Summer 2019

Greening rates and photosynthetic development of leaves in C3 and C4 plants

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GREENING RATES AND PHOTOSYNTHETIC DEVELOPMENT OF LEAVES IN C_3 AND C_4 PLANTS

being

A Thesis Presented to the Graduate Faculty

of the Fort Hays State University in

Partial Fulfillment of the Requirements for

the Degree of Master of Science

by

Tayler Kriss

B.S., Fort Hays State University

Date 8-5-2019

Approved _____
Major Professor

Approved _____
Chair, Graduate Council

This thesis for

The Master of Science Degree

By

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has been approved

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PREFACE

This thesis follows the style of the journal *Environmental and Experimental Botany*, to which a portion will be submitted for publication.

ABSTRACT

To study chlorophyll development time and overall photosynthetic development in C_3 and C_4 leaves, seeds were germinated in complete darkness and achlorophyllous leaves were then allowed to develop in lighted conditions. Corn (*Zea mays*, C_4), sorghum (*Sorghum bicolor*, C_4), green bean (*Phaseolus vulgaris*, C_3), broad bean (*Vicia faba*, C_3), and wheat (*Triticum aestivum*, C_3) were investigated for the first ten days of sunlight exposure. Chlorophyll concentration, chlorophyll fluorescence, and CO_2 gas exchange measurements were conducted daily on the first leaf that emerged after the embryonic leaves of each plant. The first five days of the experiment, days zero to four in light, had the greatest physiological impact on leaves of etiolated plants as they transitioned from an etiolated to a green state. C_3 plants developed chlorophyll and light-harvesting capacity earlier than C_4 plants. C_3 plants showed faster rates of chlorophyll development compared to C_4 plants. The majority of chlorophyll fluorescence parameters measured had developed approximately 80% of their maximum fluorescence in the first five days of light exposure, days five to ten in light had less than a 20% change. However, photochemical quenching (qP), electron transport rate (ETR), photosynthetic carbon assimilation (Photo), stomatal conductance (Cond), and internal CO_2 concentration (*C*i) were not different between C_3 and C_4 plants, suggesting that development of gas exchange abilities and capabilities of using carbon from the atmosphere in the processes of photosynthesis were similar between C_3 and C_4 plants in this experiment.

ACKNOWLEDGMENTS

I want to thank the Kansas Academy of Mathematics and Science, specifically Dr. Roger Schieferecke, Dr. Paul Adams, Mr. Ron Keller, Mrs. Ann Noble, Mrs. Angela Delzeit, Mr. Will Burns, and Mrs. Melissa Mayers, for giving me the opportunity that it is to be a KAMS student and putting up with all of the shenanigans I got into. I grew up a lot during those two years and made several great friends who I still keep in contact with. It's funny how long and difficult those two years felt while in KAMS, in retrospect I greatly miss everything about my experience in the program and would do it again if I could still enroll.

I thank the Department of Biological Sciences at FHSU. During my last semester of KAMS I decided to pursue a degree in computer science. After some reflection over the Summer I met with someone in the Biology department about switching majors, that someone was Dr. Greg Farley. After a short conversation about my interests in biology he sent me upstairs to talk to Dr. Brian Maricle. After talking to Dr. Maricle I made my decision and switched majors that day. Since then Dr. Maricle has supported me every step of the way, encouraging my undergraduate research projects, spending hours showing me lab procedures, how to use equipment, and always listening to my questions and offering suggestions. Thank you for showing me how to be a scientist, I sincerely hope that you benefitted from being my advisor as much as I benefitted from being your student.

A second round of thanks to the biology department faculty here at FHSU. Thank you to Dr. Rob Channell for helping with statistical analysis and the knowledge I gained

from Ecology, Biodiversity and Conservation, and Biostatistics. I thank my thesis committee members, Dr. Eric Gillock, Dr. Mitch Greer, Dr. Brian Maricle, and Dr. Roger Shieferecke. For their guidance and support throughout this process. I thank Dr. Bill Stark and my supervisor Mrs. Hilary Gillock for being on my orals defense committee, along with Dr. Gillock, Dr. Greer, and Dr. Maricle. I thank Ms. Liz Atwater for helping me with scheduling and making sure Workday was submitted on time. I thank the other graduate students in my cohort for their support and for making the office spaces enjoyable places to be, specifically Ms. Holly Anderson. I am so glad that studying for comprehensive exams brought us together, and I thank you for your love and support on this journey.

I thank my parents Dr. Thomas Kriss and Mrs. Michelle Hitchcock-Kriss for their support during the six years I have been away from home, I could not have done it without you two helping me, even though you did not have to. I thank my sister Ms. Tessa Kriss for being by my side these past two years and being the best sister and roommate I could ask for, I really am sorry about all the messes…

Last but certainly not least, I thank Mr. Mike Messeck for his help in the greenhouse. Providing seeds and greenhouse space, offering to take care of and water the plants in my study, and for the fruits and vegetables he gifted me. Especially the tomatoes, seriously thank you for those tomatoes.

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INTRODUCTION

Many physiological processes in plants are light dependent, including seed germination, stomatal movement, and chlorophyll development (Kami et al., 2010). Once a seed germinates it is exposed to one of two growth patterns: In the presence of light plant cells develop chloroplasts capable of using light, as seen in normal green plants. In the absence of light, proplastids develop into etioplasts instead of chloroplasts (Wellburn and Wellburn, 1971). When etiolated, plants have elongated hypocotyls and use stored nutrients in metabolism, instead of producing autotrophic metabolites (Fankhauser and Chory, 1997). If never exposed to light, the plant will use all stored nutrients, rendering it unable to perform metabolism, resulting in death (von Wettstein et al., 1995; Cortleven et al., 2016).

When exposed to light, etiolated seedlings become de-etiolated (Reinbothe et al., 1999). Development of chlorophyll in young seedlings is also a light-dependent process (Malkin and Niyogi, 2000). When etioplasts are exposed to light, thylakoid membranes form from the prolamellar body that eventually develop chlorophyll and transition into chloroplasts, becoming de-etiolated (Reinbothe et al., 1999). However, development of the light reactions of photosynthesis has not been studied thoroughly in young plants while transitioning from an etiolated to a green state. Understanding how light is used during this transition is important for understanding plant growth and development.

Light usable by plants for photochemical reactions is known as photosynthetically active radiation (PAR), with wavelengths of 400 to 700 nm (McCree, 1981). When PAR reaches a leaf, the radiation is either reflected, transmitted through the leaf, or absorbed

by the leaf (Baker, 2008). The energy that is absorbed by the leaf in chlorophyll is either used in photochemistry, dissipated as heat, or re-emitted as chlorophyll fluorescence (Baker, 2008). Chlorophyll fluorescence is a measure of the light energy that was absorbed by a leaf but then re-emitted as light. Quenching refers to any process that decreases chlorophyll fluorescence, which includes the energy dissipated in a leaf as photosynthetic reactions, known as photochemical quenching (qP), or energy transferred as heat that would otherwise damage plant cells, known as non-photochemical quenching (qN) (Muller and Niyogi, 2001).

To study the development of light-harvesting systems inside leaf tissue, chlorophyll fluorescence can be used to measure photochemical processes (Maxwell and Johnson, 2000). There are many types of fluorescence measures that can be used to understand light use by the plant in photosynthesis (Baker, 2008). A fluorometer uses the light doubling technique to measure chlorophyll fluorescence (Maxwell and Johnson, 2000). A high intensity pulse of light, short enough to not increase non-photochemical quenching, saturates Photosystem II (PSII) reaction centers in chlorophyll. During this PSII saturation, fluorescence reaches a maximum in light (Fm′), that would be seen in conditions where no photochemical quenching was taking place (Maxwell and Johnson, 2000). Fm′, along with the steady-state of fluorescence (Fs) and yield of minimum fluorescence in light (Fo′), can be used to calculate the proportion of open PSII reaction centers that are able to receive PAR, qP, and a measure of the amount of light absorbed by PSII that is used in photochemistry, Φ_{PSII} (Maxwell and Johnson, 2000; Muller et al.,

2001). From these measurements, an understanding is gained of how well the leaf is using light energy in the processes of photosynthesis when exposed to light.

Etioplast development and transition to chloroplasts has been primarily studied in vitro by means that irreparably damage plant tissue (Gunning, 1965; Wellburn and Wellburn, 1971; Smillie and Nott, 1982; Sakuraba et al., 2013). Studies such as these allow for analysis of internal structure and chemical composition of plants, but are not able to study the development and efficiency of a single leaf as it transitions from an etiolated to green state. Consequently, a study with daily measures of chlorophyll concentration and chlorophyll fluorescence during a de-etiolation period could provide insight into developmental processes in photosynthesis.

The objectives of this experiment were to determine the amount of time for an etiolated plant to become photosynthetically active once introduced to light and the subsequent time needed for a plant to become efficient at photochemical quenching. To understand leaf development from an etiolated state, several species were used that exhibit different photosynthetic pathways, being C_3 and C_4 photosynthetic types. C_4 plants have compartmentalized anatomy ("kranz anatomy") that allows RuBisCO to use atmospheric CO_2 more efficiently than C_3 plants, combating photorespiration in low CO_2 conditions and high temperatures (Edwards and Walker, 1983). C_4 plants make up 3% of flowering plant species, yet account for over 20% of global primary productivity, including many plants used by humans and livestock (Sage, 2003). All photosynthetic reactions occur in mesophyll cells of C_3 plants, whereas separate carbon-fixing reactions are in mesophyll cells and bundle sheath cells of C_4 plants (Ehleringer et al., 1997). These

differences might impact the rates at which different species generate photosynthetic machinery and how well they use light during early development.

A study that analyzes the generation time of chlorophyll in etiolated plants by fluorescence was not found, so a comparison was made among several C_3 and C_4 species. The following hypotheses were tested: 1) C_4 plants were expected to require less time to become photosynthetically viable than C_3 plants, 2) C_4 plants were expected to be able to use light at a slower rate than C_3 plants, and 3) C_4 plants were expected to make use of atmospheric CO_2 at a slower rate than C_3 plants. These results were expected because of shared features of photosynthesis between the photosynthetic types, including chlorophylls and other shared metabolic pathways.

MATERIALS AND METHODS

Experimental design and greenhouse conditions

To analyze leaf development from an etiolated to a green state, plants were germinated and grown in darkness for 14 to 21 days to generate achlorophyllous leaves. This time allowed the plants to produce leaves with a large enough surface area that could be measured by a fluorometer. Plants were grown in 10 cm × 10 cm × 10 cm pots in potting soil (MiracleGro Potting Mix; 0.21% N, 0.07% P, 0.14% K; Scotts Company, Marysville, Ohio, USA), each of which was treated as a replicate. Each pot was planted with three to five seeds to ensure a survivor; the most developed leaf from a pot was used for measurement, the same leaf was used each day. Five species were used for fluorescence measurements, two C_4 plants, corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench), and three C_3 plants, green bean (*Phaseolus vulgaris* L.), broad bean (*Vicia faba* L.), and wheat (*Triticum aestivum* L.). The plants were watered to field capacity and placed in dark cabinets to germinate and grow, and were re-watered as needed, typically every 2 days. Temperature ranged from 20°C to 22°C during germination. Three treatments were conducted on corn, Corn 2017, Corn 2018, and growth chamber corn.

Only the first set of true leaves, not the cotyledons, that developed in darkness were measured. The number of replicate pots planted per species was 12 to 14, of which 8 to 14 pots survived data collection for 11 days of measurements, depending on species. When plants had developed leaves, they were taken to the Fort Hays State University

greenhouse (38.875°N, 99.244°W) immediately after being removed from darkness, where they were initially measured and left to develop in lighted conditions for 10 days, in greenhouse temperature and humidity. Temperatures ranged from 20°C to 44°C in the greenhouse. Relative humidity varied from 24% to 50%, measured in the LI-6400XT, and sunlight ranged from 40 to 400 µmol photon m^{-2} s^{-1}, depending on the time of year the plants were measured. Green bean was measured from October 23 to 31 in 2017. Corn was measured from August 9 to 19 in 2017. Corn was measured from October 26 to November 5 in 2018. Corn was measured in a growth chamber from December 28 in 2018 to January 7 in 2019. Sorghum was measured from February 13 to 22 in 2018. Wheat was measured from March 12 to 20 in 2018. Broad bean was measured from September 13 to 23 in 2018.

Photosynthesis and fluorescence measures

The LI-6400XT Portable Photosynthesis System (LI-Cor Biosciences, Inc., Lincoln, Nebraska, USA) with the 6400-40 leaf chamber fluorometer was used to measure gas exchange and chlorophyll fluorescence in leaves. Leaf measurements were made with an air flow rate of 400 µmol s^{-1}, CO_2 concentration of 400 ppm, photosynthetic photon flux density (PPFD) of PAR of 1500 µmol m^{-2} s^{-1}, and light was 90% red and 10% blue. Temperature and humidity in the leaf chamber were near ambient levels in the greenhouse.

All fluorescence measures were performed in lighted conditions. Once a leaf was ready for measurement, the surface area of the leaf covering the fluorometer chamber was recorded for calculations, then the leaf was inserted into the fluorometer chamber. Plants were acclimated to the gas exchange parameters and light intensity inside the chamber, and measurements were made once readings had stabilized, determined when the coefficient of variation (CV) had reached a maximum of 30 or lower, taking 1.5 to 3 minutes (Johnson et al., 2015). Several variables for each plant were recorded with the LI-6400XT using the same procedure, including steady state fluorescence (Fs), minimum fluorescence in light (Fo′), and maximum fluorescence in light (Fm′). Each of these parameters is a unitless number related to the amount of fluorescence from chlorophyll. From these values the proportion of open PSII reaction centers, qP, is calculated by qP = (Fm′ - Fs) / (Fm′ - Fo′). The quantum yield of PSII photochemistry (Φ_{PSII}), is calculated after Genty et al. (1989) by Φ_{PSII} = (Fm′ - Fs) / Fm′. From Φ_{PSII} the electron transport rate, ETR, through photosystem II, is calculated after Maricle et al. (2007) by ETR = (Φ_{PSII} × 0.5 × LeafAbs × PPFD), where 0.5 represents half of absorbed light energy allocated to PSII and LeafAbs represents the proportion of PAR absorbed by the leaf, calculated by the Li-6400XT as 0.875.

The plants were measured daily, with day 0 being when the plants were moved to lighted conditions then measured for 10 subsequent days as they developed in light. All measurements were performed in lighted conditions, similar to Sofo et al. (2010). The amount of light used with the Li-6400XT in the present experiment was 1500 µmol m^{-2}

s^{-1}, to ensure all reaction centers were reduced upon illumination, for optimal fluorescence yield (Kull and Kruijt, 1998).

Photosynthetic CO_2 uptake in µmol CO_2 m^{-2} s^{-1} (Photo), stomatal conductance in mol H_2O m^{-2} s^{-1} (Cond), and internal CO_2 concentration in ppm (C_i), were measured daily in the LI-6400XT at the same time fluorescence measurements were made.

Chlorophyll concentration in developing leaves was measured with the Chlorophyll Meter SPAD-502 Plus (Konica Minolta, Chiyoda, Tokyo, Japan), which provides a unitless number that corresponds with leaf chlorophyll concentration (Caudle et al., 2014). Measurements were made each day prior to measurements of fluorescence and gas exchange with the LI-6400XT for each leaf.

Growth chamber measures

Corn was grown in a Caron 7301-50-2 plant growth chamber (Caron Products & Services Inc., Marietta, Ohio, USA), with light at 500 µmol m^{-2} s^{-1} on a cycle of 12L:12D. Daytime temperature and humidity were set according to the default settings of the growth chamber at 30°C and 60%, respectively, and nighttime temperature and humidity were 20°C and 90%, respectively. Measures were conducted on the plants in the same manner as in the greenhouse. Corn was grown in a growth chamber to determine how great an effect varying light levels, temperature, and humidity in the greenhouse might have had on the development of leaves. Growth chamber measurements allowed for comparison of development of PSII and other photosynthetic factors measured via

fluorescence to measurements of plants grown in the greenhouse. Two additional replicate treatments were conducted on corn in the greenhouse, Corn 2017 and Corn 2018.

Statistical analyses

Data analysis was performed using The R Project for Statistical Computing (R Core Team, 2019; Vienna, Austria). Repeated measures Analysis of Variance (ANOVAs) were used for assessing differences between species and days during leaf development for each chlorophyll fluorescence, chlorophyll concentration, and photosynthetic gas exchange variable. Assumptions for repeated measures ANOVAs were tested using descriptive statistics, Q-Q plots, and Mauchly's sphericity test. Greenhouse-Geisser or Huynh-Feldt corrections were used for dependent variables that violated the assumption of sphericity. Post hoc comparisons were made using the kruskalmc function from the pgirmess package in R (Patrick Giraudoux 2018. pgirmess: Spatial Analysis and Data Mining for Field Ecologists. R package version 1.6.9.) with a nonparametric multiple comparison test between treatments, since the assumption of normality for parametric multiple comparison test was violated for each dependent variable. Separate repeated measures ANOVAs were used to compare photosynthetic type (C_3 vs. C_4 plants). Statistical analyses were performed with a Bonferroni correction to control the familywise error rate for the three hypotheses in this study at $\alpha = 0.05 / 3 = 0.016$.

RESULTS

Fluorescence, gas exchange, and chlorophyll concentrations were measured on etiolated corn (*Zea mays*, C_4), sorghum (*Sorghum bicolor*, C_4), green bean (*Phaseolus vulgaris*, C_3), broad bean (*Vicia faba*, C_3), and wheat (*Triticum aestivum*, C_3) for 10 days following exposure to light. At least two-thirds of the plants in each experiment survived data collection for the full 10 days of measurements. Plants that survived the experiments were capable of photosynthesis and continued to grow, producing new leaves after measurements were done.

Steady-state fluorescence in light (Fs) increased with exposure to light (Fig. 1). On day 0, Fs ranged from -80 to 125 across all species. Following this, Fs increased for 4 to 6 days of light exposure. Maximum Fs varied among species, with mean values as high as 1189 in green bean, but only as high as 129 in wheat (Fig. 1). Fs was significantly higher in C_3 than in C_4 species ($F_{1, 6} = 42.96$, $p < 0.001$) and increased at a greater rate in light ($F_{6, 6} = 18.15$, $p = 0.001$). There was a distinct plateau of Fs in sorghum, wheat, and corn, versus a more gradual tapering of Fs in green bean and broad bean. There was a significant difference within species ($F_{6, 639} = 284.02$, $p < 0.001$), time ($F_{10, 639} = 166.99$, $p < 0.001$), and their interaction. ($F_{50, 639} = 13.49$, $p < 0.001$). Wheat and sorghum had significantly lower peak values of steady-state fluorescence than other plants, with the next lowest being growth chamber (GC) corn. Post hoc comparisons showed all species were different from each other except for broad bean and corn 2018, broad bean and green bean, GC corn and corn 2017, corn 2018 and green bean, and sorghum and wheat.

Minimum fluorescence in light (Fo′) increased with exposure to light (Fig. 2). On day 0, Fo′ ranged from -82 to 121 across species. Fo′ increased from day 0 to 8 in broad bean and corn 2018 and from day 0 to 6 in the other species. Corn 2017, corn 2018, wheat, and sorghum had less than an 11 percent increase in Fo′ from days 5 to 10. Maximum Fo′ varied significantly among species, with mean values as high as 631 in broad bean, but only as high as 25 in wheat (Fig. 2). There was a significant difference in Fo′ between photosynthetic types ($F_{1, 6} = 102.33$, $p < 0.001$), with C_3 plants having higher Fo′ than C_4 plants, but no difference between days in light ($F_{6, 6} = 2.42$, $p = 0.153$). There was a distinct plateau of Fo′ in sorghum, wheat, and corn, versus a more gradual tapering of Fo′ in green bean and broad bean. There was a significant difference within species ($F_{6, 639} = 411.75$, $p < 0.001$), time ($F_{10, 639} = 142.77$, $p < 0.001$), and their interaction. ($F_{50, 639} = 14.47$, $p < 0.001$). Wheat and sorghum had significantly lower values of Fo′ than other plants. The interaction of time and species was significant, meaning species changed in different ways over time. Fo′ reached a maximum in corn 2017 on day 7, and corn 2018 on day 8, the other five series of measurements reached a maximum Fo′ by day six of light exposure. Post hoc comparisons showed species were all different from each other except there was no difference between broad bean and corn 2018, broad bean and green bean, GC corn and corn 2017, corn 2018 and green bean, and sorghum and wheat.

Maximum fluorescence in light (Fm′) increased with exposure to light (Fig. 3). On day 0, Fm′ ranged from -80 to 124 across species. Maximum Fm′ varied among species, with mean values as high as 1414 in green bean, but only as high as 135 in

wheat. There were significant differences between photosynthetic types ($F_{1, 6} = 25.21$, $p = 0.002$), with C_3 plants having higher Fm′ than C_4 plants, and between days in light ($F_{6, 6} = 13.72$, $p = 0.003$). Fm′ reached a maximum in all species around day six to eight of light exposure. There was a distinct plateau of Fm′ in sorghum, wheat, corn 2017, and GC corn versus a more gradual tapering of Fm′ in green bean, broad bean, and corn 2018. There was a significant difference within species ($F_{6, 639} = 310.20$, $p < 0.001$), time ($F_{10, 639} = 178.50$, $p < 0.001$), and their interaction. ($F_{50, 639} = 14.00$, $p < 0.001$). Wheat and sorghum had significantly lower values of maximum lighted fluorescence than other plants. Post hoc comparisons resulted in differences between all species except broad bean and corn 2018, broad bean and green bean, GC corn and corn 2017, corn 2018 and green bean, and sorghum and wheat (Fig. 3).

Electron transport rate (ETR) varied with exposure to light (Fig. 4). On day 0, ETR ranged from -80 to 125 μmol electrons m^{-2} s^{-1} across species, but ETR increased for 4 to 6 days of light exposure. All species reached a maximum at day four to ten of light exposure. ETR was not different between photosynthetic types ($F_{1, 6} = 0.00$, $p = 0.990$), nor among days in light ($F_{6, 6} = 0.435$, $p = 0.833$). ETR did not vary significantly among species, with mean values as high as 93 μmol electrons m^{-2} s^{-1} in sorghum, but only as high as 15 μmol electrons m^{-2} s^{-1} in GC corn (Fig. 4). There was a significant difference within the interaction of species and time ($F_{50, 639} = 2.622$, $p < 0.001$), but not within species ($F_{6, 639} = 1.36$, $p = 0.227$) nor within time ($F_{10, 639} = 1.55$, $p = 0.118$). GC corn had significantly lower values of ETR than other plants. Post hoc comparisons showed

differences between broad bean and corn 2017, GC corn and all other species, and corn 2017 and wheat.

Chlorophyll concentration (SPAD) increased with exposure to light (Fig. 5). On day 0, SPAD was unmeasurable. By day one of light exposure, however, SPAD values ranged from 1.8 in sorghum to 23.9 in broad bean. Maximum SPAD varied significantly among species, with mean values as high as 48.6 in broad bean, but only as high as 22.2 in GC corn (Fig. 5). There was a slight significant difference in SPAD between C_3 and C_4 photosynthetic types, with C_3 plants having a higher chlorophyll content than C_4 species ($F_{1, 6} = 8.89$, $p = 0.025$), but not between days in light ($F_{6, 6} = 1.22$, $p = 0.406$). Mean SPAD values for each day reached a maximum in all species during day three to ten of light exposure. From days five to ten of the experiment, the greatest difference was seen in GC corn, a 25 percent increase in SPAD. There was a significant difference within species ($F_{5, 529} = 406.89$, $p < 0.001$), time ($F_{9, 529} = 132.83$, $p < 0.001$), and their interaction. ($F_{41, 529} = 2.55$, $p < 0.001$). There was a distinct plateau of SPAD in sorghum, wheat, corn 2018, and broad bean versus a more gradual tapering of SPAD in GC corn and corn 2017 (Fig. 5). Post hoc comparisons resulted in differences between all species other than GC corn and sorghum, corn 2017 and sorghum, and corn 2018 and wheat.

Photochemical quenching (qP) varied with exposure to light (Fig. 6). On day 0, qP ranged from -0.07 to 0.25 across species. Corn 2017 and green bean had less than a 12 percent difference between their day 1 and day 8 measures. There was no difference in qP between photosynthetic types ($F_{1, 6} = 0.03$, $p = 0.879$), nor between days in light ($F_{6, 6} =$

2.27, p = 0.171). qP did not vary among species, with mean values as high as 0.60 in broad bean, but only as high as 0.25 in GC corn (Fig. 6). GC corn, corn 2018, broad bean, and sorghum had decreases in qP from day 2 to day 8. There was a significant difference within species ($F_{6, 639} = 28.04$, $p < 0.001$), time ($F_{10, 639} = 20.488$, $p < 0.001$), and their interaction. ($F_{50, 639} = 9.93$, $p < 0.001$). Post hoc comparisons displayed differences between broad bean and all other species.

Photosynthetic carbon assimilation (Photo) increased with exposure to light (Fig. 7). On day 0, Photo ranged from -12.9 to 21.1 μmol CO_2 m^{-2} s^{-1} across species. Photo mean values were as high as 28.3 μmol CO_2 m^{-2} s^{-1} in GC corn, but only as high as 5.6 μmol CO_2 m^{-2} s^{-1} in broad bean (Fig. 7). Photo was not different between photosynthetic types ($F_{1, 6} = 0.01$, $p = 0.943$), nor days in light ($F_{6, 6} = 0.10$, $p = 0.993$).Green bean, broad bean, sorghum, wheat, and GC corn reached a maximum on day 7 to 9 but decreased after. Corn 2017 and corn 2018 displayed increases in Photo until day 10 of the experiment, corn 2017 increased less than 2 μmol CO_2 m^{-2} s^{-1} from day 4 to day 10 and corn 2018 increased 7 μmol CO2 m^{-2} s^{-1} from day 4 to 10. Corn 2017 and GC corn had the highest average Photo measurements. There was a significant difference within species ($F_{6, 639} = 25.30$, $p < 0.001$), time ($F_{10, 639} = 11.18$, $p < 0.001$), and their interaction. ($F_{50, 639} = 7.29$, $p < 0.001$). Post hoc comparisons showed differences between broad bean and all other species.

Stomatal conductance (Cond) varied with exposure to light (Fig. 8). Cond increased in all species until day 10 except in wheat until day 8, broad bean until day 5,

and GC corn until day 7. On day 0, Cond ranged from 0.019 to 0.372 mol H_2O m^{-2} s^{-1}, but did not vary among species over the period of 10 days of lighted development (Fig. 8). There was no significant difference in Cond between photosynthetic types ($F_{1, 6}$ = 2.08, p = 0.200), nor days in light ($F_{6, 6}$ = 2.30, p = 0.167). There was a significant difference within species ($F_{6, 639}$ = 100.91, p < 0.001), time ($F_{10, 639}$ = 5.78, p < 0.001), and their interaction. ($F_{50, 639}$ = 10.79, p < 0.001). Post hoc comparisons resulted in differences among species other than broad bean and corn 2018, corn 2017 and wheat, and green bean and sorghum.

Internal CO_2 concentration (C_i) generally decreased with exposure to light (Fig. 9). C_i values on day 0 were erratic. On day 1, C_i values varied significantly across species, with 161 ppm in corn 2018 to 377 ppm in broad bean. There was not a significant difference in C_i between C_3 and C_4 photosynthetic types ($F_{1, 6}$ = 0.10, p = 0.762), nor between days in light ($F_{6, 6}$ = 1.24, p = 0.401). C_i varied among species, with mean values as high as 584 ppm in broad bean, but only as high as 398 ppm in green bean. More importantly, mean minimum values for species were seen during days 6 to 8 of light exposure (Fig. 9). There was a significant difference within species ($F_{6, 639}$ = 310.20, p < 0.001), time ($F_{10, 639}$ = 178.50, p < 0.001), and their interaction. ($F_{50, 639}$ = 14.00, p < 0.001). Post hoc comparisons showed differences between all species except corn 2017 and corn 2018, green bean and wheat, green bean and sorghum, and sorghum and wheat.

DISCUSSION

In this study five species were measured in seven experiments to assess the development of plants from an etiolated to a green state. To examine how a single leaf of a plant transitions from an etiolated state to a green state in vivo, chlorophyll concentration, chlorophyll fluorescence, and gas exchange measurements were conducted each day for 10 days during lighted development. Chlorophyll concentration and light harvesting were quicker to develop in C_3 species than in C_4 species, but development of CO_2 fixation capabilities among species were similar for the 10 days of measurement.

Upon light exposure etioplasts immediately evolve oxygen and begin the initial stages in the processes of photosynthesis (Smith, 1954). The chemical composition and structure of etiolated seedlings has been studied thoroughly (Reinbothe et al., 1999; Gabruk and Mysliwa-Kurdziel, 2015). What has not been explored in detail is how long it takes different species to develop the ability to use light in photosynthesis when transitioning from an etiolated state.

Development of etiolated seedlings into photomorphic plants is a complicated process involving many physiological and anatomical steps (Wellburn and Wellburn, 1971; Cortleven et al., 2016). Chloroplast formation from etioplasts once a dark grown plant is exposed to light is very rapid, taking only a few days to reach a chlorophyll concentration capable of efficiently collecting energy from light (Babani and Lichtenthaler, 1996). In the present experiment, development of abilities to harvest light and fix CO_2 took 4 to 8 days, depending on the species and the measurement involved.

Development of C_3 and C_4 species in light

The first hypothesis tested was that C_4 plants were expected to require less time to become photosynthetically viable than C_3 plants. Photosynthetic viability was determined by measurements of chlorophyll concentration, chlorophyll fluorescence, and photosynthetic gas exchange.

In this experiment, the first five days of measurement (days zero to four in light) saw greater levels of increasing chlorophyll concentration, whereas the last half of the experiment (days five to ten in light) showed the least increase in chlorophyll concentration. This indicates the majority of chlorophyll in young leaves is generated during the first four days of light exposure. Five of the seven series of chlorophyll concentration measurements had less than a twenty percent increase in SPAD from days five to ten, meaning eighty percent of chlorophyll in the first set of leaves was generated in the first four days following light exposure. Broad bean and wheat had higher SPAD levels on day five of the experiment than other species, indicating that C_3 plants in the experiment generated greater amounts of chlorophyll than C_4 plants. For most species comparisons there was a difference between SPAD values, but not for growth chamber (GC) corn and sorghum, or corn 2017 and sorghum, suggesting that C_4 species generated chlorophyll in a similar manner, whereas C_3 species all displayed different SPAD values from each other.

Mean SPAD levels following 10 days of light exposure in this experiment ranged from a low of 21 in growth chamber corn up to a maximum of 48 in broad bean. These

are similar to measures of mature rice by Swain and Sandip (2010), mature *Fragaria vesca* L. (C_3, strawberry) by Roiloa and Retuerto (2006), mature *Andropogon gerardii* Vitman (C_4) by Caudle et al. (2014), and of 35 day old corn by Apostol et al. (2003). Furthermore, SPAD has been documented to provide an accurate measure of chlorophyll in leaves. Previous work by Uddling et al. (2007) and Caudle et al. (2014) investigated the relationship between chlorophyll concentration and SPAD values. Uddling et al. (2007) found SPAD accounted for 84% of the variation in chlorophyll concentration by leaf area and Caudle et al. (2014) showed a saturating relationship between SPAD and chlorophyll content that was highly significant.

In the present experiment, chlorophyll development during the first ten days of light exposure in etiolated seedlings reflected a pattern similar to previously-published research. Babani and Lichtenthaler (1996) studied 7 day old etiolated *Hordeum vulgare* L. (C_3) seedlings and found chlorophyll concentrations in leaves of 0.2 to 0.3 μg cm^{-2} after 10 minutes of light exposure and 12.6 to 17.4 μg cm^{-2} after 30 hours of light exposure, supporting the rapid generation of SPAD and fluorescence data in the present experiment. In vitro measures by Shaver et al. (2008) found isolated plastids from 5 day dark grown *Medicago truncatula* Gaertn. (C_3) to have increased chlorophyll autofluorescence one hundredfold in the first 9 hours of illumination. Croxdale and Omasa (1990) found new leaves of *Cucumis sativus* L. (C_3, cucumber) to develop chlorophyll *a* fluorescence similar to mature leaves, in 6 or more days of growth. The same results were found in leaves of plants that were 4 and 8 weeks old, suggesting that

leaves of cucumber plants display the same developmental procedure independent of plant age.

With greater chlorophyll concentration in broad bean and wheat and higher chlorophyll fluorescence in green bean and broad bean, this hypothesis was rejected as the C_3 plant species in this experiment produced greater amounts of chlorophyll than C_4 species. C_4 plants segregate carbon fixing anatomy to more efficiently harvest CO_2 for photosynthesis than C_3 plants (Brown and Hattersley, 1989) and have lower chlorophyll concentrations than C_3 leaves (Taylor et al., 2011). Kranz anatomy of bundle sheath cells may be responsible for C_4 plants not generating chlorophyll at a comparable amount to C_3 plants, potentially due to the additional time needed to generate C_4 specific bundle sheath cells (Nelson and Langdale, 1989).

Plants were measured for 10 days in light as previous studies have found a slowing of development after this time period. Jucknischke and Kutschera (1998) found the amount of chlorophyll *a*, chlorophyll *b*, and carotenoids in *Helianthus annuus* L. plants all decreased from days 10 to 14 after sowing. The decrease in pigment was seen in cotyledons and the primary leaves. Roiloa and Retuerto (2006) found SPAD values to decrease from week 1 to week 6. These studies suggest a decrease of development in leaves and cotyledons after 10 days of growth.

Light use in C_3 and C_4 species

The second hypothesis tested was that C_4 plants were expected to be able to use light at a slower rate than C_3 plants. This was determined by comparing fluorescence data among species. There were differences in Fo′, Fm′, and Fs among species in 16 of the 21 post hoc comparisons. Variation in these fluorescence parameters was more attributable to the different species than C_3 and C_4 photosynthetic types.

In this experiment, the first five days of light exposure saw greater levels of increasing chlorophyll fluorescence, whereas the last half of the experiment showed the least increase in chlorophyll fluorescence. Fm′, Fo′, and Fs are the three fluorescence parameters of importance used to quantify chlorophyll fluorescence data in this study in addition to photochemical quenching, qP, a combination of these three parameters. Corn 2017, corn 2018, wheat, and sorghum had mean fluorescence parameter differences of less than 20 percent from days 5 to 10, green bean, broad bean, and growth chamber corn had a 20 to 40 percent difference. Green bean had less than a five percent difference in fluorescence parameters from day 6 to 8. This indicates that the light harvesting capabilities of young leaves is achieved during the first five days of growth in light for corn, sorghum, and wheat, and by six days in broad bean and green bean.

Mean Fo′, Fm′, and Fs in the present experiment following 10 days of light were similar to measures of qP in mature *Solanum lycopersicum* L. (C_3) by Thwe et al. (2014) and *Nicotiana tabacum* L. (C_3, tobacco) seedlings by Guo et al. (2006) as well as ETR measures in C_4 estuarine grasses by Maricle et al. (2007). Measures in the present study

were also similar to measures of Fs, Fo′, and Fm′ in 70 day old corn plants by Sheng et al. (2008), but these fluorescence measures were less than half of what was seen in the three corn trials in the present experiment, suggesting decreased photosynthetic activity in more mature leaves of corn. Meng et al. (2012) found strawberry leaf chlorophyll fluorescence Fm′ and Fo′ similar to corn 2017 and growth chamber corn in the present study, while the other species had greater fluorescence parameter values.

In the present experiment, light use abilities during the first ten days of light exposure in etiolated seedlings reflected a pattern similar to Tarakhovskaya et al. (2013), where *Fucus vesiculosus* L. (C_3) embryos had increases in chlorophyll fluorescence and ETR from days 6 to 8 of growth, but then decreased thereafter. This same trend was seen in all species in the present experiment other than corn 2017 and corn 2018, with maximum values seen on day 10 in light. With higher fluorescence values in broad bean and green bean but not wheat, the development of chlorophyll fluorescence was more related to species differences than C_3 or C_4 differences, not supporting the second hypothesis of this experiment.

Gas exchange in C_3 and C_4 species

The third hypothesis tested was that C_4 plants were expected to make use of atmospheric CO_2 at a slower rate than C_3 plants. This was determined by comparing photosynthetic gas exchange data among species. This hypothesis was tested by analyzing Photo, Cond, and C_i and was accepted for corn, as species comparisons

revealed differences between corn and all other species and similarities between corn treatments. Sorghum C_i was not different than green bean or wheat, and average C_i ppm for sorghum was close to the C_3 species tested.

In this experiment, the first 5 days of light exposure displayed the most development in Photo, while Cond and C_i were more varied. Mean Photo from day 5 to 10 increased most in corn 2018 with a difference of 32 percent and the least in sorghum, with only a 13 percent difference. Cond was more variable with corn 2017 having a 6 percent difference between day 5 and 10, growth chamber corn, wheat, green bean nearly doubled rates of Cond between the same days, and broad bean had less than half the Cond rate on day 10 than day 5. C_i increased in broad bean, remained roughly the same in green bean, and decreased in other species, with most differences being a reduction of 15 to 20 percent from day 5 to 10. Development of CO_2 fixation abilities took approximately 5 to 6 days, with more continued development in gas exchange parameters than in the fluorescence parameters.

Mean Photo, Cond, and C_i in the present experiment were similar to measures of tobacco by Guo et al. (2006), who found Photo rates of 4 to 16 μmol CO_2 m^{-2} s^{-1}, from days 16 to 18 and decreases from day 18 to 24. Similarly, Meng et al. (2012) found Photo values of 15 to 25 in strawberry plants with no significant difference between plants until 40 days into the study, suggesting young plants share more developmental similarities than older plants. Sheng et al. (2008) found Photo rates between 5 and 9 μmol CO_2 m^{-2} s^{-1}, Cond of 0.03 to 0.04 mol H_2O m^{-2} s^{-1}, and C_i ranging from 30 to 150 ppm in the

second fully expanded leaves of 70 day old corn plants. In the present experiment Photo rates in the three trials of corn were more than twice that of the Sheng et al. (2008) study, and Cond and C_i were also greater, suggesting that developing leaves make greater use of atmospheric carbon than developed leaves.

C_3 photosynthesis is heavily influenced by ambient CO_2 concentration (C_a). Previous studies have shown that C_3 plants maintain a ratio of C_i to C_a of 0.6 to 0.8 (Haxeltine and Prentice, 1996). C_4 plants use at least two more ATP molecules than C_3 plants for the fixation of carbon per CO_2 used in photosynthesis, but maintain a lower C_i to C_a ratio as a result (Ehleringer and Bjorkman, 1977). Taylor et al. (2011) found that chlorophyll concentrations in leaves of mature C_4 species were significantly lower than in C_3 plants. Their study also found C_4 species to have greater Photo rates, but lower Cond and C_i than C_3 species (Taylor et al., 2011). Chlorophyll concentration, Photo, Cond, and C_i in the present experiment follow similar trends to that seen in by Taylor et al. (2011).

Oberhuber and Edwards (1993) found the ratio of quantum efficiency of photosystem II (Φ_{PSII}) to Photo to be constant at temperatures of 15°C to 40°C in C_4 plants, while C_3 plants had a higher Φ_{PSII}/Photo ratio at higher temperatures, decreasing carbon assimilation efficiency. In the present experiment the ratio of ETR to Photo was greater in C_3 than in C_4 plants. ETR was used for comparison as it incorporates Φ_{PSII} and accounts for the electrons being used by Photosystem II, the amount of light being absorbed by the leaf, and the PPFD coming into the leaf at the time of measurement. Oberhuber and Edwards (1993) also found Φ_{PSII} to Φ_{CO2} to be higher in C_3 plants than in

C_4 under normal atmospheric conditions. These results coincide with the Φ_{PSII} to Φ_{CO2} ratio of plants in this study with the highest values seen in the C_3 species, broad bean, wheat, and green bean and the lowest seen in the C_4 species, corn and sorghum, with Φ_{PSII} to Φ_{CO2} ratios less than half that of the C3 plants. A smaller ratio of Φ_{PSII} to Φ_{CO2} suggests C_4 plants are more efficient at using CO_2 than C_3 plants, supporting the third hypothesis. There were greater differences in the light harvesting capabilities between C_3 and C_4 plants, and fewer differences in gas exchange and carbon fixation measures in this experiment. With C_3 plants in the experiment generating chlorophyll and developing chlorophyll fluorescence at a faster rate than C_4 plants. A summary of the effect of lighted development on dependent variables in this study for each species is presented in Table 1.

Conclusions and Future Directions

In this experiment, etiolated leaves generated photosynthetic abilities during the first 4 to 8 days of light exposure. The first five days resulted in the greatest increase in chlorophyll concentration, light harvesting, and CO_2 fixation rates, with smaller increases thereafter. C_3 plants had greater chlorophyll content and generated chlorophyll at a slightly faster rate than C_4 species. C_3 plants developed light harvesting ability sooner than C_4 plants. In contrast, there were no differences between photosynthetic types or days regarding development of CO_2 fixation ability. This indicates C_3 plants are quicker to generate light harvesting abilities, but not quicker than C_4 plants to use this energy for

carbon fixation. Future experiments might include imaging of leaf tissue via microscopy to visualize anatomical development alongside physiologic data.

REFERENCES

Apostol, S., Viau, A. A., Tremblay, N., Briantais, J., Prasher, S., Parent, L., Moya, I., 2003. Laser-induced fluorescence signatures as a tool for remote monitoring of water and nitrogen stresses in plants. Can. J. Rem. Sens. 29, 57-65.

Babani, F., Lichtenthaler, H. K., 1996. Light-induced and age-dependent development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO_2 assimilation rates and different kinds of chlorophyll fluorescence ratios. J. Plant Physiol. 148, 555-566.

Baker, N. R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Ann. Rev. Plant Biol. 59, 89-113.

Brown, R. H., Hattersley, P. W., 1989. Leaf anatomy of C_3-C_4 species as related to evolution of C_4 photosynthesis. Plant Physiol. 91, 1543-1550.

Caudle, K. L., Johnson, L. C., Baer, S. G., Maricle, B. R. 2014. A comparison of seasonal foliar chlorophyll change among ecotypes and cultivars of *Andropogon gerardii* (Poaceae) by using nondestructive and destructive methods. Photosynthetica 52, 511-518.

Chory, J., Chatterjee, M., Cook, R. K., Elich, T., Fankhauser, C., Li, J., Nagpal, P., Neff, M., Pepper, A., Poole, D., Reed, J., Vitart, V., 1996. From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc. Nat. Acad. Sci. 93, 12066-12071.

Cortleven, A., Marg, I., Yamburenko, M. V., Schlicke, H., Hill, K., Grimm, B., Schaller,

G. E., Schmülling, T., 2016. Cytokinin regulates the etioplast-chloroplast transition through the two-component signaling system and activation of chloroplast-related genes. Plant Physiol. 172, 464-478.

Croxdale, J. G., Omasa, K., 1990. Chlorophyll *a* fluorescence and carbon assimilation in developing leaves of light-grown cucumber. Plant Physiol. 93, 1078-1082.

Edwards, G., Walker, D. A., 1983. C_3, C_4: Mechanisms, and Cellular and Environmental Regulation, of Photosynthesis. University of California Press Berkeley.

Ehleringer, J., Bjorkman, O. 1977. Quantum yields for CO_2 uptake in C_3 and C_4 plants: Dependence on temperature, CO_2, and O_2 concentration. Plant Physiol. 59, 86-90.

Ehleringer, J. R., Cerling, T. E., Helliker, B. R. 1997. C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112, 285-299.

Fankhauser, C., Chory, J., 1997. Light control of plant development. Ann. Rev. Cell Devel. Biol. 13, 203-229.

Gabruk, M., Mysliwa-Kurdziel, B., 2015. Light-dependent protochlorophyllide oxidoreductase: Phylogeny, regulation, and catalytic properties. Biochemistry 54, 5255-5262.

Gunning, B. E. S., 1965. The greening process in plastids. Protoplasma 60, 111-130.

Guo, H. X., Liu, W. Q., Shi, Y. C., 2006. Effects of different nitrogen forms on photosynthetic rate and the chlorophyll fluorescence induction kinetics of flue-cured tobacco. Photosynthetica 44, 140-142.

Haxeltine, A., Prentice, I. C., 1996. A general model for the light-use efficiency of

primary production. Funct. Ecol. 10, 551.

Johnson, L., Olsen, J., Tetreault, H., DeLaCruz, A., Bryant, J., Morgan, T., Knapp, M., Bello, N., Baer, S. and Maricle, B. (2015). Intraspecific variation of a dominant grass and local adaptation in reciprocal garden communities along a US Great Plains' precipitation gradient: implications for grassland restoration with climate change. Evol. Appl. 8, 705-723.

Jucknischke, A., Kutschera, U., 1998. The role of the cotyledons and primary leaves during seedling establishment in sunflower. J. Plant Physiol. 153, 700-705.

Kami, C., Lorrain, S., Hornitschek, P., Fankhauser, C., 2010. Light-regulated plant growth and development. Curr. Top. Devel. Biol. 91, 30-58.

Kull, O., Kruijt, B., 1998. Leaf photosynthetic light response: a mechanistic model for scaling photosynthesis to leaves and canopies. Funct. Ecol. 12, 767-777.

McCree, K. J., 1981. Photosynthetically active radiation. In O. L. Lange, P. S. Nobel, C. B. Osmond, H. Ziegler. (Eds.), Physiological Plant Ecology I: Responses to the Physical Environment. 41-53. Berlin, Springer-Verlag.

Malkin, R., Niyogi, K., 2000. Photosynthesis. In B. B. Buchanan, W. Gruissem, R. L. Jones. (Eds.), Biochemistry and Molecular Biology of Plants. Am. Soc. Plant Physiol., Rockville, MD, 568-628.

Maxwell, K., Johnson, G. N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51, 659-668.

Meng, Q., Zhou, Q., Zheng, S., Gao, Y., 2012. Responses on photosynthesis and variable chlorophyll fluorescence of *Fragaria ananassa* under sound wave. Energy Proc. 16, 346-352.

Muller, P., Li, X., Niyogi, K. K., 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125, 1558-1566.

Nelson, T., Langdale, J. A., 1989. Patterns of leaf development in C_4 plants. Plant Cell 1, 3-13.

Oberhuber, W., Edwards, G. E., 1993. Temperature dependence of the linkage of quantum yield of photosystem II to CO_2 fixation in C_4 and C_3 plants. Plant Physiol. 101, 507-512.

Reinbothe, C., Lebedev, N., Reinbothe, S., 1999. A protochlorophyllide light-harvesting complex involved in de-etiolation of higher plants. Nature 397, 80-84.

Roiloa, S. R., Retuerto, R., 2006. Development, photosynthetic activity and habitat selection of the clonal plant *Fragaria vesca* growing in copper-polluted soil. Funct. Plant Biol. 33, 961-971.

Sage, R. F., 2003. The evolution of C_4 photosynthesis. New Phytol. 161, 341-365.

Sakuraba, Y., Rahman, M. L., Cho, S., Kim, Y., Koh, H., Yoo, S., Paek, N. 2013. The rice faded green leaf locus encodes protochlorophyllide oxidoreductase B and is essential for chlorophyll synthesis under high light conditions. Plant J. 74, 122-133.

Shaver, J. M., Oldenburg, D. J., Bendich, A. J., 2008. The structure of chloroplast DNA molecules and the effects of light on the amount of chloroplast DNA during development in *Medicago truncatula*. Plant Physiol. 146, 1064-1074.

Sheng, M., Tang, M., Chen, H., Yang, B., Zhang, F., Huang, Y., 2008. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza 18, 287-296.

Smillie, R. M., Nott, R., 1982. Salt tolerance in crop plants monitored by chlorophyll fluorescence in vivo. Plant Physiol. 70, 1049-1054.

Smith, J. H. C., 1954. The development of chlorophyll and oxygen-evolving power in etiolated barley leaves when illuminated. Plant Physiol. 29, 143-148.

Sofo, A., Dichio, B., Montanaro, G., Xiloyannis, C., 2010. Photosynthetic performance and light response of two olive cultivars under different water and light regimes. Photosynthetica 47, 602-608.

Swain, D. K., Sandip, S. J., 2010. Development of SPAD values of medium- and long-duration rice variety for site-specific nitrogen management. J. Agron. 9, 38-44.

Tarakhovskaya, E. R., Kang, E. J., Kim, K. Y., Garbary, D. J., 2013. Influence of phytohormones on morphology and chlorophyll a fluorescence parameters in embryos of *Fucus vesiculosus* L. (Phaeophyceae). Russ. J. Plant Physiol. 60, 176-183.

Taylor, S. H., Ripley, B. S., Woodward, F. I., Osborne, C. P., 2011. Drought limitation of photosynthesis differs between C_3 and C_4 grass species in a comparative experiment. Plant Cell Environ. 34, 65-75.

Thwe, A.A., Vercambre, G., Gautier, H., Gay, F., Phattaralerphong, J., Kasemsap, P., 2014. Response of photosynthesis and chlorophyll fluorescence to acute ozone stress in tomato (*Solanum lycopersicum* Mill.). Photosynthetica 52, 105-116.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., Pleijel, H., 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 91, 37-46.

von Wettstein, D., Gough, S., Kannangara, C. G., 1995. Chlorophyll biosynthesis. Plant Cell 7, 1039-1057.

Wellburn, F. A. M., Wellburn, A. R., 1971. Developmental changes occurring in isolated intact etioplasts. J. Cell Sci. 9, 271-287.

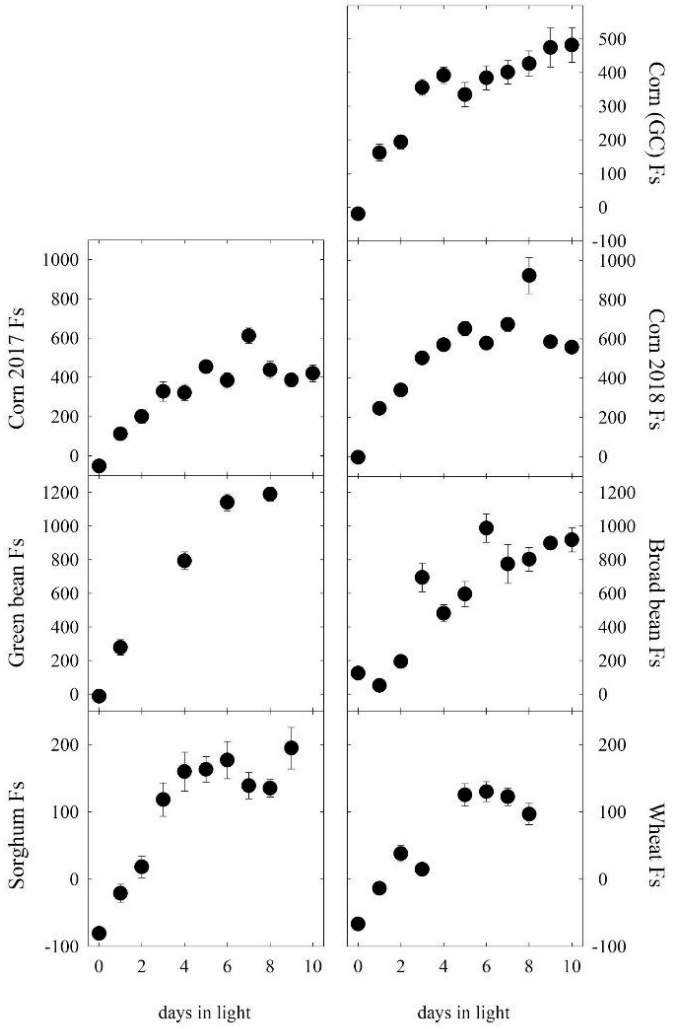

Figure 1: Daily steady-state fluorescence (Fs) measurements (unitless) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F_{(6, 639)} = 284.02$, $P < 0.001$, time: $F_{(10, 639)} = 166.99$, $P < 0.001$, species*time: $F_{(50, 639)} = 13.49$, $P < 0.001$.

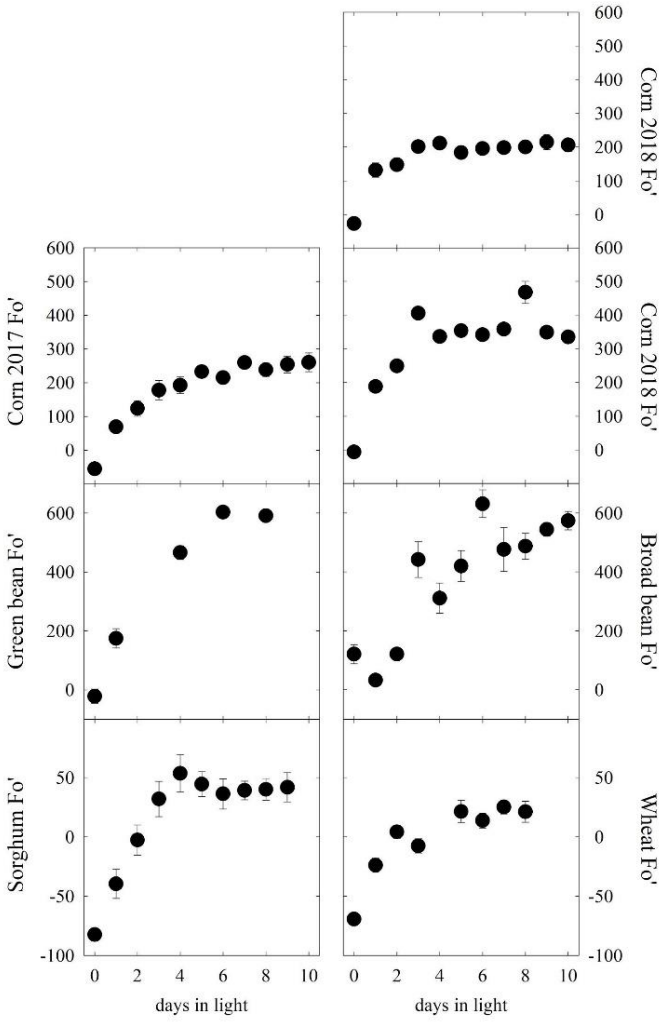

Figure 2: Daily minimum lighted fluorescence (Fo′) measurements (unitless) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Wheat and sorghum had significantly lower values of steady-state fluorescence than other plants. Repeated measures ANOVA within subject test results, species: $F_{(6, 639)} = 411.75$, $P < 0.001$, time: $F_{(10, 639)} = 142.77$, $P < 0.001$, species*time: $F_{(50, 639)} = 14.47$, $P < 0.001$.

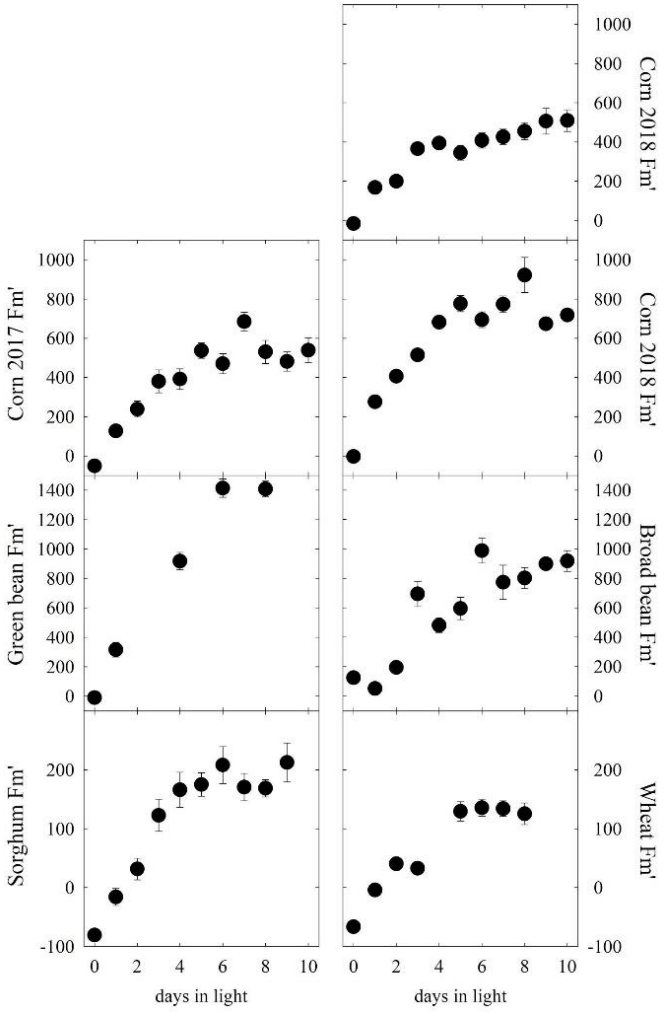

Figure 3: Daily maximum lighted fluorescence (Fm′) measurements (unitless) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F_{(6, 639)} = 310.2$, $P < 0.001$, time: $F_{(10, 639)} = 178.5$, $P < 0.001$, species*time: $F_{(50, 639)} = 14.0$, $P < 0.001$.

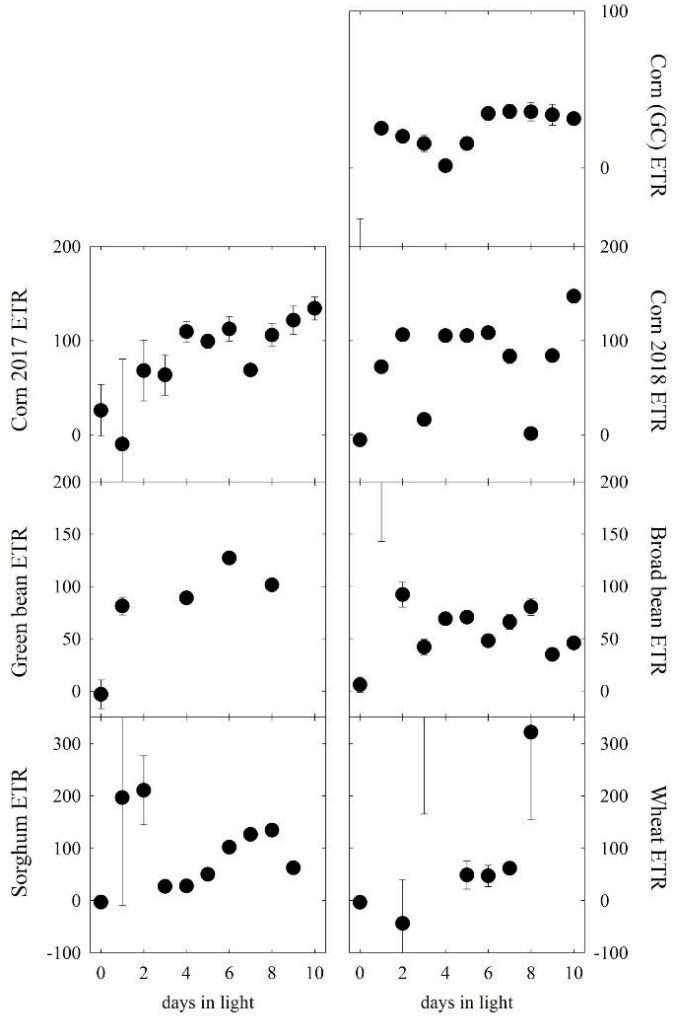

Figure 4: Electron transport rate (ETR) measurements (μmol electrons m^{-2} s^{-1}) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species*time: $F_{(50, 639)} = 2.62$, $P < 0.001$.

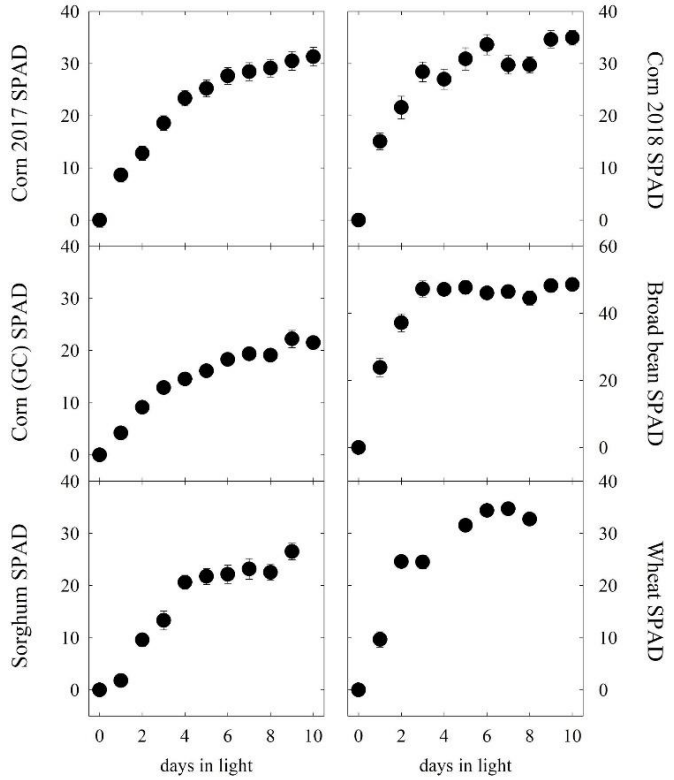

Figure 5: Daily chlorophyll concentration (SPAD) measurements (unitless) on leaves of etiolated plants following 0 days to 10 days of light exposure. Six sets of plants were measured, representing four species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F_{(5, 529)} = 406.89$, $P < 0.001$, time: $F_{(9, 529)} = 132.83$, $P < 0.001$, species*time: $F_{(41, 529)} = 2.55$, $P < 0.001$.

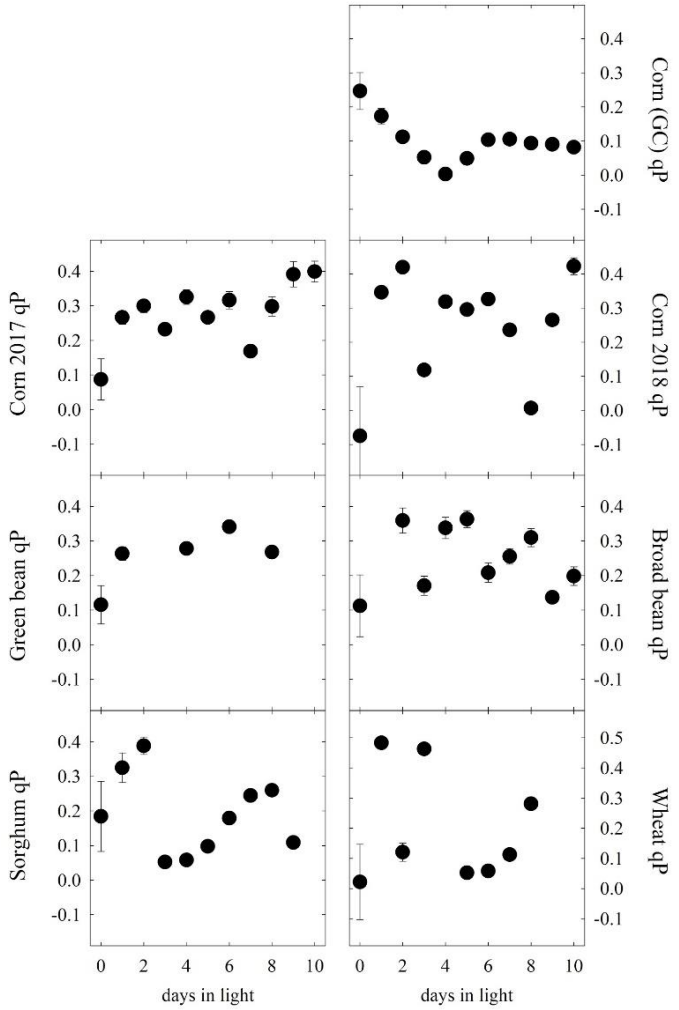

Figure 6: Photochemical quenching (qP) measurements (unitless) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F_{(6, 639)} = 28.04$, $P < 0.001$, time: $F_{(10, 639)} = 20.49$, $P < 0.001$, species*time: $F_{(50, 639)} = 9.93$, $P < 0.001$.

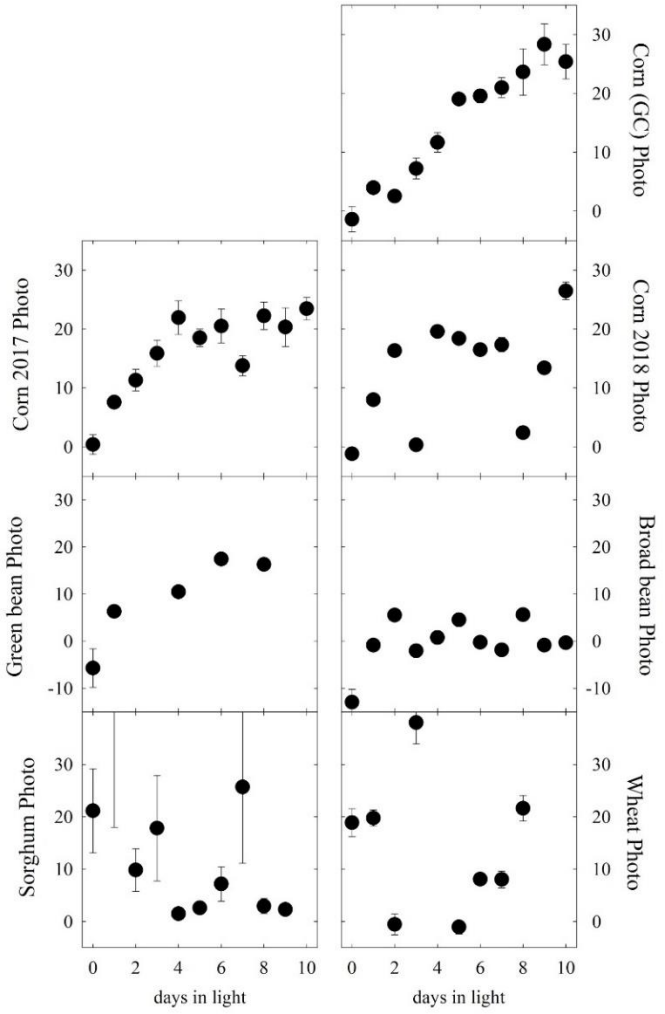

Figure 7: Daily photosynthetic carbon assimilation (Photo) measurements (μmol CO_2 m^{-2} s^{-1}) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F_{(6, 639)} = 25.30$, $P < 0.001$, time: $F_{(10, 639)} = 11.18$, $P < 0.001$, species*time: $F_{(50, 639)} = 7.29$, $P < 0.001$.

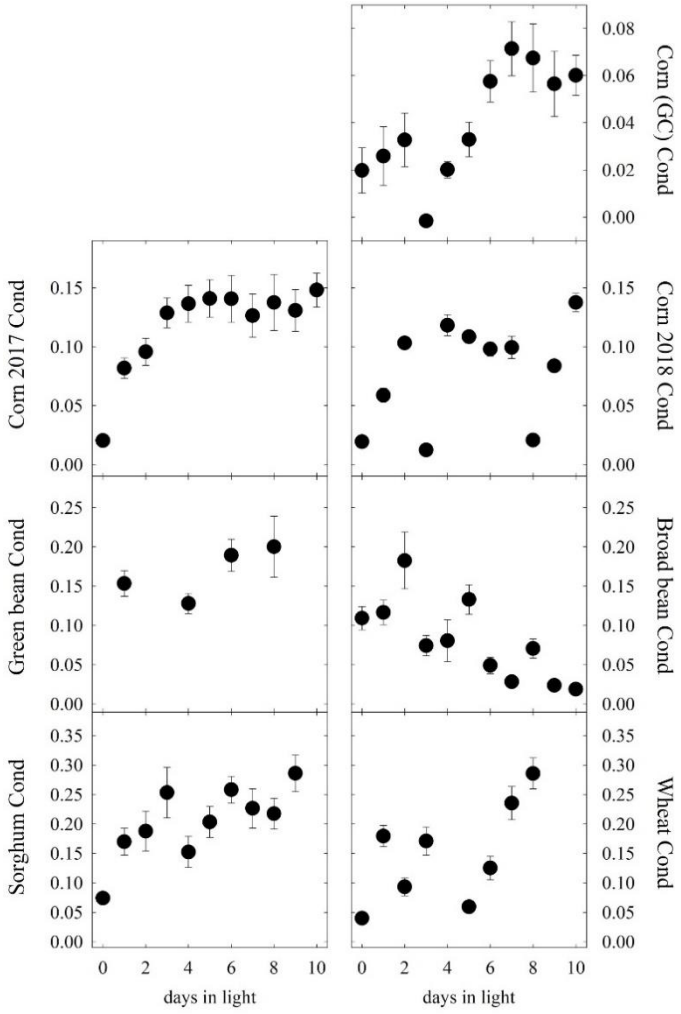

Figure 8: Stomatal conductance (Cond) measurements (mol H_2O m^{-2} s^{-1}) on leaves of etiolated plants following 0 days to 10 days of light exposure. Seven sets of plants were measured, representing five species. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F_{(6, 639)} = 100.91$, $P < 0.001$, time: $F_{(10, 639)} = 5.78$, $P < 0.001$, species*time: $F_{(50, 639)} = 10.79$, $P < 0.001$.

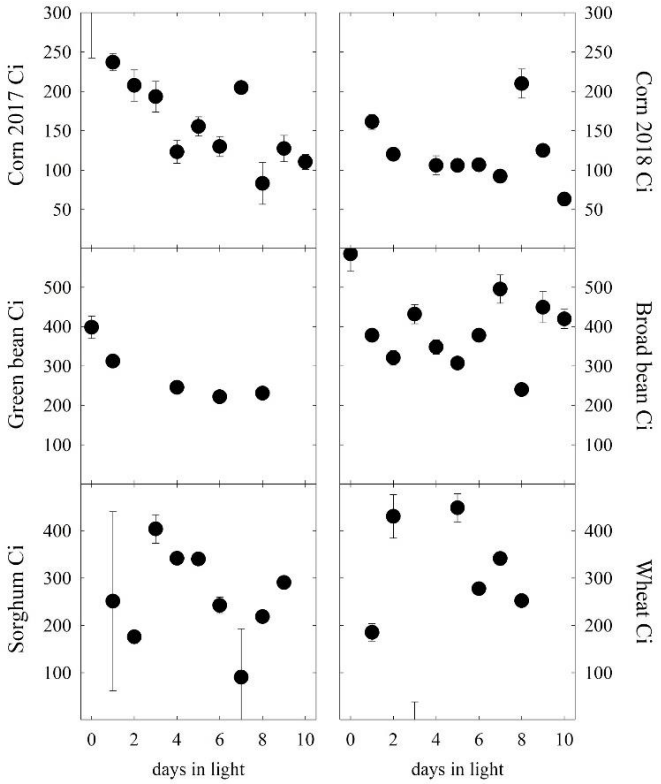

Figure 9: Internal CO2 concentration (Ci) measurements (ppm) on leaves of etiolated plants following 0 days to 10 days of light exposure. Six sets of plants were measured, representing five species. Ci for growth chamber corn was not used in calculating the results as fluctuations in recorded data were too great. Points are means of 8 to 14 replicates ± SE. Repeated measures ANOVA within subject test results, species: $F(5, 521) = 26.69$, $P < 0.001$, time was marginally significant: $F(10, 521) = 1.62$, $P = 0.098$, species*time: $F(40, 521) = 11.23$, $P < 0.001$.

Species	SPAD	Fs	Fo'	Fm'	qP	ETR	Photo	C_i	Cond
Corn 2017	+	+	+	+	+	+	+	-	+
Corn 2018	+	+	+	+	+	+	+	-	+
(GC) Corn	+	+	+	+	-	+	+		+
Sorghum	+	+	+	+	+	+	-	-	+
Wheat	+	+	+	+	+	+	0	+	+
Green bean	+	+	+	+	+	+	+	-	+
Broad bean	+	+	+	+	+	+	+	0	-

Table 1: Increase (+), decrease (-), or neutral (0) effect of light on development of dependent variables, SPAD, Fs, Fo′, Fm′, qP, ETR, Photo, and C_i for each species. Seven sets of plants were measured, representing five species.

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