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Interaction of Shading and Cytokinins in the Sun-Shade Foliar Adaptation Mechanism

Oleg S. Gross
University of Maine

INTERACTION OF SHADING AND CYTOKININS IN THE SUN-SHADE FOLIAR ADAPTATION MECHANISM

by

Oleg S. Gross

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Advisory Committee:

Dr. Michael E. Day, Associate Research Professor, School of Forest Resources, Chair
Dr. Renae Moran, Associate Professor, School of Food and Agriculture
Brad Libby, MS. Superintendent of Horticultural Facilities and Lecturer of Horticulture, School of Food and Agriculture
Dr. Rachel A. Snell, Adjunct Assistant Professor, Honors College
Dr. Bryan J. Peterson, Assistant Professor, School of Food and Agriculture

Abstract

Plants, being sessile, address environmental changes and resource constraints by means of developmental plasticity. For example, plants maximize photosynthesis driven carbohydrate production by undergoing physiological and structural changes in response to their environmental conditions. This plasticity to light environment has several potential regulatory pathways that may include light intensity and light spectral quality. Hypotheses advanced to associate foliar plasticity to light intensity include sensing products of photosynthesis and regulation by the phytohormone cytokinin. In this study, we examined the interacting roles of the cytokinin 6-Benzylaminopurine (BAP) and light intensity in the regulation foliar plasticity. Exogenous application of BAP was used on plants grown in both high and low light environments. Digital image analysis and spectrophotometric data showed a downregulation of specific leaf area ($cm^2\ g^{-1}$), chlorophyll A, and chlorophyll B by cytokinin activity. Hormone-induced downregulation of these qualities was amplified to varying degrees by light environment, suggesting an interaction between these two factors.

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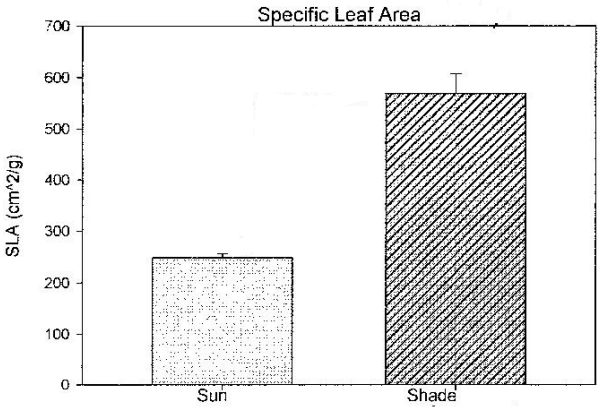
Figure 1. Map of experimental layout showing treatments and block labels.
Gray blocks indicate shaded condition. White blocks indicate unshaded condition.
+ Indicates application of BAP solution. – Indicates application of control solution.



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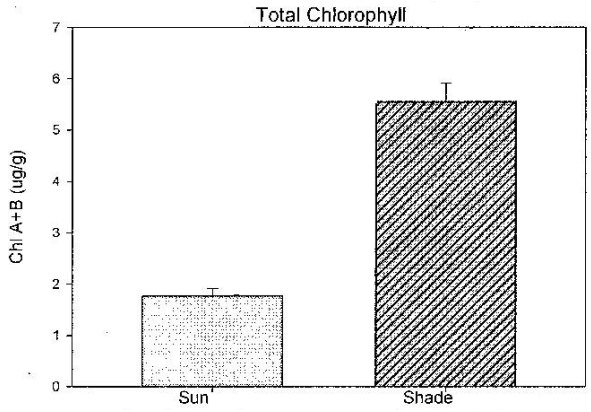

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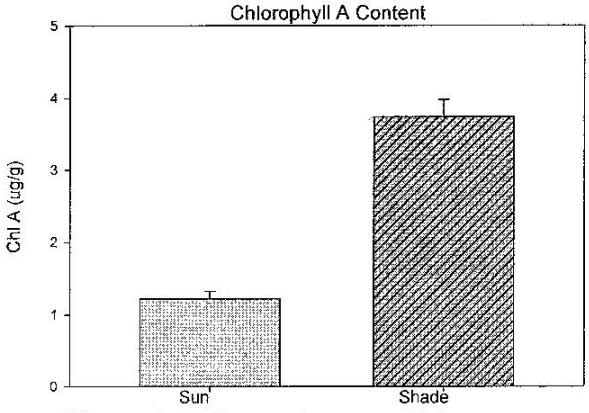

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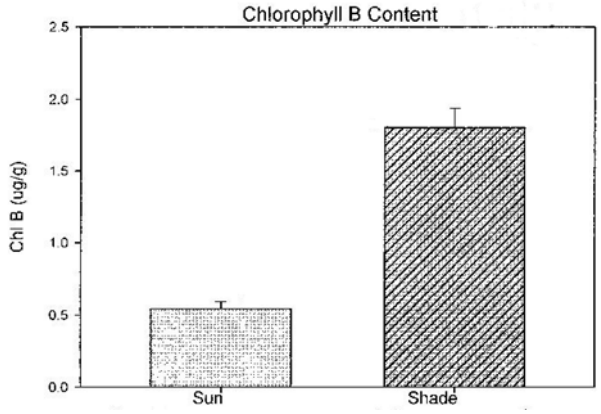

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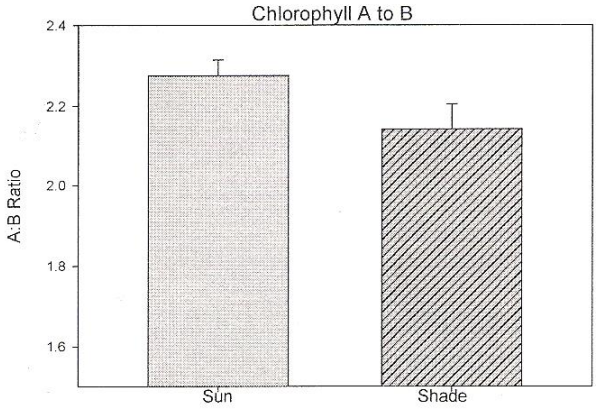
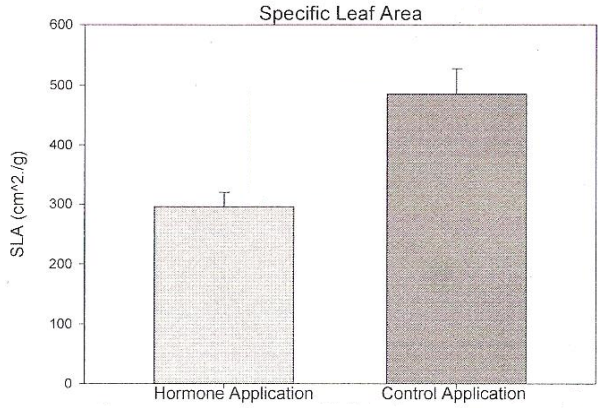


Figure 6. Effect of light treatment on chlorophyll A to B ratio.



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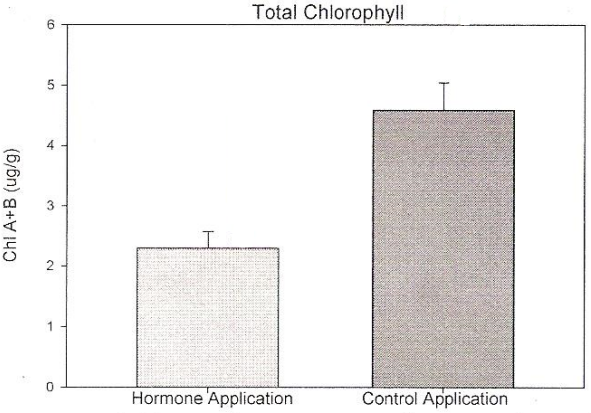

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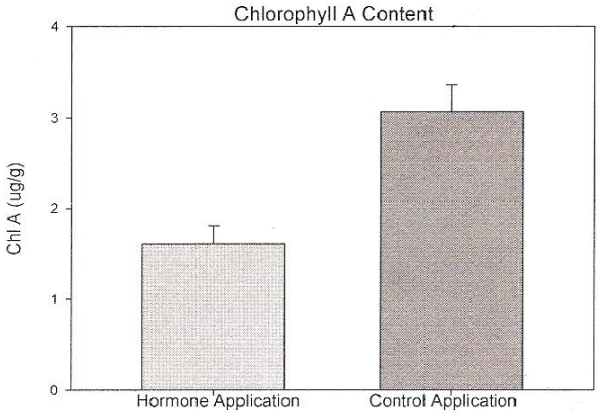

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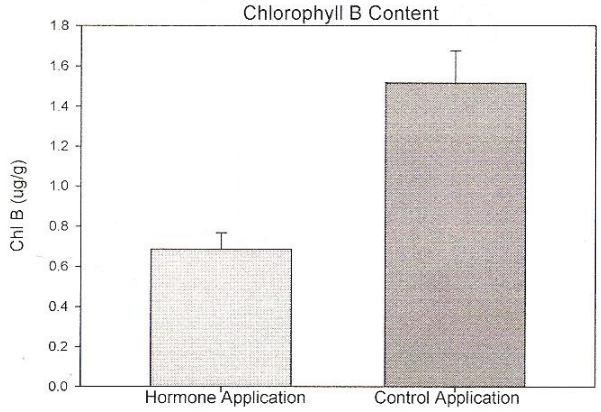

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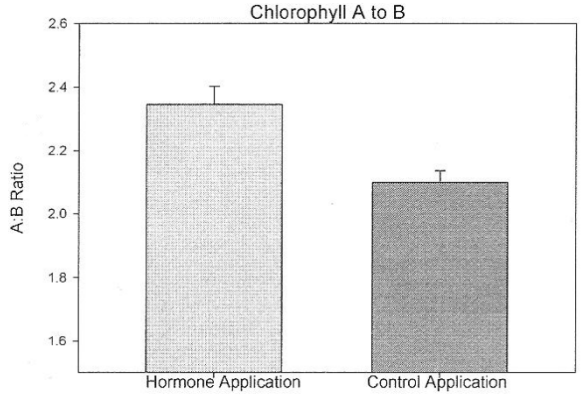

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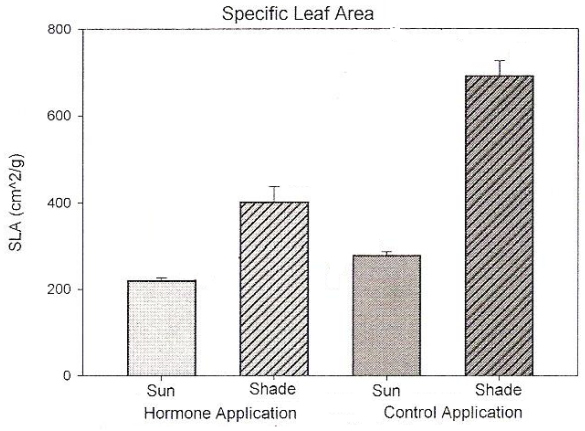

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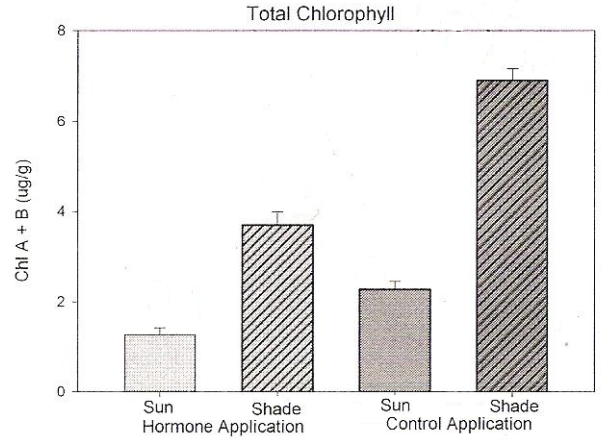

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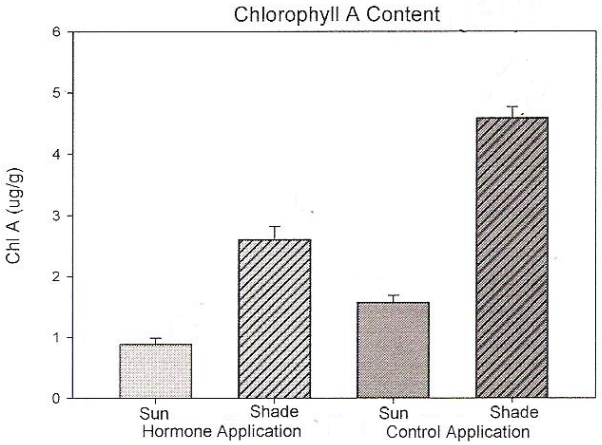

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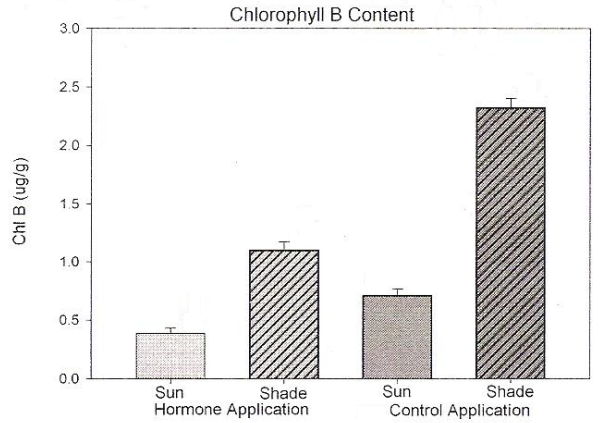

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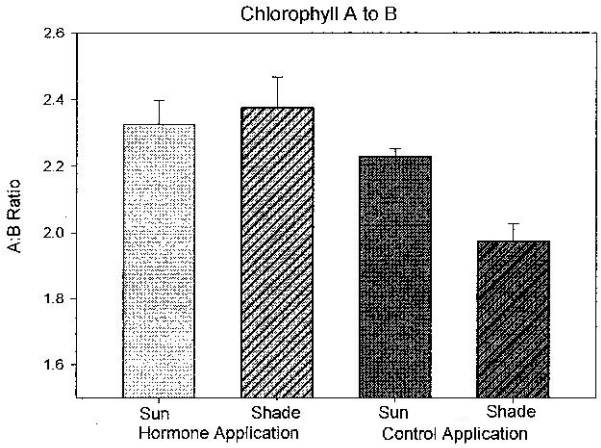

Figure 16. Composite effects of light level and BAP application on chlorophyll A to B ratio

	Block 1 Sun	Block 1 Shade	Block 2 Sun	Block 2 Shade	Block 3 Sun	Block 3 Shade	Light Intensity Reduction
Full Sun	510	60	490	90	550	60	87%
	680	60	610	150	790	60	
Partial Sun	330	60	350	60	360	60	84%
	330	40	390	70	380	50	
Full	33	6	37	5	34	5	82%
Overcast	31	7	34	7	31	6	

Table 1. Values for light intensity ($\mu m\ m^{-2}\ s^{-1}$) in shaded and unshaded experimental groups.

	SLA (cm^2/g)	Total chl (µg/g)	Chl A (µg/g)	Chl B (µg/g)	Chl A:B
Sun + Cyt +	219 ± 7	1.27 ± 0.15	0.88 ± 0.11	0.39 ± 0.05	2.33 ± 0.07
Sun – Cyt +	402 ± 35	3.70 ± 0.29	2.60 ± 0.22	1.10 ± 0.08	2.38 ± 0.09
Sun + Cyt –	278 ± 9	2.27 ± 0.18	1.57 ± 0.12	0.71 ± 0.06	2.23 ± 0.02
Sun – Cyt –	692 ± 35	6.90 ± 0.26	4.58 ± 0.19	2.33 ± 0.08	1.97 ±0.05

Table 2. Experimental values for foliar qualities of all plants in different treatment groups.

Introduction

Sun-shade adaptation is one of the major processes that allow individual plants to undergo changes in morphology to better suit an environment. This process dictates the resource allocation necessary to suit a plant to the quantity of light available to it. In sun-shade adaptation, ambient light intensity and cytokinin phytohormones control leaf structure and chloroplast development. Foliage optimized for high light levels is thicker and has less light-gathering area than foliage suited to low light levels. These features limit photon absorption in order to mitigate heat stress and water stress (Givnish 1988). Sun-optimized foliage is characterized by a lower specific leaf area (SLA—unit area per unit foliar biomass) than that of shade-optimized foliage (Pons et al. 2001). Shade-adapted foliage maximizes light interception with higher SLA, has denser chloroplasts and a higher ratio of chlorophyll B to chlorophyll A.

Chlorophylls are the pigments that allow plants to capture energy from sunlight; therefore form the basis of the photosynthetic process. Chlorophyll A and B are the primary pigments utilized for light harvesting (Gitelson et al. 2003), although several other forms of chlorophyll have been identified. These two chlorophylls have similar absorbance patterns, with maxima at the high (red to red-orange) and low (blue to violet) extremes of the visible light spectrum and relatively low absorbance throughout the midrange (Sims and Gamon 2002). Chlorophyll A absorption is greatest at 665 nm and 430 nm. Chlorophyll B absorption is greatest at 649 nm and 470 nm (Chappelle et. al. 1992). However, the maximum low-wavelength absorption for chlorophyll B is significantly greater than that of chlorophyll A, and chlorophyll A absorbs significantly more at high wavelengths than does chlorophyll B. In addition, chlorophyll B is located

only in the light harvesting complexes (LHC) of the photosystem. Chlorophyll A is located in both LHC and the reaction centers, where light energy is converted to chemical energy. As a result, chlorophyll B is more prevalent in shaded environments, where light capture is limiting to plant growth, and it can better capture the high-energy low-wavelength light that is more apt to penetrate the shade-generating canopy of competing plants (Henry and Aarssen 1997).

Cytokinins are a family of phytohormones that primarily stimulate cell division, and also contribute to regulation of cell differentiation, seed germination, and foliar senescence. These hormones are enzymatically synthesized from adenine, mainly in the roots (Xiaotao et al. 2013). Cytokinin transport is achieved by transpiration-dependent movement through the xylem (Aloni et al. 2005) and the resulting hormonal accumulation has been shown to down-regulate genes associated with chlorophyll synthesis (Pons et al. 2001). An environment with more ambient light increases xylem flow by both increasing the leaf temperature and by increasing stomatal conductance correlated with greater photosynthetic rates. Increased temperature within the leaf is compensated for by evaporative cooling, leading to increased water loss through the stomata. Heightened photosynthetic rates increase consumption of water. These factors lower foliar water concentration, thus increasing xylem flow by increasing the magnitude of the concentration gradient between the leaves and roots.

A positive-feedback mechanism linking sugar production to sun-shade adaptation has been linked to foliar growth and development, thereby optimizing individual leaves to their respective light levels (Raines and Paul 2006). Leaves exposed to higher light levels exhibit a higher transpiration rate, in turn accelerating the rate of xylem flow and

cytokinin import. Lower light incidence decreases transpiration rate; also decreasing cytokinin import (Boonman et al. 2007). It has also been shown that cytokinin activity promotes the survival of foliage exposed to high irradiance; low concentrations of cytokinins found in shaded foliage lead to reduced photosynthetic capacity, leaf senescence, and downregulation of chlorophyll B. These effects were not observed in unshaded foliage (Boonman and Pons 2007).

However, in previous studies shade environments were established by densely growing plant populations with shade-adapted foliage occurring lower on stems. This results in a shade environment that not only has lower light intensity, but also has a shift in light wavelength composition. Incident light wavelengths are selectively absorbed in upper stem foliage, resulting in a spectral shift in red (700 nm) to far-red (720+ nm) in transmitted light. It is well established that this shift is detected by plants through the pigment phytochrome and results in greater shade adaptation of developing foliage (Franklin and Whitlam 2005). Therefore, the effects of light intensity itself, and especially its interaction with cytokinin are less well understood. This study used neutral density shade cloth to minimize spectral shifts and sampled upper stem foliage to limit exposure to transmitted light to overcome these limitations and more directly test the hypotheses that:

(1) light intensity itself (independent of wavelength) is a regulator of sun-shade morphology and physiology, and (2) cytokinins independently and interactively with light intensity regulate sun-shade adaptation.

Materials & Methods

This study used bush type common beans, *Phaseolus vulgaris* Golden Wax (Chas. C. Hart Seed Co.), to examine the effects of exogenous application of 6-Benzylaminopurine (BAP) and its interaction with shading. Seeds were sown 3 per 9 cm diameter pot in Fafard 3B RSi peat/bark-based growth medium (SunGro Horticulture) and grown in a greenhouse until the emergence of the first node and first true leaves. At this time, seedlings were distributed into one of four treatment groups in one of three blocks. Each block contained the same four treatment groups, randomly assorted within the block in a randomized block design (Figure 1). Treatment groups consisted of five pots; each group was grown under one of four combinations of two variable conditions: light level and application of BAP solution.

Each experimental variable was limited to one of two categories rather than continuously varied. Two light levels were used, natural light with no shade, or natural light with 85% shade (Table 1). Shade was provided by a double layer of 25% shade cloth stretched over a 30 cm steel wire cube frame, resulting in an 85% light intensity reduction. Shade provided artificially by shade cloth does not alter the composition of wavelengths in sunlight, unlike shade provided by competing plants. However, plants are able to sense variations in light intensity through sugars and enzymes associated with sugar metabolism (Raines and Paul 2006). Light incidence was measured with a LI-COR Li-185B photometer (LI-COR, Inc.).

All plants were subject to routine exogenous application of one of two solutions, a treatment solution with 100 mg L^{-1}BAP, or a control solution with all components except BAP. Plants were sprayed until excess runoff was observed, once per 7 days for a 21 day

growth period beginning at the emergence of the first node. Before the second application, all pots were culled down to a single plant per pot to minimize mutual shading and stress from resource competition. Stock solution was formulated by suspending 2.5g BAP in 50 ml dimethyl sulfoxide (DMSO), diluted with 450 ml 95% ethyl alcohol. A diluted exogenous spray was derived from 20 ml of stock solution, 20 ml Polysorbate 20 surfactant, and 960 ml ddH_2O. The control solution described previously was formulated identically with BAP omitted.

After 21 days, all leaves above the first node was harvested from each plant. Leaves were scanned immediately upon harvest and leaf area was determined with WinSEEDLE (Regent Instruments, Inc., Quebec). Samples were dried at 65°C for 48 hours, and weighed for dry mass. A portion of each sample, (0.1g-0.15g) was then crushed, suspended in 7 ml DMSO, and incubated at 65°C for 24 hours to extract chlorophylls. Crushed leaf matter was washed with an additional 3 ml DMSO to thoroughly extract chlorophylls. Leaf sample extract was centrifuged at 2,500 rpm for 5 minutes; a spectrophotometer was used to determine light absorbance of the supernatant at 480, 649, and 655 nm wavelengths. Specific leaf area, chlorophyll A content, chlorophyll B content, chlorophyll A to B ratio, and total chlorophyll content were calculated with the equations of Wellburn (1994). The model used for this study was V_D = light+cyt+block+light×cyt+light×cyt×block+ε, where V_D = effect of interactions between independent variables on dependent variables, light = full light intensity or 85% reduced intensity, cyt = 100mg l^{-1} BAP or control, block = spatial variation, and ε = analytical error. Data were analyzed with ANOVA using SYSTAT v. 12 (Systat, Inc.)

with light environment and cytokinin treatment as main effects. Assumptions of ANOVA were tested with Levene's Test and the Wilk-Shapiro statistic.

Results

ANOVA demonstrated that main effects of both light environment and cytokinin treatments were significant, block effects were non-significant, and light environment by cytokinin interactions were significant for all dependent variables. Light intensity was measured under three different atmospheric conditions, full sun, partial sun, and full overcast. During full sun, unshaded blocks experienced an average of 605 micromoles of photons per square meter per second ($\mu m\ m^{-2}\ s^{-1}$). Shaded blocks experienced an average of 80 $\mu m\ m^{-2}\ s^{-1}$. In partial sun, unshaded blocks experienced 357 $\mu m\ m^{-2}\ s^{-1}$, and shaded blocks experienced 57 $\mu m\ m^{-2}\ s^{-1}$. On a fully overcast day, unshaded blocks experienced 33 $\mu m\ m^{-2}\ s^{-1}$, and shaded blocks experienced 6 $\mu m\ m^{-2}\ s^{-1}$ (Table 1).

Effects of Shade

The first set of results is data taken from two subsets of all experimental groups, the first group consisting of all plants grown in full sunlight; the second group consisting of all plants grown in shade. Plants grown in sun showed a specific leaf area 56.3% lower than that of plants grown in shade (Figure 2). Sun grown plants showed a decrease in total chlorophyll content per unit leaf area, 68.1% less than shade grown plants (Figure 3). Sun grown plants had 67.3% less chlorophyll A per unit leaf mass (Figure 4), and 69.7% less chlorophyll B per unit leaf mass (Figure 5) than shade grown plants. Shade grown plants showed a lower chlorophyll A to B ratio, 6.2% lower than that of sun grown plants (Figure 6). Structurally, plants subjected to shade were more elongate and featured narrower stems. Plants grown in full sun were shorter and had thicker stems. These plants

were lighter and yellow-green in color, while shade-grown plants were a deep green. No significant variation in node quantity was observed, all plants featured three to four nodes at harvest; all node quantities were represented equally and evenly distributed across all experimental groups.

Effects of Hormone Application

The second set of results is data taken from two subsets of all experimental groups: all plants treated with BAP solution, and all plants not treated with BAP. Plants exposed to BAP showed a 38.9% decrease in specific leaf area relative to plants not exposed to BAP (Figure 7). BAP-treated plants had 50.0% less total chlorophyll per unit leaf mass than untreated plants (Figure 8), as well as 47.6% less chlorophyll A per unit leaf mass (Figure 9), and 54.8% less chlorophyll B per unit leaf mass than untreated plants (Figure 10). Plants treated with BAP showed a chlorophyll A to B ratio 11.8% greater than that of untreated plants (Figure 11).

Composite Effects of Hormone Application and Shade

Sun grown plants showed a significantly lower specific leaf area relative to shade grown plants whether treated with BAP or not [$p < 0.001$]. SLA of BAP-treated shade grown plants was significantly lower than that of untreated shade grown plants, but SLA of BAP-treated sun grown plants was not significantly lower than in untreated sun grown plants (Figure 12). Sun growth and BAP application both decreased total chlorophyll per unit leaf mass [$p < 0.001$]. Plants grown in sun treated with BAP showed the lowest total chlorophyll per unit leaf mass, followed by sun grown untreated plants. BAP treated plants grown in shade showed more total chlorophyll per unit leaf mass than sun grown untreated plants, but significantly less than untreated shade grown plants (Figure 13).

BAP treatment and sun growth also decreased chlorophyll A content per unit leaf mass [$p < 0.001$]. BAP treated plants grown in shade showed the lowest content of chlorophyll A per unit area, followed by untreated sun grown plants, and then by BAP-treated shade grown plants. Untreated shade grown plants showed a significantly higher chlorophyll A per unit leaf mass than any other group (Figure 14). BAP treatment and sun growth likewise decreased chlorophyll B content per leaf unit mass [$p < 0.001$]. The pattern observed in chlorophyll A density by treatment was also observed in total chlorophyll density by treatment (Figure 15). Minimal difference was observed between the chlorophyll A to B ratio of BAP-treated sun grown plants and BAP-treated shade grown plants. Untreated sun grown plants had a lower A to B ratio than these two groups, untreated shade grown plants had a lower A to B ratio than the preceding three groups (Figure 16). Variation in light level may have had a significant effect on chlorophyll A to B ratio [$p=0.107$]; BAP treatment was shown to significantly increase chlorophyll A to B ratio [$p < 0.001$]. The combination of BAP treatment and unshaded growth condition were also shown to significantly increase chlorophyll A to B ratio [$p=0.019$].

Discussion

Plants face a unique set of challenges in survival due to their sessile nature. Since they are unable to move to a more favorable environment in times of stress, plants must adapt to cope with varying stresses and to better capitalize on available resources. This study demonstrates that both light levels and cytokinins play important roles in the process of adaptation to variations in light availability. High and low light levels place different demands on a plant's photosynthetic machinery, and so elicit responses on somatic down to subcellular levels.

The first set of results (Figs. 2-6) was used as a test for the consistency of the experiment with established information. The plants used in this study performed consistently with expectations, plants adapted appropriately to their respective light levels. Those grown in low light conditions had a greater specific leaf area, lower chlorophyll density, and a lower chlorophyll A to B ratio. This is the characteristic response of a plant grown in low light—the leaves have a broader light-intercepting surface relative to their biomass to maximize light capture where it is a limiting resource. The photosynthetic machinery is likewise adapted; more chlorophyll is present to maximize energy acquisition from incident radiation, and there is a greater proportion of chlorophyll B in order to better absorb energy from available light wavelengths.

The second set of results (Figs. 7-11) was used to examine the independent effects of BAP. All of the physical and molecular adaptations seen in the previous set of results are also observed here, but to varying degrees. Exogenous application of BAP did not depress SLA as significantly as high light level (-56.3% vs -38.9%), nor did it lower chlorophyll density as dramatically as high light level (-68.1% vs -50.0%). However, the differential in effect on chlorophyll A and B density was much greater due to BAP application than to high light level. Chlorophyll A and B were depressed about equally (-67.3% and -68.1%) in sun-grown plants, but were depressed less equally (-47.6%, -54.8%) under BAP application. A greater difference in the A to B ratio in BAP-treated plants as observed as a result of this differential. This information suggests that BAP and related cytokinins are more important in regulation of the photosynthetic systems rather than regulation of foliar growth and development. Nonetheless, cytokinin activity is a major component of the sun-shade adaptation mechanism.

The final set of results (Figs. 12-16) was used to examine the composite effects of light levels and BAP application. In this set of data, SLA was depressed significantly by BAP application in low light conditions (-41.9%), but not as significantly depressed by the same treatment in high light conditions (-21.5%). These disproportionate levels of SLA reduction further suggest that cytokinins are not solely responsible for the regulation of leaf structure and growth; instead they are likely an auxiliary component in this regulatory system. Total chlorophyll density was decreased approximately equally by BAP in both high (-44.2%) and low (-46.4%) light conditions. Chlorophyll A density was also decreased equally by BAP application in high (-43.6%) and low (-43.1%) light situations. Chlorophyll B density was depressed less by BAP in sun (-45.6%) than it was in shade (-52.9%). Due to this difference in effect, BAP application marginally increased the ratio of chlorophyll A to B in high light conditions (+4.4%), but significantly decreased this ratio in low light conditions (-20.4%). These findings suggest that cytokinins are the sole or major regulatory factor in chlorophyll A density, but are likely a major component of chlorophyll B density regulation that shares responsibility with auxiliary factors. Alternately, the effects of cytokinins may be dampened by a factor presented by high light conditions or amplified by a factor present in low light conditions.

This study was conducted to examine the role of cytokinins in the developmental plasticity of leaves, and how this plasticity is regulated. Previous literature suggests that cytokinins are indirectly regulated by light incidence, which passively regulates cytokinin levels by affecting the transpiration stream. It cannot be ruled out that light levels may cue other factors which also control the development of leaves, but the findings here strongly suggest that cytokinins are the predominant phytohormones that regulate leaf

development. This study provides evidence that light and cytokinins both cue plants to adapt to higher light intensity. This adaptation is achieved by depressing SLA, depressing total chlorophyll density, and depressing chlorophyll B more than chlorophyll A. Most likely, light and cytokinins have a dependent interaction where cytokinin activity is a direct-relationship function of light intensity.

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References

Aloni R, Langhans M, Aloni E, Dreieicher E, Ullrich C I (2005) Root synthesized cytokinin in Arabidopsis is distributed in the shoot by the transpiration stream. *Journal of Experimental Botany* **56**: 1535–1544.

Boonman A, Prinsen E, Gilmer F, Schurr, U, Peeters A J, Voesenek L A, Pons T L (2007) Cytokinin import rate as a signal for photosynthetic acclimation to canopy light gradients. *Plant Physiology* **143**: 1841-1852.

Boonman A and Pons T L (2007) Canopy Light Gradient Perception by Cytokinin (article addendum). *Plant Signaling and Behavior* **2**: 489-491.

Chappelle E W, Kim M S, McMurtrey J E (1992) Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll A, chlorophyll B, and carotenoids in soybean leaves. *Remote Sensing of Environment* **39**: 239-247.

Franklin K A, Whitelam G C (2005) Phytochromes and shade-avoidance responses in plants. *Annals of Botany* **96**: 169-175.

Gitelson A A, Gritz Y, Merzlyak M N (2003) Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology* **160**: 271-282.

Givnish T J (1988) Adaptation to Sun and Shade: a Whole-Plant Perspective. *Australian Journal of Plant Physiology* **15**: 63-92.

Hugh, H and Aarssen, L W (1997) On the Relationship between Shade Tolerance and Shade Avoidance Strategies in Woodland Plants. *Oikos* **80**: 575-582.

Pons T L, Jordi W, Kuiper D (2001) Acclimation of plants to light gradients in leaf canopies: evidence for a possible role for cytokinins transported in the transpiration stream. *Journal of Experimental Botany* **52** (360): 1563-1574.

Raines C A, Paul M J (2006) Products of leaf primary carbon metabolism modulate the developmental programme determining plant morphology. *Journal of Experimental Botany* **57**: 1857-1862.

Sims D A and Gamon, J A (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment* **81**: 337-354.

Wellburn, A R (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144**: 307-313.

Xiaotao D, Yuping J, Hong W, Haijun J, Hongmei Z, Chunhong C, Jizhu Y (2012) Effects of cytokinin on photosynthetic gas exchange, chlorophyll fluorescence parameters, antioxidative system and carbohydrate accumulation in cucumber (*Cucumis sativus L.*) under low light. *Acta Physiologiae Plantarum* **35**: 1427-1438.

Author's Biography

Oleg S. Gross was born in Teofipol, Khmelnytskyi Oblast, Ukraine on August 21, 1994. He grew up in Scarborough, Maine, and graduated from Scarborough High School in 2012. A former Chemistry and Biology double major, Oleg chose to focus on his degree in Biology. He is a University of Maine Merit Scholarship recipient. After graduation, Oleg plans to return to Southern Maine to continue his previous occupation of excavation and infrastructure construction.