NCERA-101 Station Report from Georgia, 2020

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1. New Facilities and Equipment

We have acquired and build several new imaging system for the detection of plant size and health. The TopView system (Aris, Eindhoven, the Netherlands) takes top-down images of plants under seven different colors of LEDs, including fluorescence images (plants are excited using blue light and a longpass filter in front the of the camera lens assures that the camera only captures fluorescence coming from chlorophyll. Data collection is quick and easy and allows for subsequent multi-spectral image analysis (not so quick and easy).

We have since designed and assembled three scaled down systems to only capture fluorescence images. This method allows for rapid and quick assessment of canopy size and can detect certain stresses that affect photosystem II well before any visible symptoms are present. A recording of how this works can be found at https://ashs.confex.com/ashs/2019/meetingapp.cgi/Paper/30905.

2. Unique Plant Responses

Chlorophyll Fluorescence Imaging: A Novel, Simple and Non-Destructive Method for Canopy Size Imaging

Non-destructive methods to quantify crop growth can provide a valuable tool in both research and production settings. Quantifying canopy size can be done using a variety of imaging techniques, with regular color (red/green/blue, RGB) imaging being the most common approach. However, separating canopy from background is not always easy using RGB imaging and different methods may be needed depending on the background in the image or the color of the leaves. To circumvent this issue, we developed an imaging approach that takes advantage to the fluorescence emitted by chlorophyll. The energy of about 1 to 3% of photons absorbed by leaves is re-emitted as photons in the range of ~690 to 740 nm. This fluorescence coming from plants is easy to photograph: plants are exposed to blue light and images are taken using a monochrome camera with a 680 nm long-pass filter (i.e., only photons with wavelengths > 680 nm can pass through the filter). This assures that the camera can only detect fluorescence from chlorophyll. One complication is that the chlorophyll in algae fluoresces similar to that in plants, so image processing may be needed to separate algae from leaves. This can be achieved by comparing images collected under both blue and white light: algae are more pronounced under blue than under white light. Alternatively, algicides have proven effective in suppressing algae without harmful effects on plants. Comparisons of leaf area measurements using the fluorescence imaging versus a leaf area meter indicate that the fluorescence imaging is almost perfectly correlated with standard leaf area measurements (R^2 = 0.998). Chlorophyll fluorescence imaging can also be used to monitor ripening of fruits that contain chlorophyll in their unripe state. The decrease in fruit chlorophyll levels during ripening is easily quantified using this approach. The hardware costs for a chlorophyll imaging system are ~$1,000 and the system is easy to assemble. *Researchers: Mangalam Narayanan, Marc van Iersel, Mark Haidekker.*

Light Intensity Affects Leaf-Level and Crop-Level Water Use Efficiency

The cost of dehumidification is a significant portion of the total production costs in indoor production systems. Minimizing this cost can be achieved by maximizing the water use efficiency of the plants, thus reducing the need for dehumidification. This study was performed to determine leaf- and crop-level water use efficiency of vegetative and flowering crops under various photosynthetic photon flux densities (*PPFD*). 'Purple Wave Classic' petunia and 'Green Salad Bowl' lettuce were grown in a walk-in growth chamber, under *PPFD*s ranging from 152 - 374 $\mu mol \cdot m^{-2} \cdot s^{-1}$, provided by white LED lighting. To achieve the same daily light integral (DLI) of 12 $mol \cdot m^{-2} \cdot d^{-1}$, photoperiods ranged from 21.6 to 9 h in the different treatments. The temperature in the growth chamber was 24 °C and CO_2 was maintained at 800 ppm. Leaf-level assimilation increased with increasing *PPFD* in petunias and lettuce. However, in petunias transpiration decreased with increasing *PPFD*, whereas in lettuce it increased. This led to an increase in leaf-level water use efficiency in petunias with increasing *PPFD*, whereas in lettuce, there was no correlation between water use efficiency and *PPFD.* For both lettuce and petunia, dry weight decreased with higher *PPFD*s provided over shorter photoperiods. Petunia biomass was 57.0% higher at 152 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than at 374 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and lettuce biomass was 33.9% higher at 152 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than at 374 $\mu mol \cdot m^{-2} \cdot s^{-1}$, when plants were given the same DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$. In petunia, dry weight decreased more strongly with increasing *PPFD* than water use, and thus crop-level water use efficiency decreased with increasing *PPFD* ($p < 0.001$). For lettuce, crop-level water use efficiency also decreased with increasing *PPFD* ($p < 0.001$). In conclusion, leaf-level measurements and crop-level measurements of water use efficiency did not show the same trends; leaf level measurement may thus provide misleading information. Crop-level measurements of plants grown under varying *PPFD*, but with the same DLI showed that lower light intensities and longer photoperiods resulted in higher yields and higher water use efficiency in both lettuce and petunias. *Researchers: Laura Reese and Marc van Iersel.*

Supplemental Far-Red Light Increases Final Yield of Indoor Lettuce Production By Boosting Light Interception at the Seedling Stage

Understanding crop responses to light spectrum is critical for optimal indoor crop production. Far-red light is of special interest, because it can accelerate crop growth both physiologically and morphologically. Far-red can increase photosynthetic efficiency when combined with lights of shorter wavelength. It also can induce leaf expansion, possibly increasing light capture and growth. However, the optimal amount of supplemental far-red light for crop growth and yield in indoor lettuce production is yet to be quantified. Lettuce 'Cherokee', 'Green Salad Bowl', and 'Little Gem' were grown under 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ warm white LED light with 16 levels of additional far-red light, ranging from 0 to 76 μmol·m^{-2}·s^{-1}. Supplemental far-red light increased canopy light interception (a measure of canopy size) 6 days after far-red light treatment for 'Green Salad Bowl' and 'Little Gem' and after 8 days for 'Cherokee'. The enhancement in canopy size was no longer evident after 12 and 16 days of far-red treatment for 'Green Salad Bowl' and 'Little Gem', respectively. The length of the longest leaf of all three cultivars was increased linearly by far-red light, consistent with a shade acclimation response to far-red light. Final dry weight of 'Cherokee' and 'Little Gem' were increased linearly by far-red light when harvested 20 days after the start of far-red light treatment, but dry weight of 'Green Salad Bowl' was not affected. In conclusion, adding far-red light in indoor production gives lettuce seedlings a jumpstart at capturing light. Supplemental far-red light increases crop yield linearly up to 76 μmol·m^{-2}·s^{-1} in two of the three cultivars tested. *Researchers: Jun Liu and Marc van Iersel.*

The Quantum Requirement for CO_2 Assimilation Increases with Increasing Photosynthetic Photon Flux Density and Leaf Anthocyanin Concentration in Lettuce

The quantum requirement for CO_2 fixation, or moles of photons required to fix one mole of CO_2, determines how efficiently plants can use light to produce carbohydrates. It is calculated as the amount of absorbed light (photosynthetic photon flux density (*PPFD*) × leaf absorptance) divided by gross photosynthesis. Due to the high lighting costs in controlled environment agriculture, a low quantum requirement may increase growth and profitability. Typical estimates of the quantum requirement (~10-12 mol·mol^{-1}) are based on the initial slope of photosynthesis-light response curves and do not account for non-photosynthetic pigments or changes due to light intensity. Anthocyanins, typically located in epidermal cells, are not photosynthetically active and light absorbed or reflected by them cannot be used for CO_2 assimilation. Since anthocyanins reduce how much light reaches photosynthetic pigments, anthocyanin-rich lettuce cultivars may have a greater quantum requirement than green cultivars. Additionally, photosynthetic light-use-efficiency decreases with increasing *PPFD*. We hypothesized that both higher anthocyanin levels in lettuce and increasing *PPFD* would increase the quantum requirement and quantified this using six red and three green lettuce cultivars, having a wide range of anthocyanin concentrations. Lettuce was grown in a greenhouse without supplemental lighting. The environmental conditions were a temperature of 25.2 ± 3.2 °C, a vapor pressure deficit of 1.0 ± 0.5 kPa, and a daily light integral of 24.2 ± 6.3 mol·m^{-2}·d^{-1} (mean ± SD). Leaf-level photosynthesis was measured at *PPFD*s of 0, 50, 100, 200, 400, 700, 1000, and 1500 μmol·m^{-2}·s^{-1}. An integrating sphere was used to measure leaf absorptance. Anthocyanin concentration of the lettuces ranged from 12 to 71 mg·m^{-2}. Absorptance increased linearly from 0.77 to 0.87 with increasing anthocyanin levels (R^2 = 0.72, P < 0.001). Gross photosynthesis at a *PPFD* of 1500 μmol·m^{-2}·s^{-1} was ~50% lower in leaves with the highest anthocyanin level (8.1 μmol·m^{-2}·s^{-1}) than that of those with the lowest anthocyanin level (16.2 μmol·m^{-2}·s^{-1}) (R^2 = 0.32, P = 0.004). The quantum requirement for CO_2 assimilation at a *PPFD* of 1500 μmol·m^{-2}·s^{-1} increased from 80 to 150 mol·mol^{-1} as the anthocyanin concentration increased (R^2 = 0.32, P = 0.003). With *PPFD* increasing from 200 to 1500 μmol·m^{-2}·s^{-1}, the quantum requirement increased from 30 to 110 mol·mol^{-1} (R^2 = 0.63, P < 0.001). In summary, both anthocyanins and high *PPFD* increased the quantum requirement for CO_2 assimilation to levels far above those typically cited in the literature. *Researchers: Changhyeon Kim and Marc van Iersel.*

Only Extreme Fluctuations in Lights Levels Reduce Lettuce Growth

The cost of providing supplemental lighting in greenhouses or sole-source lighting in plant factories can be high. In the case of variable electricity prices, it may be desirable to provide most of the light when electricity prices are relatively low. However, it is not clear how plants respond to the resulting fluctuating light levels. We hypothesized that plants that receive a constant photosynthetic photon flux density (*PPFD*) would produce the more biomass than those grown under fluctuating light levels. To quantify growth reductions caused by fluctuating light levels. We quantified the effects of fluctuating *PPFD* on the photosynthetic physiology and growth of 'Little Gem' and 'Green Salad Bowl' lettuce. Plants were grown in a walk-in growth chamber outfitted with three shelving units, each divided into six growing compartments. Each compartment contained two dimmable, white LED bars, programmed to alternate between high and low *PPFD*s every 15 minute. The *PPFD*s in the different treatments were ~ 400/0, 360/40, 320/80, 280/120, 240/160, and 200/200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a photoperiod of 16 hours and a DLI of ~11.5 $mol \cdot m^{-2} \cdot d^{-1}$ in all treatments. CO_2 was maintained at ~ 800 $\mu mol \cdot mol^{-1}$. Data was analyzed using linear and non-linear regression. At 400/0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 30-minute-integrated A_n (net photosynthesis integrated 15 minute at high and 15 minute at low *PPFD*) was ~65% lower than at a *PPFD* of 320/80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (or treatments with smaller *PPFD* fluctuations). 30-minute-integrated A_n in the four treatments with the smallest *PPFD* fluctuations (320/80 to 200/200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was similar. Plants grown at 400/0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ also had fewer leaves and lower chlorophyll content compared to those in all other treatments. The four treatments with the smallest fluctuations in *PPFD* produced plants with similar numbers of leaves, chlorophyll content, specific leaf area, dry mass, and leaf area. Chlorophyll content, 30-minute-integrated A_n, and dry mass were positively correlated with each other. Our results show that lettuce tolerates a wide range of fluctuating *PPFD* without negative effects on growth and development. However, when fluctuations in PPFD are extreme (400/0 or 360/40 $\mu mol \cdot m^{-2} \cdot s^{-1}$), chlorophyll levels are low, which can explain the low 30-minute-integrated A_n and poor growth in these two treatments. The ability of lettuce to tolerate a wide range of fluctuating light levels suggests that it may be possible to adjust the *PPFD* in response to variable pricing. *Researchers: Ruqayah Bhuiyan and Marc van Iersel.*

Chlorophyll Fluorescence Imaging: A Novel, Low-Cost Method for Early Stress Detection

Using non-destructive methods, like chlorophyll fluorescence imaging, to provide early stress detection in plants could augment growing methods and allow for corrective measures to minimize damage to the plants. While many chlorophyll fluorescence imaging techniques require expensive, sophisticated equipment while other techniques only take single-point measurements, the current study focuses on a scalable novel technique that provides whole plant digital images of the chlorophyll fluorescence (but not Φ_{PSII}) using blue excitation light, a monochrome camera, and a long-pass filter (> 690 nm). There are three fates of light once a photon has been absorbed by a plant: it can be used to drive photochemistry (electron transport), be converted to heat, or be reemitted as chlorophyll fluorescence. A decrease in photochemistry by stressors will typically lead to an increase in chlorophyll fluorescence and/or heat dissipation to prevent damage from excess light. Due to this relationship, chlorophyll fluorescence has been used to non-destructively diagnose the photosynthetic performance of plants, with the quantum yield of photosystem II (Φ_{PSII}) being a common indicator of photochemical efficiency. To test the performance of the system, a photosystem II-inhibiting herbicide was applied as a drench at standard field rates to lettuce (*Lactuca sativa*), impatiens (*Impatiens hawkeri*) and vinca (*Catharanthus roseus*). Chlorophyll fluorescence images were taken using the TopView Multispectral Digital Imaging System (Aris, Eindhoven, Netherlands), which also took regular RGB images. The combined reflectance and fluorescence from the leaf were measured using a spectrometer and Φ_{PSII} was measured using a chlorophyll fluorometer. These measurements were taken every 15 minutes for 8 hours. In between measurements, the plants were exposed to a photosynthetic photon flux density of 176 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by white LEDs. The pixel intensity in the fluorescence image, a measure of chlorophyll fluorescence, was negatively correlated with Φ_{PSII} ($P < 0.01$) as measured using a fluorometer. The average reflectance in the spectral range of fluorescence (670 – 760 nm) was positively correlated with the pixel intensity ($P < 0.0001$) and negatively correlated with Φ_{PSII} ($P \leq 0.07$). The results suggest that the novel chlorophyll fluorescence imaging technique is a reliable way to inexpensively detect stress to photosystem II before visible damage occurs to the plant. *Researchers: Reeve Legendre and Marc van Iersel.*

Supplemental Far-Red Light Does Not Increase Growth of Greenhouse-Grown Lettuce

The positive effects of far-red (FR) light on growth of leafy greens have been well-documented for crops grown in plant factories. However, there is a lack of information on the effects of supplemental FR on greenhouse-grown leafy greens. Therefore, we conducted a study with two cultivars of lettuce (*Lactuca sativa*, 'Green Salad Bowl' and 'Cherokee') with five lighting treatments. The treatments were supplemental lighting with a photosynthetic photon flux density (*PPFD*) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light, *PPFD* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ + 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR light, *PPFD* of 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and sunlight only. Supplemental *PPFD* was provided with 75% red and 25% blue light for 4 hours before sunrise and 4 hours after sunset. The daily light integral (DLI) received from the sun averaged 7.5 $mol \cdot m^{-2} \cdot d^{-1}$ during the study period. The treatments with supplemental *PPFD*s of 200 and 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ averaged DLIs of 13.3 and DLI of 13.8 $mol \cdot m^{-2} \cdot d^{-1}$. The FR treatments with 10 and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ received 0.29 and 0.58 $mol \cdot m^{-2} \cdot d^{-1}$ of supplemental FR light. All supplemental lighting treatments increased leaf area and plant dry weight compared to the treatment without supplemental lighting ($P < 0.0001$). However, we did not see any positive effects on crop growth by adding FR light. Similarly, the treatment with slightly higher PPFD level of 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ did not show a significant growth difference compared to the treatment with a supplemental PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Our results do not provide any evidence for positive effects of supplemental FR light on greenhouse-grown lettuce. This may be due to the presence of high levels of FR light from the sun in the greenhouses. *Researchers: T.C. Jayalath and Marc van Iersel.*

Development and Implementation of a New Optimal Supplemental Lighting Control Strategy in Greenhouses

The use of supplemental lighting is an effective way for increasing greenhouse productivity. Recently, using light-emitting diodes (LEDs), capable of precise and quick dimmability, has increased in greenhouses. However, electricity cost of lighting can be significant, and hence, it is necessary to find optimal lighting strategies to minimize supplemental lighting costs. We have modeled supplemental lighting in a greenhouse equipped with LEDs as a constrained optimization problem, and we aim at minimizing electricity costs of artificial lighting. We consider not only plant daily light integral (DLI) need during its photoperiod but also sunlight prediction and variable electricity pricing in our model. We use Markov chain to predict sunlight irradiance throughout the day. By taking sunlight prediction information into account, we avoid supplying more light than plants require. Therefore, our lighting strategy prepares sufficient light for plant growth while minimizing electricity costs during the day. We propose an algorithm to find optimal supplemental lighting strategy and evaluate its performance through exhaustive simulation studies using a whole year data and compare it to a heuristic method, which aims to supply a fixed photosynthetic photon flux density (PPFD) to plants at each time-step during the day. We also implement our proposed lighting strategy on Raspberry Pi using Python programming language. Our prediction-based lighting approach shows (on average) about 40% electricity cost reduction compared to the heuristic method throughout the year. We will test this approach in our research greenhouse in the winter of 2020-2021. *Researchers: Sahand Mosharafian, Shirin Afzali, Javad Mohammadpour Velni, and Marc van Iersel*

3. Accomplishment Summaries

In collaboration with electrical engineers, we have developed optimal control algorithms for supplemental lighting in greenhouses. These algorithms can be used from control of dimmable LED lights, HPS lights with a few discrete power levels, or non-dimmable lights. The algorithm can also predict sun light levels, and accounts for plant physiological responses to light. In the case of variable electricity prices, the algorithms can also minimize the cost of the electricity required for supplemental lighting. Simulations suggest that this may reduce lighting costs by up to 40%. The algorithms have been tested in a small testbed, using a Raspberry Pi for implementation and will soon be trialed on a larger scale in a greenhouse.

4. Impact Statement

Electricity costs for supplemental lighting can be a major cost for greenhouses. By combining plant physiological information, light measurements, and predictive modeling, we have developed optimized lighting control strategies that can reduce the cost of supplemental lighting by up to 40%.

5. Published Written Works

Refereed journal articles

Nemali, K.S. and M.W. van Iersel. 2019. Relating whole-plant photosynthesis to physiological acclimations at leaf and cellular scales under drought stress in bedding plants. *Journal of the American Society for Horticultural Science* 144:201-208. https://doi.org/10.21273/JASHS04665-19.

Kang, S, M.W. van Iersel, and J. Kim. 2019. Plant root growth affects FDR soil moisture sensor calibration. *Scientia Horticulturae* 252:208-211. https://doi.org/10.1016/j.scienta.2019.03.050

Weaver, G.M., M.W. van Iersel, and J. Mohammadpour Velni. 2019. A photochemistry-based method for optimising greenhouse supplemental light intensity. *BioSystems Engineering* 128:123-137. https://doi.org/10.1016/j.biosystemseng.2019.03.008

Weaver, G. and M.W. van Iersel. 2019. Photochemical characterization of greenhouse-grown lettuce (*Lactuca sativa* L. 'Green towers') with applications for supplemental lighting control. *HortScience* 54:317-322. https://doi.org/10.21273/HORTSCI13553-18.

Zhen, S. and M.W. van Iersel. 2019. Far-red light enhances photochemical efficiency in a wavelength-dependent manner. *Physiologia Plantarum* 167:21-33. https://doi.org/10.1111/ppl.12834.