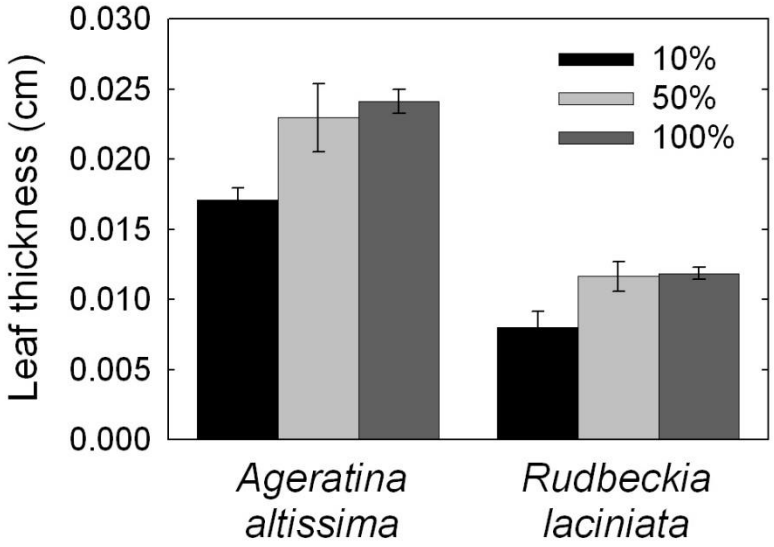

Figure 15: Leaf thickness (cm) of *Ageratina altissima* and *Rudbeckia laciniata* across light treatments after four weeks of growth. Bars represent means of 10 replicates ± standard errors.

Conversely, specific leaf area ranged from 263.76 to 672.90 cm^2 g^{-1} (Fig. 16) and although there was no statistically significant difference (p=0.191), there was a trend of increased specific leaf area for plants under shaded conditions.

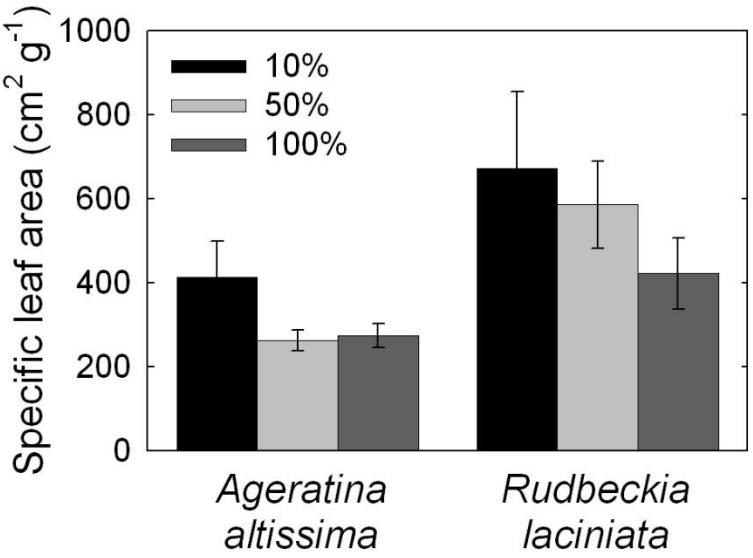

Figure 16: Specific leaf area (cm^2 g^{-1}) of *Ageratina altissima* and *Rudbeckia laciniata* across light treatments after four weeks. Bars represent means ± standard errors.

Chlorophyll *a* content in leaves was 0.25 to 0.60 µg g^{-1} leaf (Fig. 17a) and was consistent across treatments and species ($p=0.022$). However, chlorophyll *b* contents (Fig. 17b) total chlorophyll (Fig. 17c), and chlorophyll *a*/b ratio (Fig. 17d) increased with increased light intensities ($p<0.001$).

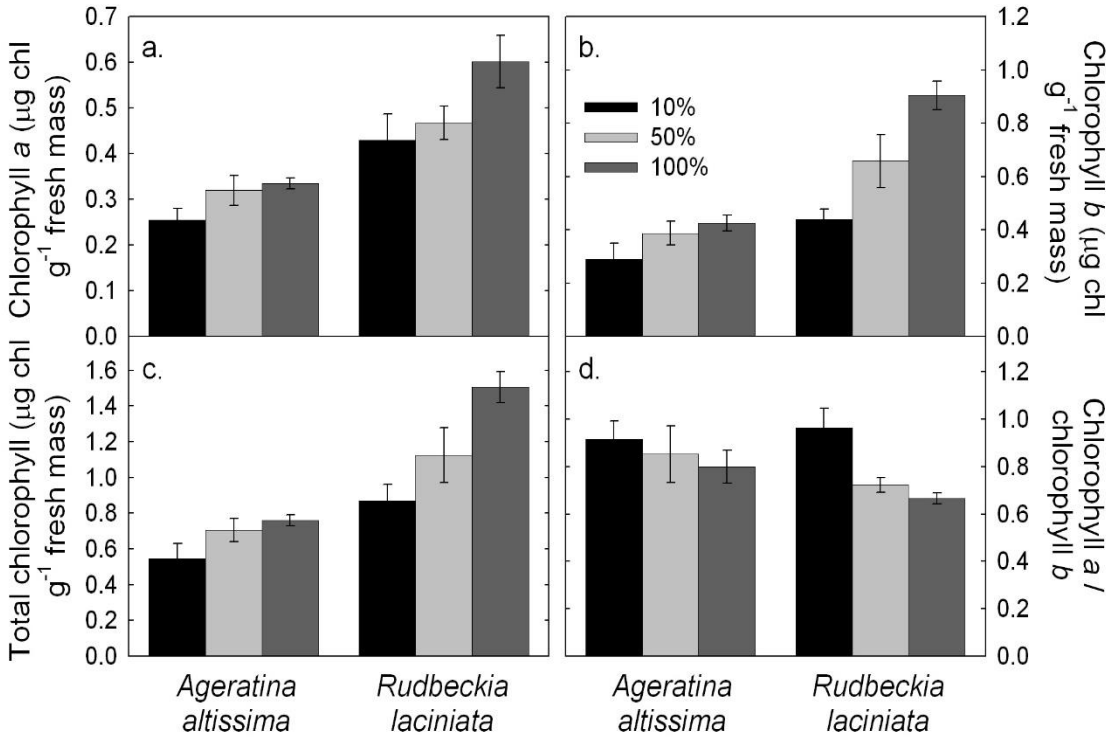

Figure 17: Pigment content (µg g^{-1} fresh mass) in leaves of *Ageratina altissima* and *Rudbeckia laciniata* across light treatments after four weeks of growth. (a) chlorophyll *a* content (µg g^{-1}), (b) chlorophyll *b* content (µg g^{-1}), (c) total chlorophyll content (µg g^{-1}) and (d) chlorophyll *a*/*b* ratio. Bars represent means of 10 replicates ± standard errors.

III. Reproduction

Shaded conditions negatively affected the percentage of plants that produced flowers (p=0.004) and the time it took for the first flower bud to develop (p=0.012). Only *A. altissima* plants exposed to 50% and 100% of light produced flowers and only 12.5% of the plants under 50% of light produced flowers, whereas 50% of the plants under 100% of

light produced flowers (Fig. 18). The time for the first flower bud to develop was 34 days for plants under 50% of light and 42 days for plants under 100% of light (Fig. 18).

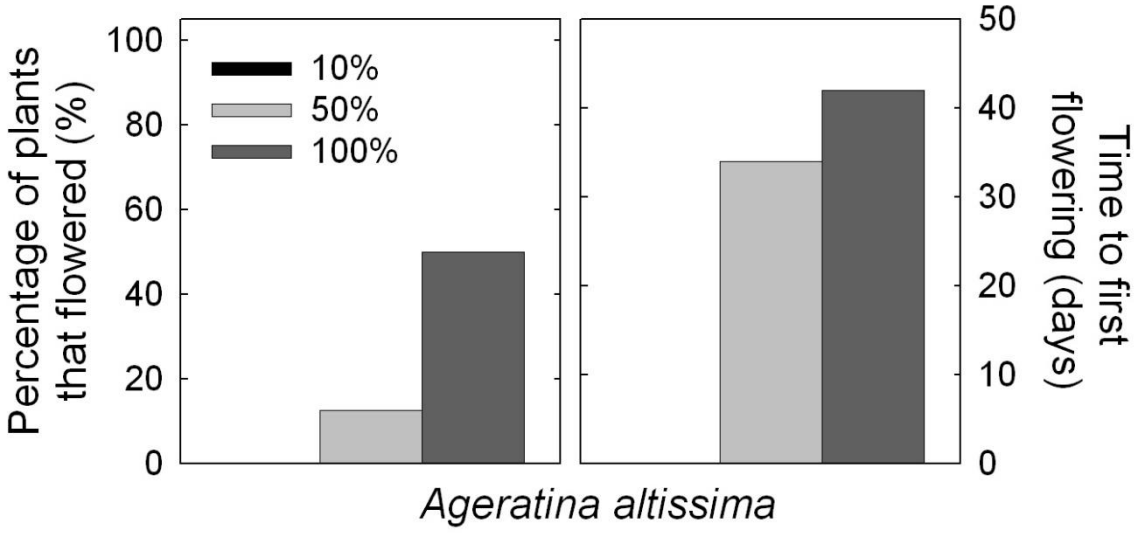

Figure 18: (Left) Percentage of *Ageratina altissima* plants that flowered by the end of eight weeks of growth across light treatments. (Right) The time it took for the first flower bud to develop within each treatment. Plants under 10% of light treatment did not develop flowers during the experiment. Bars represent means of 15 replicates.

DISCUSSION

In this experiment, the role of light was investigated in germination, growth, and reproduction in Asteraceae species that are native to Kansas. Increased light intensities enhanced growth, development, and reproduction in those species. Surprisingly, plants displayed several mechanisms related to shade tolerance.

I. Germination

Germination process in Asteraceae species was consistent with the literature on grasses, which has indicated that species vary in their light requirements during germination. Some native grasses have an obligate requirement of light for germination, while in others presence of light enhances seed germination to varying degrees, and still others do not require light for germination (Khan and Gulzar, 2003).

Presence of light is a common requirement for seed germination of Asteraceae and small-seeded species (Milberg et al., 2000; Fenner and Thompson, 2005) and shaded conditions reduced germination of all plants tested in the present study, although, the absence of light did not completely inhibit germination.

Mean germination time may be used as evaluation index of the speed of occupation for some species; for instance, seeds can be classified as fast (mean germination time less than 5 days); intermediate (mean germination time 5 to 10 days) and slow (mean germination time longer than 10 days) (Ferreira et al., 2001). Therefore, *Ageratina altissima*, *Aster drummondii*, and *Rudbeckia laciniata* are considered fast, whereas *Solidago ulmifolia* and *Eupatorium purpureum* are slow. Fast germination may indicate

that the species may rapidly establish in the environment, taking advantage of favorable conditions. However, rapid environmental change conditions may strongly impact seedling establishment (Ferreira et al., 2001), particularly conditions of light.

Light is not the only factor that regulates plant germination, other environmental factors such as temperature should be tested to provide a better understanding of the germination requirements of those species (Khan and Gulzar, 2003).

II. Shade avoidance

Shade avoidance syndrome is a common mechanism of grassland species to avoid shaded conditions and is characterized by elongation of stems and petioles to overcome the canopy and reach light; additional traits associated with shade avoidance syndrome are reduced branches, decreased leaf area, decreased shoot biomass, and increased number of ramets (Casal, 2012; Gommers et al., 2013; Ballaré and Pierik, 2017).

Surprisingly, *Ageratina altissima* and *Rudbeckia laciniata* did not display the classical shade avoidance syndrome that is largely described for *Arabidopisis thaliana* (Ciolfi et al., 2013), crops (Carriedo et al., 2016), and a few wildflowers (Du et al., 2017). Stems of *Ageratina altissima* and petioles of *Rudbeckia laciniata* were shorter in plants exposed to shaded conditions. However, both plants had some responses associated with shade avoidance syndrome.

For instance, both species had decreased leaf area and shoot biomass, and *Ageratina altissima* plants had fewer branches when exposed to shaded conditions. Also, *Ageratina*

altissima had more ramets when exposed to 50% of light than those exposed to 100% of light, which is a common mechanism for plants under competition for light, which actively places new ramets in less crowded places of their environment (Gruntman et al., 2017). However, plants under severe shade had no ramets. It is hypothesized that because plants under severe shade had very limited growth, they had no mechanical stimulation from neighbor plants. Therefore, there was no trigger for mechanisms of avoiding competition.

Besides, *Ageratina altissima* plants growing under 50% of light had fewer percentage of flowering and a reduced time for the first flowers to develop, compared with the plants growing under 100% of light. Those are common reproductive responses of shade avoiders if the shaded condition is prolonged (Morelli and Ruberti, 2002).

III. Shade tolerance

Shade tolerance is a concept that refers to a multifaceted property of plants to tolerate low light levels that is achieved by different suites of traits in different species (Valladares and Niinemets, 2008). It is usually characterized by little or absent elongation responses in stems and petioles, high specific leaf area, high chlorophyll content and high chlorophyll *a/b* ratio in leaves, low root-shoot ratio, thinner leaves, and high fractional investment of plant mass in leaves (Valladares and Niinemets, 2008). Shade tolerance responses are more common for species that occur in understory forests that are constantly exposed to shaded conditions and tend to increase carbon gain instead of avoiding shade (Gommers et al., 2013).

Unexpectedly, *Ageratina altissima* and *Rudbeckia laciniata* displayed several traits that are related with shade tolerance modifications such as little elongation of stems and petioles, higher specific leaf area, and higher chlorophyll *a*/*b* ratio, reduced leaf and root/shoot biomass, and fewer and thinner leaves, when exposed to shaded conditions. Those are mechanisms associated with shade tolerance, as these plastic phenotypic responses enhance light capture and photosynthetic utilization, increasing plant performance in the shade (Valladares and Niinemets, 2008). Tolerance mechanisms in plants, however, depend on specific structural and physiological traits, but it is also strongly affected by the status of other environmental factors (Valladares and Niinemets, 2008).

In summary, increased light conditions resulted in increased growth for both species. Plants displayed mechanisms to avoid competition when in partial shade such as actively positioning new ramets, but they also displayed mechanisms to minimize loss and maximize carbon gain and light harvest when exposed to an increased shaded environment.

Measurements on plant height and petiole length differed from measurements described on *Solidago canadensis* (Asteraceae); however, measurements of root, shoot, and root/shoot biomass in *Ageratina altissima* and *Rudbeckia laciniata* were consistent with those in *Solidago canadensis* (Du et al., 2017).

Shade avoidance and shade tolerance, however, are highly complex mechanisms and depend heavily on external biotic and abiotic factors other than light (Valladares and Niinemets, 2017).

IV. Competition for light in grassland species

The dominant grasses in tallgrass prairies characteristically have canopies ranging from 0.6 to 1.5 m tall, creating a vertical gradient in light in their environment (Jurik and Kliebenstein, 2000; Haddock, 2005).

Shade tolerance mechanisms of Tallgrass forbs may elucidate the mechanisms of competition in their environment. By displaying higher specific leaf area, and higher chlorophyll *a*/*b* ratio, reduced leaf and root/shoot biomass, fewer and thinner leaves, plants enhance light capture and photosynthetic utilization, increasing their fitness.

Furthermore, plants producing more ramets when exposed to partially shaded environments is a common mechanism for plants under competition, which plants actively place new ramets in less crowded places of their environment (Gruntman et al., 2017).

Some Asteraceae species such as *Ageratina altissima*, *Rudbeckia laciniata*, and *Solidago canadensis* are invasive in China, South Korea, and Europe (Du et al., 2017; Byun and Lee, 2017; Majewska, 2018). Because *Solidago canadensis*, an invasive in China, displays similar shade tolerance strategies as plants tested in this study, it is hypothesized that those mechanisms also play central roles in a successful invasion of *Ageratina altissima* and *Rudbeckia laciniata* in Europe (Du et al., 2017).

CONCLUSIONS

In this study, increased light intensities enhanced growth, development, and reproduction in Asteraceae species that are native to Kansas. Unexpectedly, plants displayed several mechanisms related with shade tolerance such as little elongation of stems and petioles, higher specific leaf area, and higher chlorophyll *a/b* ratio, reduced leaf area and root/shoot biomass, and fewer and thinner leaves.

Most of the studies about shade influences on plant germination, growth, vegetative development, and reproduction are about commercial crops or forest species and little is known about grassland species. Therefore, this work provides the scientific community with novel information on classic features regarding functioning and development of widely spread plant species that are native to Kansas and the Great Plains. Such information is essential and has the potential to be largely used for both conservation for endangered species and management of encroaching species.

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