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of Life Sciences

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Biovit

Tipburn in lettuce (*Lactuca Sativa L.* “*Frillice*”) – Identifying climate factors that induce tipburn and cultivation methods that mitigate tipburn in controlled environment.

Bladrandskade i salat (*Lactuca Sativa L.* «*Frillice*») –
Identifisering av klimafaktorer som induserer
bladrandskade, og produksjonsmetoder som hemmer
forekomsten av bladrandskade i kontrollert miljø.

Martin C. Knoop

Plant science – plant production systems

Forword

This thesis is written as part of the three-year research project “Control of tipburn for increased production of Frillice´ lettuce. The project is financed by the Norwegian Research Council and “owned” by 5 Lettuce growers who cooperates with the Norwegian University of Life Sciences (NMBU), the Norwegian Horticultural Growers Association (NGF), the Norwegian Extension Service (NLR) and Wagening University in the Netherlands.

The research project aims at looking into climate data from the different Greenhouses involved to gain a better understanding of what causes tipburn. In addition, there will be performed experiments at NMBU to improve the understanding of why tipburn appears and reduce the quantity and severity of tipburn. The experiments performed during this thesis work are a part of these experiments.

This thesis work is conducted the last year of a masters in Plant Science at the Norwegian University of Life Sciences (NMBU). The experiments were performed in the fall of 2018 and the writing conducted throughout the spring of 2019.

I want to convey a special thank you to my supervisor Sissel Torre for inquiring discussions and invaluable support. I also want to say thank you to Ida Hagen for all help during the experiments!

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Ås, 15.05.2019

Martin C. Knoop

Summary

This master thesis is done with the aim of adding understanding to why tipburn occurs in greenhouse production of 'Frillice' lettuce (*Lactuca Sativa* L. 'Frillice'), and to find cultivation methods can help reduce this occurrence. Tipburn is a big problem for Norwegian greenhouse lettuce growers and can account for up to 20 % losses in production, equal to almost 20 million NOK/year.

Tipburn is a form of necrosis on the outer rim of lettuce leaves, believed to be induced by a deficiency in calcium in these cells- resulting in their collapse. Tipburn is known to occur when the lettuces experience undue abiotic stress such as long photoperiods, high light sums, high light intensity, (above 16-17 moles/m²/day) and conditions that limit transpiration (high relative air humidity and low water availability).

In this thesis work, several environmental factors were tested to find a method to induce tipburn in 'Frillice' lettuce, during 5 experiments in climate-controlled growth chambers. The climate factors tested were; Elevated temperatures (20 °C → 27 °C), elevated (65 % → 90%) relative air humidity (RH), different light intensities, photoperiods and light sums.

Also, the use of white "light emitting diode" (LED) lamps (without, and in combination with LED far-red spectrum) was tested to make a comparison towards "high pressure sodium" (HPS) lamps, and see if light quality would reduce the occurrence and severity of tipburn. In addition, a "priming" of 'Frillice' lettuces during pre-cultivation was performed to see if this could help the lettuce acclimate better to environmental conditions shown to induce tipburn. This priming was performed with high light intensity (300 μmol/m²/s, HPS) and normal light intensity (150 μmol/m²/s, HPS) in combination with (100 μmol/m²/s blue LED light).

Elevated temperatures and elevated RH did not induce tipburn. Neither was there found a compounding effect on tipburn, between elevated temperatures and elevated RH. There was found a clear effect of higher light intensity/light sum on the increase in severity of inner tipburn.

The use of white LED was shown to increase outer tipburn severity. However, white LED in combination with far-red LED was found to reduce outer tipburn, compared to HPS. Priming

with high light intensity and with blue LED spectrum was ineffective in reducing the occurrence and severity of tipburn.

To identify the relationship between calcium and tipburn, nutrient analyses (Ca, K and Mg) was performed in 3 of the experiments. There was also performed an analysis to identify hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS).

A relationship between ROS and tipburn was found and indicates a link between tipburn and oxidative stress. Lower calcium levels were found in young sink leaves with inner tipburn, than in young leaves without tipburn and confirm a role of Ca in tipburn occurrence.

Sammendrag

Denne masteroppgaven er utført med det formål å tilføre en større forståelse for hvorfor bladrandskade i veksthusprodusert 'Frillicesalat' (*Lactuca Sativa L. 'Frillice'*) oppstår, og å finne dyrkingsmetoder som kan redusere skadeomfanget. Bladrandskade er et stort problem i Norsk veksthusproduksjon av salat og gir næringen tap på opptil 20 %, eller nesten 20 millioner kroner hvert år. Det er derfor viktig å finne gode løsninger på problemet med bladrandskade.

Bladrandskade er en form for nekrose på kanten av salatbladene. Man tror skaden er knyttet til kalsiummangel i disse cellene- noe som fører til at de kollapser. Bladrandskade oppstår når salaten opplever utilbørlig abiotisk stress, som for eks. lange dager, høy lyssum, høy lysstyrke (over 16-17 mol/m²/dag) og forhold som begrenser transpirasjonen (høy luftfuktighet og lav tilgjengelighet på vann).

I denne oppgaven testes det flere miljøfaktorer gjennom totalt 5 forsøk i klimakontrollerte vekstkamre, for å identifisere klima som fremmer bladrandskade i 'Frillicesalat'; økt temperatur, økt relativ luftfuktighet (RF), forskjellige lysstyrker, lysperioder og lyssummer.

Videre ble det utført forsøk med hvit LED (med og uten mørkerød LED) for å teste om lyskvalitet kan benyttes som metode for å redusere bladrandskade sammenlignet med SON-T. Det ble også utført en forbehandling («priming») av 'Frillicesalater' under oppalet for å teste om plantene kan akklimatiseres til å tolerere stress under dyrkingen. Primingen ble utført med en lysstyrke på (300 μmol/m²/s, HPS) eller (150 μmol/m²/s, HPS) i kombinasjon med (100 μmol/m²/s blå LED).

Økt temperatur og økt RF fremmet ikke bladrandskade. Det ble funnet en klar effekt av økt lysstyrke/økt lyssum som forårsaket større indre bladrandskade.

Hvit LED ga mer ytre bladrandskade, mens hvit LED i kombinasjon med mørkerød LED ga redusert ytre bladrandskade. Primingen ga ingen reduksjon i bladrandskade- hverken med høy lysstyrke, eller med lav lysstyrke og blå LED.

Det ble også utført analyser av kationer (Ca, K og Mg) for å identifisere om kalsium spiller en rolle i utviklingen av bladrandskade. I tillegg ble det utført analyse av hydrogenperoksid (H₂O₂), et fritt radikal som forårsaker oksidativt stress.

En sammenheng mellom ROS og bladrandskade ble funnet- noe som indikerer at bladrandskade er knyttet til oksidativt stress. Lavere kalsiumnivåer ble funnet i unge blader som var hardere rammet av bladrandskade, enn i unge blader som var mindre rammet. Dette bekrefter at kalsium spiller en rolle i utviklingen av bladrandskade.

Abbreviations

PPFD = Photosynthetic Photon Flux Density

HPS = High Pressure Sodium

LED = Light Emitting Diodes

FR = Far-red (light)

R = Red (light)

RH = Relative air humidity

ROS = Reactive Oxygen Species

CaM = Calmodulin (proteins)

CDPK's = Calcium dependent protein kinases

CBL's = Calcineurin B-like proteins

CIPK's = CBL-interacting protein kinases

UV = Ultraviolet (light)

EC = Electric conductivity

VPD = Vapor Pressure Deficit

W = Watts

C = Celcius

Ca = Calcium

Mg = Magnesium

K = Potassium

DAB = 3,3'-diaminobenzidine

FW = Fresh weight

DW = Dry weight

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1 Introduction

In Norway the sale of greenhouse-grown 'Frillice' lettuce (*Lactuca Sativa L.*) reached 7 617 000 lettuces in 2016. With an average price of 12.90 NOK pr. lettuce, the value of the production reached almost 100 million NOK. Tipburn is estimated to cause a loss in the production of up to 15-20 %, and will then account for a loss of up to 20 million NOK/y. There is currently about 7 hectares of lettuce production in greenhouses in Norway. This equals to about 4 % of the total greenhouse area (Torre & Sand, 2017).

An optimal production of Frillice' lettuce will yield about 2.800.000 lettuces/ha/y, and by avoiding tipburn an added value of 6,000,000 NOK/ha/y can be achieved. For a greenhouse grower with a greenhouse area of 0.5 ha, this will amount to 3 million NOK in added value/y. The participating growers have a total of 2.7 ha growing area (almost 40 % of the total lettuce growing area), and can potentially increase their income by 16 million NOK (Torre & Sand, 2017).

Tipburn is a problem in production of lettuce because the necrotic tips that is defined as tipburn (Fig. 1), is a cosmetic damage that reduce the value of the lettuce and in many cases makes the product unsellable (Torre & Sand, 2017). According to Saure (1998) and references therein, "the susceptibility to tipburn is genetically determined but influenced by environment". Because the greenhouse growers know the cultivar they grow, and this cultivar is popular with the consumer it is difficult for them to change to a cultivar less susceptible to tipburn. Understanding why tipburn occurs and to develop cultivation methods to mitigate tipburn becomes paramount.

Tipburn is not well understood, and its occurrence varies a lot under the same conditions-making it difficult to predict and understand. It also appears under conditions that promote growth and conditions that inhibit growth (Saure, 1998). A growing scheme that reduces tipburn without also reducing yields, has not yet been found (Bárcena et al., 2019).

The experiments conducted during this thesis work is performed to add to the understanding of how outer and inner tipburn is affected by temperature, relative air humidity, light intensity, light sum, light quality and photo period. They are also done with the aim of finding cultivation methods that can help mitigate the occurrence of tipburn, and its severity, within the same yield requirements. Adding understanding to why tipburn occur

and finding methods to avoid it will help growers produce quality lettuce, increase their profits, reduce food waste and increase sustainability of the greenhouse lettuce production in Norway.



Figure 1: Outer tipburn in lettuce grown at Espedal Handelsgartneri AS. Photo: Martin Knoop.

2 Theory

2.1 Frillice´ lettuce (*Lactuca Sativa* L. 'Frillice')

Frillice´ lettuce is a variant of leaf lettuce. Leaf lettuce is one of four botanical varieties of lettuce (*Lactuca Sativa*), that is cultivated (Petruzzello, 2019), and is an annual leaf vegetable of the daisy family (Asteracea). All these 4 varieties stem from a weedy plant used in ancient Egypt. This form of lettuce bolted early and gave seeds that was pressed for oil. From here lettuces spread to China and Europa and eventually the New World (with Columbus´s second journey in 1494), and was cultivated into the over 100 types we have today. The name *Lactuca* means “milk”, and *Sativa* means “common” (The Columbia Encyclopedia, 2019).

The cultivar Frillice is a type of Frillice´ lettuce that is a cross between the leaf lettuce endive, and iceberg lettuce (a type of head lettuce and another of the 4 botanical varieties cultivated (Seeds, 2019). Frillice have a sweet crispy taste and an excellent resistance to bolting. In outside conditions it can be harvested between May-October in Norway. It takes 7-8 days to germinate (at soil temperatures as low as 5°C) and 80-83 days to grow before being ready to harvest. It germinates poorly at soil temperatures above 24 °C. Lettuce prefers moderate temperatures (Ah-Chiou et al., 2015). It grows best during spring and late summer, and not

in the hottest periods during summer (Organic-seeds, 2019). Optimum temperature for lettuce is 18°C (max 17-28 °C) during day and not over 15°C at night (Vegetables.co.nz, 2019).

2.1.1 Nutritional value and use of lettuce

The most common use of lettuce is as a food. It has a high water-content ($\pm 95\%$) and mostly lesser amounts of nutrients than other green vegetables (The Columbia Encyclopedia, 2019). It is generally high in vitamin A and K (Petruzzello, 2019), vitamin C and folate (Vegetables.co.nz, 2019), minerals and fiber, but has little to no fat or protein (The Columbia Encyclopedia, 2019). Because of the high water-content lettuce is hard to preserve (dry or freeze). The optimum storage temperature is right above 0°C, and the lettuce is normally consumed fresh (Vegetables.co.nz, 2019).

Lettuce in greenhouses may accumulate high levels of nitrate, when grown under low light and low temperatures. This can be countered by using supplementary lighting. Some compounds, such as nitrosamines (van Maanen et al., 1998), that are converted from nitrate- can be carcinogenic or cause a syndrome called blue baby. Lettuce can be a source of latex and was in folk medicine used to treat some illnesses as pain and rheumatism (The Columbia Encyclopedia, 2019).

2.2 Greenhouse production of Frillice' lettuce (*Lactuca sativa*, L. 'Frillice') in Norway

Most greenhouse growers of Frillice' lettuce in Norway uses a hydroponic system where the lettuce pots are put into gutters. (The system is called Nutrient Film Technique (NFT), (Van Os et al., 2008)). This is done when the lettuce reaches the 5-leaf stadium (5 true leaves), (pers. com. Espedal, 2018). As the lettuce grows, the gutters are moved continuously from the one end of the greenhouse to the other, with an increased spacing between the gutters to optimize the amount of light the plants receive. (The spacing is adjusted 7 times during the growing period). In the beginning there is no spacing (see Fig. 2), and at harvest the gutters are about 15 cm apart.

The lettuce is sown in pots filled with peat (54 in each tray), and covered by an acrylic cloth to maintain the humidity. The lettuce is kept in a dark chamber for 4 days, at 15°C for germination, and then moved to the greenhouse where they grow for approximately 16



Figure 2: Pre-cultivated lettuce in trays (to the right) are moved over to the gutters (to the left), where the lettuce will continue growing until harvest. Picture taken at O. Espedal Handelsgartneri. Photo: Martin Knoop.

more days before being moved to the gutters. In the greenhouse the temperature is between 18 and 20 °C (pers. com. Espedal, 2018).

Under the pre-cultivation the plants are watered once every day. When inserted into the gutters the lettuces receive watering once every other hour- to once every hour depending on the time of year and the transpiration that occurs. The EC is 1,5 during summer and 2-2,5 during winter (pers. com. Espedal, 2018).

A nutrient solution is applied to the water that is used to water the lettuce. The nutrient solution is a mix of calcium nitrate, potassium nitrate, calcium chloride, a basic cucumber fertilizer and an iron chelate fertilizer (see chapter 4.8.1 for specifications), (pers. com. Espedal, 2018). If the water is recycled sand filters and UV-lighting can be used to clean the water and remove pathogens that can cause diseases (pers. com. Espedal, 2018).

During the growth period, the growers use a climate computer to control the climate in the greenhouse. Irradiance is about 110-120 W/m² (HPS-lamps), and the photoperiod can be up to 24 hours. Air humidity is held at a minimum (Fig. 3). Some growers also add CO₂ (to about 800 parts per million) to the greenhouse air, to boost the production (pers. com. Espedal, 2018).

When the lettuces are harvest-ready (>150 g weight) they are cut manually and put on a conveyor belt and moved to the packaging machine (Fig. 4). If the head is too small, two

heads can be packed together to reach the minimum 150 g required (pers. com. Espedal, 2018).



Figure 3: Lettuces grown in gutters with fans in the ceiling to move the air forward and even out the humidity. Picture taken at O Espedal Handelsgartneri. Photo: Martin Knoop.



Figure 4: Harvest-ready lettuce (to the right) is cut and put onto the conveyor belt, and packed in plastic (to the left). Picture taken at O Espedal Handelsgartneri. Photo: Martin Knoop.

There are three main cultivars; Frilice (most widely used), Danstar and Cristabell. All of these experience tipburn. For Norwegian growers it's the outer tipburn type that is the most common and commercially severe problem. Then, the outer leaves get brown tips (Fig. 1). In the wintertime the duration of a growing period is about 70-75 days until harvest (Fig. 4). In May its 49 days (pers. com. Espedal, 2018).

2.3 What is Tipburn?

As stated in the introduction, tipburn is not well understood, despite many studies on the subject. According to Uno et al. (2016) "Tipburn is a physiological disorder caused by calcium (Ca) deficiency that occur mainly in leafy vegetables such as lettuce, resulting in a reduced commercial value". It limits both appearance and shelf life. Tipburn occur as external (outer leaves) or internal (inner leaves) damage caused by insufficient calcium in the cell walls- leading to their collapse. This is seen as brown necrosis in the leaf margins (Dimsey, 2010).

Outer tipburn can to an extent be trimmed away with the outer wrapper leaves at harvest. Inner tipburn can be a gateway to bacterial breakdown and slime, and isn't necessarily apparent before harvest. Of the two, inner tipburn represents the biggest commercial problem (Dimsey, 2010). However, for Norwegian greenhouse growers inner tipburn rarely occurs, making the outer tipburn type a lot more important in a Norwegian greenhouse production context (pers. com. Espedal, 2018).

Calcium is transported from the root to the leaves through transpiration. The older, bigger leaves transpire more and therefore accumulate more calcium, than the smaller inner leaves. The young, inner leaves grow more rapidly, and with less calcium form weaker cell walls (Dimsey, 2010) making them more susceptible to tipburn (Sago, 2016).

According to Dimsey (2010), Tipburn is more a problem of calcium uptake and transportation during periods of rapid growth, than an actual deficiency. Even with plentiful supplies of calcium in the growth medium, symptoms can appear. According to Saure (1998) and Bárcena et al. (2019), tipburn is a physiological disorder caused by environmental conditions that invokes stress, and not a calcium deficiency, in other words by abiotic stress.

Tipburn is shown to be linked to a rapid growth rate (Kuack, 2017) or stress that cause uneven growth. The growth rate, as a function of climate conditions, water and nutrient

availability (Dimsey, 2010), affect the lettuces ability to take up and transport calcium to the leaf tip. This ability is impaired with a higher growth rate (Kuack, 2017).

Tibbitts and Rama Rao (1968) and Gaudreau et al. (1994) found that tipburn increases with higher light intensity and longer photoperiod. Studies at Cornell University found that increasing transpiration will help against tipburn occurrence, as this increase the uptake of nutrients (including calcium), (Ciolkosz et al., 1998). They further found that a too high light sum (above 16-17 moles/m²/day) will increase tipburn, even with measures to increase transpiration (Both et al., 1997). This because of the increased growth rate (Kuack, 2017).

A lower light intensity (at 12-13 moles/m²/day) but with an increase in CO₂-concentrations (to 1000-1200 ppm) to substitute the missing light, and maintain growth rate also was found to result in the same amount of tipburn damage as with the higher light intensity. The tipburn can occur very quickly once the conditions for it is set, even within a day- making uneven growth just as damaging (Kuack, 2017).

Bárcena et al. (2019) found that tipburn might not be an issue with a slower growth rate where nutrient uptake and transport is allowed to keep up. This is true during winter when the growth period can be as much as double the length as that during summer. This because of the lower light levels received. Even with supplemental lighting it is then easier to maintain a more stable environment, than with the higher fluctuations in light and temperatures occurring during spring, summer and fall. With supplemental lighting, subsequent increase in growth and more crop rotations are allowed during the year (Kuack, 2017), making the economic consideration one of finding the equilibrium of low enough tipburn occurrence and number of salable lettuces produced.

Carassay et al. (2012) found that the incidence of tipburn was linked to locally produced reactive oxygen species (ROS) under saline conditions. They also found that oxidative damage increased significantly before tipburn occurred. This can support the idea that tipburn is linked to stress responses.

2.4 Abiotic stress

According to Taiz and Zeiger, 2015, "the ideal growth conditions for a given plant can be defined as the conditions that allow the plant to achieve its maximum growth and reproductive potential as measured by plant height, weight and seed number, which

together comprise the total biomass of the plant". Abiotic stress can be defined as "environmental factors that affect plants and reduce growth and yield below optimal levels" (Andjelkovic, 2018). These include flooding, drought, air humidity, high and low temperatures, light intensity, duration and quality, carbon dioxide, oxygen, soil pH and nutrient content (and their availability), toxins such as heavy metals and salts (Taiz & Zeiger, 2015).

Plants are sessile organisms that cannot escape their environment. With an aim to grow and reproduce, they therefore have to adapt to the fluctuations in conditions they experience throughout their lifetime. They do this by changing physiological and developmental processes, to maintain the metabolic equilibrium- they acclimate (Taiz & Zeiger, 2015). These responses can be either elastic (reversible) or plastic (irreversible). Plants seldom experience one stress condition at a time and therefore different stress pathways overlap, making the total response a complex and difficult system to understand (Andjelkovic, 2018). (See Fig. 5).

An example of mixed stresses can be the closing of stomata because of drought, a response that will also limit the CO₂-uptake (causing reduced photosynthesis). The reduced transpiration can again limit the plants ability to cool down the leaves during warm and intense light conditions (Cramer et al., 2011). Warm weather with high amounts of light is often the reason for drought, and so the plant experiences several stresses simultaneously (Taiz & Zeiger, 2015)

As the generations go on, the adaption to balancing the processes of energy production, ion and nutrient balance and storage, growth and development, together with the impact of environmental conditions are fine-tuned into an overall fitness to the conditions of a geographical area (Taiz & Zeiger, 2015). When the plants encounter stressful conditions, this fine-tuning will help the plant decide between the trade-offs between vegetative and reproductive development (Berens et al., 2019).

Understanding the abiotic factors that induce stress in Frillice´ lettuce will help us understand how the lettuces adapt to these conditions and when and why they fail in this adaption- and in extension why tipburn occur.

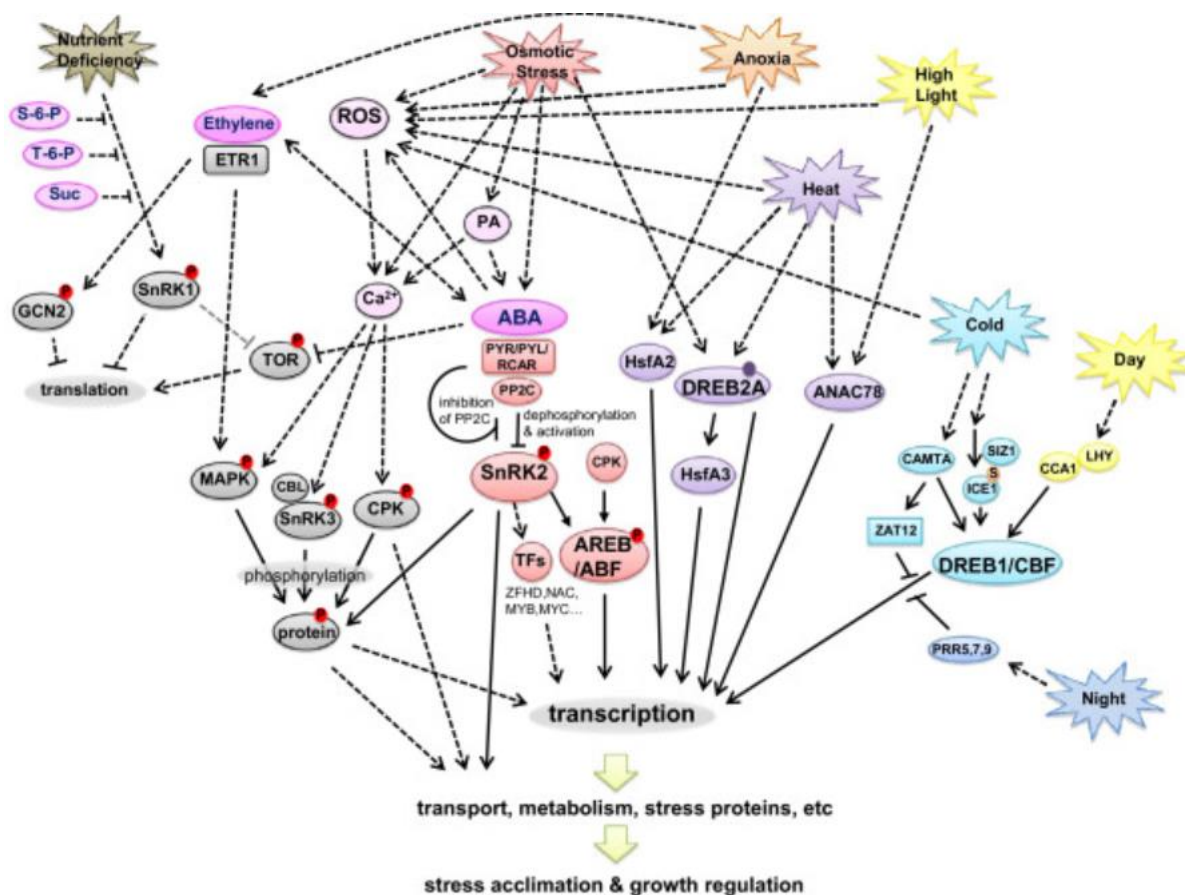


Figure 5: A simplified working model of a signaling network of plant responses to abiotic stress. Ovals represent proteins, metabolites or processes. Metabolites have magenta color. Phosphorylated proteins have red circles with a P inside. Sumoylated protein has an orange circle with an S inside. The solid purple circle indicates that DREB2 needs modification to be activated. Solid lines represent direct connections; dotted lines represent indirect connections (acting through some intermediate molecule). The gray line indicates that this reaction has not been shown in plants. Not all linkages and details of stress and hormone effects are shown in this diagram in order to simplify the model. Abbreviations: ABA (abscisic acid), ANAC (Arabidopsis NAC domain-containing protein), CAMTA (calmodulin-binding transcription activator), CBL (calcineurin B-like interacting protein kinase), CCA (circadian clock associated), CPK (calcium-dependent protein kinase), DREB/CBF (dehydration response element binding protein/C-repeat binding factor), ETR1 (ethylene response 1), GCN2 (general control non-repressible 2), HSF (heat shock factor), ICE (inducer of CBF expression), MAPK (mitogen-activated protein kinase), LHY (late elongated hypocotyl), PA (phosphatidic acid), PP2C (protein phosphatase 2C), PRR (pseudo response regulator), PYR/PYL/RCAR (ABA receptors), RNS (reactive nitrogen species), ROS (reactive oxygen species), SIZ (SAP and Miz domain protein), SnRK (sucrose nonfermenting-1 related kinase), TFs (transcription factors), TOR (target of rapamycin), ZAT (zinc finger protein). Figure and figure text taken from (Cramer et al., 2011).

2.4.1 The role of calcium in stress responses

Calcium is a macro nutrient, that in its divalent cation form (Ca^{2+}) is essential in maintaining cell wall (White & Broadley, 2003) and membrane structure (Hepler, 2005). Plants can make cell walls more rigid or plastic, and membranes more or less permeable depending on the calcium concentration (Hepler, 2005). Ca^{2+} is often grouped together with the elements K^+ , Mg^{2+} , Cl^- and Mn^{2+} as they can occur as single ionic form in plants. It generally occurs together with magnesium (Mg) in the next highest concentration in plant shoots after N and

P, and in the same Ca:Mg ratio in the plant as in the soil, making extreme ratios in soil difficult for the plant (Willey, 2016).

Calcium is transported from the root (via both apoplastic and symplastic transport) to the different parts of the plant via water transport in the xylem. It can be stored in the vacuole to attract inorganic and organic anions (White & Broadley, 2003) and is seldomly remobilized from here (Willey, 2016). Because calcium can only be delivered through transpiration and not be delivered either through storage or allocation (White & Broadley, 2003), deficiency in calcium first occur in new growth (Willey, 2016).

The intracellular concentrations of calcium are very low (Willey, 2016) allowing for signal transduction (White & Broadley, 2003). Calcium is involved in responses to different biotic and abiotic stress (Viridi et al., 2015), by fluctuating in cellular concentration- and in so doing acting as a second messenger for ex. through the calmodulin signaling system (Willey, 2016), where the signal is transduced by calmodulin (CaM)-proteins (Viridi et al., 2015). According to Viridi et al. (2015), CaM integrates “different stress signaling pathways which allows plants to maintain homeostasis between different cellular processes” (Fig. 6).

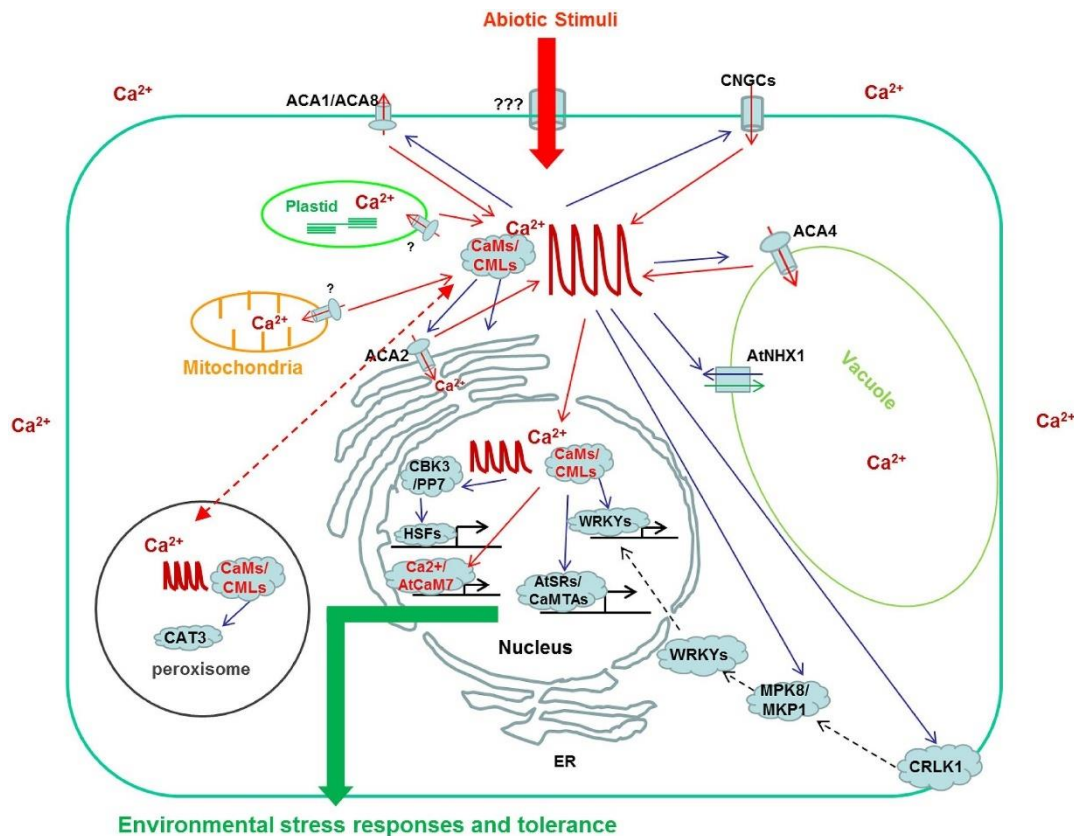


Figure 6: Schematic representation of Ca²⁺ transients and their modification and interpretation by CaM/CMLs as well as their target proteins in plant cells under abiotic stresses. This model is not exhaustive and only

includes the actions of a limited number of CaM/CMLs and target proteins; CaMs/CMLs/CBPs involved in biotic stresses and Ca²⁺ signal interpretation by other sensors such as CBLs and CDPKs are not included. Actions modifying Ca²⁺ transients or CaM/CMLs are presented by red arrows and actions regulated by Ca²⁺/CaMs or Ca²⁺/CMLs are presented by blue arrows. The dashed arrows imply multiple regulations extended to nucleus. Figure with text found in (Zeng et al., 2015).

Other ex. of proteins or enzymes linked to stress responses where calcium fluctuations are integral, are Calcium-dependent protein kinases (CDPKs), (Xiao et al., 2016) and calcineurin B-like proteins (CBLs), (Luan et al., 2002). CDPKs are involved with activating and repressing transcription factors, enzymes and channels, and in so doing triggering appropriate stress responses in the stress signaling network of the plant (Boudsocq & Sheen, 2013). CBLs interact with CBL-interacting protein kinases (CIPK) and thereby decodes calcium signals (Batistic et al., 2010).

According to White and Broadley (2003), a specific stress is thought to elicit a specific appropriate response. Mapping and understanding these signaling networks can give insights into calcium deficiency and stress tolerance in plants.

2.5 Reactive oxygen species (ROS) as a stress response

Reactive oxygen species (ROS) are harmful radicals that cause oxidative stress and can inactivate enzymes, damage membranes, cause degradation of proteins or lipids, cause damage to DNA and end up killing cells (Raja et al., 2017). From normal cell metabolism such as the reduction of O₂ to water, a small portion (estimated 1-2 %) of the O₂ is reduced into ROS (Karuppanapandian et al., 2011). According to Karuppanapandian et al. (2011), “ROS include superoxide ion (O₂⁻), hydroxyl ion (OH⁻), Hydroperoxyl radical (HO₂), Hydrogen peroxide (H₂O₂), alkoxy radical (RO⁻), peroxy radical (ROO⁻) singlet oxygen (¹O₂) and excited carbonyl (RO^{*})”.

The production of ROS is kept in balance by various antioxidant systems (Karuppanapandian et al., 2011), but when this balance is broken- due to various abiotic stress (for ex. on the photosynthetic apparatus) ROS is produced in quantity, and leads to oxidative stress (Pospíšil, 2016). Induced by abiotic stress, ROS can act as a signaling molecule that is important for acclimation processes due to these stresses, such as drought high light intensity and heat (Choudhury et al., 2017).

Each cell establishes its own ROS homeostasis and subsequent its own ROS signature that can depend on cell type, stage of development or level of stress. According to Choudhury et

al. (2017), it is likely that a specific type of abiotic stress or a specific combination of abiotic stresses can induce a distinct ROS stress-signal- its own signature, leading to a distinct stress response.

Understanding how ROS is affected by abiotic stress and how ROS acts as a signaling system inducing appropriate stress responses can give insight into plant acclimation and survival under challenging conditions (Pospíšil, 2016). These insights can help us understand why tipburn occur in lettuce production.

2.6 Priming- increasing stress tolerance in plants

“Priming” is to induce an appropriate acclimation in plants by pre-exposing them to stress- giving them a “stress memory” that can help the plants tolerate stress later in life, and also their offspring as an epigenetically inheritance (Wang et al., 2017). The priming helps the plant respond faster and stronger when experiencing stress (Conrath, 2009). According to Wang et al. (2017), “changes in hormones, metabolites, sugar signals, ROS, and other signals are induced by priming which enhance tolerance of the plants to the succeeding stressors.” Priming could be used as a cost-effective method to increase yields as it is less time consuming than other methods, such as conventional breeding (Thomas T.T & Puthur, 2017).

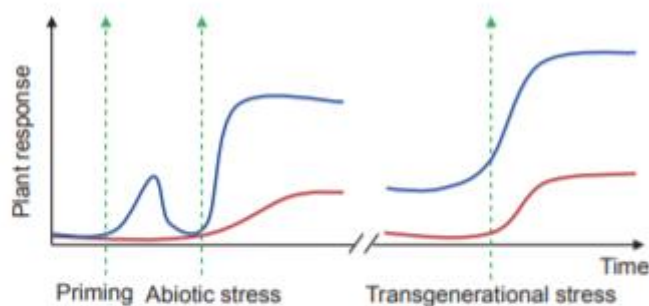


Fig. 1 Scheme of the plant priming responses in the same generation and transgeneration. The reaction level is shown in primed plants (blue) or un-primed plants (red) under different states of priming. Adapted from Balmer *et al.* (2015).

Figure 7: Schem of plant priming responses in current and coming generation, for primed (blue) and un-primed (red) plants Source: (Wang et al., 2017).

Priming with high temperatures is found to defend against heat stress in several plants (Wang et al., 2014) and (Fan et al., 2018) and wheat has been found to tolerate subsequent drought stress after drought-priming and returning to water abundant conditions (Selote & Khanna-Chopra, 2006). Thomas T.T and Puthur (2017) found that UV-priming of seeds and

seedlings of different beans, increased stress tolerance to various abiotic stresses due to higher production of metabolites and increase in antioxidant activity. According to Wang et al. (2017) evidence exist to support a theory that priming with one abiotic stress type can induce cross tolerance to several stresses (Fig. 7).

Priming could potentially serve as a strategy to reduce or avoid tipburn occurrence in a cost-effective and pragmatic manner during greenhouse production of Frillice' lettuce.

2.7 Climate control in greenhouses

The aim of controlling the climate in greenhouses is to optimize production of biomass and quality in the plants- balanced with minimizing costs of this control, by reducing energy consumption, waste of CO₂ and nutrients (Sand, 2019b). The climate in greenhouses are controlled by a climate computer system; either Hortimax, Priva or Senmatic is used in Norway, (pers. com. Sand, 2019). The climatic conditions inside the greenhouse is monitored by different sensors that are connected to the climate computer. These are ambient CO₂ levels, temperature, relative air humidity (RH), PAR irradiance from both supplementary and natural light (also outside PAR irradiance), wind (outside), water, pH and electric conductivity (EC), (Brechner & Both, 2013).

The monitored conditions are logged by the computer and the data used to activate control measures as the different conditions hit a certain set point (Brechner & Both, 2013). If it gets too hot, the computer will open the windows and hot humid air and CO₂ is vented out. Or it might start the dehumidifier, cooling the air and storing the recovered heat in a buffer tank for use during the normally colder night, and saving CO₂ and heat from being vented out the window- reducing costs (Sand, 2019b).

Controlling the climate in greenhouses is complex, and it can be challenging to optimize the use of the climate computer (Brechner & Both, 2013). Every climate condition can fluctuate dramatically during the growing period, and even during the hour- potentially causing stressful conditions for the plants. What is optimal temperature ranges, light intensity etc. also changes during the development of the plant production. What is ultimately the correct use of the climate computer changes with research and with the grower's own experience (Sand, 2019b).

2.7.1 Temperature

In greenhouses the optimal temperature for photosynthesis, growth and development of plants are dependent on cultivar and its climate requirements (Hatfield & Prueger, 2015). Physical properties of all molecules and how they behave are dependent on the temperature and temperature changes. Lipid membranes, protein conformation and subsequent function and nucleic acids are all affected by temperature (Willey, 2016). At optimal temperatures photosynthesis is optimized, due to the increased efficiency of the different photosynthesis enzymes, such as rubisco (Markings, 2018).

Temperature affect growth and development either alone or together with other climate conditions such as light and photoperiod. The different development stages often have different temperature requirements, such as seed germination, flower bud development and flowering (Willey, 2016). In production of Frillice' lettuce in ex. temperature set point for germination is set to 15°C and temperature set point to 18-20°C during growth (pers. com. Espedal, 2018). According to Bremer and Bremer (1931) "lettuce is a quantitative long-day plant"; Too high temperatures together with long photoperiod will induce bolting in iceberg lettuce (which is undesirable for a salable product), (Khan, 2018). Temperature fluctuations and extreme temperatures will cause stress that inhibits the growth and development (Taiz & Zeiger, 2015).

Leaf temperatures are often higher than the air temperature during day, due to light radiation (of which 95 % can be lost as heat), and lower during night. During transpiration the heat will dissipate from the leaves- cooling the plant (Bævre & Gislerød, 1999). Root temperature affect nutrient and water uptake. Cold roots grow slower, limiting the uptake (Willey, 2016). In general, it is best to achieve a root temp as high or a couple of degrees higher than that of the air temperature. This is also dependent on cultivar (Bævre & Gislerød, 1999).

2.7.2 Light

The foundation for greenhouse production is sunlight. Greenhouses function as big solar collectors that captures its energy to use it in the photosynthesis (pers. com. Sand, 2019).

Light from the sun that hit Earth is relative constant and this amount of solar radiation is called "the solar constant" and equals to 1370 joules/s/m² or 1370 W/m² (Willey, 2016). This light is filtered on its way to the surface resulting in a characteristic composition or

spectrum- which peak at 500 nm, wavelengths, with an irradiance of about 1000 W/m² (Bævre & Gislørød, 1999).

Of this irradiance, about 450-500 W/m² is photosynthetically active radiation (PAR), with the wavelengths of 400-700 nm (blue to red light), (Bævre & Gislørød, 1999). Light of shorter wavelengths are more energy dense than longer wavelengths (Evert & Eichhorn, 2013). For photosynthesis, the energy in a red photon is sufficient. If a blue photon hits the plant, the excess energy is lost through heat and fluorescence. It is therefore not the amount of energy within a photon but the number of photons (within the PAR spectrum) that is the basis for photosynthesis. (Bævre & Gislørød, 1999). Because we are mainly interested in the amount of photons that can be used in photosynthesis we measure the photosynthetic photon flux density (PPFD), or the amount of photons that hits a surface at any given time (Ibaraki & Shigemoto, 2013). In ex. 150 µmol/m²/s.

About 5 % of the light hitting the plants are turned into carbohydrates. This happens by oxidation of water and reduction of carbon dioxide by the help of light (Bævre & Gislørød, 1999). The rate of photosynthesis (and subsequent growth) is affected not just by light, but also by temperature, CO₂ concentration, relative air humidity, water balance and nutrient status. Thus, all climate factors have to be optimized in order to have a high plant production (Willey, 2016).

2.7.2.1 *Light intensity*

There is a saturation point in photosynthesis called the light saturation point where a continued increase in light no longer increase photosynthesis. Shade-tolerant plants will be saturated in the area of 100-200 µmol/m²/s, where light demanding plants will need about 1000 µmol/m²/s before saturation (Gislørød and Bævre, 1999). Lettuce is a shade-tolerant plant that is found to maintain relative high yields in shade (Marrou et al., 2013).

Plants are adapted to tolerating the transition from dark to light, and from low light to high light intensity. They tolerate this sudden change in light by rapid increase in chlorophyll fluorescence, followed by a gradual quenching of energy and electrons. This is known as the “Kautsky effect” (Willey, 2016). Acclimation to different light is easier for younger leaves than for older leaves (Taiz & Zeiger, 2015).

If plants are exposed to excess light, they will begin to experience photoinhibition; a reduced photosynthetic rate, due to a reduction in functional photosystem 2 complexes (PS2), caused by damage done by light (Willey, 2016). PS1 complexes are also damaged, but at a much lower rate. The repair of these photosystem-complexes is slow in shade-tolerant plants (Järvi et al., 2015). Free radicals can also be produced and then needs to be defeated by non-photochemical quenching such as by antioxidant systems (Willey, 2016).

2.7.2.2 Photoperiod

The photoperiod affects the total amount of light the plant receives and the balance between photosynthesis and respiration (Taiz & Zeiger, 2015). The plants sense the length of day (the phytochrome senses the length of night) and adjust its growth to be either vegetative or regenerative depending on the changes of photoperiod (Bævre & Gislerød, 1999).

2.7.2.3 Light sum

The plant growth and development are dependent on the total amount of light within each photoperiod; the total number of photons received during the photoperiod (Taiz & Zeiger, 2015). The light sum is calculated after this formula:

$$\text{Light sum } \left(\frac{\text{mol}}{\text{m}^2 \cdot \text{day}} \right) = \frac{X \text{ } \mu\text{mol/m}^2/\text{s} * 3600 * x \text{ h}}{1000 * 1000}$$

By controlling the light sum we can optimize growth and avoid undue stress. The amount of supplemental lighting is then dependent on the amount of natural light received (Bævre & Gislerød, 1999).

2.7.2.4 Light quality

The plants use light quality to sense their surroundings and adjust their morphology accordingly. This sensing of light is performed by photoreceptors such as the cryptochrome and the phytochrome (Evert & Eichhorn, 2013), (Fig. 8).

Photomorphogenesis, or light adjusted growth is how plants are affected by this light sensing. Plants that are grown under blue light in ex. will be more compact and have thicker leaves, while plants grown under far-red light will be long and thin (Bævre & Gislerød, 1999). This is because blue light indicates low shading and therefore low risk of competitors overshadowing the plant. Far-red light however indicates a lot of shading (lot of fluorescence

and reemitting of longer wavelengths by neighbors) and imminent risk of being out-competed (Evert & Eichhorn, 2013). Plant morphology is affected by the ratio between red and far-red light (R/FR ratio). The R/FR ratio in natural light is about 0.9-1.1. A R/FR ratio that is low indicates shade and a high ratio indicates lot of light (Bævre & Gislerød, 1999).

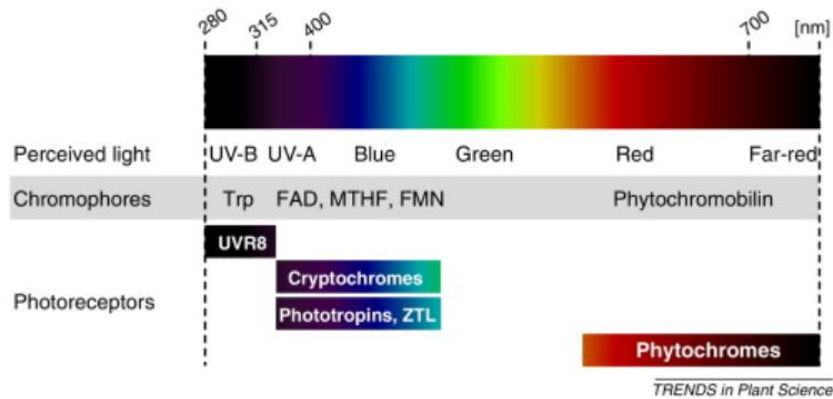


Figure 8: Photoreceptor-mediated light perception in higher plants. Plant photoreceptors perceive information from a large part of the light spectrum. UVR8 is the only UV-B photoreceptor identified to date. Tryptophans (Trp) intrinsic to UVR8 were postulated to provide a 'UV-B antenna', with a major role identified for tryptophan-285 [6]. Various proteins harbour chromophores able to absorb light in the UV-A/blue part of the spectrum. Cryptochromes bind Flavin Adenine Dinucleotide (FAD) and methenyltetrahydrofolate (MTHF) as chromophores [5]. Phototropins and the Zeitelupe (ZTL) proteins bind Flavin Mononucleotide (FMN) chromophore through their LOV (light, oxygen or voltage) domains. Phytochromes are red/far-red photoreceptors, also involved in some blue light responses, which use the plant-specific chromophore phytochromobilin, a linear tetrapyrrole. Figure and text found in (Heijde & Ulm, 2012).

2.7.2.4.1 High pressure sodium lamps (HPS lamps)

High pressure sodium (HPS) lamps is the common lamp type in lettuce production in Norway. HPS lamps give off a yellow and yellow to green dominated light and radiates a lot of heat- reducing the need for heating of the greenhouse and also causing increased growth (Sand, 2019c). Their spectral composition is low on blue light and in periods of low natural light it can be advantageous to increase the intensity to avoid elongation (Bævre & Gislerød, 1999).

HPS lamps are the most commonly used lamps in Norwegian greenhouses. Of these it's the 400 W/230V armatures that is most widely installed. Such lamps have an efficiency of 1.8 $\mu\text{mol/W}$ and have a lifetime of 10-12,000 hours before the bulbs need to be changed. If one installs a 1000 W/400V the efficiency increases to 2.1 $\mu\text{mol/W}$ (Sand, 2019d). HPS lamps use a relative long time to ignite into full brilliance (5 min) and needs at least 1-1.5 minutes before re-ignition (Bævre & Gislerød, 1999). For spectral distribution see Fig. 17.

2.7.2.4.2 Light emitting diodes (LED)

The use of LED armatures have been a greenhouse revolution in waiting. This is foremost caused by its continual high price relative to HPS (Up to 5-6 times higher). The main advantage of LED is that their spectral composition can be designed to fit the culture one wants to produce. They are also marketed as high in efficiency: between 1.6 and 2 $\mu\text{mol}/\text{W}$ for some producers, and the best LED's provides 2.4 $\mu\text{mol}/\text{W}$. Their light is cold and radiates very little heat compared to HPS (Sand, 2019c). This enables them to be used as interlighting, or in cultures that have a low temperature need- such as lettuce. Then one can reduce venting and saving CO_2 (pers. com. Sand, 2019). For spectral distribution of lamps see Fig. 18-21, and Fig. 23.

2.7.3 Relative air humidity

Relative air humidity (RH) influence plant transpiration (Willey, 2016). Furthermore, high transpiration causes an increase in humidity in closed greenhouses (Sand, 2019a). The relative air humidity (RH) is affected by the temperature inside and outside the greenhouse (Bævre & Gislerød, 1999). The air will contain more water with higher temperatures at the same RH (Sand, 2019a). This can be explained by the saturation deficit (grams of water vapor needed to saturate the air). At 18 °C and RH = 100 % the air contains 15.5 grams of water pr. m^3 . At 18 °C and RH = 80 % the air contains 12.4 grams/ m^3 and the saturation deficit is in this case 3.1 grams. If the temperature increases to 22°C and the RH is kept at 80 %, the saturation deficit increases to 4,2 grams (the air will hold 1,1 grams more pr. m^3), (Bævre & Gislerød, 1999).

When the RH = 100 % the air is saturated. Any drop in temperature would then condense and form dew on surfaces in the greenhouse, such as the leaves (Sand, 2019a). The dew point is the temperature required to condense water vapor. At RH = 80 %, a temperature drop from 22°C to 18°C will cause the need for 1.1 grams to be condensed for the RH to stay at 80 % (Bævre & Gislerød, 1999) .

The air humidity is water vapor (in gas form) in the air (Willey, 2016). This vapor influences the air pressure. The saturation deficit can therefor also be given as “vapor pressure deficit” (VPD), in pascal (Pa). VPD is the difference between the saturation pressure (pressure of

vapor needed to cause condensation) and the actual vapor pressure (Bævre & Gislerød, 1999).

Vapor pressure deficit can be calculated following this formula (Bævre & Gislerød, 1999):

$$\text{Actual vapor pressure} = \frac{\% RH * \text{Vapor pressure at given temperature}}{100} \text{ in ex: } \frac{70 * 2337}{100} = 1.6 \text{ kPa}$$

$$\text{VPD} = \text{saturation pressure} - \text{vapor pressure} \Rightarrow 2337 \text{ Pa} - 1636 \text{ Pa} = 0.7 \text{ kPa}.$$

A high RH will give plants larger in volume, with longer shoots and bigger leaves, an increased number of shoots and earlier flowering. It also affects the stomatal aperture (Bævre & Gislerød, 1999). With a higher RH the stomata are more open, but the transpiration lessens due to low VPD and the uptake, transport and translocation of calcium for can be reduced (Willey, 2016).

2.8 CO₂

Nearly half of the dry matter in plants are carbon (Taiz & Zeiger, 2015). The plants turn carbon dioxide into sugars and other metabolites during photosynthesis. Their carbon assimilation is dependent on their physiology. Several strategies exist: C₃-plants, C₄-plants and CAM-plants. Lettuce is a C₃-plant and will have great effect of elevated CO₂ concentrations above ambient levels (400 ppm), (Bævre & Gislerød, 1999).

An increase in CO₂ above ambient levels can increase the light saturation point (Willey, 2016). Carbon dioxide also compensates for light intensity when it comes to growth. For a certain interval an increase in temperature will increase the growth effects of CO₂. The transpiration will decrease with elevated CO₂ concentration. The EC can then be increased to offset the lower nutrient uptake (Bævre & Gislerød, 1999). In greenhouses CO₂ is elevated up to 1000-1200 ppm (depending on crops). The CO₂ is supplied from either a tank or by burning of gas (pers. com. Sand, 2019).

3 Main objectives

3.1 Main objectives of the study:

- i. To identify climate factor(s) that induce tipburn in lettuce.
- ii. To improve the physiological understanding of why lettuce develops tipburn and to test cultivation methods that can reduce the problem.

3.2 Sub-goals to test main objective I

- i. Test if elevated temperature and relative air humidity (RH) induce tipburn in lettuce (Exp. 1).
- ii. Test if light intensity, either by an increase in photosynthetic photon flux density (PPFD) or light sum, induce tipburn in lettuce (Exp. 2 and 3).
- iii. Test if a combination of high light intensity and high relative air humidity (RH) affects the incidence of tipburn in lettuce (Exp. 3).

3.3 Sub-goals to test main objective II

- i. Test if leaves with tipburn accumulate higher levels of reactive oxygen species (ROS) (Exp. 3).
- ii. Investigate the relationship between cations (Ca, K, Mg) and development of tipburn (Exp. 3, 4, and 5).
- iii. Test if light quality, by using different light composition during cultivation, affects development of tipburn (Exp. 4 and 5).
- iv. Test if priming during pre-cultivation affects development of tipburn during cultivation (Exp. 5).

4 Materials and Methods

4.1 Plant material and pre-cultivation

Frillice' lettuce (*Lactuca Sativa L. 'Frillice'*), supplied by Norgro (Norway) were sown (at 4-5 mm depth) in small biodegradable pots with the size of 0.08 liters- containing fertilized peat soil. (Fig. 9). The peat was of the type "Degernes torv" supplied by Degernes Torvstrøfabrikk AS (Norway). The seeds were coated as is practiced in most greenhouses. The pots were put in a dark chamber for four days until germination. The temperature was set to 15°C and the relative air humidity (RH) to 60 %.

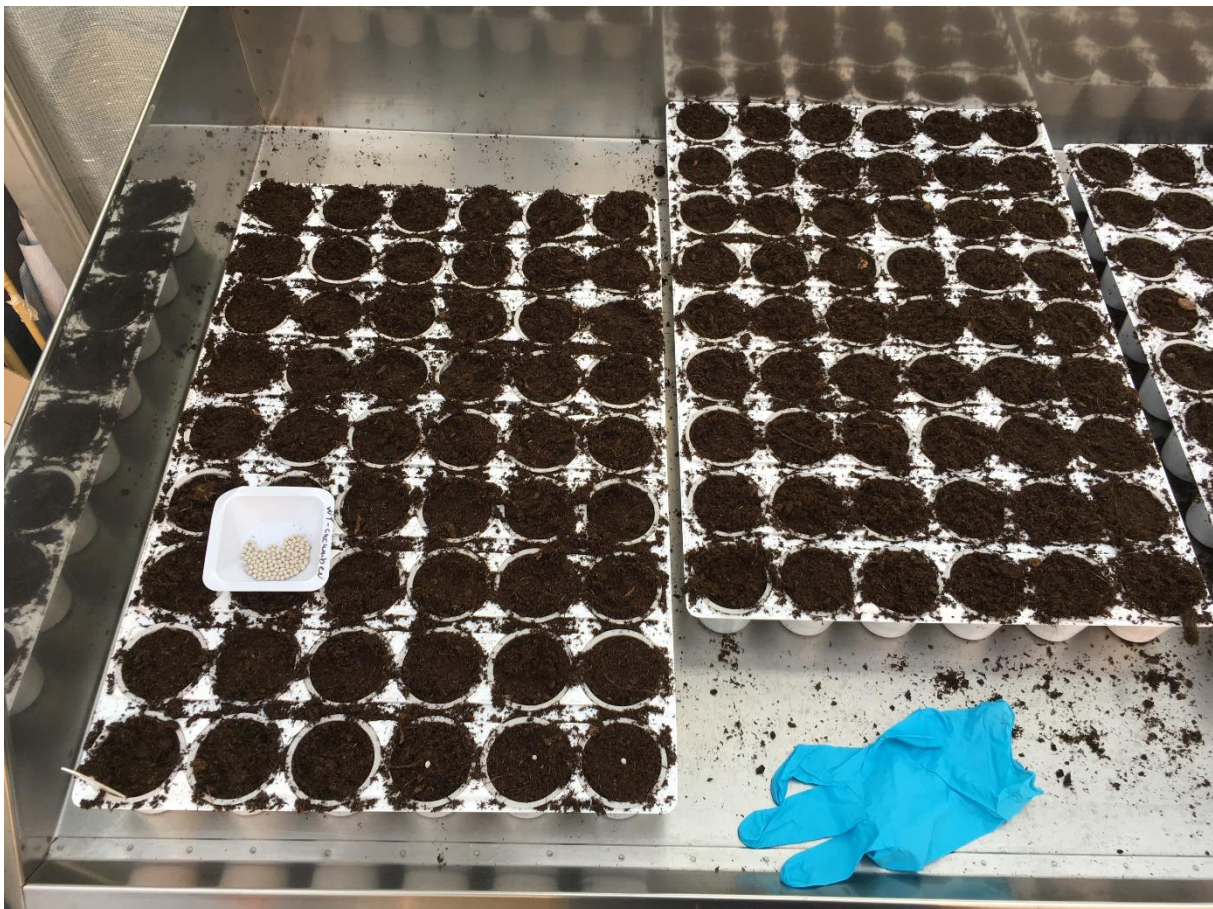


Figure 9: Trays of 54 pots each filled with peat and seeds sown at 4-5 mm depth. Coated seeds can be seen in the tray to the right. Photo: Martin Knoop.

After germination the seedlings were grown in a greenhouse compartment for approximately three weeks- until they had reached the 5-leaf stadium (5 true leaves). The seedlings were grown under 18 hours lighting from 400 Watts HPS (High Pressure Sodium lamps) supplied by Gavita (Norway). (See chapter 4.9.2 for details), with a photon flux density (PFD) of 150 $\mu\text{mol}/\text{m}^2/\text{s}$. The temperature in the greenhouse was set to 20°C and RH to 60 % day and night. The seedlings were watered once a day (more often if needed) with a greenhouse fertilizer solution of 1.5 EC, (for specifications see chapter 4.8.1).

The climate in the greenhouse was controlled by a Priva climate computer (Priva, Zijlweg, The Netherlands). The air was humidified with water from sprinklers in the roof. The threshold for turning the sprinklers on were an outside irradiance of 300 Watts, higher temperatures inside than 20°C or a RH lower than 57 %. The effervescence lasted for 10 seconds each time.

4.2 Setup for growth chambers

After reaching the 5-leaf stadium the lettuces were placed in closed growth chambers (40 lettuces in each chamber) without any natural daylight. Each lettuce was marked with a label pin so the same plant would be registered each time during the experiments. See Fig, 11 and 11.1. The lettuces were randomly distributed in the four gutters. See Fig. 12.



Figure 10: Showing growth chamber 1 with the black plastic box between the pillars the gutters rest on, and the yellow hose bringing water up to the black hoses to the right connected to the 4 gutters. To the left the water leaves the gutters and enters the transparent plastic boxes below. On top of the gutters lies the timer that starts the water pump every other hour. (The timer was hanging from the S-hook up in the right-hand corner). Photo: Martin Knoop.

Hanging from the ceiling a sensor box connected to the climate computer measured air humidity and temperature. (The sensor box had both dry and wet sensors). See Fig. 10, (Priva, Zijlweg, The Netherlands).



Figure 11: Labeling of the plants. Photo: Martin Knoop.

Figure 11.1: Plants from the greenhouse ready to be inserted into the gutters. Photo: Martin Knoop.



Figure 12: The plants inserted into the gutters at the 5-leaf stadium. This picture is from experiment 5, where there were 3 different plant stages at installment. Photo: Martin Knoop.

The growth chambers were equipped with a hydroponic system. There were four rows of gutters (Vefi AS, Norway) with 10 holes in each for the plant pots to fit into. At the one end, the gutters were connected to a hose that brought up the water with fertilizer solution from a black plastic box on the floor using a pump (Aquarium Systems Maxi-Jet 500, France) with a timer (müeller SC 28 11 pro, Germany). The other end was open and the gutters were tilted slightly towards this side for the water to leave the gutters and be collected by two other transparent plastic boxes standing beneath.

The gutters were spaced about 25 cm apart from each other for optimal lighting and close resemblance to a professional system. The gutters were 1.5 m long and 10 cm wide. The holes were 15 cm in between. See Fig. 12. The setup for the growth chambers can be viewed in Fig. 10, below. For specifications on watering during treatments in the growth chambers see chapter 4.8, below.

4.3 Experiment 1, effects of elevated temperature and humidity on tipburn

In experiment 1, four treatments were used. See Table 1 for specifications of each treatment. The pre-cultivation of the lettuces was done as explained in chapter 4.1. The first week of growth in the growth chambers, the conditions were the same in all four chambers- equal to the conditions in the MT/MRH (this to ensure the same acclimatization of the lettuces from greenhouse compartment to growth chamber- for all treatments), (Table1).

Table 1: Specifications for the treatments in experiment 1. High temperature + high RH = HT/HRH, moderate temperature + high RH = MT/HRH, high temperature + moderate RH = HT/MRH, moderate temperature + moderate RH = MT/MRH.

Treatment	Lamp type	Photo-period	Temp, day	Temp, night	Photon flux density $\mu\text{mol m}^{-2} \text{s}^{-1}$	Light sum mol/day	RH
HT/HRH	HPS	18 hr	20°C	27°C	150	9.7	90 %
MT/HRH	HPS	18 hr	20°C	20°C	150	9.7	90 %
HT/MRH	HPS	18 hr	27°C	27°C	150	9.7	60 %
MT/MRH	HPS	18 hr	20°C	20°C	150	9.7	60 %

Then, after one week the different treatments started (the conditions in MT/MRH stayed the same). The lettuces would then grow for approximately (and at least) two more weeks under the different treatments before the lettuces reached the right size (100g<) and the experiment ended.

For method of assessment of tipburn see chapter 4.10, below. The sampling of tipburn was in this experiment only done at the end of the experiment, and tipburn was only assessed for the whole plant (and not for each leaf). All 40 lettuces in each treatment was assessed.

4.4 Experiment 2, effects of increased light intensity and light sum on tipburn

In experiment 2, three treatments were used. See Table 2, for specifications of each different treatment.

The pre-cultivation of the lettuces was done as explained in chapter 4.1. The first week of growth in the growth chambers, the conditions where the same in all three chambers- equal to the conditions in HPS-LLI (this to ensure the same acclimatization of the lettuces from greenhouse compartment to growth chamber- for all treatments), (Table 2).

Then, after one week the different treatments started (the conditions in HPS-LLI stayed the same). The lettuces would then grow for approximately (and at least) two more weeks under the different treatments before the lettuces reached the right size (100g<) and the experiment ended.

Table 2: The specifications for each growth chamber in experiment 2. High light intensity (HPS) = HPS-HLI, moderate light intensity (HPS) + continuous photoperiod = HPS-MLI/ CPP, low light intensity (HPS) = HPS-LLI.

Treatment	Lamp type	Photo-period	Temp, day	Temp, night	Photon flux density $\mu\text{mol m}^{-2} \text{s}^{-1}$	Light sum mol/day	RH
HPS-HLI	HPS	18 hr	20°C	18°C	300	19.4	65 %
HPS-MLI/ CPP	HPS	24 hr	20°C	-	200	17.3	65 %
HPS-LLI	HPS	18 hr	20°C	18°C	150	9.7	65 %

As in experiment 1, the sampling where done once, at the end of experiment 2, and tipburn was only assessed for the whole plant. All 40 lettuces in each treatment was assessed. For method of assessment of tipburn see chapter 4.10, below.

4.5 Experiment 3, effects of light intensity and RH on tipburn

In experiment 3, four treatments were used. See Table 3, for specifications of each different treatment.

The preculture in this experiment differed from the other experiments. The lettuces in each treatment had been taken directly to a growth chamber (instead of having a preculture in a greenhouse compartment), from the germination room. Here they grew under the same conditions as specified for HPS-LLI (Table 3). After approximately 3 weeks, when reaching the 5 leaf-stadium, the plants where installed in the growth chambers.

Table 3: The specifications for each growth chamber in experiment 3. High light intensity (HPS) = HPS-HLI, moderate light intensity + continuous photoperiod (HPS) = HPS-MLI/CPP, high light intensity (HPS) + high RH = HPS-HLI/HRH, low light intensity (HPS) = HPS-LLI.

Treatment	Lamp type	Photo-period	Temp, day	Temp, night	Photon flux density $\mu\text{mol m}^{-2} \text{s}^{-1}$	Light sum mol/day	RH
HPS-HLI	HPS	18 hr	20°C	18°C	300	19.4	65 %
HPS-MLI/CPP	HPS	24 hr	20°C	-	200	17.3	65 %
HPS-HLI/HRH	HPS	18 hr	20°C	18°C	300	19.4	90 %
HPS-LLI	HPS	18 hr	20°C	18°C	150	9.7	65 %

The first week of growth in the growth chambers, the conditions where the same in all four chambers- equal to the conditions in the control (this to make the experiment as equal to the others as possible). Then, after one week the different treatments started (the conditions in the control stayed the same). The lettuces would then grow for approximately (and at least) two more weeks under the different treatments before the lettuces reached the right size (100g<) and the experiment ended.

For method of assessment of tipburn see chapter 4.10, below. Notice however the exception from the method explained; that the total number of leaves were not registered throughout the experiment, only at the last registration.

4.6 Experiment 4, Effect of light quality (LED and HPS spectrum) on tipburn

In experiment 4, two treatments were used. See Table 4, for specifications of each different treatment. The pre-cultivation of the lettuces was done as explained in chapter 4.1. The first week of growth in the growth chambers, the light intensity was 150 $\mu\text{mol}/\text{m}^2/\text{s}$. The other conditions as specified in Table 4. (This to ensure the same acclimatization from greenhouse compartment to growth chamber- for the lettuces in all the different treatments).

After one week the light intensity would increase to 300 $\mu\text{mol}/\text{m}^2/\text{s}$. The other conditions stayed the same. The lettuces would then grow for approximately (and at least) two more weeks under the different treatments before the lettuces reached the right size (100g<) and the experiment ended. Notice; the humidity was now set to 70 % because it was too challenging to maintain a RH as low as 65 in the growth chambers. For method of assessment of tipburn see chapter 7.10 below.

Table 4: The specifications for each growth chamber in experiment 4. High light intensity (LED) = LED-HLI, high light intensity (HPS) = HPS-HLI.

Treatment	Lamp type	Photo-period	Temp, day	Temp, night	Photon flux density $\mu\text{mol m}^{-2}\text{s}^{-1}$	Light sum mol/day	RH
LED-HLI	LED	18 hr	20°C	18°C	300	19.4	70 %
HPS-HLI	HPS	18 hr	20°C	18°C	300	19.4	70 %

4.7 Experiment 5, Priming of seedlings to limit the development of tipburn

In experiment 5, four treatments were used. See Table 6 for specifications of each different treatment. The pre-cultivation of the lettuces was done as explained in chapter 4.1, with the exceptions on light treatments explained here: 2 different priming-treatments of the seedlings were performed right after moving them from the germination chamber into the greenhouse compartment and throughout the pre-cultivation. For priming treatments, see Table 5.

Table 5: Pre-treatments of transplants in experiment 5.

Name	Priming
PT-HLI	Pre-treatment with HPS under high light intensity ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$)
PT-MLT/BLED	Pre-treatment under moderate light intensity with additional blue LED (HPS + blue LED)
PT-NORM	Normal pre-cultivation as explained in chapter 1.1.

The PT-HLI priming was performed under high light intensity ($300 \mu\text{mol/m}^2/\text{s}$), HPS, (Fig. 13). The PT-MLT/BLED priming was performed under medium light intensity ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$), HPS, and ($100 \mu\text{mol/m}^2/\text{s}$), LED (Philips, 15 W GreenPower LED module HF blue, The Netherlands), (Fig. 13). In addition to the two priming treatments a normal pre-cultivation was also performed. For the treatments in growth chambers, 10 lettuces from each of the three different pre-treatments were installed.

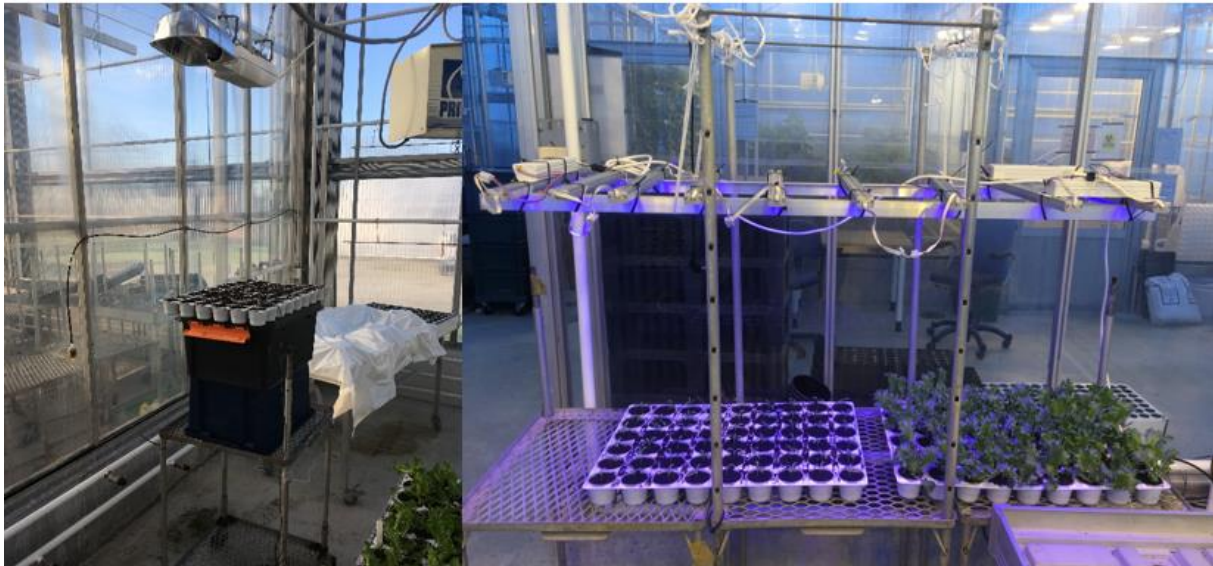


Figure 13: Priming under high light intensity ($300 \mu\text{mol/m}^2/\text{s}$) and under medium light intensity + blue LED ($100 \mu\text{mol/m}^2/\text{s}$). Photo: Martin Knoop.

The first week of growth in the growth chambers, the light intensity was $150 \mu\text{mol/m}^2/\text{s}$ in all chambers. The other conditions as specified in Table 5. (This to ensure the same acclimatization of the lettuces from greenhouse compartment to growth chamber- for all treatments). After one week the light intensity would increase to $300 \mu\text{mol/m}^2/\text{s}$ in the LED-HLI/FR treatment, and HPS-HLI treatment. The other conditions stayed the same.

The lettuces would then grow for approximately (and at least) two more weeks under the different treatments before the lettuces reached the right size ($100\text{g}<$) and the experiment ended.

For treatment LED-HLI/FR, the R/FR-ratio (red/far-red ratio) was altered when the light intensity increased after one week. We also increased the red/far-red ratio in treatment LED-LLI/FR, after the first week to be more similar to treatment LED-HLI/FR. (See Table 11 for specifications). R/FR-ratio was ensured to stay within 1 and 1.2 for both chambers. This to emulate the conditions of natural light. For explanation of the measurements of R/FR-ratio see chapter 4.9.3, below.

All in all, 30 plants per chamber, in 4 chambers were sampled and assessed for tipburn as explained in chapter 4.10, below.

Table 6: The specifications for each growth chamber in experiment 5. High light intensity (LED) + far-red (LED) = LED-HLI/FR, low light intensity (LED) + far-red (LED) = LED-LLI/FR, high light intensity (HPS) = HPS-HLI, low light intensity (HPS) = HPS-LLI.

Treatment	Lamp type	Red/Dark Red ratio	Photo-period	Temp, day	Temp, night	Photon flux density $\mu\text{mol m}^{-2}\text{s}^{-1}$	Light sum mol/day	RH
LED-HLI/FR	LED + far-red	Week 1: 1,05 Week 2-3: 1,12	18 hr	20°C	18°C	300	19.4	70 %
LED-LL/FR	LED + far-red	Week 1: 1,08 Week 2-3: 1,12	18 hr	20°C	18°C	150	9.7	70 %
HPS-HLI	HPS		18 hr	20°C	18°C	300	19.4	70 %
HPS-LLI	HPS		18 hr	20°C	18°C	150	9.7	70 %

4.8 Watering

When the lettuces were moved into the growth chambers, they were watered for one minute every second hour during the photoperiod the first week. After one week six of the watering times were extended to two minutes. This to make sure the lettuces got enough water and fertilizer as they grew. Every watering gave between 100 and 120 ml of water through the gutters (the double the amount when the timer was set to two minutes). The water contained a fertilizer solution, specified in chapter 4.8.1, below. Every few days the plastic boxes with used water had to be emptied. The water solution was not recycled.

4.8.1 Nutrient solution for fertilization

For the fertilizer solution applied to the gutters (and the seedlings in the greenhouse) two different stock solutions were mixed into the final solution. Two tanks were filled with 50 liters of water each and then applied fertilizers after the receipt in Table 7. 50/50 from each of these tanks were applied to a third larger tank filled with approximately 70 liters of water. See Fig. 14.1.

From each of the two stock solutions about 750-1000 ml were applied to the final solution until an electric conductivity (EC) of 2.0 was achieved. To measure the EC an EC-meter was used. (ScanGrow Conductivity meter, Denmark), (Fig. 14). The black plastic boxes in the growth chambers containing the fertilizer solution were refilled twice a week from the larger tank.

Table 7: Recipe for fertilizer solution used as stock solutions.

Tank 1:		Tank 2:	
Type:	Amount:	Type:	Amount:
Calcium nitrate	2.5 kg	Pioneer basic cucumber	3.125 kg
Potassium nitrate	0.625 kg	Pioneer Iron chelate, 6% EDDHA	0.025 kg
Calcium chloride	0.15 kg		

From the final solution a sample was taken and sent to Eurofins Agro Testing Norway AS for analysis of nutrient content. The result can be seen in Table 8.

Table 8: Actual content of nutrients in final solution given to the lettuce.

pH	EC (mS/cm 25°C)	Cations ppm (mg/l)						
		5	2.1	NH4 1.8	NH4-N 1.4	K 282	Na 32	Ca 148
		Anions ppm (mg/l)						
		No3 750	NO3-N 169	Cl 64	S 48	HCO3 6.1	P 37	
		Micronutrients ppb (µg/l)						ppm (mg/l)
		Fe 1843	Mn 483	Zn 275	B 292	Cu 133	Mo 86	Si 2.8



Figure 14: EC-meter used to measure the EC of the final fertilizer solution. Figure 14.1: The two stock solutions tanks seen in the back and the bigger tank with the final solution (with blue lid) standing on wheels so it's easier to move. The black troughs had to be refilled maybe 2 times a week from this tank. Photos: Martin Knoop.

4.9 About the lighting

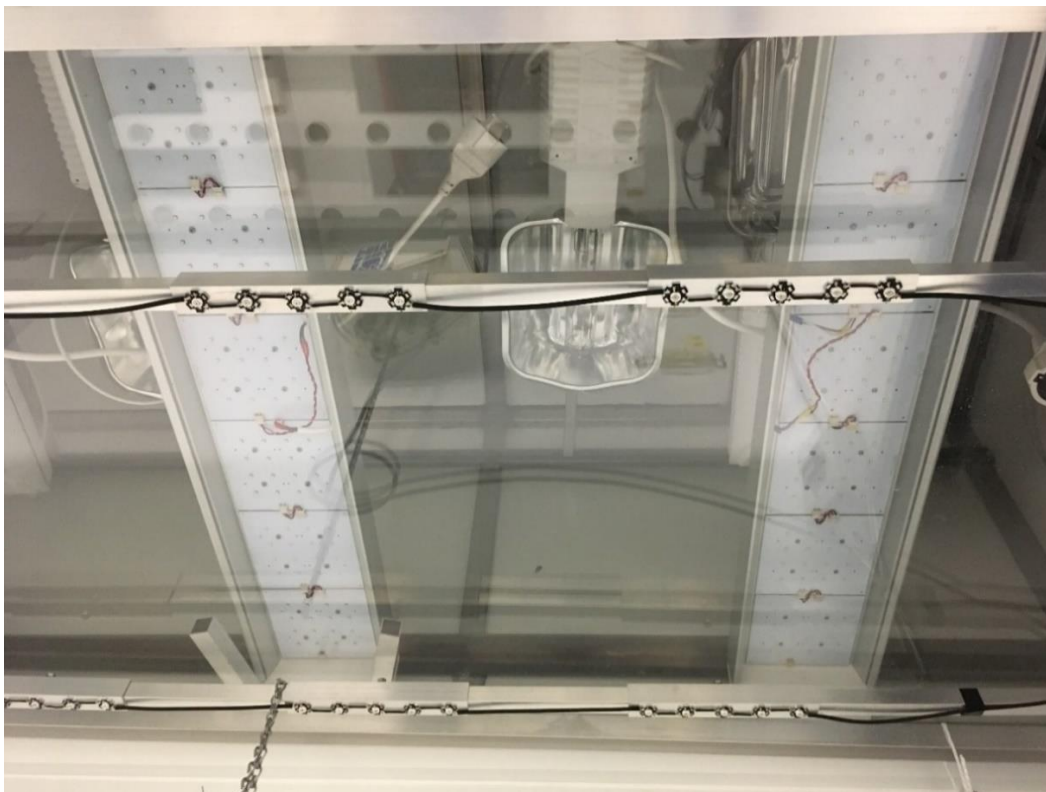


Figure 15: Showing the roof of a growth chamber with two HPS lamps (400 W each) in armatures and two dimmable LED armatures (185 W each) running vertically, and two dimmable far-red LED (80 W each) armatures running horizontally. Photo: Martin Knoop.

In the ceiling of the growth chambers there were HPS lamps or LED armatures. The HPS lamps (Fig 15) were 400 Watts each, provided by Gavita, Norway. The LED armatures were either 185 Watts (White), 80 Watts (Far-red) or 15 W (Blue), (Fig. 13), provided by Evolys, Norway.

4.9.1 Photosynthetic Photon Flux Density

To ensure the correct light intensity a net was used to cover the ceiling, and also the gutters were elevated to the correct height by building them up with crates. See Fig. 10. To control that the right photon flux was achieved, the light was measured with a quantum meter (Li-250A light meter, Li-Cor, USA), for 400-700 nm wavelengths, with the chamber doors closed. The lighting was set to either 150, 200 or 300 $\mu\text{mol}/\text{m}^2/\text{s}$. The lighting also varied from where in the chamber the measurements were done, but was ensured to always be within $\pm 10\%$ of the stated mean.



Figure 16: Optronic model 756 spectroradiometer used for measuring spectral composition of the different lighting. Figure 13.2: A quantum-meter was used to measure the achieved light intensity/photon flux. In this instance the lighting was 318 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The goal in this case was 300 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 10\%$. Photo: Martin Knoop.

4.9.2 Spectral composition

Measurements of the different spectral compositions and irradiance levels of the optical radiation sources (UV-visible-infrared) for the HPS, LED (White), LED (Blue) and LED (White) together with LED (far-red) were performed by using an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, FL, USA), (Fig. 16). After the method

explained in (Suthaparan et al., 2018). The different spectral compositions can be seen in Fig 17, to Fig. 23.

4.9.3 Red/far-red ratio

The R/FR-ratio was measured with a red/far-red sensor (Skye red/far-red sensor, The UK), at 660nm and 730 nm wavelengths- the same way the photon flux was measured with a quantum meter. The red/far-red lighting was only measured in experiment 5 and for the LED-treatments where additional lighting in the spectrum far-red was supplied. In all other treatments, the R/FR-ratio for HPS is stated to be 3.7 (Gavita, Norway) and for the LED treatments stated to be 6, (Evolys, Norway).

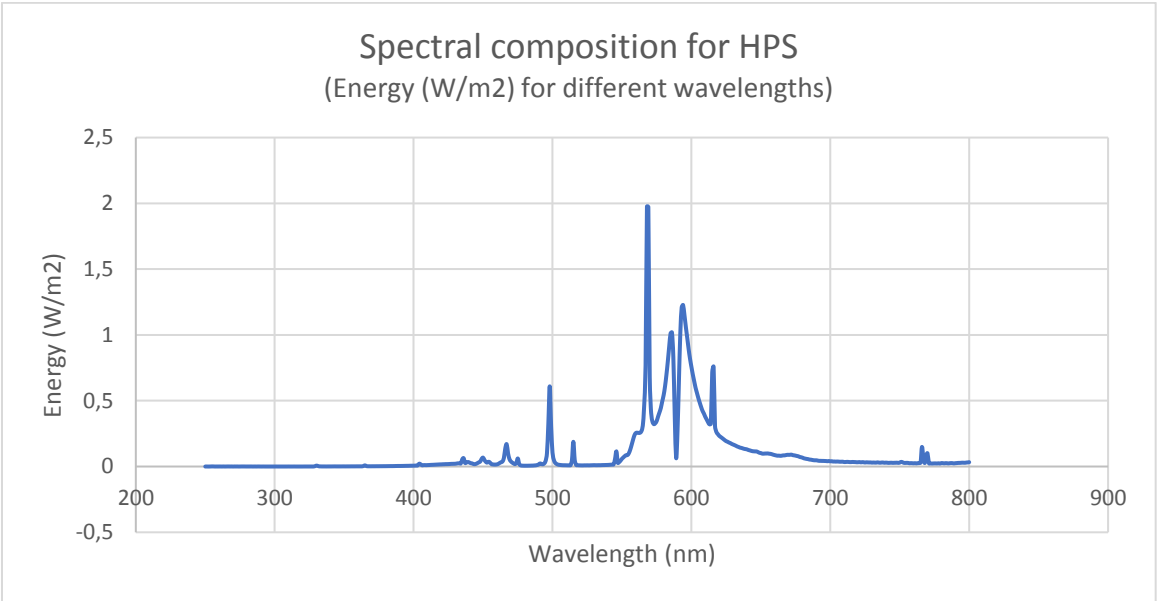


Figure 17: Spectral composition for 400 W HPS, (Gavita Norway). Used in the greenhouse compartment and the growth chambers in experiment 1,2,3, 4, and 5.

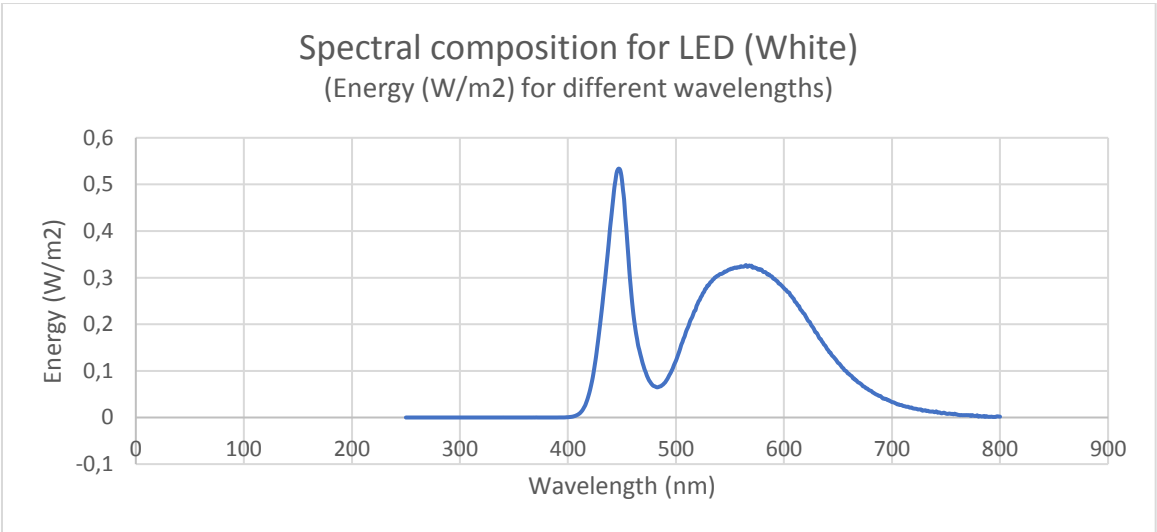


Figure 18: Spectral composition for 185 W LED, (Evolys Norway). Used in the growth chambers in experiment 4.

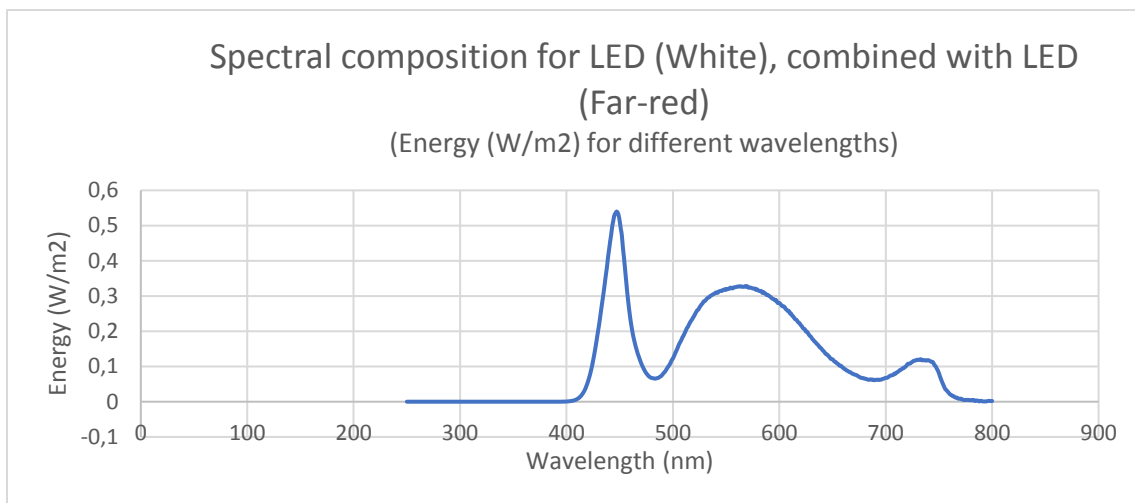


Figure 19: Spectral composition for 185 W LED (White), (Evolys Norway) in combination with 80 W (dimnable far-red) LED, (Evolys Norway). Used in the growth chambers in experiment 5.

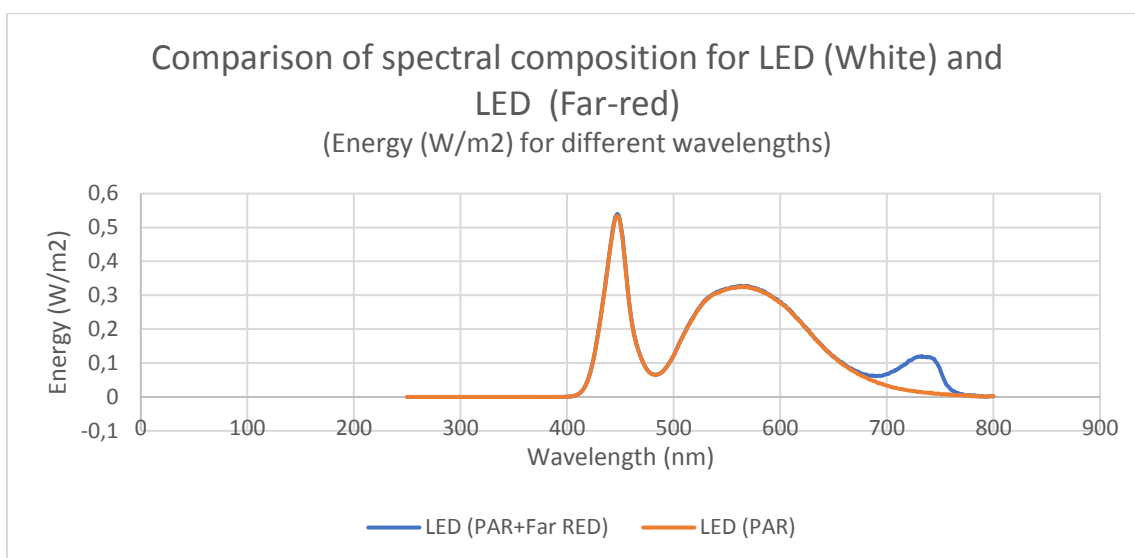


Figure 20: Comparison of spectral composition for 185 W LED (White) alone, and 185 W (White) LED, (Evolys Norway) in combination with 80 W (dimnable far-red) LED, (Evolys Norway).

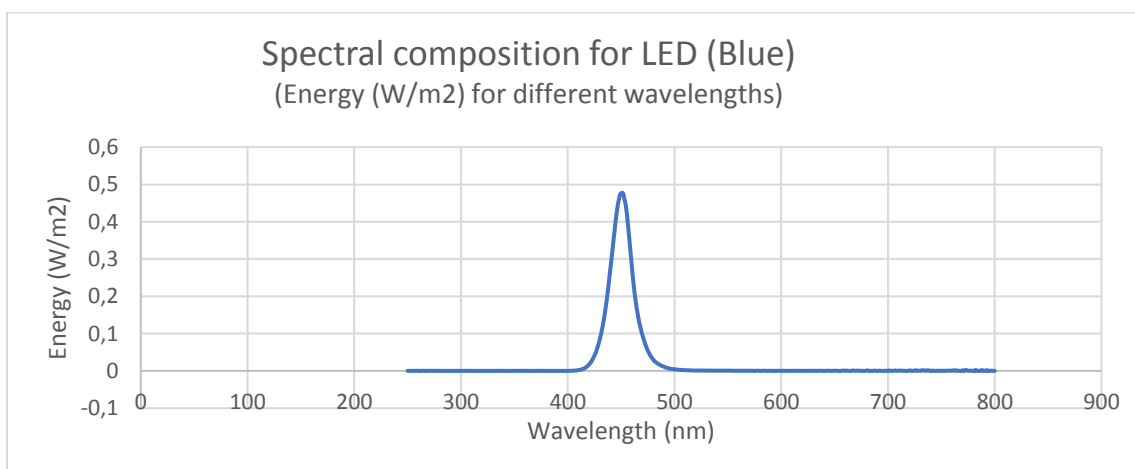


Figure 21: Spectral composition for 15 W (dimnable blue) LED, (Phillips, The Netherlands). Used in the greenhouse compartment during pre-treatment (priming) in experiment 5.

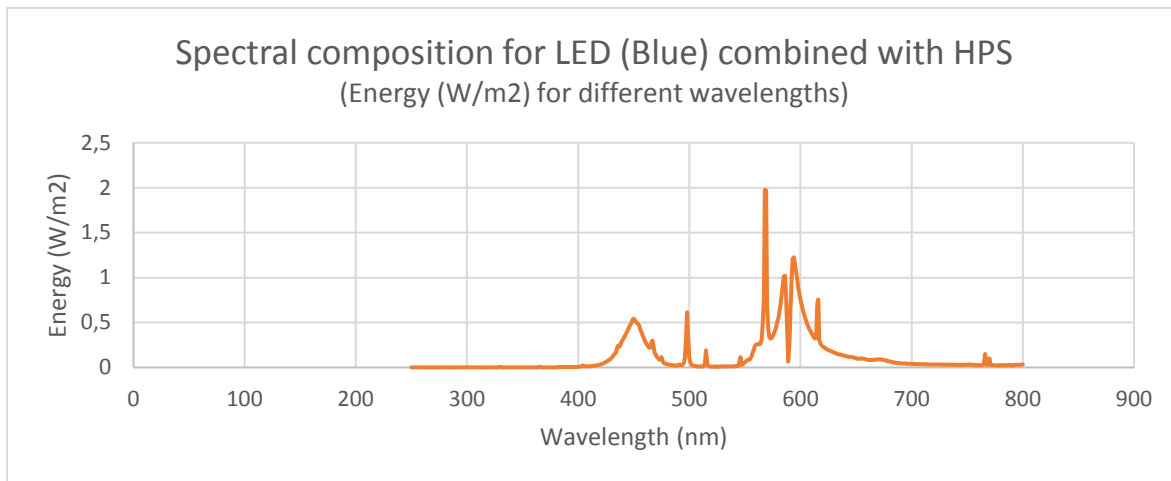


Figure 22: Spectral composition for 15 W (dimnable) blue LED, (Phillips, The Netherlands) in combination with 400 W HPS, (Gavita Norway). Used in the greenhouse compartment during pre-cultivation in experiment 5.

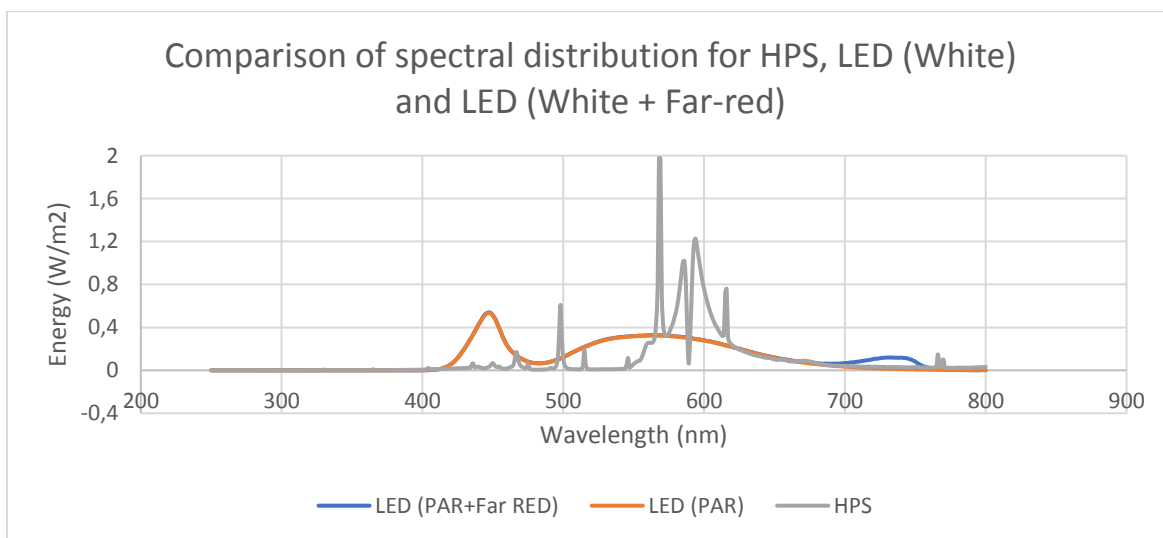


Figure 23: Comparison of spectral composition for 400 W HPS, (Gavita Norway), 185 W (White) LED, (Evolys Norway) alone, and 185 W (White) LED, (Evolys Norway) in combination with 80 W (dimnable far-red) LED, (Evolys Norway).

4.10 Registrations

The severity of tipburn development was registered for all experiments. However, the registrations for experiment 3-5 was done more thoroughly with tipburn assessed for each leaf, in addition to the whole lettuce. Leaf temperature and nutrient content was also measured for experiments 3-5. For experiment 3, a ROS (reactive oxygen species) analysis was also performed. For summary of registrations see Table 9.

Table 9: Summary of the different registrations done for each experiment.

	Tipburn		Leaf temperature	Nutrient analysis	ROS analysis
	Consecutively for 2 weeks	At end of experiment			
Experiment 1		X			
Experiment 2		X			
Experiment 3	X	X	X	X	X
Experiment 4	X	X	X	X	
Experiment 5	X	X	X	X	

Registration of tipburn

After approximately 1 week of growing (under the same conditions in all growth chambers) the different treatments started. At this time the registrations of tipburn also started (for experiments 3-5. Experiments 1 and 2 only had one registration at the end of the experiment). 10 lettuces for each treatment were registered (in experiment 5, there were 10 lettuces pr. pre-treatment (A, B or C) registered = 30 lettuces pr. treatment). The registrations were done in intervals of 3-4 days (= approximately twice a week) for 2 weeks until the plants were ready to be harvested.



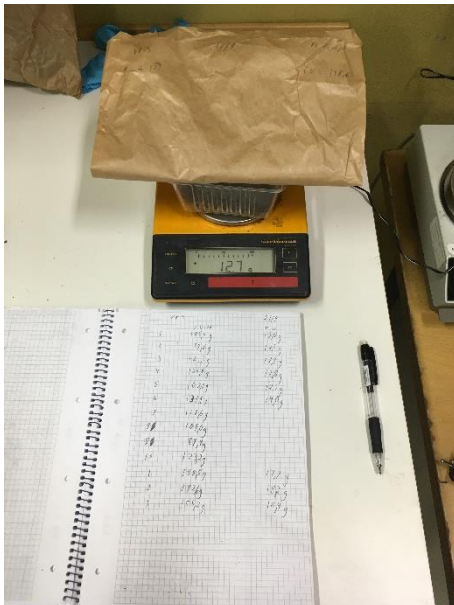
Figure 24: With the last registration the leaves were arranged from first to last and the severity of damage documented. Photo: Martin Knoop.

The severity of the tipburn damage was assessed as an interval between 1 (less severe) to 5 (most severe), for each leaf, from the first leaf after the cotyledons to the last leaf ≥ 1 cm long. 0 indicated no damage, (see appendix 1). The severity of both outer and inner tipburn was assessed. The assessment followed a scale developed by the Norwegian Extension Service (NLR). See appendix 2. Lastly, at the end of the experiment the whole plant was assessed and number of leaves were counted.

With the last registration the lettuce was cut at the base of the stem and the leaves arranged from first to last (Fig. 24). Damage was assessed as before and the lettuce put in a bag for weighing. The root development was assessed to be either bad, good or very good. Criteria's for root assessment was as follows: Thickness of roots, coloring (white to brown), elongation and evenness of growth. A "bad root" had thin roots, browning, reduced elongation and uneven growth. A "good root" had thicker white roots that grew evenly but not fully elongated. A "very good" root was as the good root, but with a good elongation. See appendix 1. All results were written down in a Table.

4.10.1 Weight, fresh weight (FW) and dry weight (DW)

The whole lettuce including the cotyledons, but excluding the rest of the stem and roots, were weighed.



Fresh weight, FW, was noted. 10 bags without any samples were also weighed and the average used to calculate the exact fresh weight of the lettuce without the bag (Fig. 25). The samples were marked with date, weight, plant nr., chamber nr., sample nr, and name.

The samples were then dried in an oven for 7 days at 62°Celsius (Fig. 26).

Afterwards the dry weight, DW, was noted. The ten empty bags were also dried and the dry weight of them used to calculate the actual dry weight without bags.

Figure 25: The bags were weighed with an accuracy of 0,01 g. Photo: Martin Knoop.



Figure 26: The bags were dried at 62°C for 7 days. Photographer: Martin Knoop.

4.10.2 Leaf temperature



Figure 27: Infrared sensor for measuring leaf temperature. Photo: Martin Knoop.

After the 10 lettuces for each treatment was harvested the leaf temperature of 10 randomly chosen of the remaining lettuces were measured. One reading for an inner and outer leaf pr. lettuce was done. To measure leaf temperature a infrared sensor was use (Fluke 62 Max IR thermometer, the USA), (Fig. 27).

4.11 Nutrient analysis of outer and inner leaves

For each treatment, 5 of the remaining lettuces were randomly selected, harvested and laid out as the plants during the registrations. 5 old “source” leaves, and 10 young “sink” leaves where then selected and put in two different bags. The 5 source leaves where chosen as the first leaves after the first 5 leaves, not counting the cotyledons. In Fig. 28, this will be nr. 6 from the upper left corner to nr. 2 from the left of the middle line. The 10 sink leaves were chosen from the last leaf >1 cm long, and counting 9 older leaves. In Fig. 28, this will be from nr. 1-10 from the bottom right. The amount of source leaves had to be at least double to have enough biomass to do a lab analysis.



Figure 28: Showing the layout of a plant with source leaves on top and sink leaves at the bottom. Photo: Martin Knoop.

The bags with source and sink leaves were labelled, weighed as before and dried at 45°C for 4-5 days and weighed again, (Fig. 29 and 29.1). Afterwards the samples were ground down by using a grinder (Fig. 30) for the larger source leaves, and a mortar for the smaller sink leaves, (Fig. 31 and 32). The powdery samples that was created was put in cylinders (Fig. 30.1), labelled and sent to the lab for nutrient analysis of the elements Mg, Ca, Fe and K, measured with ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) method (Greenfield, 1983). Nutrient analysis' where done for samples in experiments 3, 4 and 5.



Figure 29: Heating oven warming up to 45°Celsius with the samples inside. Figure 29.1: Dried samples crushed inside the bag for further processing. Photo: Martin Knoop.



Figure 30: Grinder with the lid off (in the left corner) chopping up the salad and putting it into the container glass (in the middle bottom of the picture). Figure 30.1: Cylinder containing a sample prepared to be sent off to the lab. Photo: Martin Knoop.



Figure 31: The mortars with cylinders for each sample lined up with the sample bags in the front. Photo: Martin Knoop.



Figure 32: Showing a mortar with a sample ground halfway to powder. Photo: Martin Knoop.

4.12 ROS analysis

To see if the severity of tipburn is linked to the amount of ROS in the different leaves a ROS-analysis was performed on lettuces from experiment 3, (Fig. 33). Five lettuces from each chamber were sampled. An “*In situ localization of DAB*” was performed as explained in (Thordal-Christensen et al., 1997).

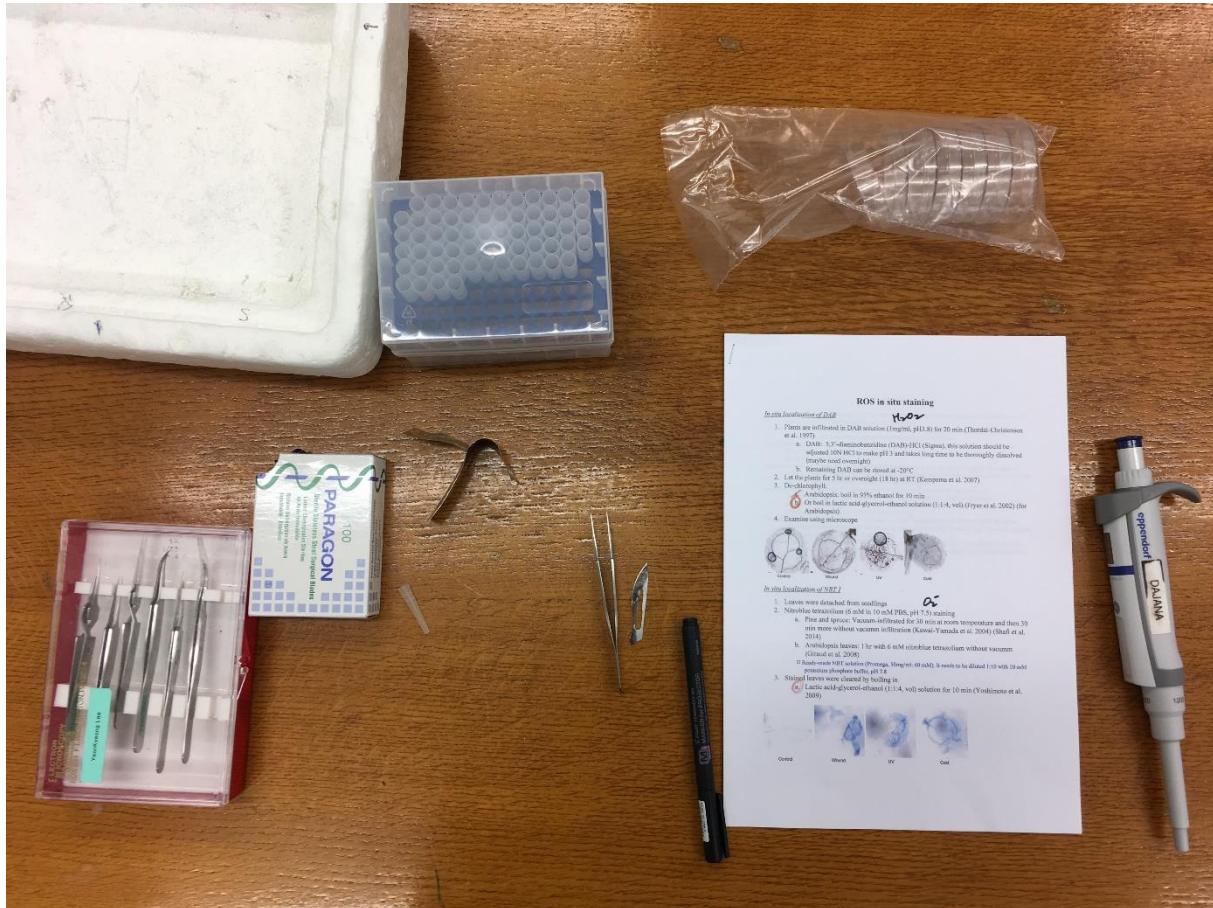


Figure 33: Equipment to do a ROS-analysis; scalpels, pincers, pipettes and petri dishes. Photo: Martin Knoop.

4.13 Results

Results were catalogued/documentated, pictures were taken and statistical analysis was performed. Excel spreadsheet was used to create graphs and treat the data before statistical analyses was performed in Minitab. In Minitab variance analyses for measured factors were performed; dry weight, fresh weight, number of leaves, leaf temperature, water content and outer and inner tipburn and cation content. A tukey test was used to separate significantly different values. A GLM two-way interaction analysis was performed to test if there was a significant difference in tipburn accumulation between treatments, attributed to pre-treatment in experiment 5.

5 Results

5.1 Experiment 1

The aim of experiment 1 was to test the growing system, to induce tipburn and develop the method for assessing the severity of tipburn. In the first experiment, it was tested if elevated temperatures and elevated relative air humidity (RH) would induce tipburn. Lastly it was to test if there would be a compounding effect of elevated temperatures together with elevated RH in regards to tipburn severity. Both temperature and RH are easy climate parameters to change in the growth chambers. The treatments were; High (27°C) temperature together with high (90%) RH (HT/HRH), high temperature (27°C) together with moderate (65%) RH (HT/MRH), and moderate temperature (20°C) together with high RH (MT/HRH) and moderate RH (MT/MRH).

Table 10 shows the results from experiment 1. Elevated temperature was not found to induce more tipburn compared to moderate temperatures. Neither did elevated RH. In fact, the treatment with moderate temperature and moderate RH (MT/MRH) was found to have significantly higher severity of outer tipburn than the other treatments. The MT/MRH treatment induced moderate outer tipburn severity and the three other treatments low severity. There was no inner tipburn found in any of the treatments. Fig. 34 shows the accumulated tipburn (outer and inner) for all treatments at the end of the experiment.

Table 10: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 1, and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). HT = High temperature. HRH = High relative air humidity. MT = Moderate temperature. MRH = Moderate relative air humidity. N = 10 in each treatment.

Treatment		Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
HT/HRH	Mean	A 16.6	A 160.04	A 16.58	A 89.39
	SD	1.26	23.30	1.11	1.00
MT/HRH	Mean	B 23.3	BC 101.68	A 15.33	B 84.22
	SD	1.63	14.21	0.62	2.13
HT/MRH	Mean	C 27.5	AB 158.9	A 15.62	A 89.96
	SD	2.12	37.7	2.63	1.86
MT/MRH	Mean	A 16.4	C 127.53	A 16.32	C 86.96
	SD	0.69	24.59	1.03	1.58
P-value		< 0.001	< 0.001	0.251	< 0.001

The moderate temperature/high relative air humidity (MT/HRH) treatment gave low (but not significantly so) fresh weight accumulation compared to the other treatments and together with the high temperature/moderate relative air humidity (HT/MRH) treatment had a significantly higher number of leaves compared to the other two treatments. The HT/HRH treatment gave significantly higher fresh weight accumulation compared to the MT/HRH and MT/MRH treatments but not the HT/MRH treatment. The HT/MRH treatment gave significantly higher biomass accumulation than the MT/MRH treatment, but not the MT/HRH treatment. High temperature gave significantly higher water content (Table 10). All roots in experiment 1 was found to be “very good”.

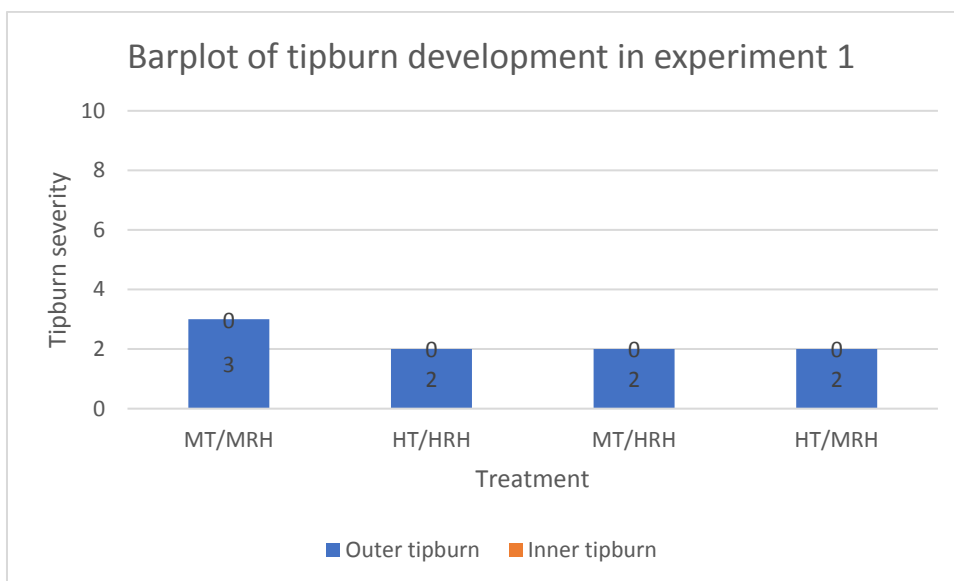


Figure 34: Barplot of tipburn severity at end of experiment 1. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. High temperature (27°C), high (90%) RH = HT/HRH, high temperature (27°C), moderate (65%) RH = HT/MRH, moderate temperature (20°C), high (90%) RH = MT/HRH, moderate temperature (20°C), low (60%) RH = MT/MRH. N = 10 in each treatment. P-value for both inner and outer tipburn = < 0.001. Tukey test outer tipburn: MT/MRH = A, HT/HRH = B, MT/HRH = B, HT/MRH = B.

5.2 Experiment 2

The aim of experiment 2 was to continue to assure that tipburn would be induced by growing Frillice’ lettuces in growth chambers and to develop the method for assessment. In this experiment the lettuces were exposed to different light sums by treating them with high (300 $\mu\text{mol}/\text{m}^2/\text{s}$) light intensity (HPS-HLI) for 18 h, with continuous lighting and moderate (200 $\mu\text{mol}/\text{m}^2/\text{s}$) light intensity (HPS-MLI/ CPP) and to test how it would affect tipburn severity compared to the low (150 $\mu\text{mol}/\text{m}^2/\text{s}$) light intensity (HPS-LLI) treatment (also 18 h). The light sums were 9.7 mol/day for LLI, 17.3 mol/day for HPS-MLI/ CPP and 19.4 mol/day for HPS-HLI.

Table 11 shows the results from experiment 2. High light intensity/light sum was found to induce inner tipburn. Higher light intensities were also found to induce significantly more tipburn (both outer and inner) than lower light intensities. The severity of tipburn in the HPS-HLI treatment was significantly higher than in the HPS-MLI/CPP treatment, and in the HPS-MLI/CPP treatment significantly higher than in the HPS-LLI treatment. Inner tipburn was not present in the HPS-LLI treatment. Fig. 35 shows the accumulated tipburn (outer and inner) for all treatments at the end of the experiment.

Table 11: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 2, and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). HPS-HLI = High light intensity (HPS), HPS-MLI = Moderate light intensity (HPS), HPS-MLI/CPP = Moderate light intensity (HPS) + continuous photo period, HPS-LLI = Low light intensity (HPS). N = 10 in each treatment.

Treatment		Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
HPS-HLI	Mean	A 21.10	A 261.6	A 12.17	A 95.32
	SD	2.08	35.5	1.24	0.38
HPS-MLI/CPP	Mean	A 21.30	A 231.8	A 11.68	B 94.95
	SD	1.25	31.9	1.50	0.30
HPS-LLI	Mean	B 19.10	B 137.09	B 7.40	B 94.60
	SD	1.29	14.13	0.80	0.31
P-value		0.008	< 0.001	< 0.001	< 0.001

High (and moderate continuous) light intensity (high light sum) resulted in significantly more biomass accumulation and higher fresh weight compared to low light intensity (low light sum). However, no significant difference was found between HPS-HLI and HPS-MLI/CPP. The same results were found for number of leaves and dry weight. Water content was significantly higher in lettuces exposed to HPS-HLI compared to HPS-MLI/CPP and HPS-LLI but the differences were small (Table 11). All roots in experiment 2 was found to be “very good”.

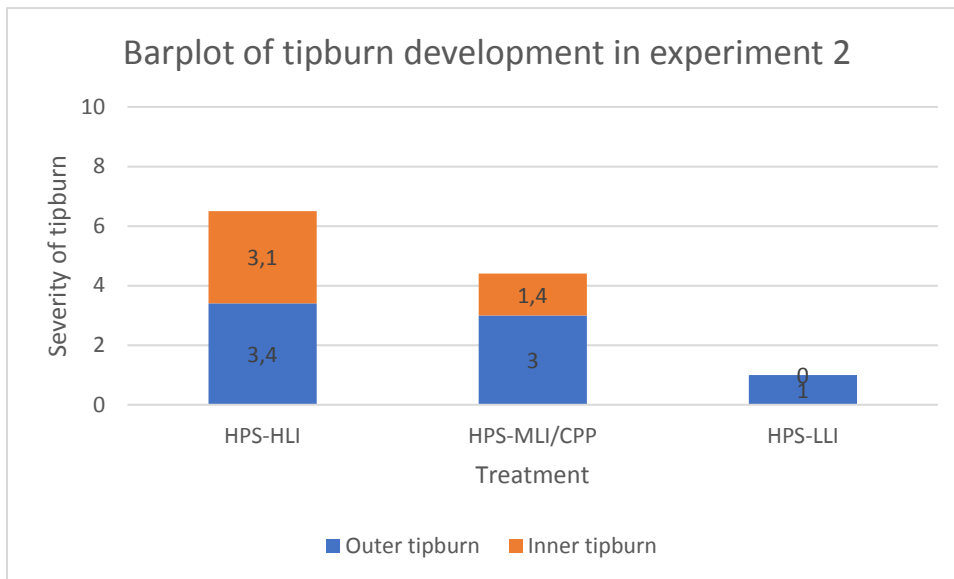


Figure 35: Barplot of tipburn severity at end of experiment 2. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. High light intensity with HPS ($300 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-HLI. Continuous moderate light intensity with HPS ($200 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-MLI/CPP. Low light intensity with HPS ($150 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-LLI. $N = 10$ in each treatment. P -value for inner and outer tipburn = < 0.001 . Tukey test outer tipburn: HPS-HLI = A, HPS-MLI/CPP = B, HPS-LLI = C. Tukey test inner tipburn: HPS-HLI = A, HPS-MLI/CPP = B, HPS-LLI = C.

5.3 Experiment 3

The aim of experiment 3 was to repeat experiment 2 (but with a different pre-treatment; Having the pre-treatment directly in growth chambers, rather than in greenhouse compartments). It was also to test if there would be a compounding effect of high light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity (HPS-HLI) and high (90%) relative air humidity (HRH). Furthermore, since experiment 2 indicates that light intensity is important for the development of inner tipburn it was of interest to test if leaves from high and low light sum accumulate different levels of hydrogen peroxide (H_2O_2) which is a reactive oxygen species (ROS). Also, measurements of cations (Ca, K, Mg) was performed on the outer (source) and inner (sink) leaves to test the relationship between incidence of tipburn and cation content.

Table 12 shows the results from experiment 3. In this experiment all treatments gave high outer tipburn severity, with no significant difference between treatments (p -value not significant on a 95 % threshold). High light intensities gave high severity of inner tipburn, while no inner tipburn in the low light intensity treatment (HPS-LLI). A significant difference between the HPS-LLI treatment and the other higher light intensity treatments was found, but not between the different high light treatments (Fig. 39). There was no significant compounding effect of high light intensity and high RH, although this treatment had the

highest severity of inner tipburn. Fig. 37 shows the accumulated tipburn (outer and inner) for all treatments at the end of the experiment.

Fig. 36 shows the development of outer and inner tipburn throughout the experiment. When the light intensity increased the development of inner tipburn was induced. The development of outer tipburn occurred regardless of light intensity, but the severity accelerated when the intensity increased.

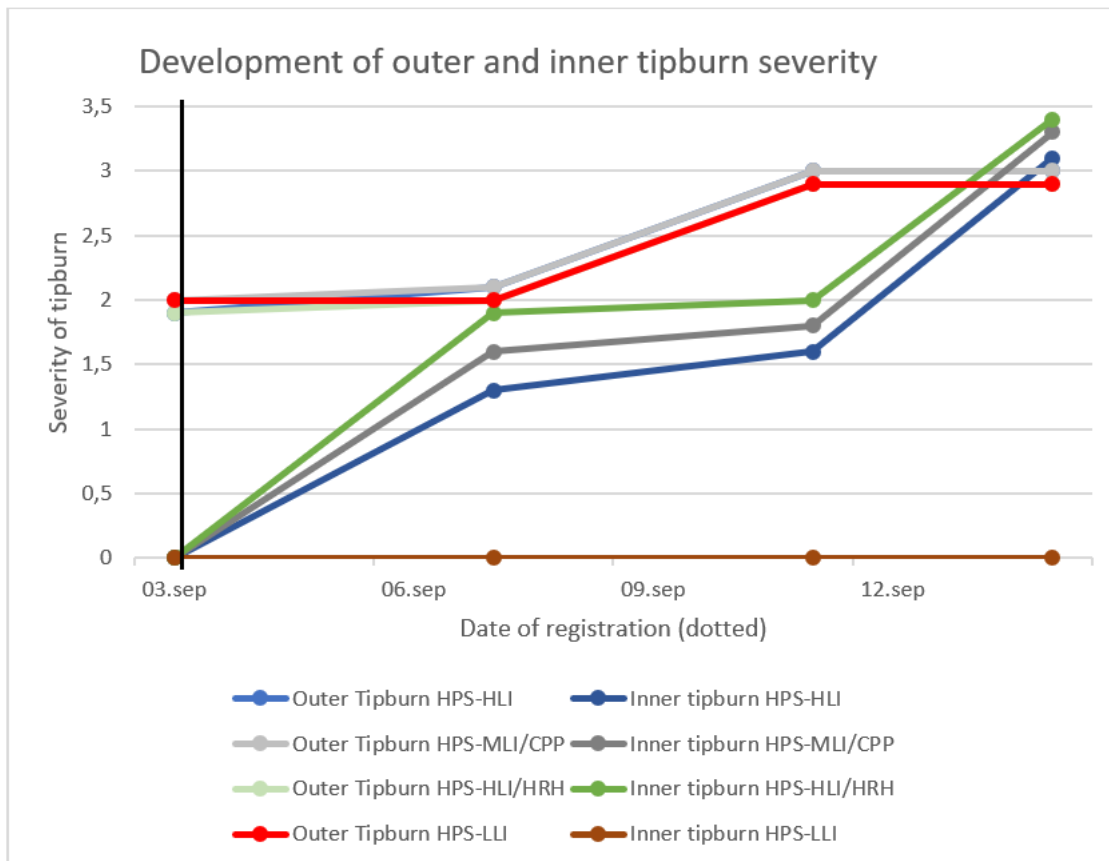


Figure 36: Development of outer and inner tipburn over time for experiment 3. The black line represents the time of increase of light intensity (from $150 \mu\text{mol}/\text{m}^2/\text{s}$ to $300 \mu\text{mol}/\text{m}^2/\text{s}$ in treatments HPS-HLI and HPS-HLI/HRH, and $200 \mu\text{mol}/\text{m}^2/\text{s}$ in treatment HPS-MLI/CPP), and humidity (from 65 to 90 %) in treatment HPS-HLI/HRH. High light intensity with HPS ($300 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-HLI. Continuous lighting, moderate light intensity with HPS ($200 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-MLI/CPP. High light intensity with HPS ($300 \mu\text{mol}/\text{m}^2/\text{s}$) + high RH (90%) = HPS-HLI/HRH. Low light intensity with HPS ($150 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-LLI. $N = 10$ in each treatment.

The morphologically biggest lettuces were found in treatment HPS-HLI. The HPS-HLI/HRH had the highest fresh weight but was also the morphologically smallest lettuce (had a more compact growth). See Fig. 37 and Fig. 38 for ex. of morphology from each different treatment. There was no significant difference in fresh weight (p -value not significant on a 95 % threshold), (Table 12).



Figure 37: From the left: Lettuces from treatment HLI, MLI/CPP, HLI/HRH and LLI seen from above.



Figure 38: From the left: Lettuces from treatment HLI, MLI/CPP, HLI/HRH and LLI seen from the side.

Table 12: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 3, and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). HPS-HLI = High light intensity (HPS), HPS-MLI/CPP = Moderate light intensity (HPS) + continuous photo period, HPS-HLI/HRH = High light intensity (HPS) + High RH, HPS-LLI = Low light intensity (HPS). N = 10 in each treatment.

Treatment		Temperature outer leaves	Temperature inner leaves	Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
HPS-HLI	Mean	A 19.08	AC 19.33	A 21.20	A 95.70	A 6.51	A 93.01
	SD	0.43	0.53	4.52	20.96	1.16	1.40
HPS- MLI/CPP	Mean	A 19.23	A 19.10	A 23.80	A 89.68	A 6.33	A 92.80
	SD	0.93	0.41	2.10	22.63	1.27	0.81
HPS- HLI/HRH	Mean	B 20.45	B 21.27	A 23.30	A 105.28	A 5.64	B 94.60
	SD	0.54	0.29	1.42	14.44	0.79	0.70
HPS-LLI	Mean	B 20.34	C 19.75	A 23.30	A 94.49	B 4.19	B 95.56
	SD	0.55	0.59	1.64	14.46	0.68	0.24
P-value		< 0.001	< 0.001	0.073	0.307	< 0.001	< 0.001

The lettuces in this experiment accumulated a low fresh weight with no significant difference between treatments (p-value not significant on a 95 % threshold). The highest dry weight accumulation was found in the HPS-HLI treatment, and the highest percentage of dry weight accumulation found in treatment HPS-MLI/CPP. There was a significant difference between the HPS-LLI treatment and the other treatments for dry weight (but not between these 3) and between the HPS-HLI and HPS-MLI/CPP and the other two for water content. There

were no significant differences between treatments for number of leaves (p-value not significant on a 95 % threshold), (Table 12). All roots in experiment 3 was found to be “very good”.

There was found a significant difference between treatments for temperature in outer leaves. The temperature in HPS-HLI and HPS-MLI/CPP was significantly lower than in the other two treatments. For temperature in inner leaves the difference was not significant between HPS-HLI and HPS-LLI, but otherwise the same (Table 12).

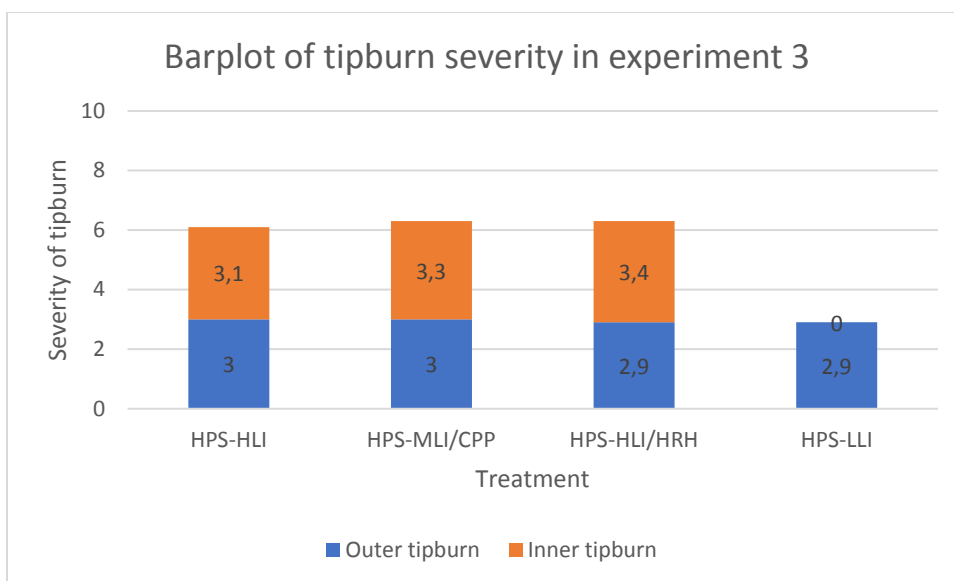


Figure 39: Barplot of tipburn severity at end of experiment 3. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. High light intensity with HPS ($300 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-HLI. Continuous lighting + moderate light intensity with HPS ($200 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-MLI/CPP. High light intensity with HPS ($300 \mu\text{mol}/\text{m}^2/\text{s}$) + high RH (90%) = HPS-HLI/HRH. Low light intensity with HPS ($150 \mu\text{mol}/\text{m}^2/\text{s}$) = HPS-LLI. N = 10 in each treatment. P-value for outer tipburn = 0.578. P-value for inner tipburn = < 0.001. Tukey test outer tipburn: HPS-HLI = A, HPS-MLI/CPP = A, HPS-HLI/HRH = A, HPS-LLI = A. Tukey test inner tipburn: HPS-HLI = A, HPS-MLI/CPP = A, HPS-HLI/HRH = A, HPS-LLI = B.

5.3.1 Results from nutrient analysis for experiment 3

An ANOVA test was performed for the accumulation of cations in source and sink leaves for lettuces between treatments. There was not found any significant difference in calcium content for source leaves (p-value not significant on a 95 % threshold). For sink leaves there was a significantly higher content in the HPS-LLI treatment compared to the other higher light treatments. There was neither found a significant difference for source leaves for potassium (p-value not significant on a 95 % threshold), but a higher accumulation in the HPS-LLI treatment for sink leaves (not significantly different from the HLI/HRH treatment).

For magnesium there was a significant difference between treatments with the lowest content in the HPS-HLI/HRH treatment for source leaves and for the HPS-MLI/ CPP treatment for sink leaves. The highest content was found in the HPS-LLI treatments for both leaf types (but there was not a significant difference between the HPS-LLI and the HPS-MLI/ CPP treatment for magnesium content in source leaves (Table 13).

Table 13: Results from ANOVA. The table shows results from ANOVA; The p-value, mean and standard deviation (SD) for content (in mg/g DW) of calcium (Ca) potassium (K) and magnesium (Mg) in source and sink leaves for each treatment. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). HPS-HLI = High light intensity (HPS), HPS-MLI/ CPP = Moderate light intensity (HPS) + continuous photo period, HPS-HLI/HRH = High light intensity (HPS) + High RH, HPS-LLI = Low light intensity (HPS). N = 10 in each treatment.

Treatment		Ca		K		Mg	
		Source leaf	Sink leaf	Source leaf	Sink leaf	Source leaf	Sink leaf
HPS-HLI	Mean	A 14.00	AB 1.86	A 82.80	A 44.40	A 4.66	A 1.92
	SD	2.12	0.79	24.60	9.56	0.23	0.164
HPS-MLI/ CPP	Mean	A 11.78	A 1.33	A 78.40	A 42.80	BC 3.94	B 1.68
	SD	1.34	0.47	9.91	5.17	0.56	0.148
HPS-HLI/HRH	Mean	A 12.80	B 2.32	A 82.40	AB 48.00	C 3.50	AB 1.88
	SD	1.30	0.44	6.15	3.08	0.28	0.08
HPS-LLI	Mean	A 13.40	C 4.02	A 103.60	B 58.60	AB 5.08	C 2.44
	SD	1.34	0.29	9.74	7.20	0.44	0.089
P-value		0.182	< 0.001	0.057	0.008	< 0.001	< 0.001

5.3.2 ROS

Analysis of hydrogen peroxide (H_2O_2) was performed in experiment 3 for sink and source leaves for each treatment. See Fig. 40 for results.

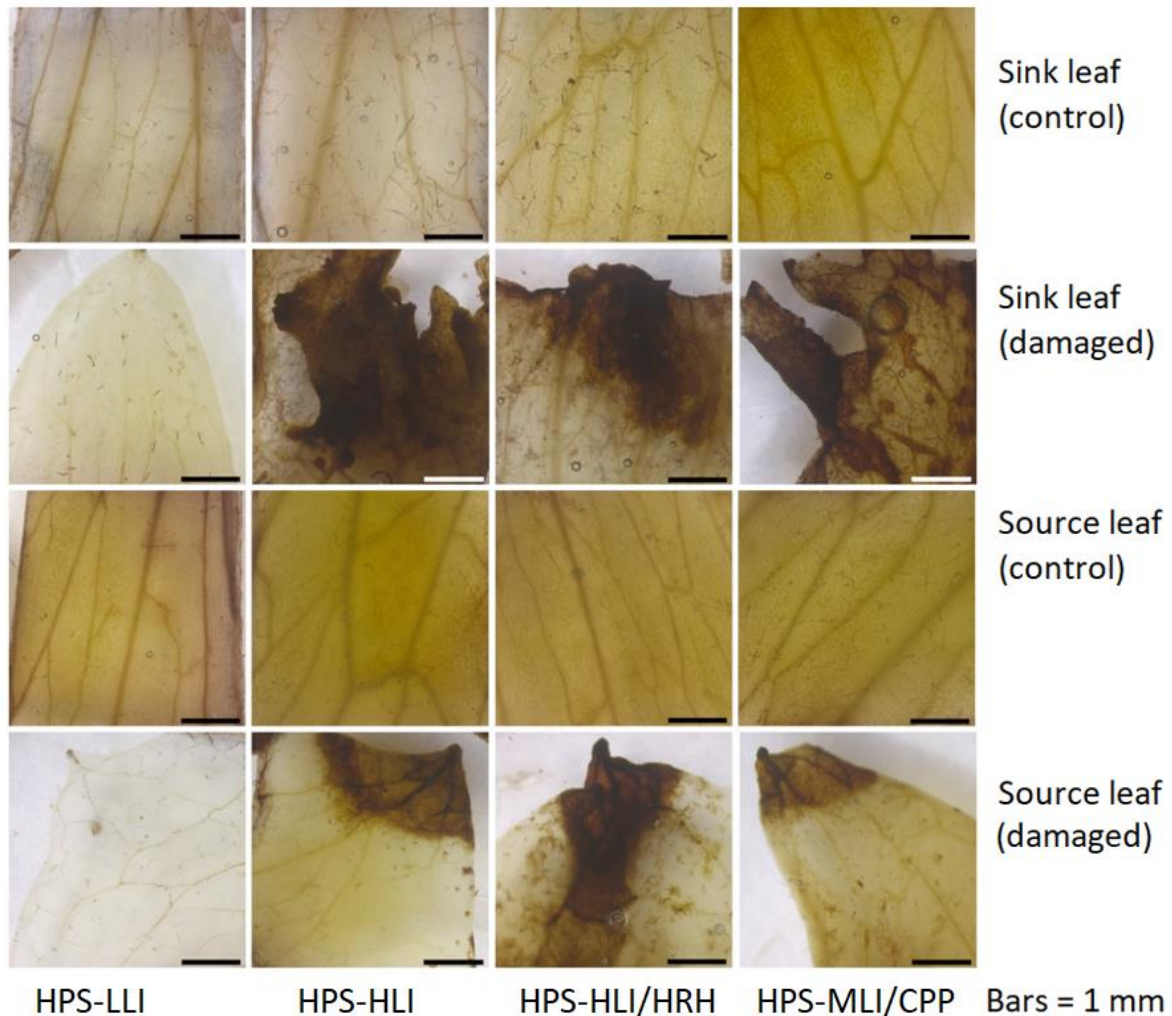


Figure 40: Hydrogen peroxide (H_2O_2)-results from DAB-analysis of ROS. Dark coloration indicates ROS accumulation. Photos: courtesy of Yeon Kyeong Lee. $N = 5$ in each treatment.

In the DAB-analysis, hydrogen peroxide (H_2O_2) was found in all treatments where leaves were damaged, except in the LLI treatment. The accumulation of H_2O_2 was strongest in the sink leaves for the three treatments with inner tipburn. The strongest ROS accumulation was in the HPS-HLI treatment for source leaves, and in the HPS-HLI/HRH for sink leaves. In the HPS-LLI treatment no H_2O_2 was found, regardless of source and sink leaves, (Fig. 40).

5.4 Experiment 4

The aim of experiment 4 was to test if white light emitting diodes (LED) could mitigate the effect of high light intensity on tipburn severity compared to the traditional HPS lamp commonly used in commercial production. It was also, to investigate the relationship

between cations (Ca, K, Mg) and development of tipburn in lettuces grown under light from the two lamp-types.

Table 14 shows the results from experiment 4. A significant difference between the two treatments for outer tipburn was found. The LED spectral distribution gave higher severity of outer tipburn. There was no significant difference for inner tipburn (p-value not significant on a 95 % threshold), but the LED spectrum was lower in severity. Fig. 41 shows the development of outer and inner tipburn throughout the experiment. As with experiment 3 the development of outer tipburn occurred regardless of light intensity in both treatments, but its severity accelerates after the increase in light intensity. Increase in light intensity induced inner tipburn and it developed up to a point before evening out for both treatments. Fig. 43 shows the accumulated tipburn (outer and inner) for all treatments at the end of the experiment.

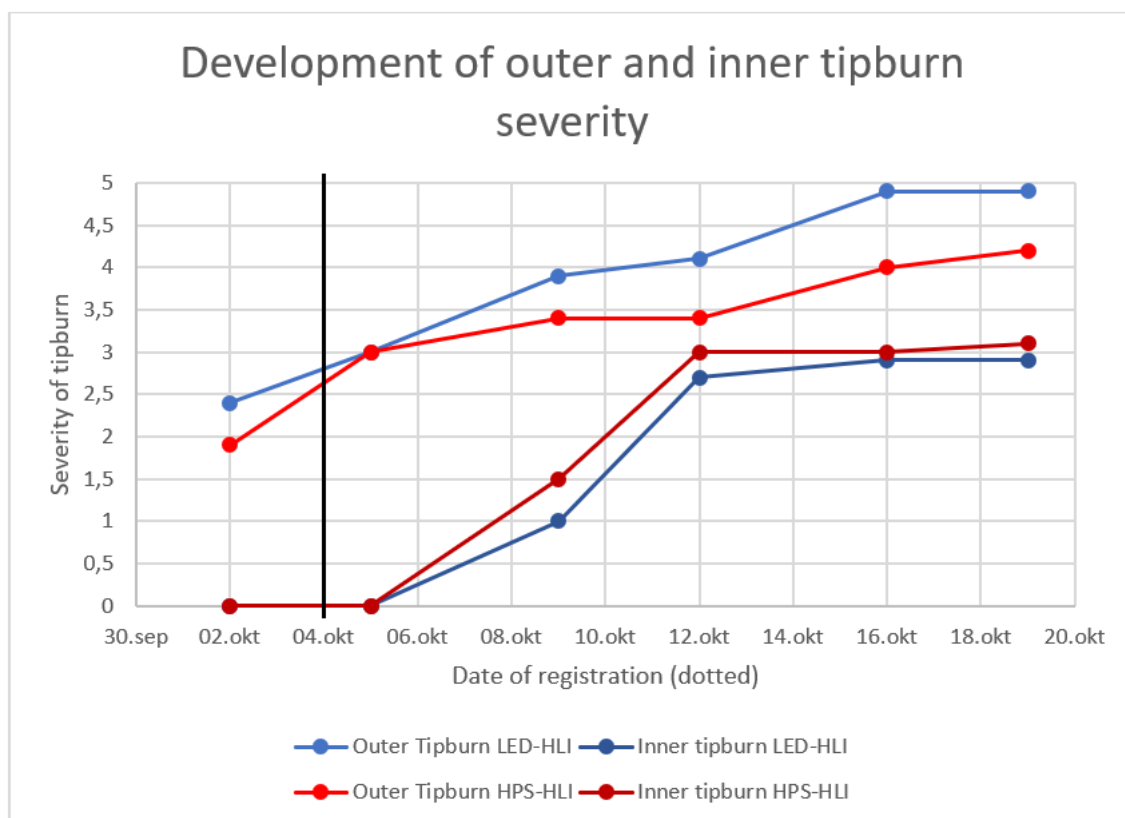


Figure 41: Development of outer and inner tipburn over time for experiment 4. The black line represents the time of increase in light intensity (from 150 to 300 $\mu\text{mol}/\text{m}^2/\text{s}$) for both treatments. High light intensity with LED = LED-HLI. High light intensity with HPS = HPS-HLI. N = 10 in each treatment.

The LED-HLI treatment gave a very compact, crispy and ugly lettuce. The HPS-HLI treatment gave a more elongated and open growth but also very damaged (Fig 42).

There was not found any significant difference for fresh weight between the treatments (p-value not significant on a 95 % threshold). There was found a significantly higher dry weight and number of leaves in the HLI treatment and the LED-HLI treatment had significantly higher water content (Table 14). All roots in experiment 4 was found to be “very good” at the end of the experiment.



Figure 42: Lettuce from experiment 4, treatment LED-HLI (the two pictures to the left) and from treatment HLI to the right. Photographer: Martin Knoop.

Table 14: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 4, and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). LED-HLI = High light intensity (LED). HPS-HLI = High light intensity (HPS). N = 10 in each treatment.

Treatment		Temperature outer leaves	Temperature inner leaves	Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
LED-HLI	Mean	A 19.17	A 19.71	A 21.30	A 96.17	A 7.55	A 92.04
	SD	0.25	0.69	1.49	17.16	0.78	0.84
HPS-HLI	Mean	B 20.87	B 21.40	B 23.00	A 105.36	B 9.62	B 90.84
	SD	0.45	0.72	0.94	6.65	0.78	0.90
P-value		< 0.001	< 0.001	0.007	0.132	< 0.001	0.007

There was found a significant difference between treatments for temperature in both outer and inner leaves. The leaf temperature was significantly lower in the LED-HLI treatment than in the HPS-HLI treatment (Table 14).

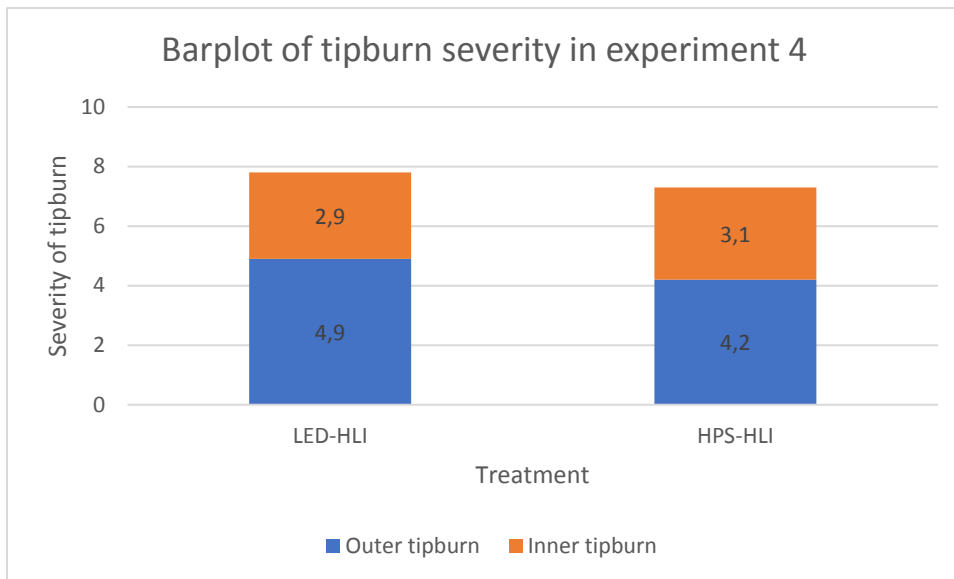


Figure 43: Barplot of tipburn severity at end of experiment 4. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. High light intensity (300 $\mu\text{mol}/\text{m}^2/\text{s}$) with LED = LED-HLI. High light intensity (300 $\mu\text{mol}/\text{m}^2/\text{s}$) with HPS = HPS-HLI. N = 10 in each treatment. P-value for outer tipburn = < 0.001. P-value for inner tipburn = 0.174. Tukey test outer tipburn: LED-HLI = A, HPS-HLI = B. Tukey test inner tipburn: LED-HLI = A, HPS-HLI = A.

5.4.1 Results from nutrient analysis for experiment 4

An ANOVA test was performed for the accumulation of cations in source and sink leaves for lettuces between treatments (Table 15). There was not found any significant difference in calcium content for either source or sink leaves (p-value not significant on a 95 % threshold). A significant difference was found between treatments for potassium content in both source leaves and sink leaves with the LED-HLI treatment having a higher content in both leaf types. There was not found a significant difference between treatments for magnesium content- for either sink or source leaves (p-value not significant on a 95 % threshold).

Table 15: Results from ANOVA. The table shows results from ANOVA; The p-value, mean and standard deviation (SD) for content (in mg/g DW) of calcium (Ca) potassium (K) and magnesium (Mg) in source and sink leaves for each treatment. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). High light intensity with LED = LED-HLI. High light intensity with HPS = HPS-HLI. N = 5 in each treatment.

Treatment		Ca		K		Mg	
		Source leaf	Sink leaf	Source leaf	Sink leaf	Source leaf	Sink leaf
LED-HLI	Mean	A 10.96	A 5.61	A 74.40	A 60.00	A 3.50	A 2.589
	SD	1.59	5.55	11.99	18.02	0.55	1.05
HPS-HLI	Mean	A 9.70	A 5.57	B 52.00	B 45.60	A 2.96	A 2.33
	SD	0.98	4.41	8.60	8.91	0.35	0.70
P-value		0.17	0.987	0.009	0.038	0.102	0.534

5.5 Experiment 5

The aim of experiment 5 was to test if light quality- by using different spectral compositions during cultivation, and pre-cultivation (priming) together with light intensity affects development of tipburn. In experiment 4, very compact lettuces appeared in the LED treatment (Fig. 43). In this experiment the same LED used in experiment 4 was added far-red (FR) light to a similar level of natural light (red/far red ratio = 1.1). Lastly, the aim was to investigate the relationship between cations (Ca, K, Mg) and development of tipburn by comparing lettuces exposed to different light qualities and light intensities during cultivation. The treatments in this experiment were; High light (300 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED light quality + far red = LED-HLI/FR. Low light intensity (150 $\mu\text{mol}/\text{m}^2/\text{s}$) with LED light quality + far red = LED-LLI/FR. High light (300 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-HLI and low light (150 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-LLI.

5.5.1 Pre-treatment normal (PT-NORM)

Table 16 shows the results for PT-HLI from experiment 5. For the lettuces from the normal pre-treatment (PT-NORM) there was found a significant difference between treatments for both outer and inner tipburn. Both LED-treatments gave significantly lower outer tipburn than the HPS-treatments. The LED-LLI/FR treatment gave higher outer tipburn severity than the LED-HLI/FR treatment but not significantly so. As did the LLI. Inner tipburn was significantly more severe in the two high light intensity treatments than compared to the low light intensity treatments. The HPS spectrum gave higher tipburn severity than the LED spectrum, but not significantly so. From Fig. 46 it is clear that the increase in light intensity induce inner tipburn. The outer tipburn continued to develop for the HPS throughout the experiment, while it stagnates for the LED spectrum. Fig. 47. show the accumulated tipburn (outer and inner) for all treatments at the end of the experiment, for lettuces from PT-NORM.



Figure 44: Examples from lettuces from PT-NORM. From the left, treated with LED-HLI/FR, LED-LLI/FR, LLI and HLI. Photographer: Martin Knoop.

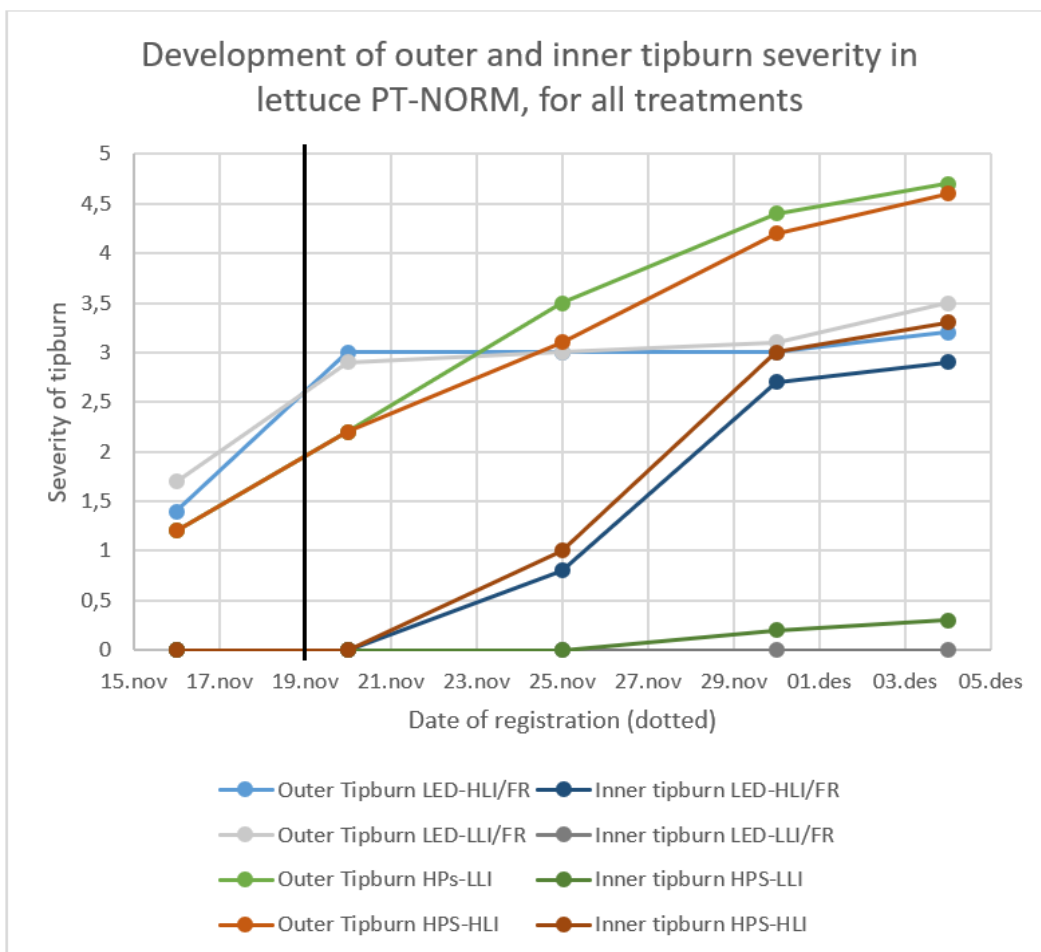


Figure 45: Development of outer and inner tipburn over time for lettuces from PT-MLI/-BLED in experiment 5. The black line represents the time of increase in light intensity. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED light quality + far red = LED-HLI/FR. Low light intensity ($150 \mu\text{mol}/\text{m}^2/\text{s}$) with LED light quality + far red = LED-LLI/FR. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS = HPS-HLI and low light ($150 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS = HPS-LLI. $N = 10$ in each treatment.

The number of leaves was mostly significantly different between treatments- with the highest number of leaves in the HLI treatment and the lowest in the LED-LLI treatment.

There was also a significant difference between treatments for fresh weight with the HLI

treatment having accumulated highest weight. There was a highest dry weight in the two high light intensity treatments with a significantly higher accumulation in the HLI treatment. The two LLI treatments were not significantly different. The water content was significantly lower in the high light intensity treatments (Table 16). All roots in lettuces from PT-NORM in experiment 5 was found to be “very good” at the end of the experiment, except in treatment HPS-LLI. In treatment HPS-LLI about half of the plants were assessed to be “good” and half was assessed to be “very good”.

Far-red light gave better morphology compared to the LED treatment in experiment 4, avoiding compact lettuces. The lettuce from treatment LED-LLI/FR gave the morphologically best lettuce with even and elongated growth compared to the other treatments. The LED-HLI/FR treatment gave a very uneven growth and the HLI treatment gave a very compact lettuce. The lettuces from the PT-NORM were the least mature lettuce and cosmetically more beautiful than from the other pre-treatments.

Table 16: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 5, for normal HPS (150 $\mu\text{mol}/\text{m}^2/\text{s}$) light intensity pretreatment (PT-NORM), and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). LED-HLI/FR = LED high light intensity + far-red lighting. LED-LLI/FR = LED low light intensity with far-red lighting. HPS-HLI = High light intensity (HPS lighting). HPS-LLI = Low light intensity (HPS lighting). N = 10 in each treatment.

Treatment		Temperature outer leaves	Temperature inner leaves	Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
LED-HLI/FR	Mean	AC 19.19	AC 19.29	A 23.00	A 110.59	A 9.73	A 91.12
	SD	0.47	0.51	0.94	9.70	0.82	0.85
LED-LLI/FR	Mean	AB 19.69	AB 19.82	B 21.60	AB 112.93	B 7.00	B 93.77
	SD	0.50	0.59	0.70	13.30	0.52	0.37
HLI	Mean	B 20.02	B 20.53	C 29.20	B 127.26	C 12.11	A 90.35
	SD	0.38	0.81	1.32	18.73	0.85	1.19
LLI	Mean	C 18.89	C 19.01	A 23.00	A 108.33	B 7.38	B 93.16
	SD	0.57	0.50	1.42	11.68	0.69	0.40
P-value		< 0.001	< 0.001	< 0.001	0.018	< 0.001	< 0.001

There was found a significant difference between treatments for temperature in both outer and inner leaves. The temperature in HPS-HLI was significantly higher than in the LED-HLI/FR treatment and the HPS-LLI treatment for both inner and outer leaves. The temperature was

lowest in the HPS-LLI treatment for both inner and outer leaves, but not significantly so from the LED-HLI/FR treatment (Fig. 47).

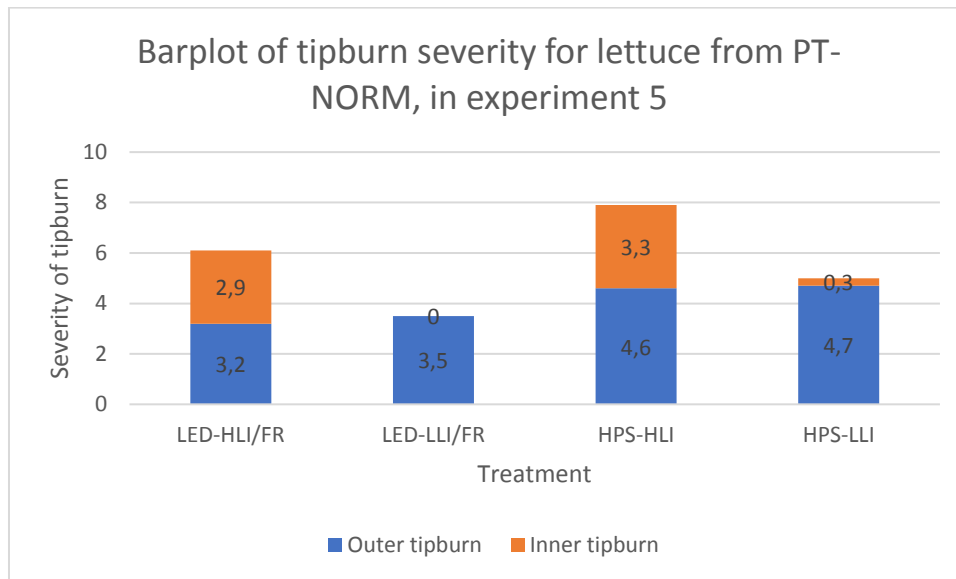


Figure 46: Barplot of tipburn severity in lettuce from PT-NORM at end of experiment 5. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. P-value for outer tipburn = < 0.001. P-value for inner tipburn = < 0.001. High light (300 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED light quality + far red = LED-HLI/FR. Low light intensity (150 $\mu\text{mol}/\text{m}^2/\text{s}$) with LED light quality + far red = LED-LLI/FR. High light (300 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-HLI and low light (150 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-LLI. N = 10 in each treatment. P-value for outer tipburn = < 0,001. P-value for inner tipburn = < 0,001. Tukey test outer tipburn: LED-HLI/FR = A, LED-LLI/FR = A, HPS-HLI = B, HPS-LLI = B. Tukey test inner tipburn: LED-HLI/FR = A, LED-LLI/FR = B, HPS-HLI = A, HPS-LLI = B.

5.5.1.1 Results from nutrient analysis for experiment 5

An ANOVA test was performed for the accumulation of cations in source and sink leaves for lettuces between treatments. There was found a significant difference in calcium content in source leaves where the HPS-HLI treatment had a lower calcium content than the other treatments. There was no significant difference between the other treatments. For sink leaves there was a significantly lower calcium content in the treatments LED-HLI/FR and the HPS-HLI. The HPS-LLI treatment was significantly different from the others and so was the LED-LLI/FR treatment that had the highest content (Table 17).

For potassium the two low light intensity treatments (LED-LLI/FR and LLI) were found to give significantly higher content of potassium in source leaves than the other two treatments. For sink leaves there was a significant difference between the HPS-HLI treatment and the LED-LLI/FR and HPS-LLI treatment. For magnesium there was found a significantly higher content in source leaves for the low light treatments (HPS-LLI and LED-LLI/FR). The content

for sink leaves were also highest in these treatments but they were in this instance only significantly higher than the HPS-HLI treatment (Table 17).

Table 17: Results from ANOVA. The table shows results from ANOVA; The p-value, mean and standard deviation (SD) for content (in mg/g DW) of calcium (Ca) potassium (K) and magnesium (Mg) in source and sink leaves for each treatment. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). LED-HLI/FR = LED high light intensity + far-red lighting. LED-LLI/FR = LED low light intensity with far-red lighting. HPS-HLI = High light intensity (HPS lighting). HPS-LLI = Low light intensity (HPS lighting). N = 5 in each treatment.

Treatment		Ca				K				Mg			
		Source leaf		Sink leaf		Source leaf		Sink leaf		Source leaf		Sink leaf	
LED-HLI/FR	Mean	A	15.00	A	1.62	A	79.60	AB	43.60	A	4.78	AC	1.82
	SD		2.24		0.30		10.97		8.73		0.59		0.27
LED-LLI/FR	Mean	A	17.40	B	4.24	B	103.40	A	50.80	B	6.30	B	2.44
	SD		0.89		0.55		9.58		4.55		0.412		0.23
HLI	Mean	B	10.94	A	1.28	C	62.20	B	34.80	C	3.40	C	1.44
	SD		1.99		0.08		7.09		4.82		0.56		0.18
LLI	Mean	A	15.40	C	2.80	B	96.00	A	51.60	B	5.68	AB	2.06
	SD		1.52		0.56		2.83		10.11		0.217		0.18
P-value			< 0.001		< 0.001		< 0.001		0.009		< 0.001		< 0.001

5.5.2 Pre-treatment high light intensity (PT-HLI)

The lettuces pre-treated with high light (300 μ mol) intensity (HPS) in the greenhouse developed significantly more tipburn than the lettuces with the normal pre-treatment ($p < 0.05$).

Table 18 shows the results for PT-HLI from experiment 5. For the lettuces from the high light intensity pre-treatment (PT-HLI), a significant difference between treatments for both outer and inner tipburn was found. Both LED-treatments gave lower outer tipburn than the HPS-treatments. Both treatments with low light intensity gave lower outer tipburn than the two with high light intensity. From Fig. 49 it appears that both outer and inner tipburn occurred before the increase in light for these lettuces. The development of inner tipburn evened out after some time. For inner tipburn it was the two high light treatments that gave the highest severity and there was no significant difference between the light spectrums. The HPS-LLI treatment was the lowest in inner tipburn severity with the LED-LLI treatment not significantly different from either the HPS-LLI treatment or the two HPS-HLI treatments. Fig. 50 shows the accumulated tipburn (outer and inner) for all treatments at the end of the experiment, for lettuces from PT-HLI.

The number of leaves was mostly significantly different between treatments- with the highest number of leaves in the HLI treatment and the lowest in the LED-LLI treatment. There was no significant difference between treatments for fresh weight (p-value not significant on a 95 % threshold). The highest dry weight was found in the two high light intensity treatments- with a significantly higher accumulation in the HPS-HLI treatment compared to the other treatments. Water content was significantly higher in the two low light intensity treatments compared to the other two. (Table 18). All roots for lettuces from PT-HLI in experiment 5, was found to be “very good” at the end of the experiment.

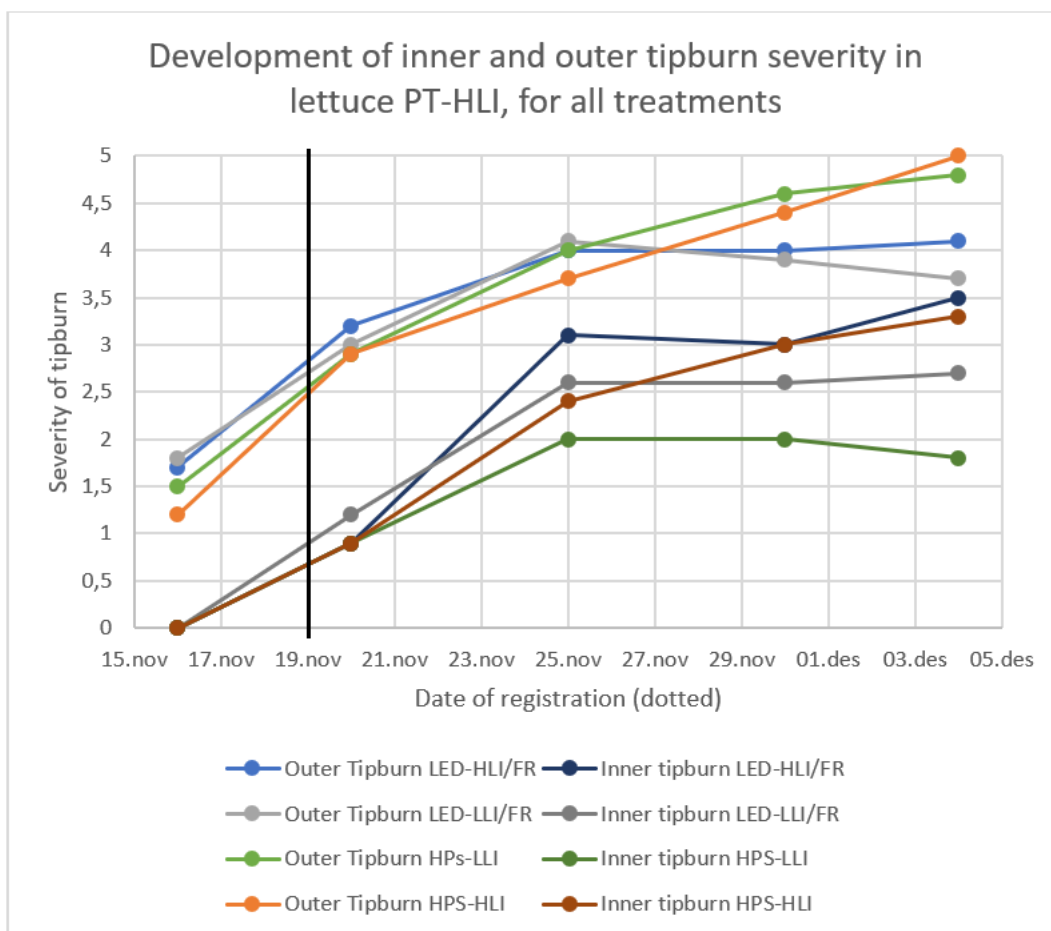


Figure 47: Development of outer and inner tipburn over time for lettuces from PT-HLI in experiment 5. The black line represents the time of increase in light intensity. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED light quality + far red = LED-HLI/FR. Low light intensity ($150 \mu\text{mol}/\text{m}^2/\text{s}$) with LED light quality + far red = LED-LLI/FR. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-HLI and low light ($150 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-LLI. N = 10 in each treatment.



Figure 48: Examples from lettuces from PT-HLI. From the left, treated with LED-HLI/FR, LED-LLI/FR, LLI and HLI. Photographer: Martin Knoop.

Far-red light induced more elongation and improved the morphology compared to the LED treatment in experiment 4, avoiding the compact lettuces. The best result was found in the LED-LLI/FR treatment where the growth was very even. The LED-HLI/FR treatment had an uneven growth with some leaves shooting well and others being stunted. The same was true for the HLI treatment. Lettuces from HPS-LLI also had an even growth. The Lettuces from the PT-HLI were longer in the development than the lettuces from other pre-treatments and was therefore morphologically more mature (older leaves with more tipburn, that were very crispy and that broke off easily).

Table 18: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 5, for pretreatment with HPS high light (300 μmol) intensity (PT-HLI), and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). High light intensity with LED light quality + far red = LED-HLI/FR. Low light intensity with LED light quality + far red = LED-LLI/FR. High light intensity with HPS light quality = HPS-HLI. Low light intensity with HPS light quality = HPS-LLI. N = 10 in each treatment.

Treatment		Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
LED-HLI/FR	Mean	A 30.40	A 143.70	A 12.20	A 91.44
	SD	1.43	17.25	1.14	1.01
LED-LLI/FR	Mean	B 27.80	A 154.45	B 8.93	B 94.13
	SD	1.545	30.84	1.26	0.63
HPS-HLI	Mean	C 33.60	A 165.59	C 14.43	A 91.19
	SD	1.58	18.63	0.76	1.05
HPS-LLI	Mean	AB 28.90	A 155.18	B 9.52	B 93.74
	SD	0.99	30.49	1.32	0.75
P-value		< 0.001	0.300	< 0.001	< 0.001

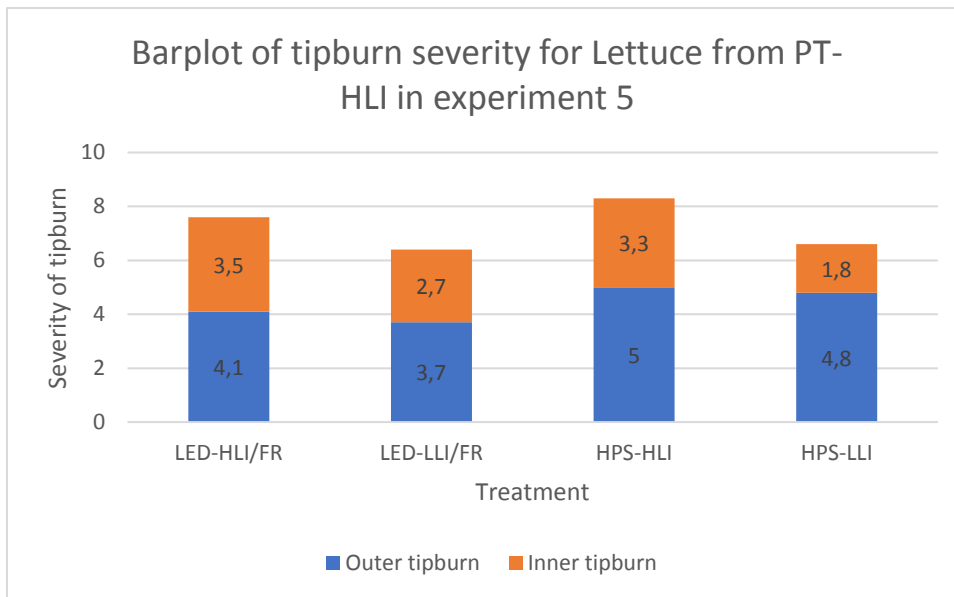


Figure 49: Barplot of tipburn severity in lettuce from PT-HLI at end of experiment 5. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED + far red = LED-HLI/FR. Low light intensity ($150 \mu\text{mol}/\text{m}^2/\text{s}$) with LED + far red = LED-LLI/FR. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS = HPS-HLI and low light ($150 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS = HPS-LLI. $N = 10$ in each treatment. P -value for outer tipburn = < 0.001 . P -value for inner tipburn = < 0.001 . Tukey test outer tipburn: LED-HLI/FR = AB, LED-LLI/FR = A, HPS-HLI = C, HPS-LLI = BC. Tukey test inner tipburn: LED-HLI/FR = A, LED-LLI/FR = AB, HPS-HLI = A, HPS-LLI = B.

5.5.3 Pre-treatment moderate light intensity and blue LED (PT-MLI/BLED)

Table 19 shows the results for PT-HLI from experiment 5. For the lettuces from the moderate light intensity with blue LED pre-treatment (PT-MLI/BLED) there was found a significant difference between treatments for both outer and inner tipburn. Both LED-treatments gave lower outer tipburn than the HPS-treatments. The LED low light treatment gave lower outer tipburn severity and the HPS-LLI treatment gave higher outer tipburn severity than the high light intensity treatments. Inner tipburn was severe in the HPS-HLI treatments and low in the HPS-LLI treatments. From Fig. 52, it is clear that the increase in light intensity induce inner tipburn. The outer tipburn continued to develop for the HPS throughout the experiment, while it stagnates for the LED spectrum. Fig. 53 shows the accumulated tipburn (outer and inner) for all treatments (of lettuces from PT-MLI/BLED) at the end of the experiment.

The number of leaves was mostly significantly different between treatments- with the highest number of leaves in the HPS-HLI treatment and the lowest in the LED-LLI treatment. There was no significant difference between treatments for fresh weight (p -value not significant on a 95 % threshold). There was a higher dry weight in the two high light intensity treatments with a significantly higher accumulation in the HPS-HLI treatment compared to the other treatments. The two low light intensity treatments were not significantly different.

The water content was lowest in the high light intensity treatments compared to the other two (Table 19). All roots for lettuces from PT-MLI/BLED in experiment 5, was found to be “very good” at the end of the experiment.



Figure 50: Examples from lettuces from PT-MLI/BLED. From the left, treated with LED-HLI/FR, LED-LLI/FR, LLI and HLI. Photographer: Martin Knoop.

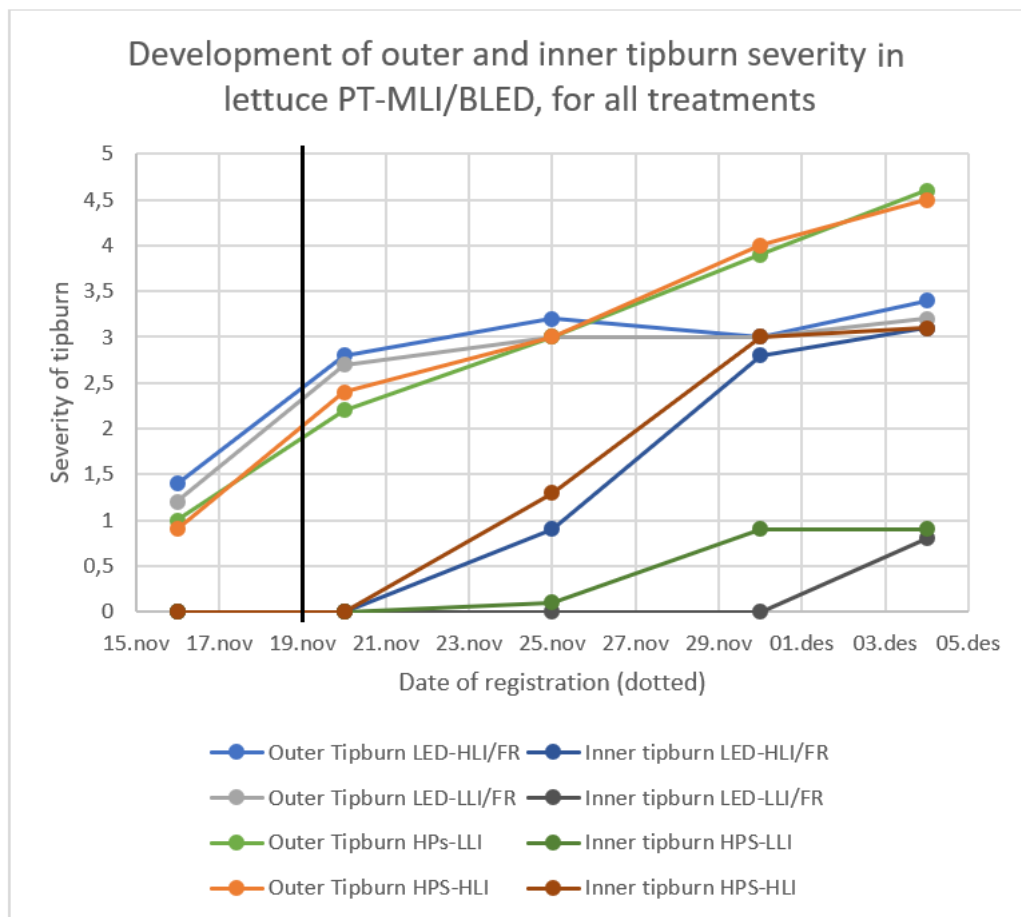


Figure 51: Development of outer and inner tipburn over time for lettuces from PT-MLI/-BLED in experiment 5. The black line represents the time of increase in light intensity. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED light quality + far red = LED-HLI/FR. Low light intensity ($150 \mu\text{mol}/\text{m}^2/\text{s}$) with LED light quality + far red = LED-LLI/FR. High light ($300 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-HLI and low light ($150 \mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS light quality = HPS-LLI. N = 10 in each treatment.

Table 19: Results from ANOVA. The table shows results from ANOVA; The mean and standard deviation (SD) for each parameter and treatment in experiment 5, for pretreatment with moderate (200 μmol) light intensity with blue LED (MLI/BLED), and also the p-value for each ANOVA test. A Tukey test was used to separate the mean values (Means in the columns that do not share a letter are significantly different). LED-HLI/FR = LED high light intensity + far-red lighting. LED-LLI/FR = LED low light intensity with far-red lighting. HPS-HLI = High light intensity (HPS lighting). HPS-LLI = Low light intensity (HPS lighting). N = 10 in each treatment.

Treatment		Number of leaves	Fresh weight, g	Dry weight, g	Water content (%)
LED-HLI/FR	Mean	AC 21.50	A 110.52	A 9.22	A 91.62
	SD	1.35	10.55	0.70	0.79
LED-LLI/FR	Mean	A 20.70	A 116.36	B 6.99	B 93.94
	SD	1.42	19.68	0.80	0.40
HPS-HLI	Mean	B 26.60	A 120.34	C 11.45	C 90.36
	SD	1.51	16.18	0.98	1.22
HPS-LLI	Mean	C 22.90	A 107.55	B 7.53	B 92.99
	SD	1.79	13.02	0.95	0.53
P-value		< 0.001	0.253	< 0.001	< 0.001

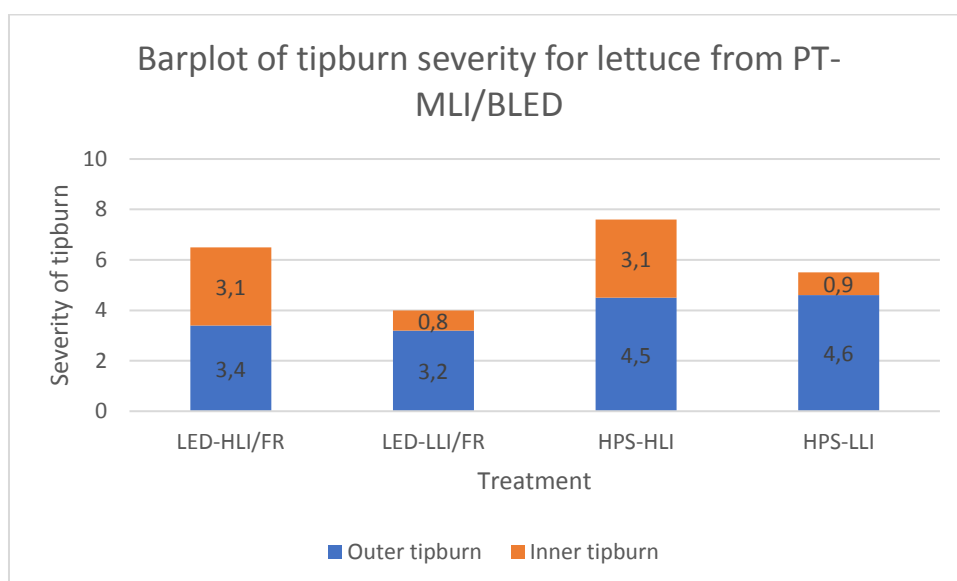


Figure 52: Barplot of tipburn severity in lettuce from PT-MLI/BLED at end of experiment 5. Maximum score is 5 for each type of tipburn = 10 in total. A tipburn severity of 2 or less is not severe, of 3 is severe and of 4 and 5 is very severe. High light (300 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with LED + far red = LED-HLI/FR. Low light intensity (150 $\mu\text{mol}/\text{m}^2/\text{s}$) with LED + far red = LED-LLI/FR. High light (300 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS = HPS-HLI and low light (150 $\mu\text{mol}/\text{m}^2/\text{s}$) intensity with HPS = HPS-LLI. N = 10 in each treatment. P-value for outer tipburn = < 0.001. P-value for inner tipburn = < 0.001. Tukey test outer tipburn: LED-HLI/FR = A, LED-LLI/FR = A, HPS-HLI = B, HPS-LLI = B. Tukey test inner tipburn: LED-HLI/FR = A, LED-LLI/FR = B, HPS-HLI = A, HPS-LLI = B.

Far-red light improved morphology compared to the LED treatment in experiment 4, and resulted in less compact lettuces (Fig. 51). The lettuce from treatment LED-LLI/FR was the least compact hand had nice crispy leaves that grew evenly and were low in damage. The LLI treatment also grew evenly while the two high light intensity treated lettuces were a bit uneven in morphology. The lettuce from the PT-MLI/BLED were between the PT-HLI lettuces and the PT-NORM lettuces in maturity.

5.5.4 Comparison between treatments and pre-treatments of outer and inner tipburn
 A general linear model (two-way interaction) analysis was performed for the accumulated tipburn damage (for both inner and outer tipburn) for lettuce from each pre-treatment (PT) and treatment (T). The results from this experiment show no significant difference in outer tipburn accumulation for treatment, attributed to pre-treatment. However, a significant difference between inner tipburn accumulation in treatments are found to be attributed to pre-treatments (Table 20).

Table 20: Fitted means and standard errors from General Linear Model (two-way interaction between pre-treatment (PT) and treatment (T). The table shows fitted means for outer and inner tipburn severity (score can be from 0-5) in treatments for each pre-treatment). P-values for treatment (T), pre-treatment (PT) and interaction between the two (PT*T) are shown for both inner and outer tipburn. N = 40 in each pre-treatment. N = 30 in each treatment.

	SE mean	Pre-treatment (PT)	Treatment (T) (Fitted mean = T*PT)				PT*T
			LED-HLI/FR	LED-LLI/FR	HPS-HLI	HPS-LLI	
Outer tipburn	± 0.167	PT-HLI	4.10	3.70	5.00	4.80	
		PT-MLI/BLED	3.40	3.20	4.50	4.60	
		PT-NORM	3.20	3.50	4.60	4.70	
P-values		< 0.001	< 0.001			0.279	
Inner tipburn	± 0.247	PT-HLI	3.50	2.70	3.30	1.80	
		PT-MLI/BLED	3.10	0.80	3.10	0.90	
		PT-NORM	2.90	0.00	3.30	0.30	
P-values		< 0.001	< 0.001			< 0.001	

Because there wasn't found a significant difference for outer tipburn between treatments attributed to pre-treatment a fitted mean for treatment alone, and for pre-treatment is presented in Table 21. A significant difference in outer tipburn is found for both different treatments and different pre-treatments.

Table 21: Fitted means and standard errors from General Linear Model (one-way interaction between outer tipburn and treatment, and outer tipburn and pre-treatment. The table shows fitted means for outer tipburn severity (score can be from 0-5) for treatment and pre-treatment P-values for treatment (T) and pre-treatment (PT) are shown. N = 40 in each pre-treatment. N = 30 in each treatment.

Treatment	SE mean	Fitted mean Outer tipburn	Pre-treatment	SE mean	Fitted mean Outer tipburn
LED-HLI/FR	± 0.1	3.57	PT-HLI	± 0.08	4.40
LED-LLI/FR		3.47	PT-MLI/BLED		3.93
HPS-HLI		4.7	PT-NORM		4.00
HPS-LLI		4.7			
P-values		< 0.001			< 0.001

The lettuces pre-treated with high light intensity in the greenhouse developed significantly more inner tipburn than plants with the normal pre-treatment ($p < 0.05$), and the lettuce pre-treated with blue light was somewhere in the middle, with significant differences between the pre-treatments for some of the treatments (table 20).

6 Discussion

6.1 Climate factors and tipburn

According to Saure (1998) several experiments have shown that high transpiration caused by elevated temperature, high light intensity or wind, can increase the occurrence of tipburn in different types of lettuce cultivars (Ende, 1954) and (Ashkar, 1971). Perhaps this is linked to growth rate or undue stress, more than to only transpiration. In this thesis, several climate parameters were tested to find a method to induce tipburn in lettuce “Frillice” which is a popular lettuce cultivar to produce in greenhouses all year round.

6.2 Elevated temperature

Elevated temperatures have been used to induce tipburn to select robust cultivars resistant to tipburn (Nagata & Stratton, 1994) but it's still unclear whether a relationship between tipburn occurrence and elevated temperatures exist (Imai, 1987). Termohlen and Van der Hoeven (1966) found that high night temperatures make lettuce more susceptible to tipburn. In experiment 1, plants were exposed to elevated temperatures but the temperature in each treatment was kept constant during day and night. Elevated temperatures did not give higher occurrence of tipburn and the severity of tipburn was very low (assessed to be either 2 or 3), compared to other tested conditions (Fig. 34). Inner tipburn did not occur in this experiment. One explanation can be that the temperature was not high enough to induce tipburn (27°C). Nagata and Stratton (1994) used temperatures between 28 and 37°C in their experiments to induce tipburn and select robust cultivars. However, in their experiment elevated temperatures was in combination with elevated (>90%) RH.

In experiments 2-5, nighttime temperature was 2°C lower than day temperature. Whether there exists a link between temperature and tipburn it might lie in the fluctuations of temperature and the subsequent stress the lettuce can experience from this (Ende, 1954). Cox and McKee (1976) and Carl (1990) found that lower temperatures might reduce tipburn occurrence. Most likely a fluctuation of 2°C is not big enough to affect tipburn development, or to create compounding effects together with other factors. In conclusion, elevated temperature did not induce tipburn, probably due to lack of temperature fluctuations during the experiment, and too low temperatures to trigger tipburn.

6.3 Elevated RH

According to Saure (1998), “a positive correlation between high ambient humidity (RH) and the occurrence of tipburn” is common. This has also been described in (Bottenberg & Tibbitts, 1968) and (Barta & Tibbitts, 1986). Tibbitts and Bottenberg (1976) also found that the growth rate increased drastically for lettuce grown under 85 % RH, compared to lettuce grown under 50 % RH. This can suggest that increased RH affect tipburn through its effects on growth rate. This is supported by Collier and Tibbitts (1984). However, their explanation was that higher RH during night reduced transpiration and increased root pressure, and that this would increase leaf calcium content. To the contrary Mason and Guttridge (1975) found that high RH reduced transpiration and calcium content in strawberry- inducing tipburn.

The results show that elevated humidity did not increase tipburn occurrence (Severity was assessed to 2 in experiment 1). There was neither found a compounding effect of elevated RH together with elevated temperatures or elevated RH together with high light intensity. However, the treatment with high light intensity and high RH in experiment 3, had the most severe inner tipburn occurrence (Table 12). The HPS-HLI/HRH treatment, had the highest fresh weight accumulation (Table 12) and a calcium content in inner leaves that was somewhere between (2.32 mg Ca/g DW) the results of the HPS-HLI (1.86 mg Ca/g DW) and HPS-LLI 4.02 mg Ca/g DW), treatments (Table 13), indicating that it grew well and accumulated calcium tolerably. As in the temperature experiment, the RH was kept constant all the time, and no variation in RH was provided during day and night. The RH was very high (90%) and the vapor pressure deficit (VPD) very low. This can explain the tolerable calcium content and why tipburn did not significantly increase in the HPS-HLI/HRH treatment.

6.4 High light intensity

Increase in light intensity or light sum have been shown to increase tipburn occurrence (Tibbitts & Rama Rao, 1968), (Cox & McKee, 1976), (Gaudreau et al., 1994), (Sago, 2016). Wissemeier and Zühlke (2002) found that light intensity was the one climate factor that correlated best with tipburn, and also the only one that did so significantly through years of experiments in the field. The effect of light was confirmed in this study. Higher light intensities/light sums induced more inner tipburn in Frillice lettuce compared to low light intensities (Figs. 35, 39, 47, 50 and 53). This is regardless of light quality. Inner tipburn mostly

did not occur under low light intensities, and when it occurred (experiment 5, PT-NORM) it was in just a few lettuces.

Ende (1954) found that sudden sunlight after periods of shade would also induce tipburn, and Wissemeier (1996) found that shading limits tipburn occurrence. Greenhouse grown lettuce receive nearly half the irradiance and almost no UV, yet still are more susceptible to tipburn than field grown lettuce (Cox & McKee, 1976), (Barta & Tibbitts, 1991a). This suggest that there are other factors in addition to light intensity that induce tipburn in greenhouse production. However, light intensity is a very important climate factor to trigger tipburn (Saure, 1998). In this study light intensity was the most convincing and safest way to induce tipburn.

Outer tipburn was found to be relatively stable for each treatment, regardless of light intensity. Higher light intensity/light sum gave higher outer tipburn occurrence but not significantly so. In experiment 5 where plants were given normal pre-treatment, outer tipburn severity was significantly higher (for comparative light quality = HPS) than in previous experiments. This was true for all pre-treatments and may be explained by less fluctuations in light during pre-treatment (late autumn gave little natural light during pre-treatment), causing less acclimation to stressful conditions later. High light intensity will increase growth and can affect tipburn occurrence by affecting growth rate (Bárcena et al., 2019).

Too high a light sum (above 16-17 moles/m²/day) is found to increase tipburn (Both et al., 1997). The results also show that higher light sum induce tipburn compared to lower light sums (Figs. 35, 39, 47, 50 and 53). The two HPS-MLI/ CPP treatments (17.3 mol/day) in experiments 2 and 3 are both significantly higher in tipburn severity than the low light sum treatments (9.7 mol/day), but inconclusively lower than the higher light sum treatments HPS-HLI (19.4 mol/day). This can be explained by the continuous photo period of these treatments which might accelerate the damage.

Koontz and Prince (1986) found that longer photoperiods may induce more tipburn than high light intensity when the total light sum was the same. My results show that the continuous photo period given in experiments 2 and 3 gave significantly higher tipburn

severity than low light intensity, but both significantly lower (experiment 2) and higher (experiment 3) inner tipburn occurrence than the HPS-HLI treatment.

6.5 Light quality

Hytönen et al. (2017) found that LED (warm-white and warm-white with blue spectra) performs just as well as conventional HPS-lamps with regards to quality and yield of Frillice´ lettuce in greenhouse production. This is supported by the findings of Chen et al. (2017).

LED with or without far-red light was not found to significantly decrease inner tipburn occurrence, but its occurrence was lower than in the HPS treatments in both experiments. Leaf temperatures were lower in the LED-treatment, than in the HPS treatment in experiment 4, and lower in the LED treatments than the HPS-HLI treatment in experiment 5. The same for FW. This can indicate lower transpiration in the LED treatments, compared to HPS (or HPS with high light intensity) and may have an effect on calcium content. However, there was no significant difference in calcium content in experiment 4, and the differences in calcium content in experiment 5 can be explained by light intensity (table 15 and 17).

White LED was found to give significantly higher outer tipburn severity (experiment 4). However, when combined with far-red LED (in experiment 5) the severity became significantly lower, when compared to HPS. This can indicate that the supplemental far-red light in experiment 5 contributed to lower outer tipburn severity. This is contrary to the findings of Kleemann (2004), who reduced tipburn by reducing far-red light in field grown lettuce. I found that LED without FR-light gave very compact plants compared to the light quality of HPS (experiment 4) and I therefore tried to promote elongation with more far-red (in experiment 5). This was very successful. The more “open growth” when adding FR-light can improve transpiration and, in that way, reduce the incidence of tipburn. However, it is also possible that FR-light as a signal affect physiology/metabolism of the lettuce rather than morphology.

6.6 Tipburn severity of inner leaves and role of calcium

According to Saure (1998), «The susceptibility to tipburn is genetically determined but influenced by environment, and plants grown in greenhouses are affected earlier and to a greater extent by tipburn than field-grown plants”. Also, according to Saure (1998), tipburn occurrence increase with more rapid growth rates. According to (Bárcena et al., 2019) tipburn shows a proclivity to occur under conditions that advances growth. Tipburn always

occurs during cell expansion (Wissemeier, 1996). All these findings support the idea that tipburn occurs when the plant cannot translocate enough calcium to expanding leaves (Collier & Tibbitts, 1982).

Barta and Tibbitts (1986) found that the concentration of calcium was lowest in leaves that develop tipburn and suggest a threshold for calcium content and tipburn occurrence to be below 1 mg of calcium/g dry weight. The results from experiments 3-5 are inconsistent with this finding as all calcium contents are above this threshold, regardless of tipburn occurrence (both outer and inner).

My results show that content of all tested cations are consistently lower in sink leaves, compared to source leaves. Calcium content is found to be higher in sink leaves from treatments inducing less tipburn and strengthens previous findings that tipburn occurrence is linked to calcium content (Barta & Tibbitts, 1991a), (Shear, 1975) and (Aloni et al., 1986).

Barta and Tibbitts (1986) suggest from their study that high magnesium content in inner leaves- could explain the develop tipburn. Mg can act antagonistically to Ca and high content of Mg in the nutrient solution can reduce the uptake of Ca (Levine & Coburn, 1984). In this study I found that magnesium content was high when calcium content was high and vice versa. However, a best subset regression analysis was performed to rank the different cations in relation to tipburn occurrence the best (table 22).

As can be surmised from table 22, Calcium is the cation that best explains tipburn occurrence. Surprisingly magnesium comes up as second. This suggests a positive correlation between magnesium and tipburn, contrary to the findings of Barta and Tibbitts (1986). Further research into this is recommended.

Table 22: Best Subset Regression for Tipburn versus Ca, K and Mg (Data from experiments 3, 4 and 5)

Vars	R ²	R ² (adjusted)	R ² (predicted)	Mallows Cp	S	Ca	K	Mg
1	79.6	77.0	72.7	0.6	0.71013	X		
1	77.2	74.4	67.4	1.3	0.74984			X
2	81.4	76.0	71.1	2.0	0.72514	X	X	
2	80.6	75.1	64.5	2.2	0.73925	X		X
3	81.4	72.0	53.9	4.0	0.78305	X	X	X

Potassium does not explain tipburn occurrence very well alone, (table 22). Barta and Tibbitts (1991b) found low calcium concentration in the presence of high potassium and magnesium content in leaves induced tipburn. From table 22 it is clear that a high R^2 appears (81.4) when both Ca, K and Mg. content of the sink leaves are included in the model. A similar high R^2 of 81.4 appears when Ca and K are included in the model. However, Ca/K ratio did not explain tipburn better than Ca alone (results not shown).

Research into other nutrients have found other links to tipburn than calcium. Crisp et al. (1976) found that boron deficiency was linked to tipburn emergence but didn't find evidence that boron affected calcium content. Sørensen et al. (1994) found that abundant nitrogen supply decreased outer tipburn in field grown lettuce. Boron and nitrogen content were not measured in the present experiment. Like Ca, boron and nitrogen uptake are connected to transpiration and low transpiration reduces the uptake of both (Heinen et al., 1991). Reduced transpiration is believed to affect tipburn severity (Saure, 1998). Furthermore, nutrient uptake is often reduced when transpiration is reduced. Thus, several nutrients can actually be involved. However, the findings in this thesis points towards Ca and strengthen the link between tipburn and calcium content (table 22).

Rached et al. (2018) found that the resistance to blossom-end rot, a disease in tomato thought to be linked to calcium, was actually linked to total amount of ascorbate, an antioxidant. Even when a link between calcium and tipburn is found, perhaps it is not the whole explanation of why tipburn occur.

6.7 ROS

Oh et al. (2009) suggests that "secondary metabolites and antioxidants are involved in environmental adaption and stress tolerance in lettuce". Carassay et al. (2012) found that treatment with "topical antioxidant applications (Tiron, DPI) reduced symptoms in treated leaves, but not in the rest of the plant". This can suggest that ROS is linked to tipburn occurrence. My results show that hydrogen peroxide was present in leaves that were experiencing tipburn, and that the effects were greatest in high light intensity treatments, reinforcing that tipburn is linked to ROS-accumulation, and is subsequently linked to stress.

The samples for ROS staining was taken 5 cm below the area with tipburn injuries. However, the ROS-accumulation observed could be a result of the injury itself, rather than the cause.

On the other hand, young sink leaves at low PPFD did not show any ROS accumulation: neither did the source leaves in high PPFD. From this, it is likely that ROS-accumulation in stressed leaves can be connected to tipburn.

6.8 Effects of priming

Since it was found that light stress induced tipburn, it was of interest to test if a pre-treatment with light stress could reduce the risk of tipburn later in the production. According to Saure (1998) plants that are growing well are not acclimated to stress. Their stress tolerance is therefore very low. Hence, even little stress can cause tipburn. The idea of priming is to help the lettuce respond faster and stronger when experiencing stress (Conrath, 2009).

I did not find that priming with either high light intensity of 300 $\mu\text{mol}/\text{m}^2/\text{s}$ (HPS) or moderate light intensity 150 $\mu\text{mol}/\text{m}^2/\text{s}$ (HPS) + blue light 100 $\mu\text{mol}/\text{m}^2/\text{s}$ (Blue LED) gave lower tipburn severity. To the contrary, the priming with high PPFD during pre-cultivation gave significantly higher inner tipburn occurrence than unprimed plants (except in the HPS-HLI treatment). For outer tipburn, priming did not give significantly lower tipburn occurrence during growing in any of the light qualities. Thus, priming with “light stress” was not found to increase tipburn resistance. The fact that it did not work can be explained by the lack of “memory” or that the light stress was not stressful enough to trigger their proactive responses for many weeks. Or, ‘Frillice’ lettuce might not have the correct epigenome to become primed by light (Lämke & Bäurle, 2017).

Ebisawa et al. (2008) found that “Supplementary UV-B together with blue light at night increased quercetin content and flavonol synthase gene expression in leaf lettuce (*Lactuca sativa* L.)”. Other priming strategies that can be cost effective and easy to implement can perhaps give better results, than priming with high light.

6.9 Tipburn assessment (method)

To be able to test to the objectives of this study, a method of assessing tipburn was developed. In the literature, it is possible to find different methods to assess severity of tipburn. Misaghi et al. (1981) assessed percentage of damage on lettuce plants and Nagata and Stratton (1994) assessed number of plants damaged in a population, but neither assessed both methods at the same time. Jonathan et al. (2004) combined the two methods mentioned here, to create a tipburn index (TI) with a score up to a 100 for assessing damage.

The problem with assessing severity of the damage as a percentage or as an index, is the identification of whether or not the lettuce is a salable product. Occurrence of inner tipburn (even with a small score) will render a product unsellable, while a few outer leaves with a high outer tipburn score can be removed and the total final damage be reduced. Also, it's the outer tipburn that is the biggest problem for Norwegian growers (pers. com. Espedal, 2018). In the experiments described in this thesis it was important to relate the damage to a commercial situation experienced by growers.

Thus, the scheme for assessment by the Norwegian extension service (NLR) was chosen. This method requires meticulous assessment of the whole plant (and for each leaf), but do not assess damage as a percentage, but as threshold of damage graded. Gaudreau et al. (1994) also assessed tipburn from 0-5, with a score higher than 1 meaning the lettuce was unsellable. The weakness of the chosen method (0-5) is the danger of assessing thresholds subjectively. However, the strength is that it enables the assessed severity to better reflect when tipburn is a problem for a grower. From the chosen method an outer tipburn occurrence of 4-5 will and/or inner tipburn occurrence of 2 will render it unsellable.

Tipburn usually occur near harvest, but can develop as early as 13 days after emergence (Saure, 1998). At which growth stage the lettuce is in, and the time of assessment is therefore important. Waiting a few days or assessing a few days too early will influence on the results. In the experiments in this thesis, tipburn was assessed with quite different final weight. However, since the tipburn was assessed on outer and inner leaves according to a scale from 0 to 5 it is possible to compare the severity between different experiments.

6.10 Practical implications

Goto and Takakura (1992) and (Lee et al., 2013) reduced tipburn by blowing air into the lettuces. The cost and simplicity/difficulty of such systems will decide their degree of implementation. They also reduced tipburn by shortening the day/night cycles (total light period was maintained in 24 h) without reduction in growth rate (Goto & Takakura, 2003). This is probably not feasible in greenhouses (even with shading), but can be applied in plant factories where day light won't interfere with light cycles.

During night, stomatal closure is found to increase root pressure. This allows calcium to reach all parts of the plant (Collier & Tibbitts, 1984), therefore increasing the relative air

humidity at night might be a simple and cheap strategy to prevent tipburn occurrence and development.

Uno et al. (2016) used a susceptible indicator cultivar as a signaling system for when tipburn occurs. This was found to be a good way of predicting tipburn occurrence and enact countermeasures to reduce the occurrence.

A new exciting field of study is the use of imaging technology together with deep learning to map and detect different factors in lettuce. Zhou et al. (2018) used this for monitoring moisture content in lettuce (which also can be used to monitor calcium content). Ren et al. (2017) found that this could be a low cost way of monitoring lettuce health, and Jiang et al. (2018) found that fresh weight also could be easily monitored and predicted. With further research this could be a low cost and effective way of detecting tipburn and monitoring its development, but also make it a lot easier to counteract.

From the findings in this thesis a climate control where the use of supplemental lighting and shading to avoid an accumulated light sum of 17 mol/day is recommended. To help do this, PAR sensors can be installed. If it is difficult to maintain an even and less fluctuating temperature, the use of LED can be applied to reduce leaf temperature.

All these measures mentioned can be adopted by growers to help produce quality lettuce and reduce the occurrence of tipburn.

7 Conclusion

- Elevated temperature (20°C → 27°C) did not affect outer or inner tipburn occurrence in 'Frillice' lettuce.
- Elevated RH (65% → 90%) did not induce inner or outer tipburn in 'Frillice' lettuce.
- High light intensity/or high light sum increased inner tipburn severity in 'Frillice' lettuce.
- Outer tipburn occurred in all treatments, but was aggravated in high light intensity/light sum
- Tipburn is linked to light stress. Increased light intensity/light sum was the strongest environmental factors tested to induce outer and inner tipburn.
- White LED without far-red will promote outer tipburn in 'Frillice' lettuce, compared to HPS, while additional far-red in white LED will reduce it.
- Priming with high light intensity and blue LED did not prevent tipburn in 'Frillice' lettuce, and is ineffective as an acclimator against tipburn.
- Presence of ROS in leaves with tipburn indicates a link between tipburn and oxidative stress.
- Lower Ca level was found in young sink leaves with inner tipburn, than in young leaves without tipburn and confirm a role of Ca.
- Climate control in greenhouses should be run with regards to light sum, and avoid a total of 17 mol/day.

8 References

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9 Appendix 1, examples of inner and outer tipburn and root assessments



Figur 1: Leaf 1, outer tipburn = 1. (The black dot where the finger touches). Photo: Martin Knoop.



Figur 2: Leaf 2, outer tipburn = 2. Photo: Martin Knoop.



Figur 3: Leaf 3, outer tipburn = 3. Photo: Martin Knoop.



Figur 4: Leaf 4, outer tipburn = 4. Photo: Martin Knoop.



Figur 5: Leaf 3, outer tiburn = 5. Photo: Martin Knoop.



Figur 6: Leaf 15, outer tipburn = 1. Photo: Martin Knoop.



Figur 7: Leaf 18, inner tipburn = 2. Photo: Martin Knoop.



Figur 8: Leaf 15, inne tipburn = 3. Photo: Martin Knoop.



Figur 9: Leaf 14, inner tipburn = 4. Photo: Martin Knoop.



Figur 10: Leaf 9, inner tipburn = 5. Photo: Martin Knoop.



Figur 11: Example of a root assessed to be «bad». Photo: Martin Knoop.

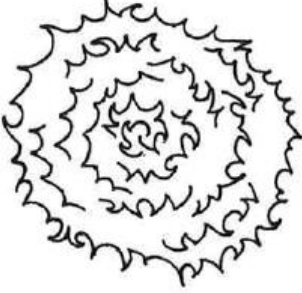
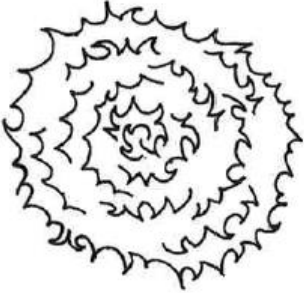

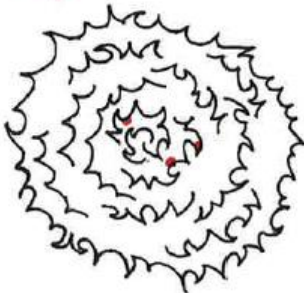
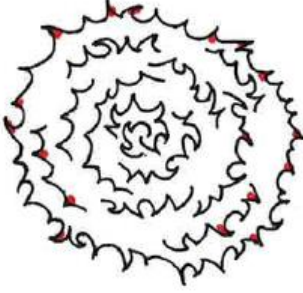
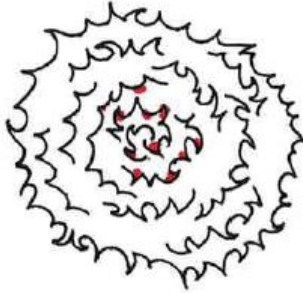


Figur 12: Example of a root assessed to be «good». Photo: Martin Knoop.

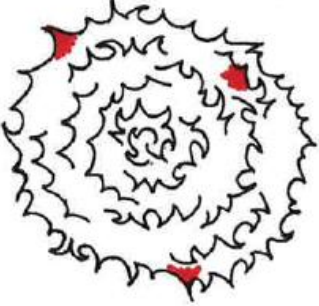


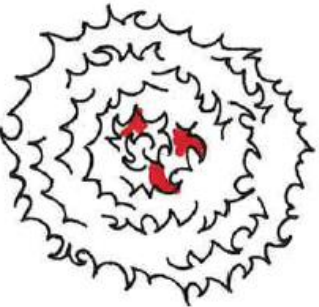
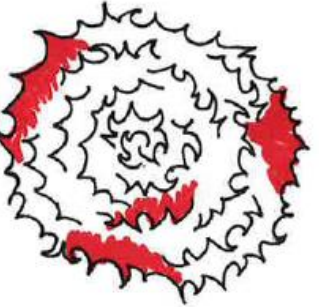
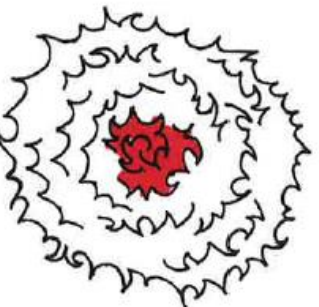


Figur 13: Example of a root assessed to be «very good». Photo: Martin Knoop.

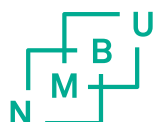
10 Appendix 2, NLR registration form for outer and inner tipburn

Skala	Forklaring	Skisse ytre bladrand	Skisse indre bladrand
0	Ingen synlig bladrandskade		
1	Små brune flekker i enkelt bladspisser		
2	De fleste bladspisser er brune i spissen		

Figur 14: Registration form by the Norwegian Extension Service (NLR).

3	Enkelte til flere bladspisser helt brune/visne		
4	Større deler av enkelte blader påvirket av bladrandskade		
5	Større dele av de fleste blader påvirket av bladrandskade		

Figur 15: Registrasjon form by the Norwegian Extension Service (NLR).



Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitenskapelige universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway