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Master Thesis

Sustainable Production of Lettuce (*Lactuca sativa* L. ´Frillice´) in a Hydroponic System: Growth and Tipburn Incidence under Different Climate Regimens

Submitted by:	Jessica Aizarani
Master Programme:	Ecosystem Services
Matriculation No.:	12353
Address:	Schliebenstr. 29, WNr.2111, 02763 Zittau
Email address:	jessica.aizarani@mailbox.tu-dresden.de
Date of submission:	09.12.2021
First supervisor:	Prof. Karsten Wesche, Senckenberg Museum
Second supervisor:	Prof. Sissel Torre, NMBU

Abstract

The contamination and depletion of natural resources have fabricated the necessity to transform food systems while considering two critical aspects; development and sustainability. Therefore, Controlled Environment Agriculture including hydroponic farming has the potential to help the human race lessen ecosystem degradation. Indoor crop production has its challenges, which include those pertaining to lettuce production.

Lettuce is one of the most consumed leafy green vegetable globally that is of high economic value. Physiological disorders cause a lot of economic loss for growers. Tipburn is one of them and is very challenging to mitigate. Calcium deficiency is usually attributed to tipburn in addition to climate factors and abiotic stress.

In this study, the aim was to further understand the causes of or factors influencing lettuce growth and tipburn occurrence in order to mitigate tipburn. The literature was reviewed thematically and the results of three experiments were analyzed relating to tipburn occurrence. The climate factors studied in relation to growth and tipburn were temperature, light intensity and relative air humidity. Calcium in nutrient solution was also studied and considered as a factor of root climate. The results showed that light is the major contributor to tipburn. Low night temperature resulted in significantly less tipburn compared to constant temperature at high light intensities. Conversely, relative air humidity and the increase in calcium content in mineral solution had no significant effect on tipburn occurrence. This study highlights the importance of investigating and identifying factors contributing to tipburn for the modulation of climatic conditions of plants grown in controlled environments to mitigate tipburn.

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List of Abbreviations

C: Celcius

Ca: Calcium

DW: Dry Weight

EC: Electric Conductivity

FW: Fresh Weight

HPS: High Pressure Sodium Lamps

K: Pottassium

LED: Light Emitting Diodes

LL: Length of Longest Leaf

Mg: Magnesium

NL: Number of leaves

ROS: Reactive Oxygen Species

SJS: Safe and Just Space

VPD: Vapor Pressure Deficit

WC: Water Content

1. Introduction

With the exponential increase in the human population that is resulting in the contamination and depletion of natural resources, it has become critically necessary to consider development using a sustainable holistic approach (Lal, 2013; Martin & Molin, 2019). Moreover, climate change is close to a tipping point and the provision of food supply, as a provisioning ecosystem service, is at risk (Wallace, 2007; Russill, 2008) Wheeler & von Braun, 2013 (Wallace, 2007) (Wheeler & von Braun, 2013) (Russill, 2008). Therefore, innovative solutions such as incorporating hydroponic systems in vertical farms to grow crops has caught the interest of many researchers and growers (Touliatos et al, 2016; Qadeer, 2020). Such solutions face challenges, among which are physiological disorders. Therefore, it is important to further understand and mitigate certain physiological disorders within crop production under controlled environments in greenhouses in order to increase productivity, protect crops and make more efficient use of water and fertilizers, reduce the emission of greenhouse gases and the use of less energy.

Agriculture over the past few decades has drastically changed globally and it is still evident that this trend continues, with it being one of the most carbon intensive sectors. There are many drivers promoting this change including the rapid need to innovatively produce more in a sustainable manner. As the human race strives for improvement in the standard of living in many countries, this in hand has created a resilient demand for high value food and more specifically, out of season, high quality produce. In a country such as Norway, where it is cold and dark most of the days of the year, with little to a couple of months to grow lettuce in arable land, this is of high relevance (Heuvelink et al., 2006). Growers are interested in becoming more sustainable while achieving a higher yield of crop.

Tipburn is one of the most prevalent disorders found in lettuce. It is commonly known that it is a disorder relating to calcium deficiency, but it is still not known how and why tipburn happens. These knowledge gaps and challenges are in part due to high tipurn variability, which can even occur within the same cultivar under the same treatment. This thesis aims to add shed light on growth and tipburn occurrence in Lactuca sativa, cuv. Frillice by examining the effect of several climatic factors including temperature, relative air humidity, light intensity and calcium content in nutrient solution as a root climate factor. Investigating ways to prevent or minimize tipburn is at the core of allowing growers to become more sustainable, since this will have the potential to allow them to reduce energy, increase their profits, and reduce food waste by producing lettuce of higher quality and investing less labor-force to deal with tipburn.

2. Literature Review

2.1. Food Systems, World Food Supply and Planetary Boundaries

Global climate change poses many threats to nature and human wellbeing via its effects on ecosystem services. Food security has become a global over the past years, due to the increase in population size and the intensification of natural resources overuse. According to the World Bank, agriculture provides employment for around 1 billion people; this includes the early stages of growing and harvesting, up until the disposal stage of food and food related items. One of the most significant threats that will exponentially show effect is water scarcity. Crop losses have exhibited an increase worldwide due to climate change and extreme land use changes, decreasing availability of arable land. One third of the total Greenhouse Gas Emissions (GHG) is driven by agriculture. (Engler & Krarti, 2021).

Conventional agriculture including but not limited to monocultures has yielded a lot of stress on nature and is by far the major driver exceeding the planetary boundaries. It is estimated that 80% of deforestation is caused by agriculture. Figure 1 shows the extent to which the human race has contributed to crossing these planetary boundaries. Genetic diversity, reported as the extinctions per million species-years (E/MSY) and biogeochemical flows, have far exceeded the safe operating space (Steffen et al., 2015)

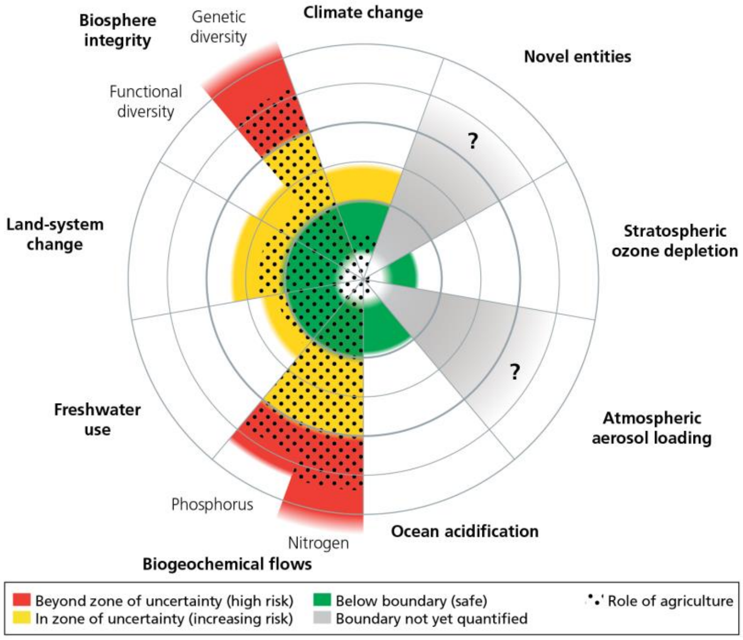

Figure 1: Planetary Boundaries and their current status. Colors green, yellow and orange present the safe operating space, zone of uncertainty (increasing risk) and beyond zone of uncertainty (high risk). (Steffen et al., 2015)

Economist Kate Raworth has developed the safe and just space (SJS) framework that dictates „the doughnut of social and planetary boundaries". This has gained a lot of interest due to an interdisciplinary approach that combines both social thresholds to assure human rights in addition to an ecological ceiling to prevent significant planetary degradation (Raworth, 2012). Figure 1 represents the planetary boundaries only as opposed to the social equity point of view of the "doughnut".

Although many countries are aiming to be more sustainable and achieving the Sustainable Development Goals (SDGs), especially advanced countries such as Norway, no country has been able to achieve securing a social threshold while providing basic needs to its people without transgressing the biophysical boundaries. In other words, and according to the knowledge we have today, countries that have high standards of living over-use natural resources, while other countries don’t reach a level of social security but are sustainable. Countries such as Germany and Norway are in the same category (Figure 2), with a high overshoot on resource use, and a low shortfall with regard to the social indicators and this is to persist if drastic change does not occur. According to Fanning et al., there also is no evidence to support the notion that nations are going in the right direction to achieve both the protection of nature and social equity. (DeFries et al., 2005; O’Neill et al., 2018)

According to Figure 1 and the literature, the negative impact of agriculture on biophysical boundaries is clear. With the global catastrophe of biodiversity loss and extensive land use, whether it is due to erosion, salinization, compaction, acidification and chemical pollution, soil has been moderately to highly degraded by 33% with the possibility of this to increase to 90% by 2050, which signifies that there is even more carbon release from the soil into the atmosphere, leading to more global warming (FAO and ITPS, 2015; Lal, 2020).

Figure 2: Germany as an example of past and projected planetary boundary transgression on the national level according to the SJS. (Fanning et al., 2021).

The economic cost of soil degradation of the EU is estimated to be in the order of tens of billions of euros annually, as it can take up to 1000 years to produce just 2-3cm of soil. In addition, soil erosion on arable or intensively grazed lands is 100-1000 times higher than natural soil erosion rates. Based on the World Bank in 2020, approximately 70% of global freshwater is used for modern agricultural purposes. Water contamination and the amount of water used will lead to a decrease in access for future prospects (DeFries et al., 2005; Boretti & Rosa, 2019). Food systems are complex interdisciplinary systems that include three pillars; environment, social and economic. An understanding of all three pillars is necessary to clarify the role and boundaries of the world food supply (Grace et al., 2021). Sustainability is similar and has the same, as we protect nature and solve climate change, ensure financial security amongst all in a functioning society, and improve social equity. As the population size increases, we are in need of more land to implement agricultural practices. Implementing ideas such as vertical farms using growing methods such as hydroponics is therefore, a good start.

2.2 Hydroponics in Greenhouse Lettuce Production

Defined as any shelter that can cover crops as they grow, greenhouses have been of keen interest ever since they were first introduced by the Romans who used them to grow exotic plants. As humans are always on the lookout for new innovative ways to become more sustainable and use the least resources possible, hydroponics was founded and coined by plant physiologist Dr. William Frederick Gericke first in 1929 as “aquiculture” then later as hydroponics in 1937 as a method to grow crops instead of just using it in a research setting. Hydroponics was found to be an innovative method to increase crop production under controlled conditions. This is accomplished by supplying the plants with the appropriate and balanced nutrients in solutions (Hershey, 1994)

The technology-based approach to crop production, called Controlled Environment Agriculture (CEA), provides an optimization of climate factors to increase crop productivity and quality (Ting et al. 2016). This also allows the flexibility in which plants and therefore, food to be grown in almost any location, creating agricultural opportunities in typically infertile areas such as cities and deserts (Nelkin & Caplow, 2008). Systems such as hydroponics are usually installed in such greenhouses or plant factories[1].

Leafy vegetables that are short seasoned such as lettuce are commonly grown hydroponically with the fairly simple design of the NFT system (Figure 3), where a nutrient solution is shallowly pumped into the gutters where the lettuces are placed, allowing the roots to absorb the nutrients from the film of water and thus supplying them with the nutrients needed for their development and growth (Mohammed, 2018; Ahmed et al., 2021). The NFT technique allows growers to use as little water as possible, since the water is being recycled back into the tray suffers from salt buildup and growing media is minimal. In Norway, most lettuce

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[1] Plant factories, also known as vertical farms, are closed and fully controlled growing systems all year round (Kozai et al., 2015)

production is grown hydroponically while using the NFT. A detailed description the NFT system can be found in the Materials and Methods section.

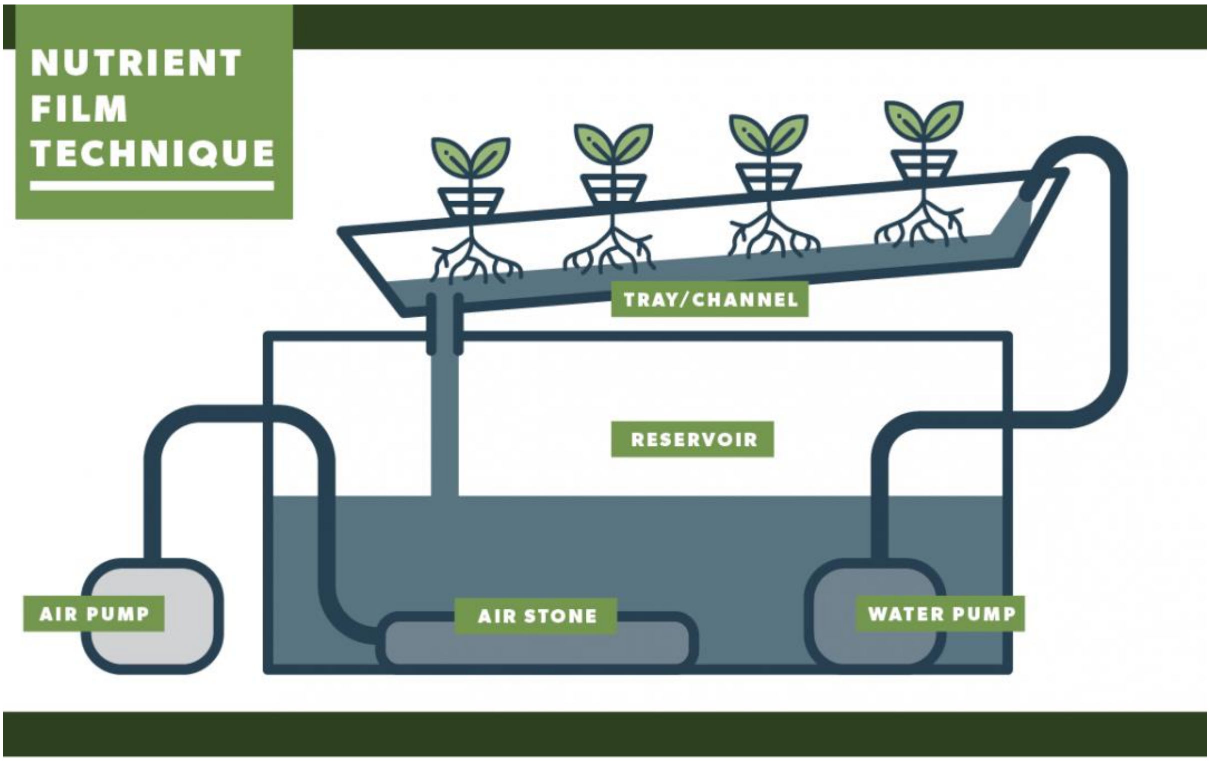

Figure 3: Nutrient Film Technique (NFT) Hydroponic Setup Illustration (Soto, 2020).

The combination of both the cost of setting up an NFT system and its potential with respect to both quality and quantity has made this technique to be commonly used by and of interest to growers and for further research to find ways to enhance the system while reducing costs.

In greenhouses, air flow techniques for optimal air distribution such as the use of fans or vents in the ceiling are very important. This air flow must be maintained between 0.2 and 0.7 meters per second. If this is not the case, then relative air humidity is impacted as well as the CO_2, which will result in negative impacts on the crop being produced.

Not only does the energy consumption of hydroponic systems pose a threat to environmental and ecological systems, but also are a pillar in deriving a sustainable business strategy in a rather competitive market in the agriculture sector. Energy consumption is by far the greatest of all operational costs of a hydroponic system. Energy consumption in a greenhouse accounts up to 50% of the total operational costs, 30% of which is related to lighting, making energy consumption the main cost factor (Shen et al., 2018) (Watson et al., 2018). A lot of research is being done on energy consumption with the aim to achieve a lower energy consumption in hydroponics and climate-controlled environments. Similar to any reduction scheme, lower energy consumption can be achieved by implementing a series of actions encompassing the appropriate usage of equipment and the right organization of production planning while considering the decreased resources.

Technological advancements are always sought when talking about indoor agriculture, however it is possible to reduce energy consumption with methods that are already available to ensure the utilization of as little amount of energy and water for a good harvest and with the least impact on the natural environment. Using LED lamps has become of great interest due to a lesser heat generation, lower demands for watering, and with a light span that is longer compared to HPS (Matysiak et al., 2021).

One method is utilizing outdoor temperatures to regulate the climate in the greenhouse, for example, high day temperature and low night temperature. to allow the climate in the greenhouse to follow the outdoor temperatures, like a high day temperature (DT) and low night temperature (NT). Using this method, operational cost savings summed ranged between 20-38% (season dependent) of the total operational costs in 2 plant factories in Denmark (Aaslyng et al., 2003).

Nowadays, growers seemingly are more concerned and critical when it comes to the growing system, pest control, structures, site selection and markets. CEA is still considered to be an expensive option, due to the heating and cooling costs associated with the energy consumption costs, as mentioned previously. However, with future advancements in raw material and energy sources, this will indefinitely become a cheaper and better option. In addition, soil borne pathogens are a major driver to delve into methods such as hydroponics.

2.3. Tipburn

Plants grown in soil and hydroponic systems are both susceptible to physiological disorders. A common irreversible physiological disorder of lettuce (Lactuca sativa) is called tipburn which occurs more in hydroponic media compared to soil, and causes economical losses as well as losses in crop quality for growers and thus consumers (Uno et al., 2016).

Tipburn, a physiological disorder commonly found in lettuce, is considered to be a form of necrosis that has the potential to impact the tip of both young and mature leaves (Carassay et al., 2012). Calcium is theorized to have an important role to play in the formation of tipburn. Moreover, such occurrences is strongly correlated to calcium deficiency in young leaves and the presence of high levels of soluble salts in mature leaves. This causes a drastic change in cell wall structure and cellular signaling, which is visible as a brown-black coloration along the leaf margins due to necrosis (Aloni et al., 1986; Périard et al., 2015).

Although a lot of research is being conducted as to why and how tipburn occurs, reliable methods to mitigate it still remain a mystery for plant scientists and growers (Uno et al., 2016). Research have sought to alter climatic conditions under controlled environment and finding the most tolerant cultivar to secure marketable, healthy and sustainable lettuce. Similar treatments result in different results and so the search persists to learn cause of and how to prevent tipburn from occurring in crops. Tipburn in lettuce usually only become visible when the harvest draws near. The increase in relative growth rate of lettuce as it matures is considered to be one biological factor that induces more tipburn development. An association between relative growth rate (RGR) and tipburn have been investigated whereby studying a variety of cultivars showed that lettuces without tipburn had low RGR while those with severe tipburn had high RGR (Lee et al., 2013).

Having mentioned that tipburn is mainly theorized to be due to calcium deficiency, calcium absorption is a key point to further discuss as the lack of Ca^{2+} seems to be tied to a lacking ability of the plant to transport said calcium to where it is needed rather than a missing ability to absorb it from the growth medium. This ability to take up calcium from the growth medium is in turn affected by many abiotic factors including pH, root temperature and water stress. In order to mitigate the occurrence of tipburn, studying and understanding the factors that cause it is key. Calcium uptake takes place from the root up into the shoot of the plant via the transpiration stream alongside the water flux. Conditions such as high light and high temperature may inhibit such a process and disrupt the transport, leading to an improper distribution of calcium to the different tissues of the plant. Another study conducted by Hartz et al., 2007 showed that it is the environment rather than availability of calcium that has the strongest impact on tipburn occurrence. Calcium foliar sprays' effectivity is variable across different species; sometimes showing positive results and other times, the opposite. There are many supporters of the idea that environmental stress has a heavier impact on the formation of tipburn. However, much of the literature mentions that environmental stresses and calcium levels are interlinked to one another in a more complex manner (Thor, 2019).

The lettuce cultivar of interest for this thesis is Frillice, which is a cross between iceberg and crisphead and is more tolerant to tipburn. According to Cox et al. (1976), crisphead lettuce is more tolerant to tipburn compared to leaf lettuce and butterhead lettuce.

Commonly, inner tipburn is more of a concern for growers globally, however, with Norwegian growers, tipburn in the outer leaves is more prominent and acts as an economic and sustainability threat. It is important to mention, nonetheless, that inner tipburn does occur in greenhouses in Norway, but it is less of a concern as its incidence is substantially less than that of outer tipburn.

2.4. Abiotic Stress and the Role of Calcium

Whether in indoor hydroponic systems or out in the field, plants including lettuce are sessile organisms that are forced to cope with the environment around them when faced with any stress, be it biotic or abiotic.

This in turn has allowed them to develop complex molecular and metabolic networks to tackle stress by exploiting certain gene regulatory networks, signaling pathways, metabolites and acclimation responses (Thor, 2019; Shao et al., 2006).

Abiotic stress allows the plant to trigger certain responses that impact growth and development both directly and indirectly. When plants experience stress, ionic and water homeostasis and cellular stability are maintained via a stress signaling response, which comprises proteins involved in ion and water transport as well as metabolic and gene expression regulation (Saddhe et al., 2020). High sodium, low potassium, excess magnesium or high pH~~,~~ lead to a stress response involving cystolic Ca^{2+} signaling. This evidence highlights the importance of calcium's role in initiating the plant's defense mechanisms against environmental and nutritional stresses (Zhu, 2016).

In both homeostatic and stress conditions calcium is one of the most important elements for plant growth and development. As a macronutrient, calcium is used as a divalent cation by the plant to maintain structural integrity of cells, providing rigidity and stabilization of the cell wall and plasma membranes. Moreover, it acts as an intracellular secondary messenger, playing crucial roles in plant growth, fertilization and stress. Calcium is dependent on the flow rate of xylem and the duration of transpiration which enables the transport of the proper amounts of calcium to all parts of the plant (Gilliham et al., 2011). Levels of calcium within organelles and the cytosol of the plant cell must always be regulated to fit into the confined range of calcium concentration. Different organelles have different calcium concentrations and limits. For example, not only does the vacuole comprise of 90-95% of the cell's volume but also is the organelle that holds the highest concentrations of calcium within a cell (1-10mM) whereas the calcium concentration within the endoplasmic reticulum can range from 1-5mM, while chloroplasts have a much lower calcium concentration (0.1–10 μM). The concentration of calcium within the cytosol needs to be maintained between 100 and 200 nM in normal conditions and increases to almost 2 μM as a result of certain stimuli or stresses (De Freitas et al., 2016). After exposure to one or more abiotic stresses, it is necessary for the cell to maintain the concentration of calcium at extremely low levels to avoid calcium toxicity and downstream cell death. As part of the defense mechanism of a healthy plant, an abiotic stress will lead to an influx of calcium into the cytosol, allowing the concentration of calcium ions to increase which will lead to the activation of defense mechanisms. This is not to elucidate the occurrence of high levels of Ca in outer leaves that do not have tipburn. Moreover, necrosis which comprises damage and rupturing of cells relies on perturbed and high levels of intracellular calcium.

Phytohormones including but not limited to absisic acid (ABA), gibberellins (GAs) and brassinosteroids (BRs) are plant hormones that regulate plant growth, organ development, and are involved in environmental responses. Developmental roles include stem elongation and leaf expansion, whereas GA's important roles in plant development and growth underlie their perturbation during abiotic stress. Calcium, hence plays an important role in phytohormone signaling pathways, through calcium-dependent protein kinases (CDPK) and thus impacting GA (Mittal et al., 2017).

2.4. Reactive Oxygen Species (ROS)

The production of ROS is an ongoing process for plants, since is involved in basic cellular metabolic processes such as respiration (Haider et al., 2021). Activation and reduction of oxygen results in ROS. The concentration of ROS within plant cells will determine its role, either as a second messenger, mediating physiological signal transduction when the concentration is low or causing damage that will lead to apoptosis or programmed cell death when the concentration is high (Tripathy & Oelmüller, 2012). For the purpose of this thesis, the latter role will be further explained. In plants under abiotic stress, by-products of metabolism and biochemical reactions include hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet -}$), hydroxyl radical ($OH\bullet$) and singlet oxygen ($^1O_2$) increase.

The ROS network/antioxidant defense systems, which comprise both enzymatic and/or non-enzymatic mechanisms, are responsible for tightly controlling the concentrations of ROS. When this network is unable to perform its function properly, oxidative stress occurs (Tripathy & Oelmüller, 2012). Defined as a disruption of the balance between oxidants and antioxidants, oxidative stress is generated endogenously and exogenously. Redox enzymes play a significant role in oxidiative stress. Two examples of redox enzymes are Superoxide dismutase (SOD) and catalase (CAT). SOD, as an oxidoreductase dismutates the superoxide anion and breaks down ROS, while CAT, a heme enzyme present in the peroxisome of aerobic cells, converts the ROS hydrogen peroxide to water and oxygen. Non-enzymatic antioxidants include ascorbate, which is a major metabolite in a cell, and glutathione, which reduces passive sodium influx, thereby increasing tolerance to salt stress.

2.5. Climatic Factors and their Significance | System Parameters

Climatic factors include factors such as light, temperature, relative air humidity and CO_2 and are essential for the healthy growth and development of all plant species with lettuce being no exception. System parameters such as EC and pH are also very important to maintain good growing conditions. Optimal conditions are highly dependent on the cultivar planted, with different species having widely varying needs. Climatic factors are very difficult to isolate as they are closely bound to each other and small variations in one can have massive impact on another, making finding the optimal growing set up for specific cultivars can be challenging in the extreme.

2.5.1. Light

Without light, photosynthesis would be an impossible task any light requiring plant, including every type of lettuce. Access to light is thus of paramount importance to any food crop, and lighting regimen for the crop is closely monitored as far as this is possible. Light is as such considered one of the most important factors affecting the growth and development of crops (Cometti et al., 2012; Wang et al., 2019). Throughout a plant's life cycle, light has a plethora of roles to play including plant development, morphogenesis, biosynthesis of cell components and regulation of gene expression (Wang et al., 2015; Jiao et al., 2007). Due to crop light demands in greenhouses and the variation of natural light conditions in the winter season in countries such as Norway, growers end up with reduction of dry matter accumulation meaning the light regimens are not as viable as those from a field. This "mismatch" between natural and light house light conditions also lead to a decrease in photosynthetic rate due to nonstomatal limitation, the carboxylation efficiency and high ribulose biphosphate[2] (RuBP) regeneration rates (Szymańska et al., 2017). The aforementioned unoptimized light stress would results in making the crop more vulnerable to both disease and stress reactions while slowing down both growth and development; this again makes the plants more susceptible to pests and results in more fallen/dead leaves and a loss of biomass production. In light of the above, studying light conditions in greenhouses is an essential part to further the enhancement/development of greenhouse technology in the context of light environment control (Ruangrak & Khummueng, 2018).

A variety of lamps has been used historically to set up indoor hydroponic systems. Originally metal halide lamps and high pressure sodium lamps, with newer development within the field of LED lighting systems having led to more energy effective lighting setups in an attempt to enhance the energy consumption of greenhouses that use artificial light. Although MH and HPS lamps are high-intensity discharge gas discharge lamps, they use differing modes of operation. To produce light, MH utilizes vaporized mercury and metal halides, while HPS lamps use sodium in an excited state. Dougher and Bugbee emphasized on the increased leaf expansion rate, increased radiation capture and increased growth by around 25% under High Pressure Sodium Lamps compared to metal halide lamps which have only 6% compared to broad spectrum light with a 30% blue light (400 to 500nm). This shows that the two light sources have varying impacts on the food

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[2] Ribulose biphosphate is a significant compound within the Calvin cycle. It contains 5 carbons and a phosphate on each end, taking part in light independent reactions of photosynthesis. With the help of RuBisCO, a 6 carbon compound is formed from atmospheric carbon.

crops they are used to illuminate. HPS lamps have a variety of benefits; they have a longer lifespan and do not produce mercury infrared or UV light (with the light produced by these having a wavelength of around 589nm). Although LEDs reduce energy consumption (decrease costs to power a growing area) while being photosynthetically efficient, it is important to note that there are many challenges especially within the context of tipburn. Experiments having shown an increase in tipburn incidents when LEDs are used as light sources (Matysiak et al., 2021).

The wavelengths of light that the plant can utilize for photosynthesis are shown in Figure 3. These are defined as the spectral range of solar radiation, i.e Photosynthetically Active Radiation (PAR) range of plants (blue to red light) (Figure 4). It is important to note that what is of interest is the Photosynthetic Photon Flux Density (PPFD), defined as "the number of photons in the 400- to 700-nm waveband incident per unit time on a unit surface" (Rabinowitz & Vogel, 2009). The unit used for PPFD or light intensity is $\mu mol/m^2/s$). A relatively high PPFD can have a negative impact on young leaves due to high transpiration rates which may result in calcium deficiency (Sago, 2016)

Some characteristics of light include light quality and light intensity. Light quality is defined by the previously described measure of PAR in nm, whereas the intensity is the PPFD, and it is important to mention the photoperiod is the period in which the plant is exposed to light in the span of a day. Photoperiodism is the functional reaction of a plant to daylength. This allows the plant to adapt as environmental changes occur, with too much variations either way resulting in stress reactions to compensate. Too little light and the plant will seek out "more" by focusing on leaf production and upwards growth to the exclusion of all else. While an overabundance of light will result in stunted growth as the plant tries to shield itself from both high transpiration rates (as the amount of photosynthetic reactions rise the need for water increases drastically) and excess levels of energy absorption by its photocenters resulting in dangerous redox reactions occurring within the leaves themselves.

An increase in light might help with creating more resilience and adaptation to light stress, as seasonal shifts occur. This allows the plant to adjust its growth and development accordingly.

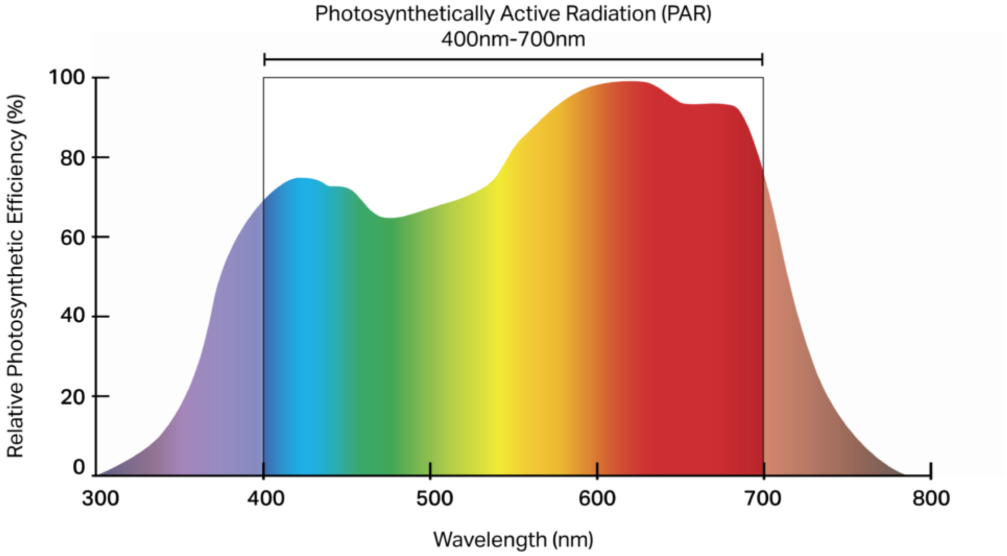

Figure 4: Relative Photosynthetic Efficiency (%) as a function of Wavelength (nm). The PAR range is indicated (Grow Light Spectrum Explained, 2020).

2.5.2 Temperature

The optimum temperature across species and cultivars differs. Similar to light quality and intensity, temperature is also an important factor that influences plant growth and development, impacting functions such as photosynthesis, transpiration, respiration, germination, and flowering (Gray & Brady, 2016). By sensing temperature changes, whether warm or cold, plants have the ability to adjust their biochemical composition as a survival and adaptation mechanism (Ruelland & Zachowski, 2010). Plant temperature (T_P) and air temperature (T_a) are dependent characteristics, and usually T_a is used as an approximation of T_P due to the complexity of measuring T_P through the meristem of a plant. However, new research has shown that T_a might significantly diverge from T_P (Savvides et al., 2013). The plant's ability to increase its internal temperature via irradiance and decrease it via transpiration is proof that T_p and T_a are indeed not equal all the time. Plant temperature is decreased via transpiration and increased via irradiance when the air temperature is high and low, respectively (Yu et al., 2015). The plant temperature at the top of the plant canopy will have larger fluctuations in temperature and this may be due to the presence of more stomata at the tips of the leaves of the lettuce (Figure).

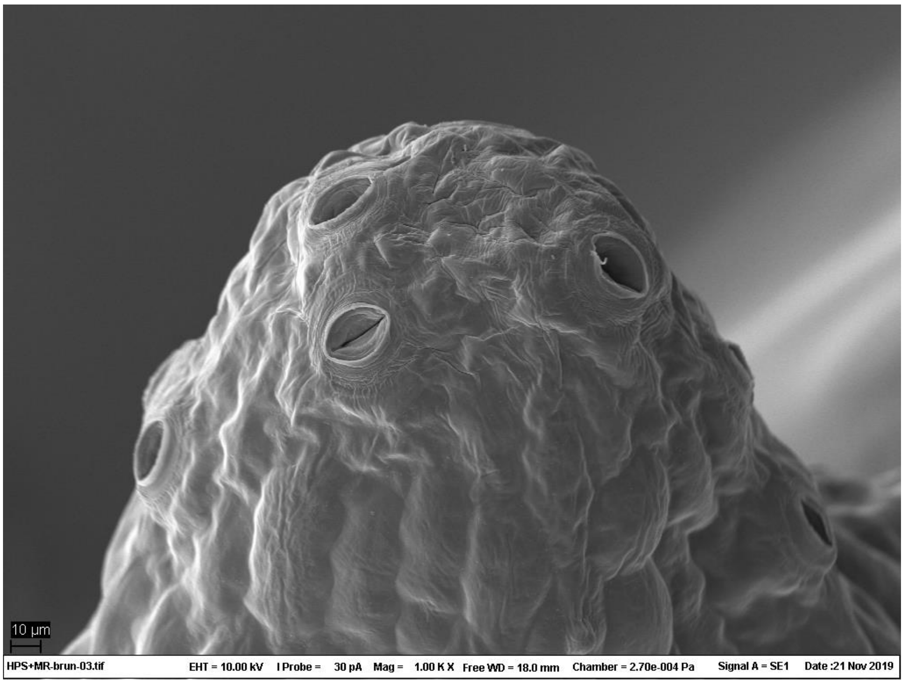

Figure 5: Stomata at the tip of a lettuce leaf.

An increase in root temperature (12°C to 25°C) have been shown to have a positive effect on plant growth, improving water and nutrient supply to the shoots, decreased R:S[3], improved gas exchange, and increased chlorophyll content (Awal & Ikeda, 2003) .

The optimal temperature for lettuce is between 15-

Enzymes and their catalytic functions are also most often temperature dependent, whereby a decrease in temperature below the threshold yields inactive enzymes and an increase beyond the threshold leads to the denaturation of the enzymes, i.e an irreversible reaction that involves the unfolding of the enzyme structure. Most proteins start losing integrity and enzymatic function certain temperature thresholds are passed, thus, halting/disturbing most biochemical and metabolic processes are very dependent on a somewhat stable temperature.

2.5.3. Humidity

Humidity is a climatic factor that is dependent on temperature and pressure. The relationship between humidity and temperature is inversely proportional. The relative air humidity, on the other hand, is an indicator of how much water vapor is in the air, compared to how much it could hold at a given temperature. It is calculated as a percentage, whereby the partial vapor pressure (P_{H20}) is divided by the equilibrium or

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[3] The ratio of root biomass to the shoot biomass (Rogers et al., 1995)

saturated vapor pressure (P^*_{H20}). Condensation occurs if RH is more than P^*_{H20} and evaporation occurs when RH is less.

Vapor pressure deficit (VPD) is a major measurement in greenhouses, since it positively correlates with transpiration rate and thus is very important for regulating/influencing photosynthesis and stomatal functions (Inoue et al., 2021). It is the difference between the amount of moisture present in the air and the potential of how much moisture the air could potentially hold when saturation is achieved. Opposed to the RH measurement, temperature and VPD are proportional if the humidity remains constant. A very high VPD is problematic since water content of air is higher with higher temperatures, allowing the plant to transpire a lot more than it can efficiently transport. On the other hand, a very low VPD prevents the plant from cooling down, impacting nutrient supply to the leaves and the transport of water. This makes condensation, even with minimal drop in temperature, very likely, whereas evaporation demand decreases. (Amitrano et al., 2020). Moreover, extreme fluctuations in VPD result in a decrease in biomass, stomatal conductance and assimilation rate[4] (Inoue et al., 2021)

Under high temperature and high relative air humidity, the guard cells allow the stomata to open, increasing stomatal aperture, which leads to an increase gas exchange (Lawson, 2009). When the humidity is low, stomatal conductance decreases along with growth rate. In butterhead lettuce, a relative air humidity of below 85% resulted in a faster growth rate compared to that below 50% (Tibbitts & Bottenberg, 1976).

2.5.4. Potential of Hydrogen (pH) & Electric Conductivity (EC)

Whether plants are growing in soil or in hydroponics, pH is a very significant factor to keep within specific set boundaries. Lettuce in hydroponics prefers a slightly acidic pH between 5.6 and 6.2; this also happens to be the case for most plants. A more challenging aspect of hydroponics, compared to conventional farming in soil is to maintain the pH within the optimal range. This challenge is due to the lack of a buffering capacity in hydroponic systems as compared to a traditional soil growing environment, which results in more and higher fluctuations within the pH scale of the system. As pH fluctuates to undesirable values, the plant tends to lose its ability to absorb the required nutrients provided by the medium it is growing in resulting in a negative impact on growth rates. The severity of the increase or decrease in pH relative to the optimal range dictates nutrient absorption and can lead to a multitude of growth problems as the plants try to cope with a hostile pH environment.

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[4] It is the assimilated CO_2/unit leaf area/time. Unit: mol/m^2/s

EC, on the other hand, is the electric flow water is capable of passing, thus it is the total amount of dissolved mineral salts in nutrient solution. Lettuce is known to be a salt-sensitive plant, which is why several studies have addressed the impact of several EC levels in the case of saline nutrient solutions. In an NFT system, fresh weight of two cultivars of lettuce (romaine and iceberg lettuce) differed significantly in Pasternak et al's study when EC levels were adjusted to 8.2 or 10.5 dS/m and an EC level less than that (5.6 and 7.6 dS/m) resulted in poor growth. Tolerance for salt concentration in iceberg lettuce is lesser than it is for romaine cultivars, though both are considered to not to not be salt-tolerant (Pasternak et al., 1986). Yields of curly endive lettuce were significantly decreased with the increase in salinity (Shannon et al., 2000). As mentioned before, Frillice is a result of iceberg x curly endive, so it can be speculated that it is also less tolerant compared to other lettuces.

3. Objectives

- To investigate the relationship between several climatic factors and the occurrence of lettuce tipburn in a hydroponic system.
- To improve the understanding of why tipburn develops in lettuce and to test cultivation methods that might mitigate or reduce the occurrence of tipburn.

3.1. Specific Aims

- To inspect if low night temperature reduces the occurrence and severity of tipburn in Lactuca Sativa 'Frillice'.
- To test if varying relative humidity (across 24 hours) reduces tipburn occurrence in lettuce.
- To test the effect of light intensity on the occurrence of tipburn in lettuce.
- To explore whether a high calcium content in the nutrient solution reduces tipburn occurrence in lettuce.

4. Materials and Methods

4.1. Plant Material and Preparation

For the purpose of this study, Frillice lettuce (*Lactuca Sativa L. 'Frillice'*) was the plant of interest. All lettuce plants that were used for the experiments explained in this chapter underwent the same pre-cultivation method mentioned below.

The seeds, supplied by Norgro in Norway, were sown 4-5mm deep in biodegradable pots. Each pot of 0.08 liters included one seed and was completely filled with fertilized peat soil ("Degernes torv"), functioning as a growing medium provided by Degernes Torvstrøfabrikk AS (Norway). It is important to note the importance of using peat soil for pre-cultivation, as it allows the lettuce to grow more resiliently. As practiced in most greenhouses, the seeds were also coated with fungicides. For germination to take place, the pots were put in a dark room at a temperature of 15 degrees Celsius and with relative humidity of 60% for a timeframe of four days.

After germination occurred, resulting in seedlings, the pots were placed in a greenhouse compartment until each of the seedlings produces five true leaves, which requires approximately three weeks. Within these compartments, the seedlings are placed and allowed to grow in a consistent climatic environment having a temperature of 20°C and relative humidity of 60 %. As for the light source, 400 Watts High Pressure Sodium lamps (HPS) were used for a cumulative of 18 hours, combined with a photon flux density of 150 $\mu mol/m^2/s$. In order to maintain the temperature and relative humidity within the greenhouse to optimize the environment for the seedlings to grow, a Priva Climate Computer was used, provided by the high-tech company, Priva, specialized in climate control alongside energy saving and optimal reuse of water.

As a mechanism to lower the temperature when it reached above 20°C or to increase RH when it reached below 57%, sprinklers in the roof were activated, which humidify the air with water for 10 seconds each time the threshold is met. This was also the case when external irradiance reached 300 Watts. The vents in the roof of the greenhouse opened when the temperature was greater than 23°C.

4.2. Growth Chambers and Experimental Design

Figure 6: Chamber setup with an NFT Hydroponic System (Knoop, 2019)

The lettuce was transferred from the greenhouse, after they reached the 5-leaf stage, to the enclosed chambers where they would get artificial light from then onwards. Each growth chamber, equipped with a hydroponic system, included 40 plants that were randomly distributed in the 10 holes of each of the four rows of gutters (Vefi AS, Norway). The distance or spacing between each of the 4 gutters was 25cm and 15cm between the holes of each gutter. The length and width were 1.5 m and 10 cm respectively. One end of the gutter was open so that water can leave and be collected by two transparent plastic boxes, whereas the other end had a hose, a pump (Aquarium Systems Maxi-Jet 500, France) and a timer (Müller SC 28 11 pro, Germany) in order to ensure the flow of water and the nutrient solution into the gutters (Figure 6)

Figure 7: Priva aspirated sensor box along with the planted lettuce in gutters. Photo: Niveditha Umesh Katyayini

An aspirated sensor box (Figure 7) was distended in the air to measure the temperature and air humidity of the chamber in which the hydroponic lettuce is growing. It is important to note that the sensor box involved both wet and dry sensors, which signifies the ability to efficiently measure the temperature and air humidity. These sensors are connected to a climate computer which monitors the climatic conditions closely so that they remain within the range of temperature/humidity presented in the experimental setup.

The hydroponic system technique used for all three experiments was the Nutrient Film Technique (NFT). For further explanation, refer to the "Hydroponics" subsection in the literature review.

Three experiments were performed, two of which are more interlinked than the third. The first two experiments were conducted at two different light intensities. In Experiment 1, the effect of two different temperatures on lettuce growth parameters and tipburn were tested, and in experiment 2, the effect of relative air humidity with high summer temperatures as a background on lettuce growth paramaters and tipburn were tested. In experiment 3, calcium's role in tipburn occurrence was investigated. To assess tipburn, which is at the core of this study, one scoring method was applied (Section 4.6.).

4.3. Lighting

The growth chambers contained the light source at the top of the ceiling and depending on the experiment, either HPS lamps (400 Watts each) or light emitting diodes (LEDs) provided by Gavita, Norway and Evolys, Norway respectively, were used. The light was measured using a quantum meter (Li-520A light meter, Li-Cor, USA) in order to ensure that the right light intensity was provided to the plants in the gutters and a photon flux density was measured between 400 – 700 nm wavelengths, while the chamber doors are closed. The correct light intensity was maintained via a net around the lights in the ceiling and by elevating the gutters to the right height with the help of crates. Light was either 150 or 300 µmol/m^2/s, depending on the experiment. The light intensity was measured and checked from different parts of the chamber at different times and so a measurement +/- 10% was acceptable (Figure 8)

Figure 8: Optronic model 756 spectroradiometer for spectral measurement composition (left) & Quantum meter used to measure the light intensity (right) (Knoop, 2019)

4.3.1. Spectral Composition

A spectroradiometer (Optronic model 756), from Optronic Laboratories in the USA, was used to measure the different spectral compositions and irradiance levels of the optical radiation sources of both HPS and LED lamps. Similar to the measurement of light intensity, the doors of the chambers were closed and the measurement was done. The method used was similar to that mentioned and explained in Suthaparan et al., 2018. The following Figures depict the spectral composition of HPS and LED White combined with Far Red. (Figure 9 and 10).

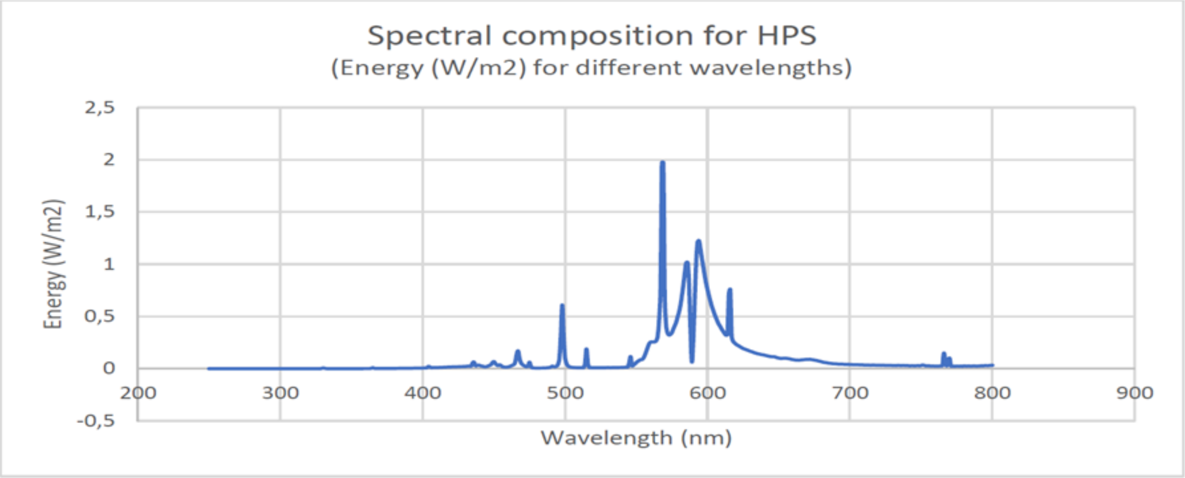


Figure 9: Spectral composition of HPS lamps (400W), used in the greenhouse and chambers in experiment 1&2. R:FR *=3.7*

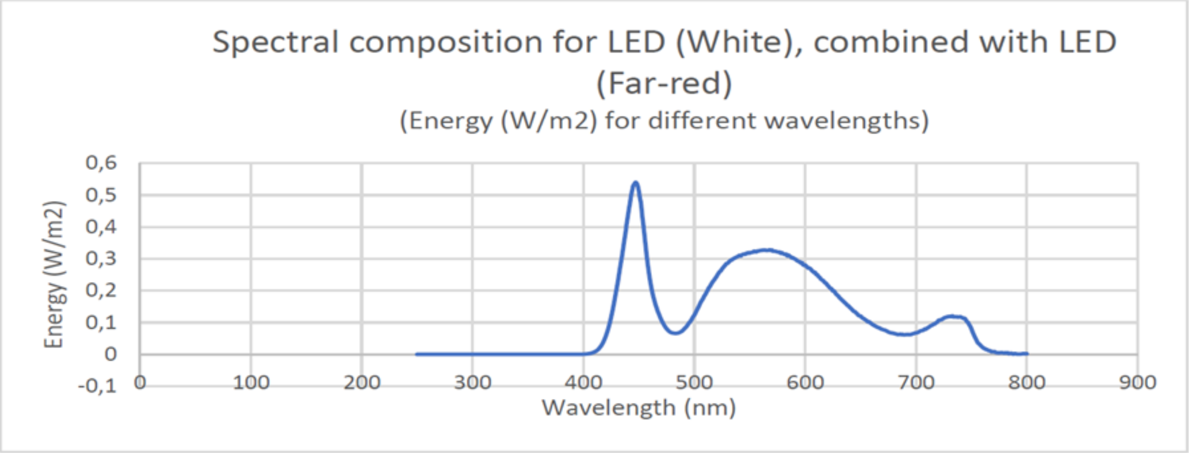


Figure 10: Spectral composition of LED lamps used in experiment 3. R:FR= 1.1

4.4. Experiment 1. Effect of different temperatures/ low night and light intensities on tipburn

In experiment 1, the effect of two different daily temperatures were tested, one treatment with constant daily temperature and another treatment with a higher day temperature (DT) than night temperature (NT) but an equivalent temperature sum (Table X). Each treatment consisted of 40 plants, each representing a replicate of the other with varying number of leaves, however, only 10 were randomly selected and assessed for tipburn.

Both conditions were subject to two different light intensities, 150 and 300 µmol/m^2/s, while using HPS lamps as a light source. A photo-period of 16h and temperature sum of 416 ^{o}C were similar in both treatments (Table 1)

Table 1: Treatments in experiment 1. Moderate Light Intensity= MLI, High light intensity= HLI, Constant Temperature = CT, Low Night Temperature=LNT

Treatment	Photo-period (hr)	Day Temperature (°C)	Night Temperature (°C)	Photon Flux Density (µmol/m^2/s)	Relative Air Humidity (%)	Temperature Sum (°C)
CT/ML	16	18	17.5	150	80%	416
LNT/ML	16	20.3	14.5	150	80%	416
CT/HL	16	18	17.5	300	80%	416
LNT/HL	16	20.3	14.5	300	80%	416

The plants were grown with a complete nutrient solution, which was a result of the combination of two stock solutions mentioned in Table. Two tanks were prepared by filling 50 liters of water each and adding stock 1 in one of the tanks and stock 2 in the other. From each of the prepared tanks, a 50/50 ratio from each of the tanks was added to a third tank that included 70 liters of water.

The addition of 750-1000mL of stock 1 and stock 2 (Table 2) was necessary in order to achieve an electric conductivity of 2.0, measured by an EC meter (ScanGrow Conductivity Meter, Denmark).
The black boxes in the growth chamber containing the nutrient solution that was pumped up and into the gutters were refilled twice every week

Table 2:Fertilizer solution contents of Stocks 1 and 2.

Stock Solution 1		Stock Solution 2	
Compound	Quantity (kg)	Compound	Quantity (kg)
$CaNo_3$	2.5	Pioneer basic cucumber	3.125
KNo_3	0.625	Pioneer Iron chelate, 6% EDDHA	0.025
$CaCl_2$	0.15		

A sample of the final solution was taken and analyzed by Eurofins Agro Testing Norway AS to distinguish final pH, EC, micro and macronutrients of the solution that will flow through the gutters (Table 3 and 4).

Table 3: Nutrient Solution Contents in ppm

pH	**EC**
5.0	2.1

Cations ppm (mg/L)	NH_4	NH_4^-N	K	Na	Ca	Mg	
	1.8	1.4	282	32	148	29	
Anions ppm (mg/L)	No_3	NO_3^-N	Cl	S	HCO_3	P	
	750	169	64	48	6.1	37	
Micronutrients ppm (mg/L)	Fe	Mn	Zn	B	Cu	Mo	Si
	1.84	0.48	0.27	0.29	0.13	0.09	2.5

4.5. Experiment 2. Effect of relative air humidity with high temperatures and different light intensities on tipburn

In experiment 2, the conditions were considered as a summer simulation, whereby the temperature was higher than that of the first experiment. The effect of relative air humidity was studied based on one condition having a constant RH and another where it was varying, to check whether or not it will have an impact on tipburn occurrence. The temperature sum in both was 546 °C, whereas the nutrient solution used was similar to experiment 1. Since it was a summer simulation, the light exposure time frame representing longer day light was greater by 4 hours and thus altering the diurnal cycle (Table 4 and Figure 11).

Table 4: Treatments for all growth chambers in experiment 2. Moderate Light Intensity= MLI, High light intensity= HLI, Constant Relative Humidity = CH, Varying Relative Humidity=VH

Treatment	Photo-period (hr)	Day Temperature (°C)	Night Temperature (°C)	Photon Flux Density (µmol/m^2/s)	Relative Air Humidity (%)	Temperature Sum (°C)
CH/ML	19	25.4	16.5	150	86	546
VH/ML	19	25.5	16.5	150	74 - 92	546
CH/HL	19	25.4	16.5	300	86	546
VH/HL	19	25.5	16.5	300	74 - 92	546

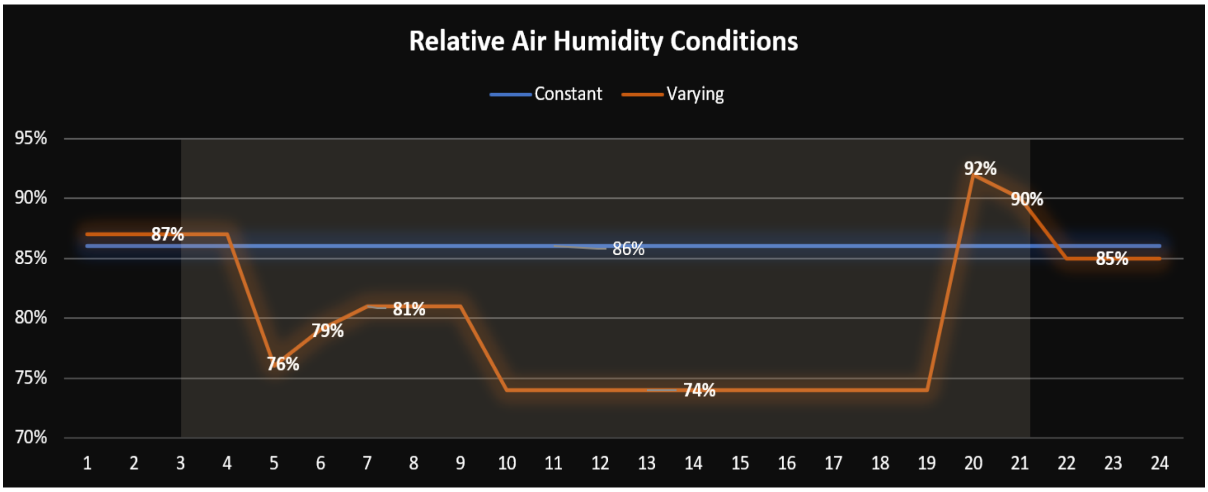


Figure 11: Relative Air Humidity (%) as a function of time. 1=1:00 and the bright shading represents day time and thus, the diurnal cycle.

4.6. Experiment 3: Effect of Calcium on Tipburn

In experiment 3, the effect of increasing calcium content in nutrient solution was investigated. As opposed to the previous experiments, two nutrient solutions were prepared to study whether or not the concentration of calcium in the nutrient solution being supplied to the lettuce in the gutters has an impact on the occurrence of tipburn. Three stock solutions, mentioned in Table 5, were prepared in order to create the two final nutrient solutions, whereby the stocks were combined as Stock 1 + Stock 2 and Stock 2 + Stock 3 to get the final nutrient solutions of high calcium and low calcium, respectively.

Table 5: Contents of Stock Solutions of experiment 3

Stock Solution 1		Stock Solution 2		Stock Solution 3	
Compound	Quantity (kg)	Compound	Quantity (kg)	Compound	Quantity (kg/50L)
$CaNo_3$	2.5	Pioneer basic cucumber	3.125	$CaNo_3$	1.5
KNo_3	0.625	Pioneer Iron chelate, 6% EDDHA	0.025	KNo_3	1.625
$CaCl_2$	0.15			NaCl	0.15

Analysis of both low and high calcium nutrient solutions of the combined stocks, conducted by Eurofins Agro Testing Norway AS, showed the pH, EC, micro and macronutrient concentrations as shown in Table 6 and 7.

Table 6: Nutrient Solution Contents in ppm for Low Calcium Treatment

pH	**EC**
6.3	2.2

Cations ppm (mg/L)	NH_4	NH_4^-N	K	Na	Ca	Mg	
	3.6	2.8	352	44	92	27	
Anions ppm (mg/L)	No_3	NO_3^-N	Cl	S	HCO_3	P	
	738	167	67	48	31	33	
Micronutrients ppm (mg/L)	Fe	Mn	Zn	B	Cu	Mo	Si
	1.68	0.39	0.23	0.26	0.08	0.07	2.5

Table 7: Nutrient Solution Contents in ppm for High Calcium Treatment

pH	**EC**
5.2	2.1

Cations ppm (mg/L)	NH_4	NH_4^-N	K	Na	Ca	Mg	
	1.8	1.4	266	34	152	27	
Anions ppm (mg/L)	No_3	NO_3^-N	Cl	S	HCO_3	P	
	738	167	60	51	31	33	
Micronutrients ppm (mg/L)	Fe	Mn	Zn	B	Cu	Mo	Si
	1.68	0.48	0.24	0.26	0.09	0.12	2.8

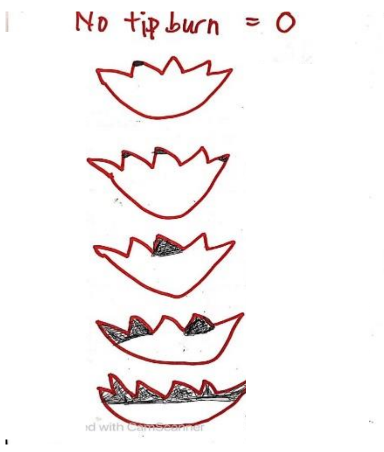
The experiment included 20 plants for each treatment, resulting in a total of 40 Frillice lettuce. Environmental conditions for both low and high calcium were identical. The lettuce plants were allowed to grow under low light of 163 $\mu mol/m^2/s$ with an LED lamp situated in the middle, 70% relative air humidity and a temperature of 18 degrees from 05:00 till 11:00 and 20 degrees Celsius from 11:00 till 05:00 for the first two weeks. For the following week, conditions remained the same, except for the light intensity. Knowing that high light intensity induces inner tipburn, the light intensity was increased from 163 $\mu mol/m^2/s$ to 300 $\mu mol/m^2/s$. The plants were watered every 2 hours for 1 minute every day, and this was increased to double once the light intensity was increased. After growing under low and high light, harvesting took place on day 22, whereby tipburn was assessed using the scoring method mentioned above. Only 10 plants per treatment were assessed for growth parameters and tipburn.

A mineral analysis was then conducted to measure the amount of calcium in the tips of the grown lettuce, as well as other minerals including potassium, magnesium and manganese. This was done by cutting tips with and without tipburn (1.0-1.5 cm) from > 15 random plants. The tips with tipburn always included a green part below the damaged part. The tips were dried for one week, then grinded with a mortar and the elements were measured in the plant tissue using the Inductively Coupled Plasma-Atomic Emission Spectroscopy, also known as the ICP -AES method (Greenfield, 1983).

4.6. Tipburn Assessment: Registration and Scoring

Following harvest, all leaves were evaluated for tipburn, both sink and source leaves. Sink leaves are the inner leaves, that are newly growing and source leaves are the outer leaves that are undergoing photosynthesis. The method used to register and to a certain extent quantify tipburn is through the qualitative scoring system for tipburn (Table 8).

Table 8: Tipburn Scoring System. A score of 0 is no tipburn, 1 or 2 represent non-severe tipburn, 3 severe and 4 or 5 extremely severe.

Tipburn Score	Description	Illustration
0	No visual evidence of any tipburn; leaves look completely healthy.	
1	1-2 of the leaf tips exhibit a brown or burnt coloration at the extremities	
2	More than 50% of the leaf tips exhibit a brown coloration at the extremities	
3	1-2 of the entire leaf tips exhibit a completely brown coloration	
4	More than 50% of the entire leaf tips exhibit a brown coloration	
5	All the leaf tips exhibit a brown coloration	

Registration for experiments 1, 2 and 3 were only registered once at the end of the experiments, which was also the timepoint at which harvesting took place. Knowing that the experimental setup of the first two experiments included 4 different treatments, all placed under two different light intensities and each treatment containing 10 randomly selected lettuces, this results in a total of 80 lettuces for all conditions. In addition to the 40 lettuces of experiment 3, 120 lettuces were registered and held a specific rank for each of its leaves. The oldest was number one and the youngest/smallest leaf was the greatest number.

After the end of each experiment, the leaves were organized from oldest to youngest leaf, and a number was given to each leaf, the oldest being number 1. A tipburn score was assigned to each leaf while excluding the dead leaves but taking note of those leaves that were dead. Based on the registration and the allotment of tipburn score for each leaf post harvested, growth data was collected which included the number of leaves, the longest leaf, length of the longest leaves measured in cm, fresh and dry weight for both inner and outer leaves in addition to the water content percentage.

4.7. Growth Data

The number of leaves were manually counted and the longest leaf was selected to be measured. As for the fresh and dry weight, several steps were necessary. Excluding the rest of the stem and the roots, the entire lettuce, including the cotyledons were weighed. The bags used to weigh the fresh weight were tared, making the balance reset to zero so that a measurement of the actual weight of the lettuce can be seen on the display of the balance. Having marked the samples with the date, weight, plant number, chamber number, sample number and name, the samples were ready for drying. The bags were put in an oven for 7 days at 62 degrees Celsius. After the mentioned time was over, the ten samples were weighed and then the bags were weighed too to get the final dry weight. By knowing the fresh and the dry weight, it was possible to calculate the water content in grams and in % using the following equations.

$$Water\ Content = Fresh\ Weight\ (g) - Dry\ Weight(g)$$

$$Water\ content\ (\%) = \frac{water\ content\ (g)}{fresh\ weight\ (g)} \times 100$$

The experiments mentioned above were conducted by Niveditha Umesh Katyayini.

4.8. Statistical Analysis & Data Visualization

In order to determine whether or not a significant difference exists between the different environmental conditions within the experimental setup, statistical analyses were conducted in R. For the growth data of experiment 1, a two-way ANOVA was performed. As for Experiment 2, due to the nature of the data, it was not possible to conduct a two-way ANOVA and as such, the experiment was split, considering RH as the only factor among the 2 groups. Hence, a pairwise comparison was done for the growth data. The same was done for experiment 3. Tipburn data was exclusively dealt with as qualitative data and so, to check for significance, Kruskal Wallis test was used since itt was necessary to use a nonparametric test for the ranking system for all of the experiments mentioned above.

The climate data collected from the Priva Climate computer of all three experiments was also reported in order to check whether or not the climate conditions and values for temperature and relative air humidity were within the right range in the experimental setups. (refer to Results)

5. Results

5.1. Experiment 1

The goal of experiment 1 was to study the effect of two different daily temperatures with an equal temperature sum on lettuce growth and tipburn incidence. The effect of a higher day temperature and a lower night temperature was investigated to see whether or not this has a positive impact on the occurrence of tipburn, that is a decrease in the frequency of tipburns in 'Frillice' lettuce. Knowing that high light intensities induce tipburn, the treatments were allowed to grow under two different light regimes, medium light and high light intensities (see Methods Experiment 1).

5.1.1. Growth Data

In order to assess the effect of the treatments on lettuce growth, we measured the lettuces' fresh weight, dry weight, water content, longest leaf length, and leaf number. A two-way ANOVA test was conducted for all of the growth parameters to infer significant interactions between the parameters across the different treatments. TukeyHSD was performed to determine whether there are significant differences between the different treatment groups.

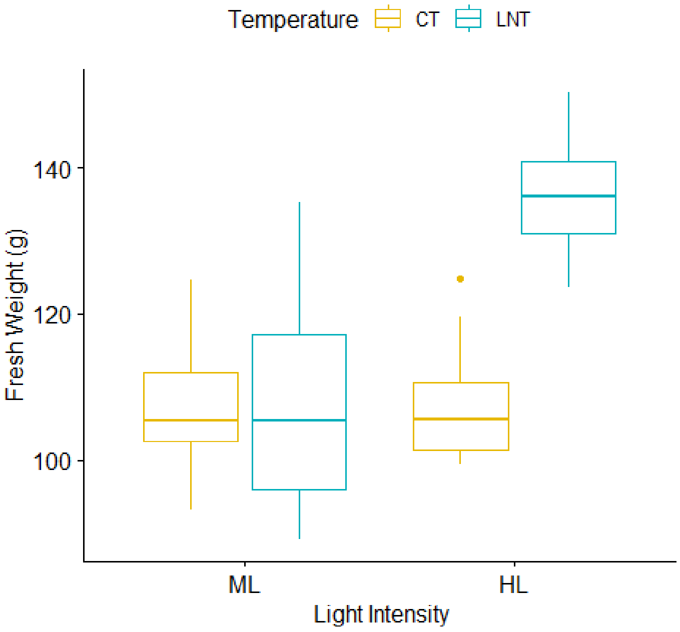


Figure 12: Fresh weight (g) under 4 different treatments, p-value=0.00063. Moderate Light= ML, High light= HL, Constant Temperature = CT, Low Night Temperature=LNT

Fresh Weight (FW) There is a significant interaction between light and temperature with respect to the fresh weight. The interaction between both factors is highly significant (two-way Anova $p \leq 0.05$). The HL/LNT treatment group, shown in Figure 12, exhibited a 1.3 fold significant increase in fresh weight relative to the other treatment groups (TukeyHSD $padj \leq 0.05$). This indicates the positive effect of higher day temperature in combination with lower night temperature on lettuce fresh weight. Interestingly, the median fresh weights of the lettuces under moderate light is very similar to the median fresh weight of the lettuces under the condition of high light and constant temperature.

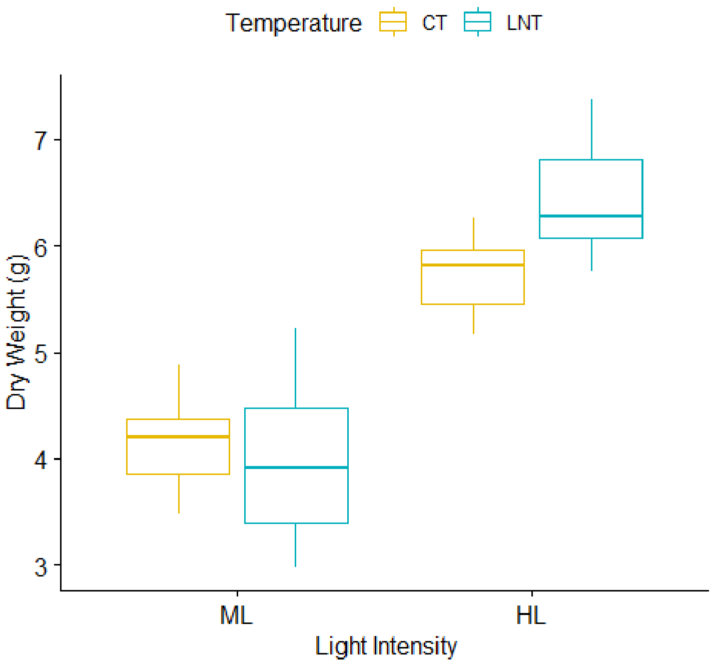


Figure 13: Dry weight (g) under 4 different treatments, p-value=0.027. Moderate Light= ML, High light= HL, Constant Temperature = CT, Low Night Temperature=LNT

Dry Weight (DW) To complement the FW data, DW was measured. The results showed is a significant interaction between light and temperature with respect to the dry weight (two-way Anova p ≤ 0.05 , p=0.027). As shown in Figure 13, an increase in biomass is evident with increased light intensities. There is no significant difference in dry weight between the two ML treatment groups. For the HL treatment groups, a small significant difference was observed (padj≤0.05) with the LNT group exhibiting a 1.1 fold increase compared to the CT group. All the other pair-wise comparisons performed resulted in significant differences in DW (pad≤0.05). The CT treatment groups under the two light conditions exhibited a fold change difference of 1.4, while the LNT treatment groups under the two light conditions exhibited a fold change difference of 1.6. LNT/ML and CT/HL exhibited a fold increase of 1.5.

Water Content ML treatment groups reveal a higher water content percentage than that of HL treatment groups (Figure 14). A significant interaction between light and temperature with respect to water content was observed between (p=0.012). In each of the two light intensity conditions, the median water content of the plants in the LNT group is higher than that in the MT groups. The highest water content observed was in treatment group LNT/ML and the lowest in CT/LNT. The TukeyHSD test revealed that the p-adj values for all pair-wise comparisons were less than 0.01,

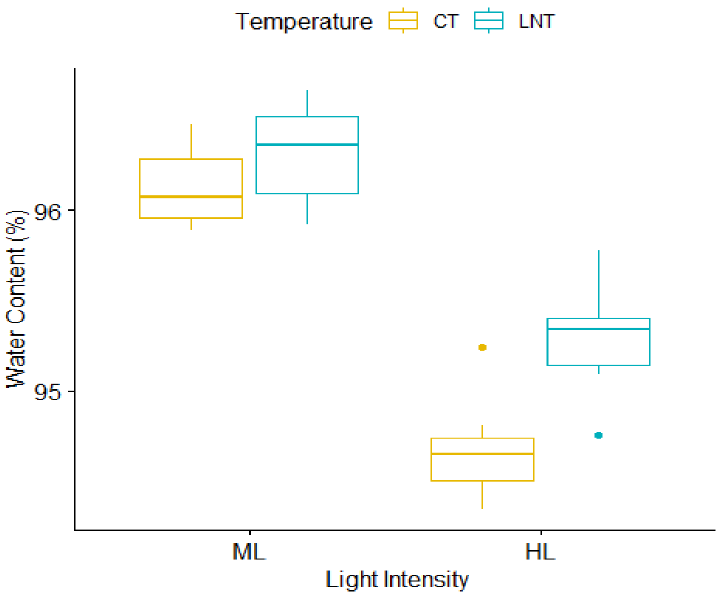


Figure 14: Water Content (%) under 4 different treatments, p-value=0.012. Moderate Light= ML, High light= HL, Constant Temperature = CT, Low Night Temperature=LNT

except for the comparison between LNT/ML and MT/ML treatment groups. Although statistically significant differences were observed between the different treatment groups, the differences were very small with the maximum fold change difference being 1.02. The values of the water content were calculated from values that were obtained from measurements of the fresh and dry weights of the lettuce (refer to Methods section) and the calculated values of water content contained several digits after the decimal point. The subtle differences observed in water content may be attributed to technical variability in the data resulting from experimental variability/error (for example variability in the measurements of fresh and dry weights).

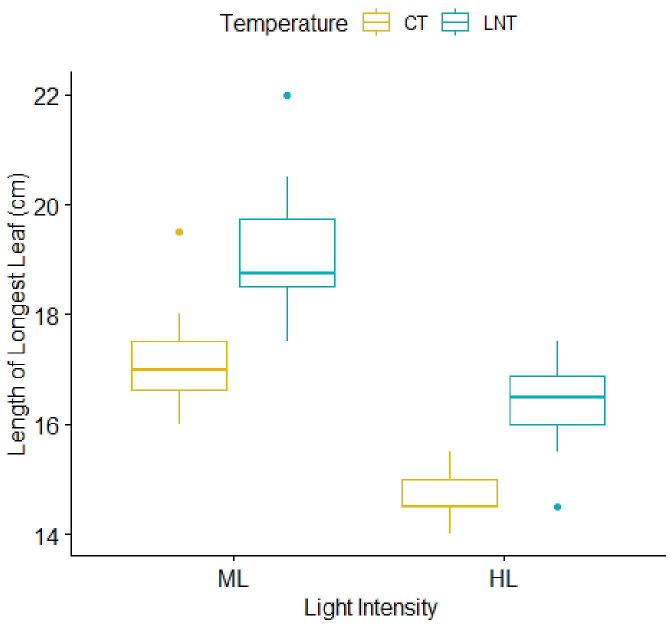


Figure 15: Length of Longest Leaf (cm) under 4 different treatments, p-value=0.56. Moderate Light= ML, High light= HL, Constant Temperature = CT, Low Night Temperature=LNT

Length of Longest Leaf (LL) As depicted in Figure 16, the LL is greatest in the ML/LNT treatment group. Tukey test showed that there is a pair-wise significance difference between all treatment groups, except between CT/ML and LNT/HL. Both ML conditions show higher LL values than those of the HL groups under the two temperature regimens. At higher light intensities, a drop is observed in the length of longest leaf in both CT and LNT treatments. The observation that longest leaf is lower in HL treatments groups compared to the ML treatment groups may be attributed to the plants' adaptive response to high light while trying to limit water loss, so a smaller surface area results in less light and so less water loss. Interestingly, the length in the LNT group is 1.1 fold higher than that in the CT in both light conditions. A 2cm LL exists between the HL treatment groups, while a 1.7cm difference was found between the ML treatment groups. The greatest fold change difference (1.3) was observed between the CT/HL and LNT/ML with 4.25cm difference in median longest leaf length. Although as factors, light and temperature exerted significant effects on longest leaf length, the interaction between these factors was not statistically significant (p= 0.56).

Number of Leaves (NL) The number of leaves depicted includes the number of dead leaves counted at the end of the experiment. Even though the number of dead leaves was generally low (Figure 15), an estimate of 1.5 leaves across four treatments, significant differences in the number of leaves were observed between the CT/HL and LNT/HL (padj≤0.05) treatment and the CT/ML and CT/HL treatment groups (padj≤0.05). The difference in the medians was between 1 and 2 leaves. Therefore, statistically significant, the differences in the NL were very minor across the plants in the different treatments.

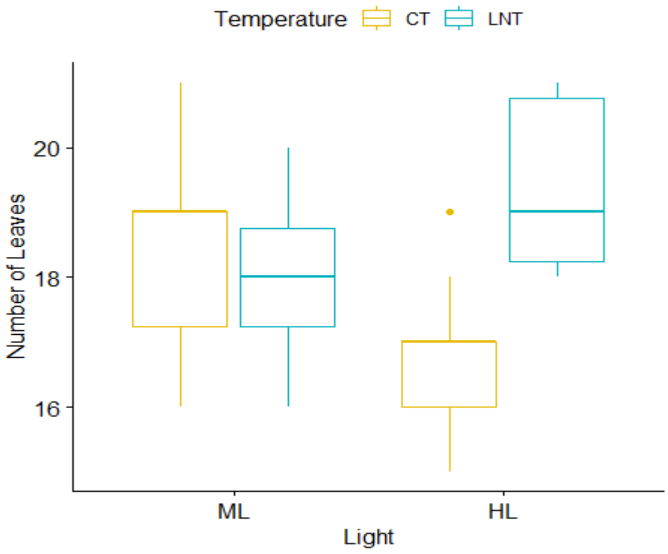


Figure 16: Number of Leaves (with dead-leaves) under 4 different treatments, p-value=0.0008. Moderate Light= ML, High light= HL, Constant Temperature = CT, Low Night Temperature=LNT

5.1.2. Tipburn

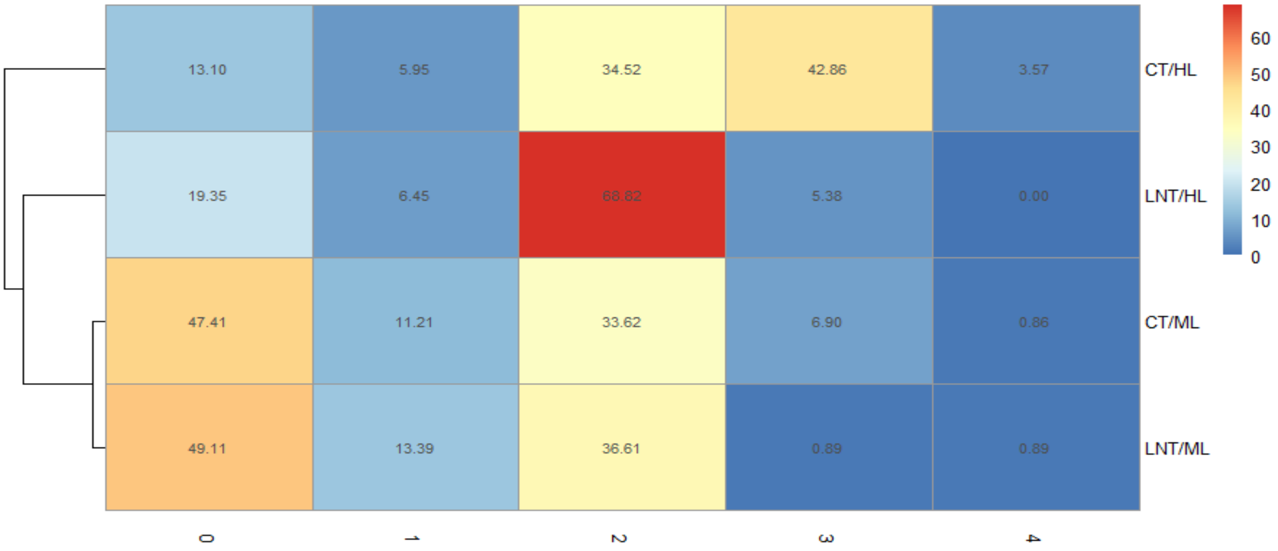
All leaves from the 10 different selected plants were scored and ranked on the basis of tipburn at the end of the experiment, with a score of 0 being the lowest score indicating no tipburn and a score of 4 indicating the most severe tipburn (see Methods) The clustered heatmap shown below in Figure 18 shows the percentage of each tipburn score for outer tipburn within each of the four different treatments. As shown in Figure 18, lettuce under the two moderate light treatments have the least outer tipburn, as the majority of leaves scored had a score of 0.

As shown in the heatmap, hierarchical clustering of the treatment groups shows that the ML treatment groups are the most similar to each other. Furthermore, ordering the scores in descending order of their respective percentages shows that the order is the same for both ML treatment groups (Score 0 > Score 2 > Score 1 > Score 3 > Score 4). The HL treatment groups exhibited more tipburn, which is not surprising since higher light intensities are known to cause more tipburn.

As shown in the heatmap and by the hierarchical clustering of the treatment groups, the HL treatment groups are more variable with respect to each other, and unlike the ML treatment groups, there is divergence in terms of the descending order of the score percentages. The highest score was that of Score 2 at 68.82% and Score 3 at 42.86% for LNT and CT, respectively. LNT/HL exhibits higher percentages for Score 0 and 1 than those of CT. Interestingly, 3.57% of the leaves had the highest tipburn severity score of 4 in the CT/HL treatment group, but no leaves were observed to have a tipburn score of 4 in the LNT/HL treatment group. Furthermore, in descending order, the scores for CT are Score 3 > Score 2 > Score 0> Score 1 >Score 4 and for LNT Score 2> Score 0 >Score 1> Score 3 >Score 4. This data indicates that LNT/HL treatment results in less outer tipburn in lettuce compared to the CT/HL treatment.

To further support this observation, inner tipburn was then assessed under high light conditions. The results are shown in Figure 8. The predominant tip burn score was different for the two treatments; it was Score 2 for the CT treamtent group and Rank 0 for the LNT treatment group. Strikingly, Score 0 comprises the highest percentage of 44% in LNT, while it was the lowest at 9% in CT. All other scores had less percentages in LNT than in CT. Scores 2 and 3 make up 65% (33%+32%) of the inner tipburn score in CT.

In light of the above, under high light conditions LNT treatment leads to a lower inner and outer tipburn incidence in lettuce compared to CT treatment.



	0	1	2	3	4
CT/HL	13.10	5.95	34.52	42.86	3.57
LNT/HL	19.35	6.45	68.82	5.38	0.00
CT/ML	47.41	11.21	33.62	6.90	0.86
LNT/ML	49.11	13.39	36.61	0.89	0.89

Figure 17: Cluster heatmap showing the percentage of outer tipburn scores in each of the treatments of experiment 1. The columns and rows represent the measurement of tipburn according to the qualitative ranking system and the treatments, respectively. Score 5 was represented due to it being 0% in all treatments. Score 2 or less is not, Score 3 is severe and Score 4 and 5 is very severe. Moderate Light= ML, High light= HL, Constant Temperature = CT, Low Night Temperature=LNT.

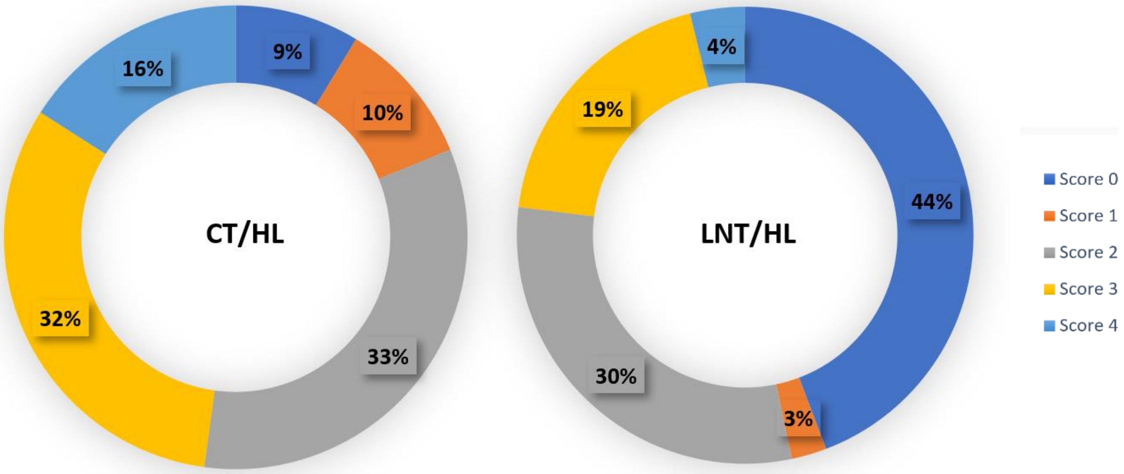


Figure 18: Percentage of each inner Tipburn Score for inner leaves under high light conditions.

In order to determine whether or not there was a significant difference in tipburn score with outer as well as inner tipburn, a Kruskal Wallis Rank Sum Test was employed. The test revealed a non-significant difference in outer leaf tipburn scores between the ML treatment groups (p-value=0.43). However, this was not the case for outer leaf tipburn under HL, where a significant difference was reported with a p-value of $2.837e^{-6}$. Similar to the outer tipburn under HL treatments, inner leaf tipburn under HL conditions also resulted in high significance. The inner leaf tipburn scores under ML conditions were not considered for this test since there was no tipburn of inner leaves in the two treatments. In light of the above, under high light conditions LNT treatment leads to a lower inner and outer tipburn incidence in lettuce compared to CT treatment.

The mineral analysis performed for the tips of leaves under ML conditions showed a higher concentration of calcium, potassium and manganese in necrotic tips compared to non-necrotic tips. However, the concentration of magnesium was less in necrotic tips versus non-necrotic tips.

Table 9: Mineral Analysis of Necrotic and Non-Necrotic Tips of Lettuce Leaves for Experiment 1. All values have a unit of mg/100g of DW

Treatment	Tipburn	Ca	K	Mg	Mn
CT/ML	Necrotic Tips	16.4	8.5	36.4	1.75
	Non-necrotic Tips	10.6	8.2	60.3	0.60
LNT/ML	Necrotic Tips	15.8	9.1	44.8	1.82
	Non-Necrotic Tips	10.2	8.9	55.1	0.73

5.2. Experiment 2

The aim of experiment 2 was to study the effect of varied relative air humidity on the occurrence of tipburn while having high temperatures and under two different light conditions. Both treatments under 2 different light intensities of 150 µmol/m^2/s and 300 µmol/m^2/s were performed. Due to the nature of the data collected and the fact that both summer simulation under high light intensity had to be stopped before the expected harvest day, the number of days differed greatly (Table 2). To avoid false quantitative data analysis, an ANOVA test similar to that conducted in experiment 1 or any statistical testing was not possible across all four treatments. Normalizing to the number of days and conducting the tests would also have resulted in invalid results from a biological and statistical perspective since the data cannot be properly compared.

The experiment under high light conditions included the harvesting of 10 plants out of 40 that grew for 16 days. The experiment was halted due to the severity of the tipburn in the leaves of the lettuce. While on the other hand, the experiment with moderate light temperature, lettuce was grown for 22 days. It is important to note that although the difference in the number of days was 6, the number of leaves per day were comparable with 1.35 and 1.20 for moderate light and 1.21 and 1.19 for high light even though the average number of leaves was 29.8/26.5 and 19.4/19. Nonetheless, the data from each experiment or light treatment condition was analyzed separately, whereby a pairwise comparison was performed using the student t-test for the growth data and a Kruskal Wallis test for the tipburn data.

Table 10: Number of Leaves compared to number of days of experiment 2

Treatment	Average Number of Leaves	Number of Days	Number of leaves / day
CH/ML	29.8	22	1.35
VH/ML	26.5	22	1.20
CH/HL	19.4	16	1.21
VH/HL	19	16	1.19

Table 3 shows that differences fresh weight, dry weight, water content, and length of longesters between the ML treatment groups were no significant. Nevertheless, a significant difference was observed in number of leaves with a 1.12 fold increase in the CH group relative to the VH group. The number of dead leaves for these two treatment groups was an estimated average of 2.5 leaves. Comparisons between the high light intensity treatment groups yield significant differences in fresh weight, dry weight, and water content. The fresh weight, dry weight and water content were higher in VH than in CH.

Table 11: Growth data of Experiment 2. Average of each growth parameter, standard deviation and p-value from each pairwise comparison. CH= Constant Relative Humidity, VH= Varying Relative Humidity, ML= Moderate Light Intensity, HL= High Light Intensity

Treatment		Fresh Weight (g)	Dry Weight (g)	Water Content (%)	Number of Leaves	Length of Longest Leaf (cm)
CH/ML	Mean	121.24	4.772	97.79174	29.80	19.00
	SD	25.52	0.94	0.40	1.34	1.56
VH/ML	Mean	130.27	4.88	97.92	26.50	19.90
	SD	30.07	0.97	0.62	1.53	1.32
Significance p-value		p>0.05	p>0.05	p>0.05	p<0.05	p>0.05
CH/HL	Mean	71.28	3.94	93.66	19.40	14.92
	SD	9.19	0.38	0.59	1.23	0.94
VH/HL	Mean	79.56	4.641	94.15	19.00	15.00
	SD	6.70	0.32	0.21	0.92	0.47
Significance p-value		p<0.05	p<0.05	p<0.05	p>0.05	p>0.05

In terms of tipburn, the majority of leaves scored from the plants of treatment CH and VH under ML light condition have a ranking of 0, at 72.25% and 73.63%, respectively. Furthermore, Score 3 and 4 in the VH/ML and CH/ML group had minimal or no contribution to the percentage. Although the data from the ML groups follows the same descending order pattern of ranks, Score 1 is less prevalent in VH compared to CH and Rank 2 is more prevelant in VH compared to CH.

In the HL treatment groups, Score 2 was the most predominant outer tipburn score for both CH and VH, with its percentage being around 10% higher in VH relative to CH. Score 0 had a higher percentage in VH compared to CH by 8.4%, whereas Score 1 had a lower percentage (12.32%).

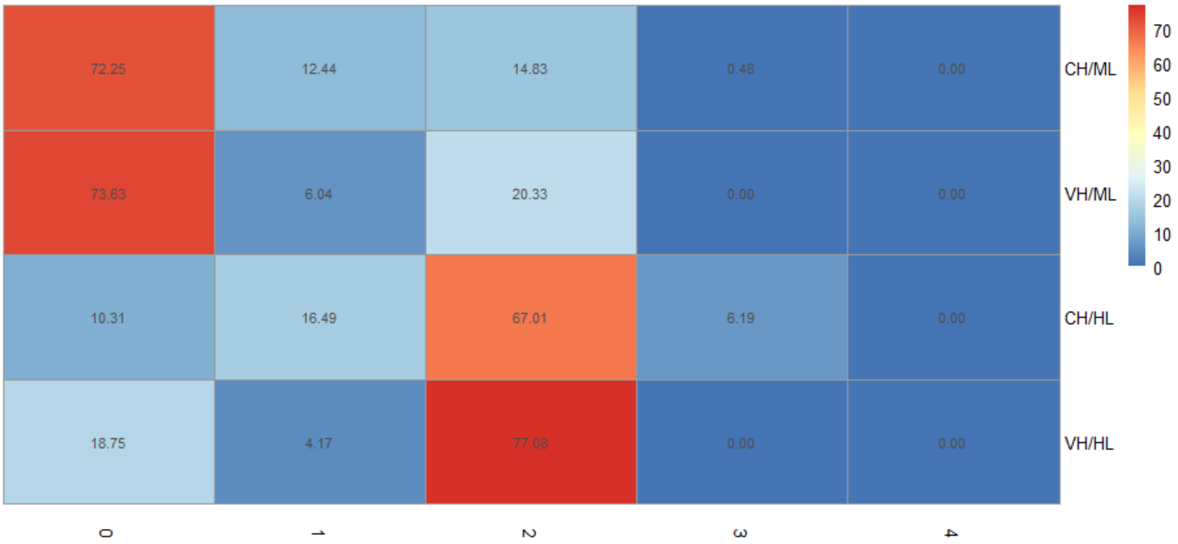


Figure 19: Cluster heatmap representing the percentage of the tipburn scores in each of the treatments of experiment 2. Numbers 0,1,2,3,4 represent the tipburn score. VH= Varying Relative Air Humidity, CH= Constant Relative Air Humidity, ML= Moderate Light Intensity, HL= High Light Intensity.

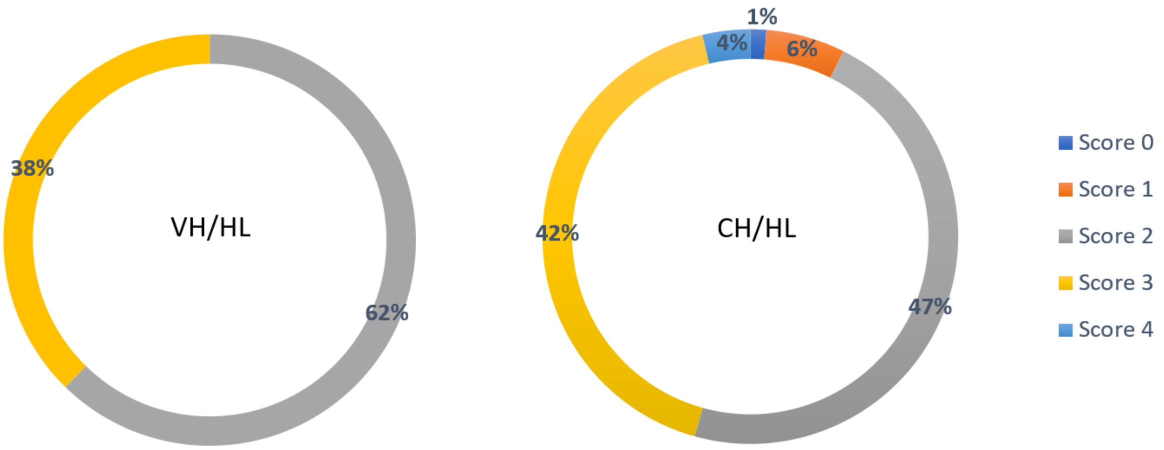


Figure 20: Inner Tipburn Score Percentage Experiment 2. VH= Varying Relative Air Humidity, CH= Varying Relative Air Humidity, HL= High Light Intensity.

The percentages of inner tipburn scores for the HL treatment groups are shown in Figure 10. Only two scores were scored in the VH/HL treatment group, scores 2 and 3 with a predominance of Score 2 at 62%. However, all 4 scores were scored in the CH/HL treatment group. The predominant inner tipburn score was 2 (47%), which is the same as in VH, followed by Score 3 at 42% and the rest of the scores comprised 11% of the total inner tipburn percentage of experiment 2.

Even though no statistical analysis was conducted to compare ML and HL treatments, significance was tested for each light regimen separately. For HL treatments, a significant difference was reported for inner tipburn at a p value= $5.678e^{-6}$. However, this was not the case for outer tipburn data.

5.3. Experiment 3

The aim of experiment 3 was to study the effect of increased calcium content in the nutrient solution on tipburn occurrence. Tipburn was induced by increasing the light intensity during the experiment.

The growth data shown that all measured growth parameters had higher values in the low calcium treatment group compared to the high calcium treatment group but the differences were not significant (Figure 21).

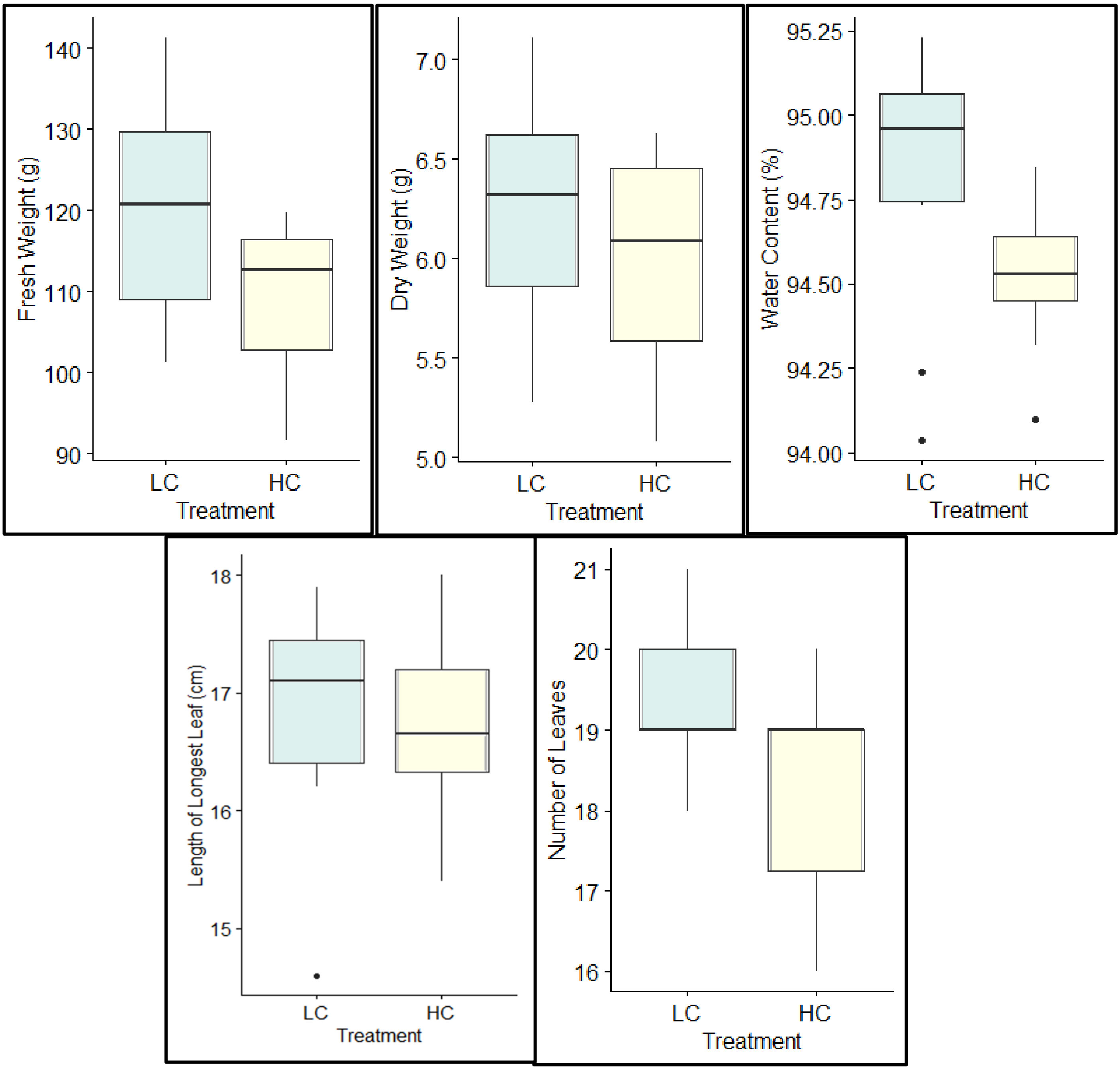


Figure 21: Growth Data of Experiment 3. LC= Low Calcium, HC= High Calcium.

According to Figure 22, the severity of outer tipburn was less than that of LC. As for inner tipburn, Scores 0, 1 and 4 showed a higher percentage opposing to Scores 3 and 5, whereas Score 2 was equal. Evaluation of the differences across the two calcium treatments and tipburn incidence rate was tested using the nonparametric Kruskal Wallis Test. The test revealed insignificant differences (p=0.17). Thus, the null hypothesis was accepted.

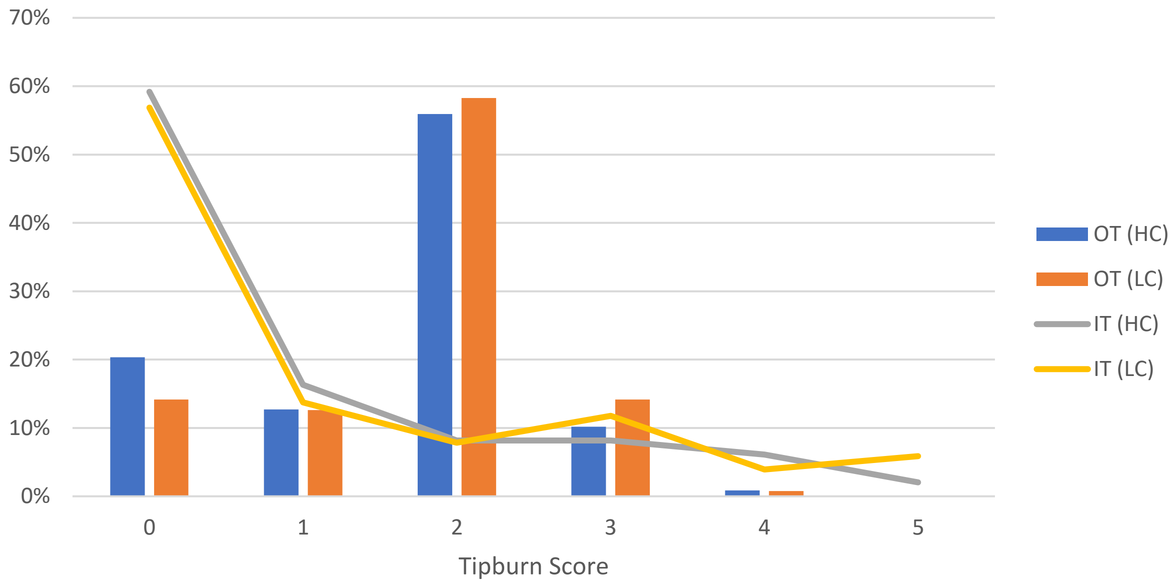


Figure 22: Percentage of Tipburn Score for Inner and Outer Tipburn. OT= Outer Tipburn, IT=Inner Tipburn, HC=High Calcium. LC= Low Calcium

Mineral Analysis of necrotic and non-necrotic tips of the lettuce leaves (Table 12) revealed that Ca levels, in addition to K, Mg and Mn were always lower in leaves with no tipburn compared to those with tipburn. Moreover, there was no significant difference in mineral composition between the HC and LC treatment groups.

Table 12: Mineral Analysis of Necrotic and Non-Necrotic Tips of Lettuce Leaves for Experiment 3. All values have a unit of mg/100g of DW

Treatment	Tipburn	Ca	K	Mg	Mn
HC	Outer tipburn	11.8	6.2	38.9	0.47
	No tipburn	3.3	3.6	25.7	0.06
LC	Outer Tipburn	11.4	5.7	36.8	0.53
	No Tipburn	3.2	3.3	31.5	0.05

5.4. Evaluation of Climate Data of Growth Chambers

The data from the Priva Climate computer was compared with the expected temperature and humidity mentioned in the experimental designs of all experiments. Utilizing R, the average of each 45 minutes was calculated for every day for the entire timeframe of the experiments. A total of 24 averages was the result. From those averages, a total average was calculated for both temperatures and treatments, shown in the tables below (Table 13,14 and 15). As shown in the experimental design (Appendix 1), there is a range in which the temperature must be within, expressed as heat and cold, thus an average was calculated for each experiment. There was no significant difference between the expected temperature and the actual temperature from the Priva Climate computer.

Table 13: Expected and Actual Average Temperature Data of Experiment 1

Treatment	ML/CT	ML/LNT	HL/CT	HL/LNT
Expected Temperature (°C)	17.8	18.3	17.8	18.3
Actual Temperature (°C)	18.28	18.21	18.36	18.57

Table 14: Expected and Actual Average Temperature Data of Experiment 2

Treatment	ML/CH	ML/VH	HL/CH	HL/VH
Expected Temperature (°C)	23.6	23.6	23.6	23.6
Actual Temperature (°C)	23.96	23.65	24.45	23.88

Table 15: Expected and Actual Average Temperature Data of Experiment 3

Treatment	Low Calcium	High Calcium
Expected Temperature (°C)	19.4	19.4
Actual Temperature (°C)	19.64	19.88

Similar to the of the average temperatures across the three experiments, there was also no significant difference between the expected and actual relative air humidity across all three experiments. The maximum difference between the expected and actual RH was ~ 3% with an exception in the case of experiment 1, HL/CT treatment, where the difference was 5.25% higher (but this is higher than 3%) than expected. The standard deviation of both factors was not included due to multiple timepoint data of the actual versus the expected temperature and relative air humidity. Statistical tests were therefore also not possible.

Table 16: Expected and Actual Average Relative Air Humidity Data of Experiment 1

Treatment	ML/CT	ML/LNT	HL/CT	HL/LNT
Expected Relative Air Humidity (%)	78	79	78	79
Actual Relative Air Humidity (%)	76.33	76.94	83.25	78.22

Table 17: Expected and Actual Average Relative Air Humidity Data of Experiment 2

Treatment	ML/CH	ML/VH	HL/CH	HL/VH
Expected Relative Air Humidity (%)	86	80	86	80
Actual Relative Air Humidity (%)	83.29	76.88	88.90	79.05

Table 18: Expected and Actual Average Relative Air Humidity Data of Experiment 3

Treatment	Low Calcium	High Calcium
Expected Relative Air Humidity (%)	70	70
Actual Relative Air Humidity (%)	69.71	69.73

6. Discussion

Light is one of the key factors that impact the growth rate of lettuce (Saure, 1998). As the irradiance increases, the relative growth rate also increases, while taking into account the photosynthetic saturation point (Miles, 1974; Mckenna & Houle, 1999). According to experiment 1, lettuce grown in a hydroponic system under high light intensity had a higher biomass compared to lettuce grown under moderate light intensity. Although an increase in light increases photosynthesis, an increase beyond the saturation point causes light induced stress and an increase in ROS production. This in turn leads to oxidative stress and ultimately necrosis (Carassay et al., 2012; Shikanai et al., 2020). The development of inner tipburn in experiment 1 supports this claim, whereby an increase in light intensity from 150 $\mu mol/m^2/s$ to 300 $\mu mol/m^2/s$ yielded not only worsened outer tipburn in both experiments 1 and 2, but also generated inner tipburn as compared to the absence of it under moderate light conditions. The inner tipburn occurring in LNT and CT exhibited a highly significant difference, as Score 0 held the majority of 44% as compared to 9% in CT and all scores associated with low, moderate to severe tipburn were higher in CT. A high percentage of the lettuce under ML treatment groups had no tipburn (Score 0), whereas the lettuce in HL treatments had higher “not severe” and “severe” tipburn incidence in LNT and CT conditions, respectively. Hence, light stress triggers the incidence of both outer and inner tipburn.

The biomass of a plant is increased as the DIF[5] increases, hence a negative DIF (DT<NT) would result in reduced biomass and a positive DIF (DT>NT) would result in more biomass (Stavang et al., 2010). Experiment 1 had the same temperature sum across all treatments, but the DIF differed due to varying day temperature and low night temperature (DT>NT). In terms of morphology, lettuce under LNT treatment in both light conditions were seen to have more elongated leaves and stems, which can be explained by the increased content of giberellins (GA) due to the increased expression levels of their biosynthesis genes, as shown by Stavang et al. (2010). High content of GA changes the properties of the cell wall, allowing cells to elongate, explaining the difference in morphology among CT and LNT treatments. LNT/HL treatment resulted in a higher biomass accumulation ($p<0.05$) among the two HL treatment groups, however that was not the case for ML treatment groups, whereby the difference was not significant. Moreover, this implies that light is the major factor contributing to the increase in fresh weight and biomass accumulation. This conclusion align with previous light treatment experiments on Frillice (Knoop, 2019). It also shows that the interaction between temperature and light is important for the response. This means that plants respond differently to temperature depending on the light level, which is common in a lot of different plant species (Myster and Moe, 1995).

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[5] DIF refers to the difference in temperatures during day and night. $T_{daytime}$-$T_{nighttime}$

Using the same technique, the NFT, Vanhassel et al. (2016) describes the potential of the positive effects of low night temperatures to butterhead lettuce with respect to the reduction of tipburn occurrence. The drop in temperature during the night allows the plant to decrease respiration compared to during the day. This can be supported by the water content results that showed a higher water content in ML compared to HL. This can lead to tipburn development.

Experiment 2 failed to meet its original purpose due to purely practical reasons. The experiments had to be stopped because the leaves of the lettuce had severe inner tipburn and stopped growing~~.~~The alternative aim was then to test the effect of varying RH on the occurrence of tipburn, while not taking account light as a factor. The results showed that the treatments under high light, whether under constant or varying humidity, led to high tipburn occurrence. What was highly prevalent was the incidence of inner tipburn, which advised against the continuation of the experiment. Inner tipburn is more problematic than outer tipburn, since from a practical perspectve outer tipburn leaves can be removed without severely compromising the quality of the whole lettuce, while the removal of inner tipburn compromises the lettuce's quality and makes it unsuitable for the market. The results showed non-significant differences between the treatment groups under moderate light-with variable humidity group (VH/ML) exhibiting more outer tipburn than the constant humidity group (CH/ML). Although the results were not statistically significant, the literature supports the notion that high RH during night time induces less tipburn, and thus can be used to control and limit tipburn occurrence (Vanhassel et al., 2016). This can be explained by the root pressure as an effect of the decrease in transpiration, resulting in a decrease in transpiration of an equal transport of calcium and other nutrients to the entirety of the plant including the young inner leaves.

Wien & de Villiers (2005) found out that the importance of transpiration is far higher than that of root pressure in terms of calcium distribution to and within young lettuce leaves. Whereas Hartz et al. (2007) claims that calcium does not play an important role in tipburn occurrence after assessing 15 commercial romaine lettuce fields in Central California, as there was no impact of leaf or soil calcium concentration on the occurrence of tipburn and the addition of calcium fertilizers did not make a difference. Thus, they concluded that the environmental conditions are the key drivers to tipburn occurrence. Growing lettuce in the field is very different than CEA, but this knowledge is beneficial for further understanding tipburn occurrence in hydroponically grown crops.

Nutrient uptake is positively correlated to transpiration and it has been documented that climate factors such as high temperature, high light intensity or wind are prone to increase the possibility of tipburn occurring (Cramer et al., 2008; Saure, 1998). Calcium is directly attributed to the occurrence and severity of tipburn, which is why many researchers have attempted to increase calcium content via foliar spray or nutrition solution, like in experiment 3. The results showed that there is no significant difference in inner and outer tipburn occurrence in low and high calcium concentrations in nutrient solution. According to a study relating to the effects of foliar application of calcium twice a week (400 or 800 mg/L) on tipburn in Salanova lettuce (‘Red Butter’, ‘Green Butter’, and ‘Red Oakleaf’), foliar spray treatment resulted in a reduction of the severity of tipburn (Samarakoon et al., 2020) (Samarakoon et al., 2020). It is likely easier to increase Ca content in lettuce by spraying on the leaves than to add more Ca in the nutrient solution. This route of penetration allows the leaf to not rely on the transport of calcium through the xylem.

The results of the mineral analysis conducted in experiment 3, Barta & Tibbitts (2000) showed that K concentration is highest at the site where tipburn occurred. Ca concentrations exhibited a severe decrease during enlargement and Mg concentrations remained constant. We cannot say whether or not the data collected to be the same since the data does not represent a multi-point dataset, it was only the tips that were used for the mineral analysis. It was also found that there are other benefits to Ca foliar application such as relating to pest control, whereby growers can avoid specific fungal diseases.

Examining other minerals such as potassium and measurements like pH, Bres and Weston’s experiments showed that the increase in K concentration or pH levels in three lettuce cultivars resulted in no significant effect on tipburn occurrence, but climate conditions had an impact. In contrast, after comparing lettuce (*Lactuca sativa* L. var. Acephala) growth and yield via two experiments in greenhouses (light source versus solar radiation) during the spring and summer growing seasons using same nutrient solution with different proportions of macro-cations and macro-anions, the results from Fallovo et al. (2009) showed that the combination of seasonal changes and an increase in the concentration of fertilizer solution had positive implications on hydroponic (DWC/floating system) lettuce growth and yield. A high calcium level in the nutrient solution yielded higher calcium content, chlorophyll, glucose and fructose concentrations. This is likely due to the vital role calcium plays in the growth and development of plants, such as cellular integrity in new leaf and root tips. Nevertheless, the season still had a more statistically significant impact on leaf growth and development, than did the calcium concentrations. The results from experiment 3 did not include a mineral analysis for entire leaves, but rather the tips of the lettuce. Similar to tips with tipburn, tips with no tipburn also exhibited no significant difference in calcium content in both LC and HC treatments. Knowing that the systems used were different in that DWC immerses the plant roots entirely in nutrients, compared to the NFT system, which provides a greater gas exchange. The results from experiment 3 strengthen the notion that the cause of tipburn is independent of the increase in calcium content in the medium, but rather the inability of the plant to transport Ca especially knowing that Ca is an immobile element in plants and the fact that the calcium to the leaves as calcium transport is dependent on the xylem flow rate and the duration of transpiration.

All experiments included 10 out of 40 selected lettuce plants for the assessment of tipburn only at the end of the experiment and as such, the method would benefit from further development. For instance, time course experiment would be beneficial in actually distinguishing the intermittent effect of each treatment.

Quantitative ordinal scales are usually used to describe and assess the severity of a disease relating to plants. However, there are limitations to the scoring method used to assess tipburn in this thesis. The scoring system was comprised of 2 scores of opposing extremes, non-severe and extremely severe with only one score representing severe tipburn. Although this system makes the classification of tipurn severity easy and simple, the identification of more moderate tipburn states would be required for a more accurate and holistic phenotypic classification of tipburn severity. However, from a grower's perspective, a simple and clear scoring system would be favorable, since this would easily allow growers to determine whether or not the lettuce produced can be put on the market or not. Knowing that the experiments comprised of assessing inner and outer tipburn, this gives us a clearer view of the problem since Norwegian growers' main concern revolves around outer tipburn and not inner tipburn. There are many other ways to assess tipburn severity, compared to the scoring method mentioned and used in the experiments. (Birlanga et al., 2021) also used a more complex scoring method comprising 9 scores instead of 5 and providing a more detailed scoring system to abide by. The scoring system contained 2 scores for each severity level and included light, moderate, severe and highly severe tipburn scores. Furthermore, the tipburn incidence was calculated using the following formula

$$TBI = \frac{\text{n plants severe tipburn} \times 5 + \text{n plants medium tipburn} \times 3 + \text{n plants light tipburn}}{\text{n plants x5}} \times 100$$

While Martinis et al. (2020) utilized a binary approach where only presence and absence of tipburn was reported as 1 and 0, respectively. On the other (Uno et al., 2016) calculated a percentage after counting the leaves that had tipburn and the total number of leaves on a daily basis. An unconventional and also new concept on the rise is machine learning in greenhouses and plant factories. Utilizing deep learning through self-learning and data augmentation, Gozzovelli et al. (2021) further extended the concept of assessing tipburn fast and accurately. Their approach, while detailing classification and segmentation, allows plant factories to assess tipburn severity automatically when dealing with a dense canopy of plants.

Frillice is a very good and important cultivar of lettuce for the Norwegian growers, and according to all the experiments conducted, no bolting occurred which means that it is a bolt tolerant cultivar. However, tipburn remains a problem for the Norwegian growers for outer leaves more than inner leaves the main concern of Norwegian growers is outer tipburn and not inner tipburn, of which the latter is mostly addressed in the literature.

Tipburn resistance in lettuce has a complex genetic architecture (Macias-González et al., 2019), which is why future research should include understanding gene expression regulation, genetic interactions and networks. More research should also be done involving different climate factors, while utilizing the newest technologies as mentioned before. If we understand the mechanism and factors driving tipburn occurrence, we can exploit them to minimize tipburn. By doing so, systems such as hydroponic systems will potentially gain even more interest and allow growers to produce higher quality plants to put on the market. The energy consumption of greenhouses and vertical farms is a lot due to the lighting, however, LED lights are being investigated as means to reduce high tipburn incidence compared to HPS lamps. By partially shifting farming to a CEA through facilities such as vertical farms, we will allow ecosystems to recover from the intensive use of crop land.

Hydroponics, as well as other techniques in CEA will not be the innovative technology that will override all other agricultural methods, which is what lots of people assume to be the case with the rise of new techniques to farm. However, it will complement the various methods, for example, in the agroecology field which is currently undergoing research.

We cannot make a change if we as a human race do not change our way of life; we need a drastic shift in mentality which would consist of eating healthier and plant-based diets. It is also important to note that government intervention will also be required, in addition to improved financing within the food system domain. The greatest battle during the 21st century is securing natural resources for the future generation without depriving our own.

Upscaling the food system to accommodate the steep increase in human population density will not be a solution, but a transformation is necessary. Renewable energy solution, hydroponic systems and other controlled environment agriculture techniques are very promising tools to be included in the transformation of the world food system.

Chronic growth is not in compliance with the natural way of life. Similar to any organism, humans and the global economy they have developed abide by the same cycle, which is why growth is nowadays problematic. What is required is to strike a balance, socially, economically, and environmentally. Thus, alongside a transformation in the global food system, which comprises a lot of the global economy, transformation of entire economies should take place whereby economies are designed to thrive instead of grow.

7. Bibliography

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Declaration of Authorship

I do solemnly declare that I have completed the submitted master thesis independently without undue help from others and without using tools other than those specified.

Where I have used thoughts from external sources, directly or indirectly, published or unpublished, this is always clearly attributed. The presented intellectual work of this master thesis is my own.

Furthermore, I certify that this master thesis or any part of it has not been previously submitted for a degree or any other qualification at the Technische Universität Dresden or any other institution in Germany or abroad.

The submitted electronic version of the thesis matches the printed version.

Place, Date Zittau, 09.12.2021 Signature