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Nighttime Blue Lighting and Downward Airflow to Manage Tipburn in Indoor Farm Lettuce

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Abstract. **Indoor vertical farms (IFs) have gained popularity in the United States within the last decade. Lettuce is the most frequently produced crop in these systems, but it often suffers from the calcium (Ca) deficiency called tipburn because of poor airflow, which causes low transpiration of young leaves near the meristem. We hypothesized that a low-intensity blue light can open stomata and enhance whole-plant transpiration without increasing the overall plant growth rate. Dim light of blue (B100) or blue/red (B80R20) were applied at a photosynthetic photon flux density of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 'Klee' and 'Rex' lettuce plants during the nighttime for the final 14 days of the 28 days of growth after transplanting. A leaf gas exchange analysis showed that application of B100 lighting alone increased nighttime leaf conductance by more than 50% and transpiration by 25% over nighttime darkness, while the results of B80R20 treatment were not different from those of the B100 or control treatments. However, increased whole-plant transpiration driven by dim nighttime lighting was ineffective for targeting Ca transport to areas of high tipburn risk, and tipburn severity (percentage of leaves with tipburn) was not reduced at harvest (28 days after transplanting). In a second experiment combining B100 lighting or nighttime darkness with downward vertical airflow (0.4 $m \cdot s^{-1}$), tipburn was eliminated regardless of nighttime lighting. Nighttime dim lighting seems to be an ineffective strategy, and downward vertical airflow is likely the most practical and effective tipburn prevention mechanism for IFs.**

Indoor vertical farms (IFs) commonly encounter tipburn of lettuce (*Lactuca sativa*) during the fast-growing phase near harvest. Tipburn manifests as a localized calcium (Ca) deficiency, with visible symptoms appearing as necrosis along the leaf margins near the meristem. This physiological disorder is primarily linked to environmental conditions that suppress transpiration, such as elevated daytime humidity and low air speed, thereby limiting the Ca transport essential for the growth of young leaves (Ahmed et al. 2020; Collier and Tibbitts 1984). The presence of tipburn significantly reduces the marketability of lettuce and often leads to rejection of the entire crop by packing companies even if as little as 5% of the plants are affected (Jenni and Hayes 2010). In some instances, affected leaves can be manually removed to salvage part of the harvest for sale as cut leaf products, although this is labor-intensive and diminishes profitability (Shimamura et al. 2019). Therefore, there is a need for IF-specific strategies that can effectively prevent the occurrence of lettuce tipburn.

Similar to IFs, greenhouse lettuce growers frequently combat tipburn through the use of downward airflow fans, foliar Ca sprays, or slowing the plant growth rate. However, these strategies are not always suitable for IF conditions. Specifically, targeted airflow strategies such as downward vertical air have been demonstrated to effectively reduce tipburn in lettuce by reducing the boundary layer thickness over susceptible young leaves, thereby enhancing transpiration and supporting Ca transport to expanding tissues (Frantz et al. 2004; Goto and Takakura 1992a). This directed airflow method is commonly used in greenhouses with ceiling fans positioned to prevent tipburn (Brechner and Both 1996). However, implementing such strategies in vertically stacked cultivation systems presents challenges caused by the limited headspace above the crop.

Foliar applications of Ca chloride ($CaCl_2$) are effective for distributing Ca to expanding leaves, thus averting the tipburn risk (Samarakoon et al. 2020). Yet, in IFs, this approach is less favorable because of the risks associated with spraying water and salts on electrical equipment and structures, which may shorten their lifespan (Tan and Singh 2014). Moreover, the practice of wetting plants can inhibit gas exchange and may promote disease (Ishibashi and Terashima 1995), while additional aerial humidity following sprays increases the load on and energy cost of heating, ventilation, and air conditioning (HVAC) systems in IFs (Ahamed et al. 2023).

Other approaches to avoiding tipburn involve slowing the plant growth rate by limiting light intensity, reducing the CO_2 concentration, or lowering air temperature. While effective at mitigating tipburn risk, such strategies can adversely affect the annual productivity of an IF by limiting biomass production and the number of cropping cycles (Both et al. 1997).

One unique tipburn solution includes the application of high nighttime relative humidity (RH; <95%) via aerial humidification or small plastic covers placed on the meristem and young inner leaves. With this method, high RH under the plastic covers or whole canopy can increase hydraulic pressure of the xylem, promoting guttation of the tipburn-susceptible tissues and increasing Ca transport (Frantz et al. 2004; Palzkill and Tibbitts 1977; Palzkill et al. 1976). While effective for strawberries (Kroggel and Kubota 2017) and lettuce (Vanhassel et al. 2015), maintaining a near-saturation humidity environment makes crops susceptible to the same foliar disease issues as those associated with foliar $CaCl_2$ applications. Ultimately, increasing mass flow through transpiration enhancement or restriction can increase Ca transport within plants, thus mitigating the risk of Ca deficiency disorders.

Given these constraints, there is a strong demand for better alternative tipburn mitigation strategies that can be incorporated into IF systems. Based on our understanding that tipburn is a result of unbalanced transpiration and growth (Sago 2016), our study explored the potential of using dim nighttime lighting to increase transpiration and, consequently, Ca transport during periods when plant growth (biomass production) is limited. We also hypothesized that the transpiration rate can be increased by optimizing the light quality of nighttime lighting. Blue light is known to open stomata in many species even at relatively low photon flux densities, thus enhancing stomatal conductance for transpiration (Inoue and Kinoshita 2017). The mechanism involves the excitation of phototropins and cryptochromes in the guard cells, thereby triggering a cascade of reactions that lead to the hyperpolarization of the plasma membrane and the opening of potassium channels, thus swelling the guard cells and opening the stomata (Matthews et al. 2020; Shimazaki et al. 2007). This process is enhanced by the presence of red light in some species, which stimulates photosynthesis in the guard cells' chloroplasts, thus producing sucrose that, in turn, increases turgor pressure and widens the stomatal aperture (Assmann 1988; Matthews et al. 2020). In these studies, stomatal opening was enhanced by treatments comprising red light at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and blue light additions between 5 and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

In this study, we selected a dim nighttime lighting strategy (photon flux below the plant light compensation point) with the primary objective of determining whether increased

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stomatal opening and transpiration rates during the night could enhance the Ca supply to expanding leaf and meristematic tissues, thereby reducing tipburn severity. In the first of two experiments, we compared either nighttime blue light or a mix of blue and red light to determine if light quality influenced stomatal opening and transpiration as a secondary objective. In the second experiment, we compared a blue nighttime lighting strategy with the known practice of vertical downward airflow to prevent tipburn.

Materials and Methods

Plant materials and seedling growing conditions. Two commercial lettuce cultivars, Klee and Rex (Rijk Zwaan, BV, the Netherlands), were selected for the experiments. According to our previous studies (Ertle and Kubota 2023), 'Klee', a red leaf-type lettuce, and 'Rex', a green butterhead-type lettuce, exhibited high and moderate sensitivity to tipburn under conditions promoting tipburn, respectively. Seeds were germinated in rockwool cubes (3.8 × 3.8 cm; Grodan Inc., Kingsville, ON, Canada) for 2 d at 20 °C in a growth chamber (E15; Conviron, Winnipeg, MB, Canada) continuously lit at a photosynthetic photon flux density (PPFD) of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After germination, seedlings were grown for an additional 12 d in the same chamber under a 16-h photoperiod at day and night temperatures of 23 °C and 19 °C, respectively. Lighting was provided by white, fluorescent lamps (F72T12-CW-HO, 85W; GE, Boston, MA, USA) at a PPFD of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and plants were fertigated daily with a half-strength version of a nutrient solution.

Growth chamber setup and nutrient management. The 14-d old seedlings were transplanted to grow an additional 28 d in two identical walk-in growth chambers located in Columbus, OH, USA, each with a floor area of 2.74 × 3.32 m and a growing area of 2.83 m^2 (Conviron, Winnipeg, ON, Canada). The chambers were equipped with four wire-rack carts, with each hosting four nutrient film technique (NFT) channels [9.5 (width) × 4.0 (height) × 123.0 (length) cm] made from food-grade polyvinyl chloride (PVC) (CropKing, Lodi, OH, USA). Each channel had six (2.5 × 2.5 cm) square holes to accommodate the plants.

During the initial week after transplanting, the NFT channels were kept filled with nutrient solution to maximize root contact. To maintain sufficient oxygen levels (>5 $mg \cdot L^{-1}$), the solution was drained and replenished daily. After the first week, a continuous flow was established to create standard NFT hydroponic conditions. The full-strength nutrient solution, which was specifically formulated for leafy green hydroponics (M. Jensen, unpublished), contained (in $mg \cdot L^{-1}$) 185 total nitrogen (N) (178 NO_3-N, 7 NH_4-N), 46 phosphorus (P), 149 potassium (K), 188 Ca, 46 magnesium (Mg), 64 sulfur (S), 91 chloride (Cl), and micronutrients. During the seedling growth phase, this solution was reduced to half-strength. The solution's pH and electrical conductivity (EC) were maintained at 6.0 ± 0.5 and 2.0 ± 0.2 $dS \cdot m^{-1}$, respectively, with complete replacements occurring weekly.

Table 1. Growth chamber environmental averages ± standard deviation (after transplanting). Daytime setpoints were 23 °C, 75% relative humidity, 0.87 kPa vapor pressure deficit, and a CO_2 concentration of 1000 $\mu mol \cdot mol^{-1}$. Nighttime setpoints were 19 °C with venting (no setpoints) to reduce humidity and CO_2 concentration.

Expt.	Rep[i]	Chamber	Photoperiod[ii]	Temp °C[iii]	Relative humidity %	VPD[iv] kPa	CO_2 $\mu mol \cdot mol^{-1}$
1	1	1	D	23.5 ± 0.6	71.9 ± 14.0	0.81 ± 0.40	1014 ± 144
1	1	1	N	18.9 ± 0.7	68.7 ± 12.7	0.73 ± 0.29	—
1	1	2	D	23.6 ± 0.8	72.6 ± 12.6	0.80 ± 0.35	998 ± 140
1	1	2	N	19.1 ± 1.0	56.0 ± 15.0	1.03 ± 0.36	—
1	2	1	D	23.2 ± 0.6	75.0 ± 12.6	0.71 ± 0.31	1052 ± 120
1	2	1	N	19.1 ± 0.9	68.6 ± 12.1	0.68 ± 0.20	—
1	2	2	D	22.7 ± 1.8	73.4 ± 13.6	0.71 ± 0.26	1046 ± 117
1	2	2	N	19.4 ± 1.8	72.0 ± 13.3	0.61 ± 0.20	—
2	1	1	D	23.2 ± 0.7	76.2 ± 10.0	0.72 ± 0.29	1014 ± 140
2	1	1	N	19.2 ± 0.8	62.6 ± 10.2	0.88 ± 0.24	—
2	1	2	D	22.8 ± 0.9	69.2 ± 10.1	0.81 ± 0.29	935 ± 142
2	1	2	N	19.2 ± 1.6	63.3 ± 9.2	0.74 ± 0.24	—

[i] Rep = experimental replication.
[ii] D = daytime; N = nighttime.
[iii] Average temperature of all four carts within each chamber and pooled standard deviations.
[iv] VPD = vapor pressure deficit (air temperature-based).

A sensor used for controlling the air temperature, relative humidity, and CO_2 concentration (EE872; E+E Elektronik, Engerwitzdorf, Austria) was placed in an aspirated shield positioned 1.4 m above the floor in the center of the chamber. Additionally, a CR1000X datalogger (Campbell-Scientific, Logan, UT, USA) recorded canopy-level air temperature at the center of each cart using calibrated T-type thermocouples (gauge: 36). Air temperature and relative humidity were also measured with an aspirated HMP-60 probe (Vaisala; Vantaa, Helsinki, Finland) located centrally between the carts opposite the growth chamber door. Relative humidity was maintained in the chambers using an ultrasonic humidifier (Optimus U-310002; Anaheim, CA, USA). Additional heaters (3757105; Utilitech, Cincinnati, OH, USA) were added to achieve lower relative humidity while maintaining the same air temperature (Expt. 2 only). Air speeds, both horizontal and vertical, averaged 0.19 ± 0.18 $m \cdot s^{-1}$ and 0.07 ± 0.02 $m \cdot s^{-1}$ respectively, across the growing area in both chambers (192 points measured before and after each experimental replication using a hotwire anemometer; A004, Kanomax, Osaka, Japan). The environmental conditions were selected so that they would limit plant transpiration rates while achieving a high growth rate. Actual growth chamber aerial environmental conditions are summarized in Table 1. To assess the aerial environment affecting in situ plant transpiration and water evaporation rates under the chamber conditions, methodology involving both petri dishes and deep-dish tools developed by Papio (2021) was used.

Lighting was adjusted using light-emitting diode (LED) light-bar modules (GPL PM 168 DRBWFR L120 G3.0 C4 NA; Phillips Signify, NB, the Netherlands) mounted 50 cm above the NFT channels. The daytime light spectrum consisted of 19% blue (400 to 499 nm), 10% green (500 to 599 nm), 71% red (600 to 699 nm), and a small amount of far-red (700 to 750 nm) (Fig. 1C), as well as a daily light integral (DLI) of 15.6 ± 2.7 $mol \cdot m^{-2} \cdot d^{-1}$ (16 $h \cdot d^{-1}$). Nighttime treatments varied, providing either 100% blue light (treatment B100) (Fig. 1A) or a mix of 80% blue and 20% red light (treatment B80R20) (Fig. 1B) at a PPFD of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (0.9 ± 0.3 $mol \cdot m^{-2} \cdot d^{-1}$ for the 8 $h \cdot d^{-1}$ lighting). This PPFD was selected so that it would not exceed the light compensation point (>50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) determined for lettuce leaves grown for 19 d and 27 d after transplanting.

Considerations of the growth chamber design and planting density were made to minimize the temporal and spatial errors. To mitigate canopy edge effects and maintain uniformity within the plant growing area, four additional planting spaces per cart were used as border plants and were not included in the analysis. Additionally, NFT channels were rotated daily to mitigate the spatial environmental differences. The density of plants was intentionally reduced to 17 plants/m^2 in Expt. 1, below the seemingly typical indoor farming density of 40 to 50 plants/m^2, to improve airflow and humidity management within the growth chambers. In Expt. 2, additional airflow and humidity control in the chamber permitted a more conventional plant density (34 plants/m^2).

Expt. 1: Dim nighttime lighting of lettuce to prevent tipburn. The experiment was repeated twice, with the first run from 24 Feb to 7 Apr (Expt. 1_1) and the second run from 28 Apr to 9 Jun (Expt. 1_2), in Columbus, OH, USA. The experimental design was a split-plot within a randomized complete block design (RCBD). Each of the two walk-in growth chambers served as a main plot, with the four wire-rack carts within each chamber constituting individual blocks. Three treatments (B100, B80R20, and control) were randomly assigned to the carts, with two carts per chamber receiving a nighttime lighting treatment and the other two serving as controls with nighttime darkness. The lighting treatments were initiated 14 d after transplanting

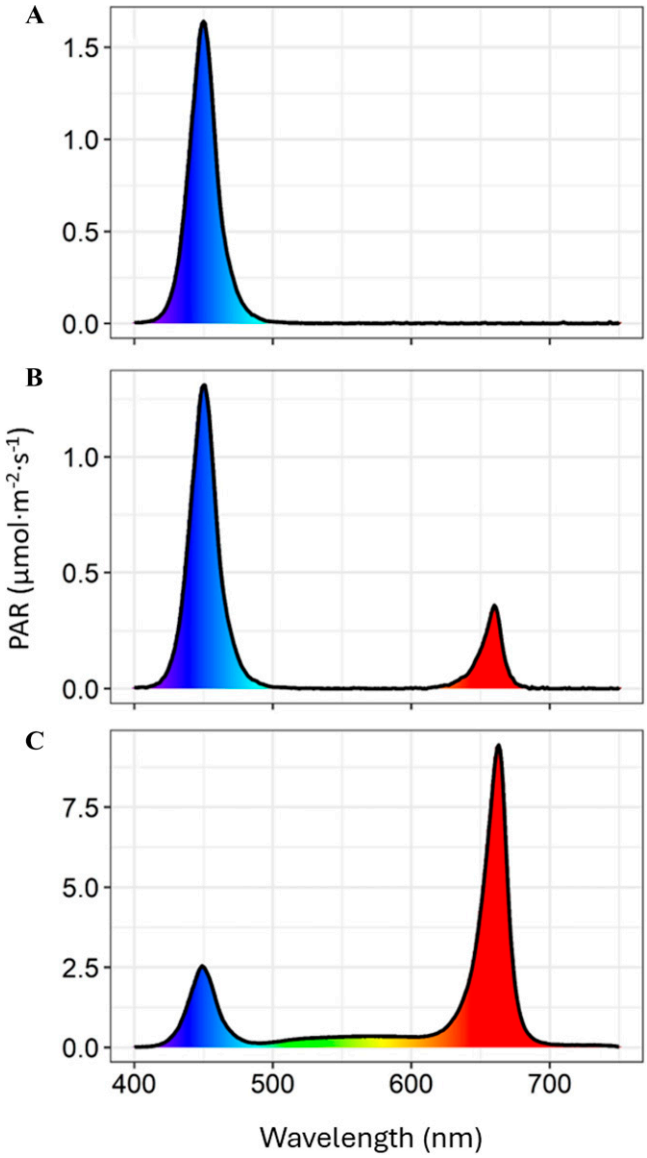

Fig. 1. Light spectra were measured using an Apogee Instruments spectroradiometer (BLK C-25, Tampa, FL, USA) at 30 cm from the light source, which is approximately the height of the lettuce canopy at the time of harvest. (**A**) Nighttime lighting of 100% blue light (B100). (**B**) Nighttime lighting of 4:1 red and blue light (B80R20). (**C**) Daytime light spectra for the entire experiment of 2:1:7 blue, green, and red light.

for the remaining 14 d to harvest. Two cultivars (Klee and Rex) were randomly assigned to planting spaces (subplot) within each cart in a completely randomized design (CRD). The experimental model was designed to account for sources of variability and treatment effects. We treated the experimental repeats, which took place during two separate periods, as a random effect with 1 degree of freedom (df) (df = 1). The growth chambers were also considered a random effect (df = 1). The light treatments (df = 2) and cultivars (df = 1) were analyzed as fixed effects. The residual error encapsulates unexplained variance within the experiment (df = 158). Individual plants were considered experimental units, with n = 10 for each cultivar in each treatment (n = 80 for the entire experimental repeat, with n = 20 plants for each lighting treatment and n = 40 for the control).

Expt. 2: Dim nighttime lighting and downward airflow to prevent tipburn of lettuce. The second experiment was conducted from 6 Oct to 17 Nov 2022, using a 2 × 2 factorial RCBD. The two primary factors studied were the presence or absence of dim nighttime lighting (B100 only) and the presence or absence of enhanced downward airflow, creating four distinct treatments: nighttime blue lighting only (B100); nighttime blue lighting with fans (B100_F); a control with no nighttime lighting (Dark); and a control with fans (Dark_F).

Each of two walk-in growth chambers was divided into four wire-rack carts (blocks) to which one of the four treatments was assigned, ensuring that all treatments were simultaneously replicated in both chambers. Box fans (B2041; Lasko, West Chester, PA, USA) were placed on top of the carts (at 0.4 m above the NFT channel surface), achieving an average downward airflow of 0.41 ± 0.2 $m \cdot s^{-1}$ over the plant canopy, and curtains were installed on all carts to prevent airflow cross-over between treatments. The control treatment airflow stayed the same as the levels described previously.

As in Expt. 1, cultivars were randomized to planting spaces on each cart in the CRD. The experimental model considered the two growth chambers as random effects (df = 1), the four carts as a random effect (df = 3), the four treatments as a fixed effect (df = 3), the two cultivars as a fixed effect (df = 1), and the residual error for capturing unexplained variance (df = 146).

Data collection and statistical analysis. After transplanting, the plants were visually assessed daily by the same person for signs of visible tipburn. Tipburn incidence was considered the presence or absence of visible tipburn symptomology (necrotic lesion forming along the leaf margin). Tipburn emergence was considered the number of days after transplanting to visible symptomology. Tipburn severity was measured as the percentage of leaves larger than 1 cm^2 expressing symptoms at the time of harvest.

At harvest, plant growth measurements were obtained, including the number of leaves, number of tipburned leaves, and shoot and root fresh mass. Then, the plants were dried at 55 °C for at least 3 d to determine the dry mass. Leaf tissue samples were collected for the mineral nutrient composition analysis and evaluated using dry ash and inductively coupled plasma methodologies (JR Peters, Allentown, PA, USA).

Leaf gas exchange was measured during the daytime using a portable gas exchange measurement system equipped with an LED lighting module that matched the blue:green:red photon flux density ratio daytime spectra used and reported previously (Fig. 1C) (Ciras-3; PP Systems, Amesbury, MA, USA). Data collected included the net photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*), and water use efficiency (WUE; formula = *Pn*/*E*). Instrument settings matched the light spectra (Fig. 1) and intensity setpoints using the CFM-3 LED light module. Control settings for CO_2 matched the ambient condition within the chamber at the time of measurement, and H_2O controls targeted 70% of ambient humidity. Temperature was not controlled during gas exchange measurement to capture leaf temperature variation within the growth chamber. Daytime and nighttime gas exchange measurements were performed 26 d and 27 d after transplanting. Nighttime gas exchange matched the light intensity (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) and quality (100% blue or 4:1 blue:red) (Fig. 1A, 1B) of each treatment. For the control (darkness), LEDs were turned off. Nighttime measurements were conducted at least 90 min after darkness. For each lighting treatment in the first experimental design, four to eight plants (equal numbers for each cultivar) were randomly selected for nighttime gas exchange, resulting in 28 total measurements (out of 80 plants). In the second experimental design, eight plants (four of each cultivar)

were sampled from each of the four treatments for 32 total measurements (out of 80 plants).

Statistical analyses were conducted using R and RStudio (R Core Team 2022; RStudio Team 2022). Mean separation was conducted using Tukey's honestly significant difference, and mixed linear model analysis tests were used to determine significance ($\alpha \leq 0.05$). Packages "lme4" and "ggplot2" were used for mixed linear models and plotting regression analyses (Bates et al. 2015; Wickham 2016).

Results

Evaporation rates as affected by growth chamber conditions

The petri dish method allowed for the evaluation of evaporation driven by both horizontal and vertical airflow, while the deep-dish tool, with its high sidewalls, provided a more focused estimate of vertical airflow effects. During the daytime, evaporation rates on channel surfaces equipped with fans were measured at 12.3 ± 0.8 mol·m^{-2}·h^{-1} for the petri dishes and 9.6 ± 0.6 mol·m^{-2}·h^{-1} for the deep dishes (Table 2). Conversely, channels without fans exhibited significantly lower evaporation rates, with recordings of 6.2 ± 0.5 mol·m^{-2}·h^{-1} and 4.5 ± 0.4 mol·m^{-2}·h^{-1} for petri and deep dishes, respectively. These findings suggest that low-evaporative conditions (<10 mol·m^{-2}·h^{-1}) correspond to reduced transpiration potential, highlighting the impact of airflow on the plant microclimate. Additionally, while nighttime lighting contributed slightly to increased evaporation rates, the primary influence on evaporation was clearly the use of fans.

Table 2. Mean ± standard deviation of evaporation rates during the day and night. Experimental repetitions were nonsignificantly different; therefore, they were combined.

Expt.	Trt[i]	Photoperiod[ii]	Petri dish evaporation rate mol·m^{-2}·h^{-1}	Deep dish evaporation rate mol·m^{-2}·h^{-1}
1	B100	D	8.6 ± 1.9	7.1 ± 2.0
		N	7.2 ± 1.4	6.4 ± 0.8
	B80R20	D	8.5 ± 1.6	7.1 ± 1.8
		N	5.0 ± 1.5	5.4 ± 0.5
	Control	D	8.5 ± 1.8	7.0 ± 1.9
		N	2.4 ± 0.4	2.3 ± 0.4
2	B100	D	8.7 ± 1.3	7.1 ± 1.2
		N	5.2 ± 0.9	4.6 ± 1.3
	B100_F	D	17.0 ± 3.1	15.4 ± 3.0
		N	9.8 ± 1.2	9.6 ± 0.9
	Control	D	7.3 ± 1.4	5.9 ± 0.5
		N	3.8 ± 0.4	3.3 ± 0.9
	Dark_F	D	16.0 ± 5.1	14.2 ± 3.5
		N	9.0 ± 2.2	8.3 ± 1.7

[i] Trt = treatment.
[ii] D = daytime; N = nighttime.

Expt. 1: Dim nighttime lighting of lettuce to prevent tipburn

Tipburn incidence, emergence, and severity. The analysis of tipburn incidence, emergence, and severity revealed no significant differences among the three treatment groups—100% blue light (B100), 80% blue light and 20% red light (B80R20), and control (nighttime darkness)—for both Klee and Rex lettuce cultivars. For both cultivars, tipburn symptoms typically appeared around the same time, with average emergence times of 22 ± 0.6 d after transplanting for Klee and 22 ± 0.5 d after transplanting for Rex (Table 3). Despite similar emergence times, the severity of tipburn varied slightly between the two cultivars. For 'Klee', the average percentage of leaves affected by tipburn was 42.0% ± 24.1% (Fig. 2A), while 'Rex' showed a lower and more consistent tipburn severity of 32.7% ± 3.5% of leaves affected (Fig. 3B).

Plant growth. The growth of the Klee cultivar was unaffected by the dim nighttime lighting treatments. For the Rex cultivar, variations in the number of leaves and root fresh mass were observed when exposed to nighttime lighting treatments compared with those of the control (Table 4). Specifically, with the B80R20 lighting treatment, 'Rex' displayed an increase of approximately 18 more leaves compared with the control (a 44% increase) (Table 4). Additionally, root fresh mass of 'Rex' was approximately18.4% higher with either nighttime lighting treatment compared with the control. However, the root dry mass remained similar across all treatments for 'Rex' (Table 4).

Leaf gas exchange. Daytime leaf gas exchange variables showed no significant differences between the lighting treatments (Table 3). However, during nighttime conditions, some differences were observed in *E* and *gs* across treatments. At night, the control treatment exhibited the lowest *E* for both cultivars. In contrast, plants under the B100 treatment demonstrated the highest *E*. 'Klee' showed a nighttime *E* of 1.5 ± 0.17 mmol·m^{-2}·s^{-1}, which was 55% higher than that of the control, while 'Rex' had a rate of 1.7 ± 0.02 mmol·m^{-2}·s^{-1}, which was 64% higher than that of the control (Fig. 2E, 2F). Stomatal conductance at night also increased under the B100 treatment, with 162.0 ± 30.6 mmol·m^{-2}·s^{-1} for 'Klee' and 193.3 ± 23.8 mmol·m^{-2}·s^{-1} for 'Rex', representing increases of 34% and 44% over the control, respectively (Fig. 2C, 2D). Notably, for 'Klee', there was no significant difference in *gs* between the nighttime lighting treatments (Fig. 2C). The *E* for plants under the B80R20 treatment during nighttime was not statistically different with either B100 lighting or the control, but it trended closer to that under B100 compared with that of the control (Fig. 2E, 2F).

Additionally, both nighttime *Pn* and WUE were negative for all measured plants across all treatments, confirming that the plants were respiring rather than photosynthesizing during the nighttime. However, specific data for these measurements are not shown.

Tissue calcium. The Ca concentrations in both inner and outer leaf tissues revealed no significant differences among the treatments (Table 3). However, the difference between inner and outer leaves seemed to be cultivar-specific. For the cultivar Klee, the average Ca concentration in the inner leaves of the control was 6.1 ± 1.6 g·kg^{-1} (dry mass), while that of the outer leaves was higher at 13.0 ± 1.8 g·kg^{-1}. Cultivar Rex of the control displayed a lower average Ca concentration in its inner leaves at 5.3 ± 1.6 g·kg^{-1}, and a significantly higher average concentration in the outer leaves at 17.1 ± 6.3 g·kg^{-1}. The ratio of inner to outer leaf Ca concentrations was consistent across all treatments for both cultivars. 'Klee' had a ratio of 0.51 ± 0.16, indicating that its inner leaves contained approximately half the Ca of that in its outer leaves. 'Rex' had a lower ratio of 0.33 ± 0.18, suggesting even greater disparity between the Ca contents of inner and outer leaves.

Expt. 2: Dim nighttime lighting and downward airflow to prevent tipburn of lettuce

Tipburn emergence and severity. Downward airflow using fans effectively prevented tipburn. No instances of tipburn were observed in any treatment group that used fans (Fig. 3A, 3B). The only exception was a single 'Klee' plant under the B100_F treatment (100% blue light with fans), which developed one tipburned leaf (1.02% of 'Klee' plants).

For the treatments without fans, namely B100 (100% blue light) and the control (nighttime darkness), tipburn occurred in both cultivars, with average incidences of 75% and 50% for Klee and Rex plants, respectively, when B100 and control treatments were combined (Table 3). There were no significant differences in tipburn incidence, emergence, or severity within these treatments for either cultivar (Fig. 3A, 3B).

Plant growth. Plant growth metrics exhibited notable variations primarily influenced by the presence or absence of fans. For the cultivar Klee, growth was generally reduced in the B100_F treatment (100% blue light with fans) compared with that of the control. Specifically, the number of leaves decreased by 19%, shoot fresh mass decreased by 6%, and shoot dry mass decreased by 17% under the B100_F treatment (Table 4).

In contrast, 'Rex' experienced a 9% reduction in the number of leaves under the B100_F treatment compared with that of the control, indicating a less pronounced

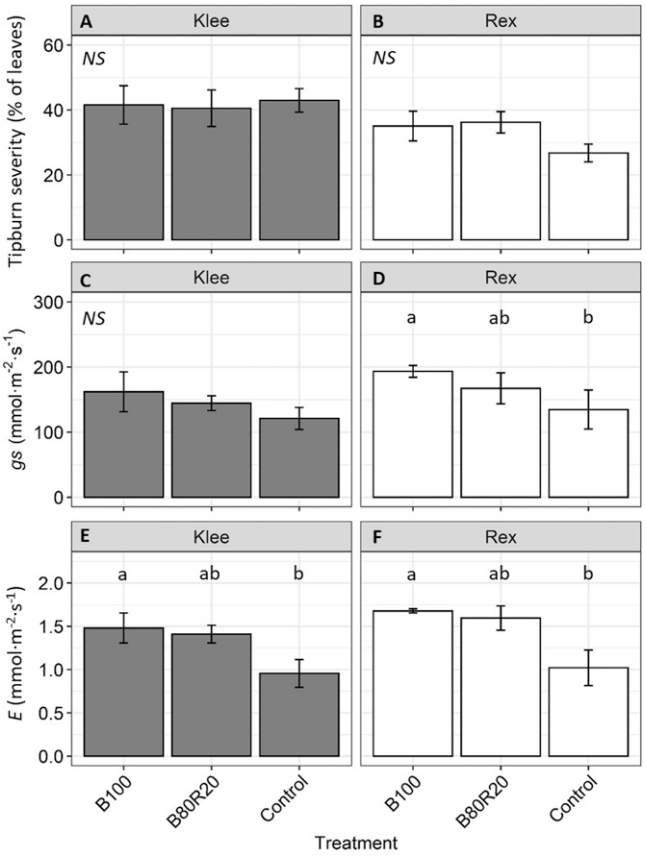

Fig. 2. Multiplot figure of tipburn severity and nighttime gas exchange results from the first experiment across the nighttime lighting (B100, B80R20) or darkness (control) treatments. Error bars represent the standard error. Differences within each cultivar are significant according to the analysis of variance and Tukey's honestly significant difference ($\alpha \leq 0.05$). NS = nonsignificant. (**A**) Tipburn severity of 'Klee'. (**B**) Tipburn severity of 'Rex'. (**C**) Nighttime stomatal conductance (*gs*) of 'Klee'. (**D**) Nighttime *gs* of 'Rex'. Nighttime transpiration rates (*E*) of 'Klee' (**E**) and 'Rex' (**F**).

response to the combination of blue light and fan-induced airflow. However, when exposed to the B100 treatment, 'Rex' displayed a 20% increase in shoot fresh mass compared with that of the control, although the shoot dry mass was not significantly different between the B100, B100_F, and control treatments. Additionally, both root fresh and dry mass values for 'Rex' increased by approximately 25% under conditions of nighttime lighting, regardless of the presence of fans (Table 4).

Leaf gas exchange. Under the B100_F treatment, the mean of daytime *gs* was 63% lower than that of the control for 'Rex'. For 'Klee' in the B100_F treatment, *gs* was 43% lower than that of the control, although nonsignificantly different from that of the other treatments (Table 3). During the nighttime, *Pn* and *WUE* were negative for both cultivars in all treatments (data not shown). Nighttime *gs* did not differ for 'Rex' among the treatments (Fig. 3D) but was lowest under either fan treatment (B100_F, Dark_F) for 'Klee' (Fig. 3C). Nighttime lighting without fans produced similar rates of *gs* for 'Klee' as the control treatment (Fig. 3C). For 'Rex', there were no significant differences in *gs* among the treatments (Fig. 3D).

Under the B100_F treatment, the mean *gs* was significantly reduced, with a 63% decrease for 'Rex' and a 43% decrease for 'Klee' compared with that of the control; however, the decrease for 'Klee' was not statistically significant from that of other treatments (Table 3).

During nighttime, both *Pn* and WUE were negative across all treatments for both cultivars regardless of nighttime lighting, suggesting respiratory activity rather than photosynthesis. Specific data for these measurements are not shown.

Nighttime measurements of *gs* showed no significant differences among treatments for 'Rex' (Fig. 3D). For 'Klee', however, the lowest *gs* rates occurred under treatments involving fans (B100_F, Dark_F) (Fig. 3C). In contrast, nighttime lighting without fans yielded *gs* rates for 'Klee' that were similar to those of the control (Fig. 3C).

Tissue calcium. The Ca concentration in the inner leaves did not show any significant differences between treatments for both cultivars (Table 3). Similarly, the ratio of the Ca concentration between inner and outer leaves was consistent across all treatments, with 'Klee' exhibiting a ratio of 0.65 ± 0.06 and 'Rex' exhibiting a ratio of 0.54 ± 0.05. However, the outer leaf Ca content for 'Klee' was slightly affected by the fan treatments, showing a 9% decrease in treatments involving fans, with an average concentration of 12.7 ± 0.2 $g \cdot kg^{-1}$. No significant differences were observed in the outer leaf Ca concentration for 'Rex', which maintained a consistent average of 13.6 ± 0.7 $g \cdot kg^{-1}$ across all treatments (Table 3).

Discussion

The present environmental conditions induced high incidences of tipburn for both Klee and Rex lettuce cultivars, as we have previously reported (Ertle and Kubota 2023). The combination of high daytime humidity, low airflow speed, supplemented CO_2, and high DLI allowed rapid growth rates of lettuce but also caused tipburn to occur in cultivars with low tipburn sensitivity. Additionally, the nutrient solution EC of 2.0 $dS \cdot m^{-1}$ could have contributed to the tipburn incidence in comparison with that associated with a lower EC (fertilization rate) (Langenfeld et al. 2022). Based on petri and deep-dish evaporation analyses, all treatments in both experiments without the use of fans resulted in low dish evaporation rates, indicating that plant transpiration would be suppressed under these conditions.

Lettuce tipburn was observed in all treatments in both experiments except for those using downward vertical airflow fans. Contrary to our hypotheses, the application of dim nighttime lighting, whether using blue or a combination of blue and red light, did not reduce tipburn incidence or severity (Fig. 2A, 2B). Interestingly, while fan treatments effectively eliminated tipburn, they did not lead to increased Ca concentrations in the inner leaves of lettuce (Table 3). However, the nighttime lighting treatments did result in increased *gs* and *E*, partially supporting our hypothesis that dim lighting, through the blue light mechanism, could enhance water flux through the leaves (Fig. 2C–2F). These findings suggest that while specific physiological responses (i.e., increased transpiration) to nighttime lighting were achieved, they did not translate into the expected reduction in tipburn.

In the first experiment, nighttime lighting did not affect shoot growth in either cultivar (Table 4), indicating that the nighttime light intensity of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was low enough to avoid additional shoot growth in either cultivar. Interestingly, 'Rex' under B80R20, but not under B100, lighting developed 44% more leaves than the control treatment (Table 4). A previous study found that extending the photoperiod from 16 to 24 $h \cdot d^{-1}$, while maintaining a PPFD of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$, increased the leaf number of 'Rex' by 8%. (Kelly et al. 2020). In our experiment, it is unclear why 'Rex' exhibited such a substantial increase in leaf initiation under B80R20 nighttime lighting, especially considering that the DLI was only slightly increased by the additional photon flux and was not seen under the B100 lighting treatment. Additionally, 'Rex' showed an 18.4% higher fresh root fresh mass under both nighttime lighting treatments compared with that of the control, although root dry mass remained consistent across all treatments (Table 4). The lack of correlation between increased root fresh mass

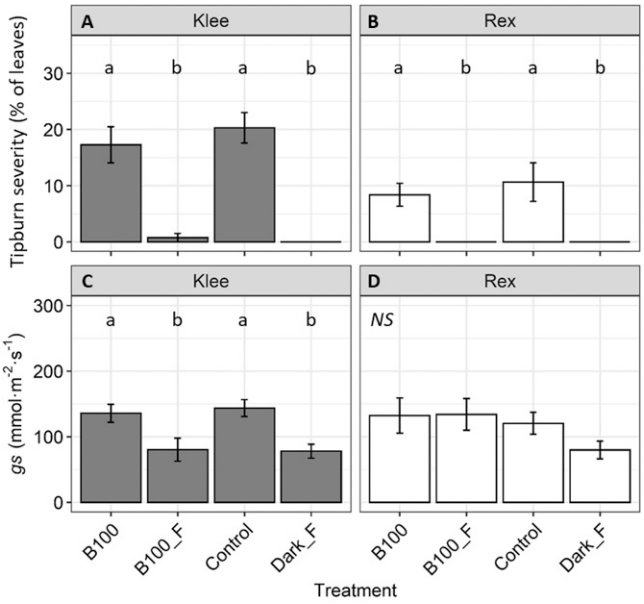

Fig. 3. Multiplot figure of tipburn severity and nighttime gas exchange results from the follow-up experiment across the nighttime lighting (B100, B100_F) or darkness (Dark_F, control) treatments and presence of fans (denoted by "_F"). Error bars represent the standard error. Differences within each cultivar are significant according to the analysis of variance and Tukey's honestly significant difference ($\alpha \leq 0.05$). NS = nonsignificant. (**A**) Tipburn severity of 'Klee'. (**B**) Tipburn severity of 'Rex'. (**C**) Nighttime stomatal conductance (*gs*) of 'Klee'. (**D**) Nighttime *gs* of 'Rex'.

and dry mass was puzzling; however, it may be attributed to potential measurement errors during the harvest process, such as inadequate separation of rockwool from the roots before recording the fresh mass.

In the second experiment, differences in plant growth trends of shoot growth and leaf number were not the same for the two cultivars. For 'Klee' grown under the B100_F treatment, shoot growth and the number of leaves were reduced compared with those of all other treatments (Table 4). While lettuce grown under higher fractions of blue light from 0% to 100% reportedly have reduced growth (Izzo et al. 2021), the application of 100% blue nighttime lighting in this experiment did not reduce fresh mass under the B100 treatment in either experiment. It is unclear why, for 'Rex', plants under the B100 treatment had the highest shoot fresh mass in the second experiment. Nighttime lighting added a total of 12.6 $mol \cdot m^{-2}$ (0.9 $mol \cdot m^{-2} \cdot d^{-1}$) over the final 14 d of growth, which accounted for 2.9% of the total photon flux provided over the entire experiment and should not contribute to a 19.75% increase in shoot growth compared with that of the control group. Therefore, it is unclear why this result was found for 'Rex' only in the second experiment (Table 4). Possibly, differences in the environmental conditions or the experimental setup could have caused this result, but environmental variation seemed minimal, and carts were set-up identically other than their assigned treatments (Table 1).

Daytime measurements of *Pn*, *E*, *gs*, and WUE of both cultivars in the first experiment were similar between all treatments (Table 4). However, nighttime *E* for both cultivars and nighttime *gs* for 'Rex' were increased by B100 lighting (Fig. 2C–2F). Opposite to our hypotheses, nighttime lighting treatments had no effect on tipburn in both cultivars and did not affect the leaf Ca concentration despite an increase in nighttime *gs* and *E* (Fig. 2C–2F). The blue light effect is known to increase opening of stomata for some species through a mechanism that excites the phototropin in guard cells (Inoue and Kinoshita 2017), and it is known to be enhanced by the addition of red light to drive further stomatal opening (Shimazaki et al. 2007). However, this mechanism is not present, or has minimal effect, in some species (Doi et al. 2006), and it has not been well-examined for lettuce. In this experiment, the magnitude of stomatal opening to blue light or blue and red light seemed to be cultivar-specific. Nighttime lighting treatments of B100 increased nighttime *E* significantly over that of the control treatment for both cultivars (Fig. 2E, 2F), but only significantly increased nighttime *gs* for 'Rex' (Fig. 2C, 2D). However, tipburn severity was not decreased by this strategy (Fig. 2A, 2B). Although young expanding leaves were directly exposed to this nighttime lighting, it is likely that the treatments increased *E* for the whole plant. Because *E* was increased by nighttime lighting, the boundary layer resistance under low airflow conditions was likely restricting transpiration of young leaves. Photosynthesis measurement devices create a turbulent aerial environment within the leaf chamber to reduce the boundary layer to best measure the gaseous fluxes of the leaf (PP Systems 2023). Therefore, in situ leaf gas exchange was likely

Table 3. Mean ± standard error for (daytime) gas exchange rates, tipburn incidence (%, means only), and calcium concentrations of inner and outer leaves. Letters indicate significant differences ($\alpha \leq 0.05$) within cultivars.

Expt.	Cultivar	Trt	Tipburn incidence % of plants	*Pn* $\mu mol \cdot m^{-2} \cdot s^{-1}$	*E* $mmol \cdot m^{-2} \cdot s^{-1}$	*gs* $mmol \cdot m^{-2} \cdot s^{-1}$	WUE $mmol \cdot mol^{-1}$	Inner Ca $g \cdot kg^{-1}$	Outer Ca $g \cdot kg^{-1}$
1	Klee	B100	80	9.6 ± 1.0	2.1 ± 0.3	187.8 ± 29.7	4.9 ± 0.7	6.0 ± 0.6	11.8 ± 1.0
		B80R20	85	12.2 ± 1.0	2.5 ± 0.1	228.0 ± 17.0	5.0 ± 0.6	6.4 ± 1.4	11.7 ± 0.7
		Control	92.5	11.1 ± 0.7	2.5 ± 0.1	236.5 ± 15.2	4.5 ± 0.3	6.1 ± 0.5	13.2 ± 0.6
	P		NS	NS	NS	NS	NS	NS	NS
	Rex	B100	85	13.7 ± 0.7	2.7 ± 0.1	266.4 ± 19.2	5.2 ± 0.2	5.7 ± 1.1	19.2 ± 3.7
		B80R20	100	12.8 ± 0.7	2.7 ± 0.1	265.4 ± 14.9	4.7 ± 0.4	4.1 ± 0.6	15.0 ± 1.5
		Control	80	12.2 ± 0.4	2.4 ± 0.1	219.9 ± 11.3	5.2 ± 0.3	5.7 ± 0.5	17.2 ± 2.4
	P		NS	NS	NS	NS	NS	NS	NS
2	Klee	B100	75.0 b	6.5 ± 1.2	2.6 ± 0.3 ab	188.0 ± 45.9	2.5 ± 0.5	8.1 ± 1.2	12.6 ± 0.1 ab
		B100_F	5.3 a	7.5 ± 10	2.1 ± 0.2 b	140.8 ± 17.5	3.9 ± 0.8	8.2 ± 0.5	11.6 ± 0.4 b
		Control	85.0 b	5.9 ± 1.6	3.4 ± 0.3 a	246.3 ± 29.7	1.9 ± 0.5	7.3 ± 0.6	12.9 ± 0.3 a
		Dark_F	0.0 a	6.9 ± 1.9	3.0 ± 0.4 ab	254.0 ± 40.3	2.5 ± 0.9	7.9 ± 0.7	11.6 ± 0.2 b
	P		<0.0001	NS	0.037	NS	NS	NS	0.0049
	Rex	B100	55.0 b	9.0 ± 1.3 ab	3.3 ± 0.3 a	258.7 ± 36 ab	3.1 ± 0.7 ab	7.5 ± 0.8	13.7 ± 0.6
		B100_F	0.0 a	8.4 ± 1.2 ab	1.9 ± 0.2 b	119.5 ± 22.3 b	4.5 ± 0.8 a	6.5 ± 0.5	12.6 ± 1.0
		Control	45.0 b	6.6 ± 1.6 b	3.7 ± 0.4 a	323.2 ± 65.6 a	1.9 ± 0.4 b	8.1 ± 0.9	14.5 ± 0.6
		Dark_F	0.0 a	9.5 ± 1.9 a	3.1 ± 0.2 ab	256.3 ± 34.4 ab	3.3 ± 0.7 ab	7.4 ± 0.9	13.7 ± 0.7
	P		<0.0001	0.029	0.0041	0.015	0.0006	NS	NS

Ca = calcium; *E* = transpiration rate; *gs* = stomatal conductance; NS = nonsignificant; *Pn* = net photosynthetic rate; WUE = water use efficiency.

Table 4. Mean ± standard error for all plant growth values. Letters indicate significant differences ($\alpha \leq 0.05$) within cultivars.

Expt.	Cultivar	Trt[i]	Leaves no.	Shoot fresh mass g	Shoot dry mass g	Root fresh mass g	Root dry mass g
1	Klee	B100	71 ± 3.4	140 ± 13.7	4.6 ± 0.5	12.9 ± 0.9	1.8 ± 0.1
		B80R20	58 ± 6.7	154.7 ± 7.1	5.6 ± 0.5	14.9 ± 0.6	1.9 ± 0.1
		Control	69 ± 3.0	145.2 ± 7.6	4.9 ± 0.3	13.2 ± 0.5	1.8 ± 0.1
	P[ii]		NS	NS	NS	NS	NS
	Rex	B100	37 ± 1.6 ab	174.1 ± 14.6	5.0 ± 0.5	14.8 ± 0.8 a	1.8 ± 0.1
		B80R20	59 ± 4.4 a	155.6 ± 11.0	5.4 ± 0.5	14.8 ± 0.7 a	2.0 ± 0.1
		Control	41 ± 1.6 b	156.8 ± 6.5	4.7 ± 0.3	12.5 ± 0.4 b	1.8 ± 0.1
	P		0.0013	NS	NS	0.0039	NS
2	Klee	B100	56 ± 1.8 a	90.3 ± 3.7 a	3.9 ± 0.1 a	9.8 ± 0.6	1.2 ± 0.1
		B100_F	48 ± 1.7 b	64 ± 4.9 b	3.0 ± 0.2 b	8.9 ± 0.6	1.1 ± 0.1
		Control	59 ± 1.7 a	83.4 ± 3.5 a	3.6 ± 0.1 ab	9.4 ± 0.4	1.2 ± 0.2
		Dark_F	58 ± 2.4 a	74.7 ± 4.7 ab	3.4 ± 0.2 ab	8.6 ± 0.5	1.1 ± 0.1
	P		0.00033	0.0020	0.0031	NS	NS
	Rex	B100	27 ± 0.5 ab	106.7 ± 3.5 a	4.1 ± 0.2 a	10.3 ± 0.4 ab	1.4 ± 0.1 a
		B100_F	27 ± 0.6 b	83.4 ± 3.5 b	3.7 ± 0.1 ab	11.0 ± 0.5 a	1.4 ± 0.1 ab
		Control	30 ± 1.1 a	89.1 ± 5.5 b	3.4 ± 0.2 b	8.7 ± 0.5 b	1.1 ± 0.1 ab
		Dark_F	28 ± 0.7 ab	80.4 ± 4.9 b	3.1 ± 0.2 b	8.3 ± 0.7 b	1.0 ± 0.1 b
	P		0.009	0.00015	0.0099	0.0018	0.0047

[i] Trt = treatment.

[ii] Significance value (*P* value; $\alpha \leq 0.05$) determined by the linear mixed model one-way analysis of variance. Letters indicate significant differences between treatments according to Tukey's honestly significant difference ($\alpha \leq 0.05$). NS = nonsignificant ($\alpha > 0.05$).

suppressed by the present boundary layer in the growing environment, and increases in *gs* and *E* measured by a photosynthesis system were plant responses with the boundary layer removed.

In the second experiment with B100 lighting and fans, only treatments with fans were able to eliminate tipburn of lettuce (B100_F, Dark_F) (Fig. 3A, 3B). Using the method of Papio (2021) to measure evaporation rates, those of petri and deep dishes under the fan treatments were approximately double those of treatments without fans during the day and night (Table 2). Papio (2021) also found that *E* of lettuce grown under their conditions was approximately equivalent to 11% of the petri dish evaporation rate or 17% of the deep dish evaporation rate. Unlike the first experiment, B100 lighting alone did not increase *gs* compared with that of the control for either cultivar (Fig. 3C, 3D). The nighttime evaporation rates of the B100 treatment were 37% and 39% higher when measured by petri and deep dishes compared to the control treatment (Table 2). We expected that this large difference in the measured evaporation rate of the dishes, in addition to the radiation of nighttime blue lighting, would greatly increase both *gs* and *E* in both lettuce cultivars. However, *gs* was not significantly affected by airflow and nighttime lighting in Expt. 2. Possibly, *gs* was similar between the B100 and control treatments in the second experiment, but not the first experiment, because of the doubled planting density used (34 plants/m^2), potentially creating a thicker boundary layer attributable to the dense canopy.

Treatments with fans in the second experiment reduced nighttime *gs* of 'Klee', but not of 'Rex', compared with experiments without fans (Fig. 3C, 3D). The Dark_F and B100_F treatments resulted in lower *gs* compared with treatments without fans (Fig. 3C, 3E). When evaporation rates were measured by petri and deep dishes, Papio (2021) found that increased air speed enhanced the evaporation rate, whereas increased photon flux from electric lighting had a much smaller effect. In our experiment, for example, petri dishes deployed under the nighttime B100 treatment had a 37% increase in the evaporation rate, while those under the Dark_F treatment had a 137% increase (Table 2). In another study, long-term exposure to higher air speeds have been shown to reduce *gs* in IF-grown lettuce when the velocity is increased from 0.25 to 0.50 or 0.75 $m \cdot s^{-1}$ (Ahmed et al. 2022). However, lettuce grown under increasing DLI has been shown to produce a small increase in *E* and had no effect on *gs* (Gavhane et al. 2023). Therefore, it appeared that *gs* may have been increased by nighttime lighting but decreased by higher air speed, resulting in the conflicting responses seen in 'Klee' between the first and second experiments.

Consistent with previous findings (Ertle and Kubota 2023), outer lettuce leaves in both cultivars generally had two- to three-times the Ca concentration found in inner leaves (Table 3). This pattern aligns with those reported by Sago (2016), who observed similar Ca distributions between inner and outer leaves. Despite the elimination of tipburn under treatments with downward vertical airflow fans, none of the treatments led to an increased accumulation of Ca in the inner leaves (Table 3, Fig. 3A, 3B). Other studies have found that increasing downward vertical air velocity over IF-grown lettuce can significantly boost Ca accumulation in inner leaves (Goto and Takakura 1992a).

While horizontal air speeds between 0.5 and 0.75 $m \cdot s^{-1}$ can mildly suppress lettuce growth in indoor farms (Ahmed et al. 2022), many studies have shown that vertical airflow speeds of 0.5 $m \cdot s^{-1}$ can reduce or eliminate tipburn without affecting growth (Frantz et al. 2004). In our experiment, horizontal and vertical air velocities did not exceed 0.16 $m \cdot s^{-1}$ and 0.41 $m \cdot s^{-1}$, respectively. Interestingly, lettuce grown under fan treatments in the second experiment exhibited less shoot growth than that under the B100 lighting treatment (Table 4). This suggests that mechanical stress from high air speed immediately after transplanting may have stunted early growth. We initiated the fan treatments immediately after transplanting and suspect that the higher velocity airflow may have initially stressed the plants, leading to suppressed growth compared with that of the B100 and control treatments.

Although our methodology of dim nighttime lighting was unsuccessful, modifying a light source to provide targeted photon flux to the inner leaves could potentially reduce the tipburn risk. Enhancing localized transpiration and mass flow through photon flux at the critical young leaves and meristem while whole-plant transpiration remains restricted by typical nighttime stomatal closure might increase Ca transport and prevent tipburn. However, constructing a light source for this purpose presents significant challenges, and the risk of tipburn could increase if the photon flux is too high, vertical airflow is too low, or targeted lighting is poorly directed.

Guttation has been demonstrated as an effective strategy for mitigating tipburn in lettuce (Collier and Tibbitts 1984). Unlike whole-plant transpiration, guttation relies on enhanced xylem pressure caused by high root pressure and nighttime stomatal closure, which forces apoplastic water transport through the hydathodes (Singh 2014). This process allows xylem-transported nutrients like Ca to become accessible through the symplastic pathway, thereby preventing tipburn by increasing leaf Ca content in leaves that experience nighttime guttation, as has been demonstrated in other species (Kroggel and Kubota 2017). However, inducing guttation in indoor farming systems is practically challenging because of the high humidity requirements (90% to 95% relative humidity) throughout the nighttime. High humidity can lead to condensation on electrical and structural components, necessitating HVAC systems capable of handling significant dehumidification

loads and conflicting with growers' anecdotal reluctance to wet plants because of disease risks (Ahamed et al. 2023).

In our second experiment, treatments with downward vertical fans resulted in slightly lower Ca accumulation in the outer leaves compared with that of either B100 lighting or control treatments without fans. The difference in concentration was minor, with only an 8% to 11% reduction in Ca under fan treatments compared with that of treatments without fans (Table 3). We expected that the inner leaf Ca concentration would increase with downward fan treatments because other studies have found that inner leaves under perforated air tubes can have a 4.6-fold increase in Ca with air speeds estimated near 1.33 $m \cdot s^{-1}$ (Goto and Takakura 1992b). Furthermore, nighttime airflow has been shown to increase Ca concentration in inner leaves in similar studies (Goto and Takakura 1992a). However, in our experiment, we did not observe an increase in Ca concentration in inner leaves under fan treatments despite the average downward air speed over the growing surface being 0.41 ± 0.2 $m \cdot s^{-1}$. While the reason for the lack of increased Ca is unclear, it is evident that the additional airflow prevented tipburn in lettuce, suggesting that adequate Ca was present to support normal growth.

Our hypothesis that dim nighttime lighting could enhance Ca transport to young expanding leaves was not supported by the results. While dim lighting increased stomatal conductance and promoted transpiration, the anticipated improvement in Ca transport did not occur. The broad impact of increased whole-plant transpiration, driven by the blue light mechanism, likely mitigated any potential benefits. Specifically, the opening of stomata on all leaf surfaces illuminated by blue light likely reduced xylem pressure, which, in turn, suppressed guttation and the transport of Ca to tipburn-susceptible tissues. Guttation is crucial for driving Ca to these critical areas by increasing xylem pressure, although liquid water accumulation on the tips of lettuce leaves is not commonly seen in IF production. Nighttime stomatal opening has been shown to reduce hydraulic pressure in the xylem, potentially suppressing guttation and nutrient transport (Assmann 1988). Therefore, it is likely that in our experiment, nighttime dim lighting reduced xylem pressure, suppressed guttation, and ultimately increased the risk of tipburn. As a result, dim nighttime lighting does not appear to be a viable strategy for preventing tipburn in lettuce.

Conclusion

Tipburn is a problematic nutrient disorder affecting most IFs producing lettuce. Although several methods exist to avert this Ca deficiency in greenhouse-grown lettuce, most strategies have limited application in vertical farms. We examined a dim nighttime lighting approach using a unique application of dim nighttime blue light to increase stomatal conductance via a blue light physiological mechanism. However, this strategy was ineffective for increasing the Ca concentration in tipburn-susceptible inner leaves because of the combination of high boundary layer resistance over the crop and whole-plant increase in the transpiration rate, which ultimately minimized Ca transport to the targeted region. In contrast, downward airflow at 0.4 $m \cdot s^{-1}$ eliminated tipburn entirely throughout the crop regardless of nighttime lighting. This result confirms that continuous application of downward vertical airflow fans increases transpiration of tipburn-susceptible inner leaves and minimizes tipburn risk, as has been previously reported by many other studies of greenhouse applications. Therefore, we suggest that designing and evaluating appropriate airflow strategies for IFs should be research priorities to minimize the risk of lettuce tipburn at IFs.

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