



Article

Selection of Heat Tolerant Lettuce (*Lactuca sativa* L.) Cultivars Grown in Deep Water Culture and Their Marketability

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Abstract: Lettuce is a cool season vegetable often produced in greenhouses and other protective structures to meet market demands. Greenhouses are being increasingly adopted in warm climate zones where excessive heat often leads to physiological disorders of lettuce, such as tipburn and premature bolting. Greenhouse lettuce growers in warm climates need cultivar recommendations that can help improve production without ignoring marketability. In the current study, eighteen lettuce cultivars were grown in deep water culture and evaluated for growth, bolting, and tipburn in a greenhouse in Auburn, AL, starting on 30 June and 19 August 2016. Based on the severity of bolting and tipburn, nine cultivars were then selected and evaluated on 17 November 2016 for sensory attributes and marketability by 50 untrained consumer panelists. Cultivars ‘Adriana’, ‘Aerostar’, ‘Monte Carlo’, ‘Nevada’, ‘Parris Island’, ‘Salvius’, ‘Skyphos’, and ‘Sparx’ were selected as having higher heat tolerance than cultivars ‘Bambi’, ‘Buttercrunch’, ‘Coastal Star’, ‘Flashy Trout Back’, ‘Green Forest’, ‘Green Towers’, ‘Jericho’, ‘Magenta’, and ‘Truchas’. Higher crispness, lower bitterness, higher overall texture, and higher overall flavor each correlated to higher marketability, regardless of cultivar, but the strongest predictor of marketability was overall flavor. Overall flavor and overall texture were more strongly correlated to marketability than bitterness and crispness, respectively, suggesting that broader sensory categories may better capture human sensory perceptions of lettuce than narrower categories. Cultivars ‘Aerostar’, ‘Monte Carlo’, ‘Nevada’, ‘Parris Island’, ‘Rex’, ‘Salvius’, and ‘Sparx’ performed well in a hot greenhouse and were preferred by consumers. This step-wise experiment could be an adaptable tool for determining highest performing cultivars under any given production constraint, without ignoring marketability.

Keywords: greenhouse vegetables; hydroponics; lettuce; bolting; tipburn; sensory evaluation; heat tolerance

1. Introduction

Lettuce (*Lactuca sativa* L.) is a cool season crop susceptible to physiological problems including tipburn, bolting, ribbiness, rib discoloration, and the development of loose, puffy heads when grown at supra-optimal temperatures [1]. At sub-optimal temperatures, growth can be slowed or plant death can occur. Optimal temperatures range from 17 to 28 °C in the day and 3 to 12 °C at night [2], while optimal pH and electrical conductivity (EC) are 5.8 to 6.5 and 1.5, respectively [3]. There are five commonly grown types of lettuce including Butterhead, Cos or Romaine, Crisphead, Leaf, and Summer Crisp [4].

In warm climate zones, such as the southeastern U.S., greenhouse lettuce production is often limited by occurrence of tipburn and premature bolting. Tipburn is a physiological disorder resulting from a calcium deficiency in young leaves [1,5], which is usually caused by high relative humidity and temperature [6], high light [7], and/or pH and water stress [8], not by a lack of calcium in

the soil or nutrient solution [9]. Some of these factors, specifically high temperature and light, often lead to growth rates with which calcium translocation cannot keep pace [10]. While these negative factors can be mitigated under idealized, controlled growing conditions [11], they are often encountered in greenhouses, particularly in the summer months in warm climates. Bolting is rapid stem elongation followed by flowering and is caused by excessively high temperatures and long photoperiods. Both tipburn [12] and bolting [13] can ruin the marketability of lettuce by causing undesirable appearance and/or taste. Since both disorders are directly related to heat and are often responsible for marketable yield losses, resistance to either or both can be considered heat tolerance.

Susceptibility to both tipburn and bolting is related to genetic diversity in lettuce [14–16]. Crisphead lettuce cultivars typically have a greater genetic variation for tipburn resistance compared to Romaine and leaf types, possibly due to breeding efforts for this type [17]. Breeding for tipburn resistance in other lettuce types could be a successful strategy to mitigate tipburn severity in greenhouse-grown lettuce, but selective breeding has not yet produced a fully tipburn-resistant cultivar for growers. In greenhouse and field applications, calcium foliar sprays relieved tipburn symptoms in some cases [18], but were not effective on tight heads (crisphead) and had limited success on other types. Production strategies that increase transpiration and reduce temperature, like blowing air across young leaves as heads start to form, have also reduced tipburn severity in some cases [19], but are not fully effective for all lettuces. Multiple genes have also been identified that are directly linked to bolting [16], and selective breeding efforts have produced lettuce cultivars that are marketed as slow-bolting. While a combination of selective breeding and cultural practices is likely needed to fully eliminate tipburn and bolting incidence in greenhouse lettuce production, the selection of the best available cultivars offers an easily adopted production solution for growers. Better cultivar selection can also help greenhouse growers meet year-round market demands and better utilize their expensive greenhouse structures instead of halting production when outdoor temperatures rise [20].

Although growth and development conditions are important to greenhouse lettuce growers, marketability of their produce is paramount. Butterhead, and its sub-type Bibb, are the most commonly grown greenhouse lettuce types [20], but local markets often demand other types of lettuces. For example, Romaine lettuce is often in high demand [21], but little information regarding the heat tolerance or marketability of greenhouse-grown Romaine lettuces is available in the literature. The same is true for other lettuce types.

Consumer preference surveys, or sensory evaluations, have been conducted for multiple foods, ranging from fresh produce [22] to processed foods like mayonnaise [23], and can be important tools for deciding what crops to grow and market. Sensory perceptions affect consumer preferences for foods, whether consciously or subconsciously, and can change over time or due to changes in expectations. For example, sweet-tasting foods are often more desirable than bitter-tasting foods, but as humans age, they may desire more bitter foods due to acquired tastes. The concept that taste can be acquired indicates that other factors can affect preferences, like beliefs and expectations of food [24]. Most consumers expect lettuce to be crisp [25] and, in general, consumers expect lettuce to not be bitter [26]. However, it is unclear in the literature to what extent other sensory criteria affect the marketability of greenhouse-grown lettuce. Determining which organoleptic qualities (taste, sight, smell, and touch) consumers most desire in lettuce could help lettuce growers and breeders select the best cultivars for production.

The objectives of this study were to use a step-wise experiment to select heat-tolerant, marketable lettuce cultivars for greenhouse production in warm climatic zones and to determine which sensory criteria most influence lettuce marketability.

2. Materials and Methods

On 30 June and 19 August 2016, eighteen cultivars of lettuce (*Lactuca sativa* L.) were grown in deep water culture (DWC) in a greenhouse at Auburn University (32.5970° N, 85.4880° W). The greenhouse was a stand-alone hoop-style house with 2-m-tall fiberglass sidewalls covered with a double layer

of polyethylene film and equipped with a pad-and-fan cooling system in which only the exhaust fans worked. The cooling pad was non-functioning throughout the experiment so cooling was only achieved through air exchange and movement through the greenhouse. Cultivars of different types of lettuce, marketed as heat-tolerant, slow-bolting, or both, were selected from popular seed companies. Eleven Romaine, two Bibb, three Butterhead, and four Summer Crisp cultivars were selected (Table 1). Cultivars ‘Muir’ and ‘Teide’ were originally selected but were not included in the trial due to low seed germination percentages and poor seedling vigor. Seeds were sourced from Johnny’s Selected Seeds (Fairfield, ME, USA) with the exception of ‘Green Towers’ which was sourced from Paramount Seeds (Stuart, FL, USA).

Table 1. The cultivars selected for heat tolerance trial based on their being marketed as heat tolerant and/or slow-bolting.

| Type ¹ | Cultivar |
|-------------------|---|
| Romaine/Cos | Aerostar Coastal Star Flashy Trout Back Green Forest Green Towers Jericho Monte Carlo Parris Island Salvius Sparx Truchas |
| Bibb | Bambi Buttercrunch |
| Butterhead | Adriana Rex Skyphos |
| Summer Crisp | Magenta Muir ² Nevada Teide ² |

¹ Types are indicated based on marketing information from seed companies. ² Not included in experiments due to low germination percentages and lack of seedling vigor.

Lettuce seeds were sown on 16 June and 5 August 2016, grown for two weeks in OASIS[®] Horticultubes (OASIS[®] Grower Solutions, Kent, OH, USA) (2.54 cm × 3.18 cm × 3.81 cm), and fertilized with nutrient solution from municipal water (Auburn, AL) containing 150, 80, 200, 150, and 35 mg/L⁻¹ N, P, K, Ca, and Mg, respectively from water-soluble 8N-6.5P-30K (Gramp’s Original Hydroponic Lettuce Fertilizer, Ballinger, TX, USA), calcium nitrate (15.5N-0P-0K), and magnesium sulfate (10% Mg). Seedlings were irrigated overhead with a nutrient solution after emergence every other day for two weeks before being transplanted to one of four separate DWC pools, each containing the same nutrient solution used to irrigate the seedlings.

Each DWC pool measured as 1.2 m × 2.4 m and was framed with treated lumber planks (5 cm × 30.5 cm × 2.4 m). Frames were stabilized by 10 cm × 10 cm × 30.5 cm treated lumber posts in each corner and were connected to each post using 10-cm deck screws. Pools were lined with a 6-mL black construction film and filled with nutrient solution to a 254-cm depth to conform the liner to the pool sidewalls. Each pool was filled with 7315 L of nutrient solution. The excess liner was then rolled and fastened to the pool frames using wooden slats and deck screws. Sixty-eight evenly-spaced (20-cm, center-to-center) circular holes, each measuring 2.2 cm in diameter, were drilled into four polystyrene foam boards (2.54-cm thick, R 5 Unfaced Polystyrene Foam Board Insulation, Kingspan

Insulation, Atlanta, GA, USA), which had been cut to fit the dimensions of each pool in order to serve as DWC rafts. One raft was floated on top of the nutrient solution in each pool and the lettuce plants were transplanted onto the floating rafts by fitting Oasis[®] Horticubes snugly into each hole and aligning the top of the root cube to the surface of the raft. 'Rex' lettuce plants were planted as a border row along the entire edge of each raft. Two plants of each cultivar were then randomly placed into two of the 40 remaining holes. Each pool was supplied with 30 L min⁻¹ air pushed through four airstones using one of two pumps (Hailea ACO-9730 Air Pump, Guangdong, China). Greenhouse air temperature at plant height, and nutrient solution temperature were measured hourly for the duration of the experiment using HOBO Pendant[®] dataloggers (Onset Computer Corporation, Bourne, MA, USA). The solution pH and EC were monitored weekly using a LAQUA Twin pH Meter and a LAQUA Twin EC Meter (Spectrum Technologies, Inc., Aurora, IL, USA), respectively, to ensure they were within acceptable ranges. The solution pH remained within an acceptable range of 5.8–6.5 for the duration of the experiment. At 15 days after transplant (DAT), nutrient solution was replaced in all pools to ensure adequate concentrations of each nutrient and appropriate nutrient ratios. At 30 DAT, a size index (SI) was measured ($[\text{height} + \text{width}_1 + \text{width}_2]/3$), bolting was quantified by assigning each plant a visual rating (0 = no visible internode elongation; 1 = visible internode elongation; 2 = internode elongation with dense head forming; 3 = internode elongation and inflorescence forming; 4 = flowering), and tipburn was quantified according to USDA-AMS guidelines [12] by assigning each plant a rating (0 = no visible tipburn; 1 = tipburn visible with an aggregate area less than 3.2 cm²; 2 = tipburn visible with an aggregate area exceeding 3.2 cm² but smaller than 19.4 cm²; 3 = tipburn visible with an aggregate area exceeding 19.4 cm²). The tipburn ratings of 2 and 3 correlated to the USDA-AMS [12] ratings of "damage" and "serious damage", respectively, for the Butterhead lettuce type at shipping. Tipburn ratings of 0 and 1 were included to account for all visible damage. The lettuce heads were then cut at the base and immediately weighed to determine head fresh weight.

An analysis of variance was performed on all responses using a PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC, USA). The experimental design was a generalized randomized complete block with the experimental run in the model as a random variable. Four blocks contained eight replicates per cultivar per experimental run. Each pool was a block and each block contained two plants of each cultivar for a total of 40 experimental units per block. The experimental units were completely randomized within each block. Head fresh weight and size index were analyzed using the normal probability distribution. Least squares mean comparisons between the cultivars within types were determined using Tukey's Honest Significant Difference Test. Where there were only two cultivars within a type, differences were determined using the main effect T-test. Bolting and tipburn ratings were analyzed using the multinomial probability distribution with a cumulative log link. All possible paired comparisons among treatments were estimated with medians reported. All significances were at $\alpha = 0.05$. Data were analyzed by lettuce group to allow for appropriate comparisons of size and mass. Cultivars within each group having median bolting and tipburn ratings ≤ 2.5 and ≤ 1 , respectively, from the greenhouse experiment were selected for the following sensory evaluation experiment.

On 17 November 2016, nine cultivars of lettuce, grown in DWC using the previously described methods, were evaluated for sensory attributes and marketability by 50 untrained consumer panelists recruited from students, faculty, and staff members at Auburn University. The selected cultivars were 'Adriana', 'Aerostar', 'Monte Carlo', 'Nevada', 'Parris Island', 'Rex', 'Salvius', 'Skyphos', and 'Sparx'. The sensory evaluation experiment was approved by the Institutional Review Board (IRB) as "Exempt" under federal regulation 45 CFR 46.101 (b)(6). Sensory evaluation was conducted in Auburn University's Research Kitchen & Sensory Laboratory (Poultry Science Department, Auburn University, AL, USA). Prior to sensory evaluation, samples of each lettuce cultivar were thoroughly rinsed with tap water, dried with paper towels, and stored for 24 h at 4 °C in zipper-sealed plastic bags. Each cultivar was randomly assigned a 3-digit numerical code. The order of presentation of each cultivar to each participant was completely randomized. Unsalted crackers and water were provided to cleanse the participants' palates between samples. The samples were evaluated under fluorescent lighting

within compartmentalized sensory evaluation booths. The sample portions were individual leaves that did not include the innermost or outermost leaves. Samples were provided to each panelist individually and in succession. Sample ballots prompted panelists to rate their perceptions of crispness, bitterness, overall texture, overall flavor, and marketability utilizing a 5-point scale with descriptive anchors for each criterion. Scores of “1” indicated “not crisp”, “very bitter”, “poor texture”, “poor flavor”, and “unlikely to buy” for crispness, bitterness, overall texture, overall flavor, and marketability, respectively, while scores of “5” indicated “very crisp”, “not bitter”, “excellent texture”, “excellent flavor”, and “likely to buy”. Overall texture and overall flavor were included to accommodate any perceptions of texture and taste that may not have been directly perceived as crispness or bitterness, respectively. For example, we realized that butterhead lettuces are often crisp, but not as rigid as Romaines and other types, so we decided that an additional criterion called overall texture may capture some differences that may be missed by only including crispness. Similarly, we knew that some flavor differences exist between lettuces that may not be directly related to bitterness, so overall flavor was included as a sensory criterion to capture those perceived differences as well.

An analysis of variance was conducted in SAS using PROC GLIMMIX (SAS Institute, Cary, NC, USA) and panelist was treated as random variable. Estimate statements within the simulate method were used to compare panelist responses. Spearman correlation coefficients of medians were determined in SAS using PROC CORR. The experiment was a Randomized Complete Block Design with panelists serving as blocks. All significances were at $\alpha = 0.05$.

3. Results

3.1. Heat Tolerance of *Lactuca Sativa* L. cultivars Grown Hydroponically

The average air temperatures were 33 and 24.5 °C for day and night, respectively, and the average water nutrient solution temperatures were 29.5 and 27.5 °C for day and night, respectively (Table 2). Daytime and nighttime air temperatures were much higher than the recommended ranges of 17–28 °C and 3–12 °C, respectively [2].

Table 2. Average temperatures during a lettuce heat tolerance trial conducted in deep water culture in a greenhouse from 30 June to 29 July and from 19 August to 19 September, 2016.

| Time ¹ | Temperature (°C) | |
|-------------------|-----------------------------|--------------------------------|
| | Greenhouse Air ² | Nutrient Solution ³ |
| Day | 33.0 ± 0.9 | 29.5 ± 0.9 |
| Night | 24.5 ± 0.9 | 27.5 ± 0.9 |

¹ Daytime temperature was determined by averaging temperature values from the first datum after sunrise to the first datum after sunset each day. Remaining data from each day were used to determine average nighttime temperature.

² Greenhouse air temperature was recorded hourly for the duration of the experiment at plant height using a HOBO Pendant® datalogger (Onset Computer Corporation, Bourne, MA, USA). ³ Nutrient solution temperature was recorded hourly for the duration of the experiment using a HOBO Pendant® datalogger (Onset Computer Corporation, Bourne, MA, USA) suspended midway in the water column of each deep-water pool.

Within the Romaine group, ‘Monte Carlo’ had a higher SI (26.2) than ‘Truchas’ (20.6), but a lower SI than all the other Romaine cultivars (avg. = 34.4) except for ‘Aerostar’ (31.1). ‘Green Forest’, ‘Salvius’, and ‘Sparx’ had the highest head fresh weight (HFW) of 384.8 g, 410.5 g, and 402.5 g, respectively (Table 3). ‘Flashy Trout Back’ had the highest bolting rating (4.0), while all the other Romaine cultivars had bolting ratings of at least 2.0. The median tipburn ratings for ‘Monte Carlo’ (0.5) and ‘Truchas’ (1.0) were lower than those for ‘Green Towers’ (2.0) and ‘Jericho’ (2.0) but were similar to all the other Romaine lettuces. Within the Bibb group, ‘Buttercruch’ had a higher SI (22.9) than ‘Bambi’ (18.9), but HFW, bolting rating, and tipburn rating were nonsignificant. Within the Butterhead group, ‘Adriana’ had a higher SI (27.1) than ‘Rex’ (20.7) and ‘Skyphos’ (22.3), but HFW, bolting rating, and tipburn rating were nonsignificant. Of the two Summer Crisp cultivars trialed, there were no differences

in SI or HFW, but ‘Magenta’ had higher bolting (2.0) and tipburn (1.0) ratings than ‘Nevada’, at 1.0 and 0, respectively.

Table 3. Differences in size index, head fresh weight, bolting rating, and tipburn rating among cultivars of four lettuce types ¹ grown in deep water culture in a greenhouse for a 30-d period.

| Cultivar ² | Size Index ³ | Head Fresh Weight (g) ⁴ | Bolting Rating ⁵ | Tipburn Rating ⁶ |
|---------------------------|-------------------------|------------------------------------|-----------------------------|-----------------------------|
| Romaine Group | | | | |
| Aerostar | 31.1cd ⁷ | 278.5b | 2.0bc | 1.0ab |
| Coastal Star | 34.9abc | 242.4bc | 3.0b | 1.5ab |
| Flashy Trout Back | 34.7abc | 241.5bc | 4.0a | 2.0ab |
| Green Forest | 37.4a | 384.8a | 3.0b | 1.0ab |
| Green Towers | 33.3abc | 230.8bc | 3.0b | 2.0a |
| Jericho | 31.9bc | 244.8bc | 3.0b | 2.0a |
| Monte Carlo | 26.2d | 174.0c | 2.0c | 0.5b |
| Parris Island | 32.8abc | 196.4bc | 2.5bc | 1.0ab |
| Salvius | 37.1a | 410.5a | 2.5bc | 1.0ab |
| Sparx | 37.2a | 402.1a | 2.5bc | 0.5ab |
| Truchas | 20.6e | 78.0d | 3.0b | 1.0b |
| Bibb Group | | | | |
| Bambi | 18.9 | 112.3ns | 3.0ns | 2.0ns |
| Buttercrunch | 22.9 | 126.4 | 3.0 | 2.0 |
| Butterhead Group | | | | |
| Adriana | 27.1a | 179.8ns | 2.0ns | 0.0ns |
| Rex | 20.7b | 135.2 | 2.0 | 0.0 |
| Skyphos | 22.3b | 132.1 | 1.0 | 0.0 |
| Summer Crisp Group | | | | |
| Magenta | 25.8ns | 171.1ns | 2.0 | 1.0 |
| Nevada | 22.3 | 137.4 | 1.0 | 0.0 |

¹ Types are indicated based on marketing information from seed companies. ² Cultivars that were marketed as heat tolerant and/or slow bolting were selected for the trial. ³ Means are reported ($n = 16$). Size index was recorded at 30 DAT for each experimental run and was measured in cm as: [(Height + Widest Width + Perpendicular Width)/3]. ⁴ Means are reported ($n = 16$). ⁵ Medians are reported ($n = 16$) because discrete visual rating data are non-normally distributed. Bolting rating scale was 0 = no visible internode elongation; 1 = visible internode elongation; 2 = internode elongation with dense head forming; 3 = internode elongation and inflorescence forming; 4 = flowering. ⁶ Medians are reported ($n = 16$) because discrete visual rating data are non-normally distributed. Tipburn rating scale was based on USDA-AMS inspection instructions for tipburn and was 0 = no visible tipburn; 1 = tipburn visible with an aggregate area less than 3.2 cm²; 2 = tipburn visible with an aggregate area exceeding 3.2 cm² but smaller than 19.4 cm²; 3 = tipburn visible with an aggregate area exceeding 19.4 cm². ⁷ Values in column, within each group, sharing a letter were nonsignificantly different according to Tukey’s Honest Significance Difference Test at $\alpha = 0.05$ for Size Index and Head Fresh Weight data and estimate statements within the simulate method at $\alpha = 0.05$ for Bolting and Tipburn rating data. In the Bibb and Summer Crisp groups, which had only two cultivars each, lack of letter designation indicates statistical differences. Some medians are numerically equal but do not share common letters because statistical differences were determined by counts of discrete rating data.

3.2. Using Sensory Evaluation to Assess Consumer Preferences for Hydroponically-Grown Lettuce

Any cultivars, regardless of type, having median bolting and tipburn ratings ≤ 2.5 and ≤ 1 , respectively from the greenhouse experiment, were selected for the sensory evaluation. Criteria were chosen based on what we thought captured all the marketable lettuce and would make the sensory evaluation manageable for the participants. Selected cultivars were ‘Adriana’, ‘Aerostar’, ‘Monte Carlo’, ‘Nevada’, ‘Parris Island’, ‘Rex’, ‘Salvius’, ‘Skyphos’, and ‘Sparx’.

Median crispness ratings for ‘Monte Carlo’ and ‘Salvius’ (4.0 each) were statistically higher than those for all other lettuces, while ‘Adriana’ had a lower median crispness rating (2.0) than all the other lettuces (Table 4). ‘Skyphos’ had a lower median overall texture rating (2.0) than all the other lettuces. There were no significant differences within median bitterness or overall flavor ratings. Median marketability ratings for ‘Adriana’ (2.0) and ‘Skyphos’ (2.5) were lower than for all other lettuces, while all the others were statistically similar.

Table 4. Differences in five sensory criteria between nine lettuce cultivars tasted by 50 untrained panelists in a sensory evaluation.

| Cultivar ¹ | Crispness ² | Overall Texture ³ | Bitterness ⁴ | Overall Flavor ⁵ | Marketability ⁶ |
|-----------------------|------------------------|------------------------------|-------------------------|-----------------------------|----------------------------|
| Adriana | 2.0e | 3.0c | 3.0ns | 3.0ns | 2.0c |
| Aerostar | 3.0c | 4.0ab | 4.0 | 3.0 | 4.0ab |
| Monte Carlo | 4.0a | 4.0ab | 3.0 | 3.0 | 4.0ab |
| Nevada | 3.0d | 3.5ab | 4.0 | 3.0 | 3.5ab |
| Parris Island | 3.0d | 3.0c | 3.0 | 3.0 | 3.0ab |
| Rex | 3.0bc | 3.0bc | 3.0 | 3.0 | 4.0ab |
| Salvius | 4.0a | 4.0a | 4.0 | 4.0 | 4.0a |
| Skyphos | 3.0d | 2.0d | 3.0 | 3.0 | 2.5c |
| Sparx | 3.0c | 4.0ab | 3.0 | 3.0 | 3.0ab |

¹ Cultivars were selected for sensory evaluation based on bolting and tipburn ratings in a heat tolerance experiment conducted in a greenhouse in Auburn, AL in the summer of 2016. ² Crispness was rated on a scale from 1 to 5 with 1 being “not crisp” and 5 being “very crisp”. ³ Overall Texture was rated on a scale from 1 to 5 with 1 being “poor texture” and 5 being “excellent texture”. ⁴ Bitterness was rated on a scale from 1 to 5 with 1 being “very bitter” and 5 being “not bitter”. ⁵ Overall Flavor was rated on a scale from 1 to 5 with 1 being “poor flavor” and 5 being “excellent flavor”. ⁶ Marketability was rated on a scale from 1 to 5 with 1 being “unlikely to buy” and 5 being “likely to buy”. ⁷ Medians are reported ($n = 50$) because discrete sensory rating data are non-normally distributed. Values in columns sharing a letter were nonsignificantly different according to Estimate statements within the simulate method at $\alpha = 0.05$; ns = nonsignificant. Some medians are numerically equal but do not share common letters because statistical differences were determined by counts of discrete rating data.

Crispness and bitterness ratings were not statistically correlated, but all other rating pairs were correlated (Table 5). As expected, crispness correlated positively and most strongly with overall texture (174.8), but also correlated positively with overall flavor (43.9) and marketability (100.7). Similarly, bitterness was most strongly correlated with overall flavor (154.9), but also correlated positively with overall texture (28.7) and marketability (129.4). Overall texture correlated most strongly with marketability (223.4), but also correlated positively with overall flavor (133.9). The strongest correlation was between overall flavor and marketability (259.6).

Table 5. Correlations between sensory criteria for greenhouse-grown lettuce from a sensory evaluation conducted by 50 untrained consumer panelists.

| | Bitterness | Overall Texture | Overall Flavor | Marketability |
|------------------------|--------------------------------------|-----------------|----------------|---------------|
| Crispness | 2.28 ¹ ns ² | 174.8 *** | 43.9 *** | 100.7 *** |
| Bitterness | | 28.7 *** | 154.9 *** | 129.4 *** |
| Overall Texture | | | 133.9 *** | 223.4 *** |
| Overall Flavor | | | | 259.6 *** |

¹ Reported statistics are Spearman correlation coefficients derived using PROC CORR in SAS (SAS Institute, Cary, NC). ² ns = nonsignificant; $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***)

4. Discussion

By design, temperatures in the greenhouse experiment were higher than what is typical. We selected the hottest greenhouse we could use for this experiment in order to test the lettuce cultivars in a “worst-case” scenario. Even though all the cultivars trialed were marketed as “heat-tolerant” and/or “slow-bolting”, there were large differences in growth characteristics and in bolting and tipburn incidence, even within lettuce groupings. In the Romaine group, for example, a large range in biomass was measured, with the largest difference (136%) being between ‘Salvius’ (410.5 g) and ‘Truchas’ (78.0 g). More notably, large differences were observed in bolting and tipburn responses, which we

referred to collectively as heat tolerance. In the Romaine group, the most notable difference in median bolting response may have been between 'Flashy Trout Back' (4.0) and 'Monte Carlo' (2.0) while for median tipburn response, the largest differences were between 'Green Forest' and 'Jericho' (both 2.0) and 'Monte Carlo' (0.5). Numerically, these differences do not appear large, but since medians are much less sensitive than means, small numerical differences often indicate large biological differences. We attribute these differences to genetic diversity. The same patterns due to genetic diversity were seen in the other lettuce groups as well but were most pronounced in the Romaine group.

Some direct comparisons of growth and development responses reported by other researchers are possible since a few lettuce cultivar trials have been conducted using some of the same cultivars as the current study. An experiment conducted in West Virginia evaluated the heat tolerance of several lettuce cultivars, five of which were included in our greenhouse experiment ('Buttercrunch', 'Coastal Star', 'Nevada', 'Rex', and 'Skyphos'). The major differences between the West Virginia trial and our experiment were that the West Virginia trial was conducted in a high tunnel without active ventilation, and the lettuce seedlings were transplanted onto white plastic mulch instead of being grown hydroponically [27]. For their trial, two runs were completed from April to September. The five cultivars common to both experiments appeared to perform well and suffered little from heat, with no bolting reported [27]. In addition, the overall texture and flavor for all five of the cultivars did not receive a rating below 4 (using a scale 1 to 5; 1 = poor flavor/texture, 5 = excellent flavor/texture) [27]. These results were not similar to the results of our greenhouse experiment, in which all of these cultivars suffered at least some degree of heat-related problems. However, the two experiments differed in location and method of growing. The author did not report air or soil temperatures from the experiment, but mentioned that "record heat" was recorded during the timeframe. It is possible that lower air temperatures were experienced by the lettuce the West Virginia experiment [27] compared to the lettuce in our experiment. It is also possible that the soil and mulch temperatures in the West Virginia experiment [27] were lower than the nutrient solution temperatures in our experiment. Soil-based systems may help improve heat tolerance, but recommendations are still needed for greenhouse hydroponic systems, which allow for shorter crop cycles and greater location flexibility.

Greenhouses are more expensive structures than high tunnels and require the added fixed and variable costs of specialized growing systems and electricity. However, quicker crop cycles and easier harvesting and post-harvest handling requirements are some of the benefits of greenhouses compared to soil-based controlled environments such as high tunnels. In our experiment, 'Buttercrunch', 'Coastal Star', 'Nevada', 'Rex', and 'Skyphos' weighed 126.4 g, 242.4 g, 137.4 g, 135.1 g, and 132.1 g, respectively after 30 d. The same cultivars weighed an average of 139.5 g, 195 g, 132.5 g, 153.5 g, and 90.5 g, respectively in the West Virginia experiment [27]. While the author did not specify the exact experimental run times, he did report that two runs were conducted between April and September, meaning that each run lasted approximately 70 d. Similarly, in his conclusions, the author mentions that under normal conditions, lettuce takes 60–80 days to produce. Lettuce is not often marketed based on weight, but these data do allow a rough comparison of relative growth rates in two types of modified environments during hot weather. The trade-off of faster growth rates in our experiment compared to the West Virginia experiment [27] may, however, come at the expense of quality. The author also reported 0% bolting for 'Buttercrunch', 'Coastal Star', 'Nevada', 'Rex', and 'Skyphos', whereas those same cultivars received median bolting ratings of 3.0, 1.5, 1.0, 2.0, and 1.0, respectively, in our experiment. High temperature clearly increases bolting incidence [28], but it may also be possible that increased growth rates increase the tendency for lettuce to bolt. More research is needed to directly address this question.

Another experiment, conducted in Indiana [29], had similar results to our greenhouse experiment and used several of the cultivars that we used; however, the study did not use a soilless growing method. The author evaluated bolting and taste, along with other criteria, and reported that 'Green Towers' and 'Aerostar' suffered 50% bolting after 70 d from seed. The cultivars that did not bolt 100% until after 76 d or more were 'Aerostar' and 'Nevada'. The cultivars in the present study all suffered a

degree of bolting or tipburn by the end of each experimental run (45 days from seed). Similar to the results from the West Virginia experiment, these differences between our greenhouse experiment and the Indiana experiment may have resulted from a different growing method or from the faster growth rates in our experiment.

Using Sensory Evaluation to Assess Consumer Preferences for Hydroponically-Grown Lettuce

In the literature, sensory evaluation has been reported to be a good way to assess consumer preferences for lettuce in response to fertilization regime [30], postharvest treatment [31] and plant genetic differences [29], among others. While individual tastes certainly vary, general consumer expectations of lettuce are that leaves be crisp [25] and not bitter [26]. The results of the current sensory evaluation agree with these general consumer attitudes towards lettuce. Both crispness and bitterness, though independent of each other, correlated well to marketability (Table 5). The lower the crispness rating (lower crispness) and the lower the bitterness rating (more bitter), the lower the marketability rating (less likely to buy) and vice versa (Tables 4 and 5). Overall texture and overall flavor were also positively correlated with marketability. Since the literature indicates crispness as a quality indicator for lettuce [25] we hypothesized that crispness would correlate well with marketability. We included overall texture to account for any perceived textural differences that may have been broader than crispness because some lettuce types are known to have softer textures than others. For example, Butterhead lettuces, while crisp, are generally softer than Romaine lettuces, but are often highly desired by consumers. Overall texture and marketability had a higher correlation coefficient (223.4) than did crispness and marketability (100.7), which indicates that overall texture was a better predictor of a consumer's willingness to buy than crispness. Since consumers expect lettuce to not be bitter [25] the observed correlation between bitterness and marketability (129.4), in which lettuce that was less bitter was more marketable and vice versa, was expected. A stronger correlation was observed between overall flavor and marketability (259.6), indicating that overall flavor was a better predictor of consumer preference than bitterness, crispness or overall texture. While these findings are certainly interesting, a more comprehensive study of how specific sensory criteria affect consumer preferences for lettuce is needed.

5. Conclusions

Through an iterative process of screening for heat tolerance in a "worst-case" scenario greenhouse and evaluating consumer preferences for lettuce via sensory evaluation, seven of the original eighteen lettuce cultivars trialed can be recommended to greenhouse growers in warm climates that desire to market lettuce year round and who wish to offer more options than standard Butterhead/Bibb cultivars. The recommended cultivars from this study are 'Aerostar', 'Monte Carlo', 'Nevada', 'Parris Island', 'Rex', 'Salvius', and 'Sparx'. In addition, we found that flavor was a better predictor of consumers' willingness to buy than texture, and that overall texture and overall flavor were better criteria for evaluation than crispness and bitterness, respectively, likely due to the nuances of human palates. The step-wise nature of this study can be adapted and used to select cultivars under various production constraints to ensure both high performance and consumer acceptance.

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