Norwegian University
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Master's Thesis 2020 30 ECTS
BioVit

Impact of light regimes on growth and tipburn formation in lettuce (*Lactuca Sativa* L. 'Frillice') and the use of NDVI for early detection of tipburn.

Påvirkningen av lysforhold på vekst og bladrandskade i salat (*Lactuca Sativa* L. 'Frillice') og bruk av NVI analyse for å gjenkjenne tidlige tegn på bladrandskade.

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Lectorate Degree in the Sciences

Foreword

This thesis work was done as a part of the project "Control of tipburn for sustainable production of lettuce" financed by the Norwegian Research Council (NFR) and Grofondet, in cooperation with the Norwegian University of Life Sciences (NMBU). LED lamps were provided by EvoLys.

I want to convey my deepest gratitude to my supervisor Sissel Torre for all her help and encouragement throughout this period of my life.

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A final thank you goes to Martin Knoop, for forging ahead and being a benevolent senior in my work.

Ås, 16.8.2020

Herman Bang

Sammendrag

Arbeidet i denne oppgaven ble utført for å undersøke påvirkningen av lysstyrker, lysperioder og endret forhold mellom rødt (R) og mørkerødt (MR) lys på bladrandskade hos 'Frillice' salat dyrket i et vekstkammer. I tillegg ble det undersøkt om «Normalisert Vegetasjons Indeks» (NVI) analyse av bilder tatt med et nær-infrarødt kamera kan brukes for å forutse bladrandskade før det kan observeres med det blotte øye. I Norge er det i perioder opp til 20 % svinn på grunn av bladrandskader i 'Frillice', så metoder for å hindre eller minske omfanget av bladrandskader vil øke effektiviteten og profitten samt bidra til en mer bærekraftig produksjon av 'Frillice'.

Bladrandskade er en type nekrose som oppstår på bladkanten hos eldre blad, og unge blader under utvikling. I Norge er ytre bladrandskade den største utfordringen. Den ledende teorien for hvorfor bladrandskader oppstår peker på en kalsiummangel. Kalsium er et viktig næringsstoff som blant annet styrker celleveggene i levende planteceller og lite kalsium kan lede til total cellekollaps og nekrosedannelse som sett i bladrandskader. I tillegg kan flere andre abiotiske faktorer også påvirke en plantes motstandsdyktighet mot bladrandskader. Disse inkluderer temperatur, relativ luftfuktighet, lysperioder og lysstyrke og R/MR forhold. I denne masteroppgaven ble påvirkningen lysperioder, lysintensitet og en forhøyet R/MR forhold undersøkt.

Eksperimentene for lysperioder og lysstyrke ga veldig varierende resultater, da de to forsøkene endte opp med motstridende konklusjoner på lysperiode avhengig av styrken på ledetallet. Ved normalt ledetall (2.0 mS) viste resultatene mer alvorlig bladrandskade hos planter eksponert for 12 timer lysperiode, men ved lavt ledetall (1.2 mS-1) viste planter dyrket med 12 timer lysperiode mindre bladrandskade, spesielt på unge blader.

For eksperimentene som undersøkte påvirkningen av forhøyede ratioer av rødt til mørkerødt lys var dataene enklere å tyde. De statistiske forsøkene for begge eksperimenter viste ingen sammenheng mellom den gjennomsnittlige bladrandskaden observert på individuelle planter og de forskjellige lysbehandlingene med p verdier godt over 0.05 i begge forsøk (tabell 6 og 7).

I tillegg viste NVI analysen av bildene tatt under forsøkene ingen relevant bladrandskader enkelt kunne observeres på de voksende plantene, noe som betyr at NVI ikke gir informasjon som kan benyttes til å forutse dannelsen av bladrandskader før de er synlige.

Summary

The work in this master thesis was undertaken in order to add to invesitage how light schedules impact the growth and the formation of tipburn in 'Frillice' lettuce when grown hydroponically in controlled growth chambers, and if the use of the Normalized Difference Vegetation Index (NDVI) analysis on images taken with a Near Infra-Red (NIR) capable camera could be used to predict the early formation of tipburn. The ability to lessen or even eliminate the formation of tipburn would, in Norway, prevent the annual loss of almost 20 % of all greenhouse 'Frillice' crops resulting in a large efficiency and economical boost and a more sustainable production for the farmers growing the cultivar.

Tipburn is a form of necrosis that can affect the inner and outer part of lettuce cultivars, with outer tipburn presenting the largest challenge for Norwegian 'Frillice' growers. Tipburn is thought to mainly be induced by a calcium deficiency in the affected leaves, leading to cell walls weakening and resulting in eventual necrosis. Several other abiotic factors such as temperature, relative humidity, photoperiods and light intensity have been shown to impact the severity of the tipburn observed during growth. In this thesis the impact of photoperiods, light intensity and red to far-red (R:FR) ratios on 'Frillice' were tested with a total of four experiments conducted.

The two experiments regarding photoperiods and light intensity yielded very varied results, with the two parallel experiments data opposing each other. With a normal electrical conductivity (EC) in the nutrient solution the observed average tipburn was the heaviest in the plants grown under 12-hour high intensity light, but with a lower EC value of 1.2 the observed tipburn was heavier in the plants growing under 24 hour lower intensity light.

For the latter two experiments the statistical test done for the average tipburns found on the plants at the end of the growing period showed no statistical differences between the two treatments with high (2.8) and low (0.8) R/FR ratio, meaning that the R/RF ratio does not seem to impact the formation of tipburn, only growth. Plants grown in high R/RF developed longer but fewer leaves compared to plants exposed to a more standard R/RF ratio.

The NDVI analysis of the plants was unable to predict the formation of tipburn, with the first severe drop in the ratio of R/RF light being reflected coinciding with the visual formation of tipburn in the plants, though this may have to do with the experimental setup work and the lack of a truly specialized camera more than the actual NDVI analysis.

Abbreviations:

LED – Light Emitting Diodes

PPFD – Photosynthetic Photon Flux Density

R – Red (light)

FR – Far-Red (light)

NIR – Near Infra Red

Ca – Calcium

EC – Electrical Conductivity

ROS – Reactive Oxygen Species

NDVI - Normalized Difference Vegetation Index

FW – Fresh Weight

DW – Dry Weight

W – Watts

Table of contents

1 Introduction

As the amount of land available for agriculture in the world is seeing more and more threats, like the great aquifers of the Midwest drying up and rising global temperatures turning farmable land unsuited for most crops, the focus on greenhouse growth of food is rising globally. Such growing methods have the potential to be more effective, less work intensive and more ecologically sound with a focus on recycling nutrients used instead of the usual leak into the surrounding biosphere found in traditional large/scale farming.

In Norway the focus on greenhouse production is on the rise as well, as year around production and so income is to many farmers an attractive prospect. In 2016 the production of ‘Frillice’ lettuce grown in greenhouses in Norway was seven and a half million heads, with a combined value of almost 100 million NOK. Almost 20 % of these crops were lost to a phenomenon called tipburn, meaning a net loss of almost 20 million NOK, leading to a large economic incentive to lessen or possibly even eliminate this threat to the production and sale of ‘Frillice’ grown in a greenhouse setting (Knoop. 2019). While plants usually are not lost wholesale to the appearance of tipburn, the cosmetic damage to the plant often makes them unsellable as produce, forcing the farmer to discard the aforementioned 20 % of grown plants.

Tipburn, a type of necrotic damage to the leaves, can be found on the outer or inner part of any leaf in a ‘Frillice’ head, though in Norwegian greenhouse production tipburn to the outer parts of the leaf are the most predominant (Knoop. 2019). The conditions which make tipburn appear, and the mechanisms behind it, are not very well understood despite decades of research at this point, and to make things more complicated the appearance of tipburn can vary widely between identical experiments, making drawing decisive conclusions difficult (Saure. 1998).

In this thesis the impact of light regimens on the formation of tipburn have been tested, with two experiments concerning themselves with photoperiods and two with modified R/RF ratios to see if the formation of tipburn varied in any statistically meaningful way between the parallels of the experiments. In addition, during the latter experiments, a (NIR) capable camera was used to ascertain if the occurrence of tipburn could be predicted using NDVI to inspect the R/RF ratios reflected from the growing plants. This technology could theoretically be used to warn a farmer of impending tipburn formation and allow him or her to adjust the growing environment for the batch in hopes of avoiding tipburn entirely.

2 Theory

2.1 Lactuca Sativa L. ’Frillice’; Its history, use and greenhouse production in Norway

’Frillice’ lettuce is a cultivar of the common lettuce, of which four botanical variants are commonly cultivated in the world today: augustana, capitata, longifolia and crispa (Enclyclopædia Brittanica, 2018) the latter of which ’Frillice’ belongs to. The scientific name of lettuce stems from the latin word *Lactuca*, meaning “milk”, and Sativa, meaning “common”. The common name in english of “lettuce” is an abbreviation of the latin “lactuca” (Oxford dictionary of word histories, 2002).

It is believed that all four of these varieties stem from a single predecessor found in ancient Egypt. This proto lettuce wasn’t cultivated for food, but rather its seeds were pressed, and the oil used in cooking. From Egypt the plant was spread with human migration and trade as far as China and the northern parts of mainland Europe, and eventually also landed in northern and southern America with the advent of colonialism (The Columbia Encyclopedia, 2019) and later local cultivation and crossing has resulted in the over 100 types of lettuce seen in the world today.

The cultivar called ’Frillice’ specifically is the result of a cross between the leaf lettuce Endive and an iceberg lettuce (Seeds, 2020). The resulting plant has a crisp texture and a sweet taste to the leaves, making it excellent for use in a large variety of dishes. The seeds require a low temperature to germinate, at about 5 °C, and once the plant has reached the five leaf stage prefers temperatures between 22-25 °C during the day and 15 °C while growing (Agricultural Marketing, 2018).

Like most leafy greens (with high water contents), ’Frillice’ contains little in the way of nutrients such as fat and protein, but contains valuable minerals, a high fiber count and several vitamins such as A, K and C (Enclyclopædia Brittanica, 2019) and as such can be a valuable part of a balanced diet. In Norway ’Frillice’ is commonly grown in greenhouses utilizing the Nutrient Film Technique (NTF) for watering and growing stabilisation. The NTF hydroponic system utilizes growing racks in which individual plants are spaced into a gutter that contains flowing nutrient solution (Figure 1):

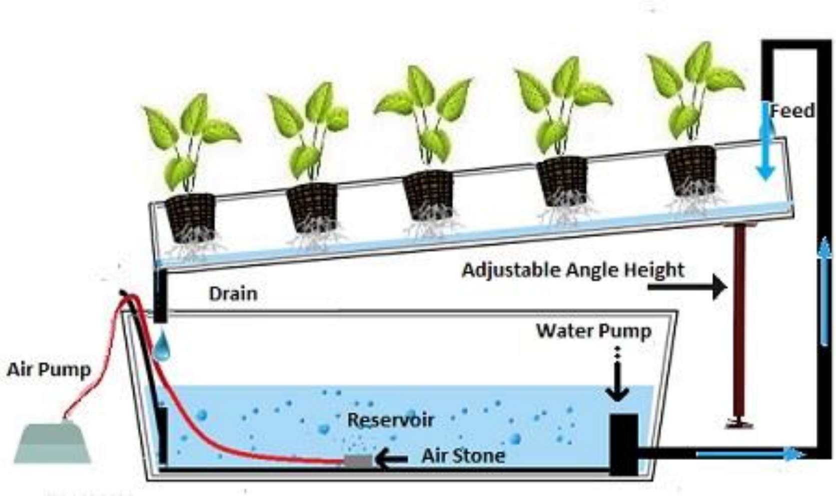


Figure 1: An example of a simple NTF setup, with plants growing in a gutter with nutrient solution flowing through. Such systems can either get rid of the excess solution, or it can be recycled for less release to the environment. Image: Luv2Garden.com

While NTF systems are effective at growing simple leafy greens and herbs, its worth noting that such production requires more insight into plant physiology and a tighter oversight of the different variables impacting growth. As paraphrased from "Hydroponic Production of Vegetables and Ornamentals":

> "*The ability to control the root zone increases with the move from soil to organic substrates and then to hydroponics. But correspondingly increase in knowledge and skill is required to manage the crop successfully, since the number of factors that can be controlled economically increases with this progression from soil to hydroponics*" (P. Adams, 2002)

'Frillice' is today one of the main greenhouse productions in Norway, and as such research into increasing production values and decreasing loss to diseases or poor nutrient or light management is of high importance.

2.2 Tipburn in 'Frillice' and other leafy vegetables

One of the main physiological problems in the production of 'Frillice' is the formation of tipburn. The formation of tipburn in leafy greens such as 'Frillice' is characterized by the formation of necrotized tissue either at the tips of leaves old and new, or at the base. In the case of this article, only tipburn formed at the edge of leaves is focused on. This tissue forms brown patches that usually spread to encompass the entire leading edge of the leaf, given sufficient time. Plants with such defects cannot be sold on the market, as they are viewed as undesirable by both customers and, in extreme cases, health and safety laws. Loss to tipburn can at times tip over 20 % of net production, leaving it non-viable both economically and sustainability wise.

As such research programs are endeavouring to find out what factors impacts the formation of such tipburns, and how to avoid or at least lessen the frequency of them forming, both to lessen the financial loss of crops, but also to make greenhouse production of ’Frillice’ more effective, less energy demanding and more sustainable (Uno et al, 2016).

2.2.1 Defining tipburn and its formation

Tipburn as seen in leafy greens take the form of brown necrotized patches on leaves, either at the edge of leaves or at the base. As mentioned above, in Norwegian greenhouses tipburn on the outer areas of the leaves (outer tipburn) are a more serious concern than inner, young developing leaves (inner tipburn) and as such is what will be focused on in this thesis.

Though the exact physiological reasons behind the formation of tipburn is currently not wholly understood, several strong theories have been developed over the last decade or so. The main theory states that the mechanism to induce tipburn seems to be bound to a calcium ion (Ca^{2+}) deficit in the affected leaves leading to the collapse of several critical cellular wall functions (Saure, 1998). Such deficits have proven difficult to counteract, as even growing lettuce in soils with plentiful supplies, or giving regular supplementary applications with watering does not seem to impact the plants ability to absorb enough calcium to counteract the formation of tipburn (Murdoch et al, 2000).

The formation of both variants of tipburn also seems tightly tied to the rate at which plants are growing, with higher production levels heightening the risk of inducing tipburn significantly as compared to slower growing “winter” adapted variants of lettuce (Murdoch et al, 2000). It is believed that this is due to the need for transportation of ions to the leaves overwhelming the plants innate transport mechanisms when the growth rate tips a certain unknown threshold, leading to the critical shortage tied to the emergence of tipburn. As such, higher intensities of light, usually considered a positive for the yield of a crop can in the case of leafy greens often be more of a detriment than a boon as large parts of the crop can end up being lost to tipburn. From this it is also clear that the light conditions during growth is of paramount interest in the ongoing battle against tipburn losses, and as a greenhouse offers better control over the total light sum a growing plant has access to over a day when compared to open field growing plants it is of even more interest to farmers growing hydroponically in greenhouses to know what rate a plant can grow at while avoiding tipburn and maximise production efficiency.

2.3 Lighting conditions and light signal perception

The connection between higher light intensities and the emergence of tip urns have been the subject of several research works, such as that of Gaudreau et al (1994):

> "The highest tipburn ratings were associated with treatments involving the high light levels (i.e those whose growth was most rapid)"

In addition, its also been shown experimentally that the photoperiod of the growth regimen also impacts the formation of tipburn even when grown under normal light intensities, with photoperiods longer than the usual daylight hours for outside growing having a measurable negative impact (Koontz & Prince. 1986). In other words, both the intensity of the light provided to the plants and the length of exposure to said light have diverse impacts on the health of the plant regarding the emergence of tipburn of both variants.

Most, if not all, plants have mechanisms to sense the environment surrounding them. Chief amongst these sensory mechanisms is the ability to read the lighting conditions, and any fluxuation in these conditions, as the availability and quality of light is of critical important to all photosynthetic plants to secure growth and in extreme cases just mere survival. To aid in this, specialized sensory proteins called photoreceptors have evolved, with the ability to sense specific wavelengths of light and in many cases their intensity, to produce signals that impact the growth of the plant in question (Taiz et al. 2018). While plants can register both blue light and UV-B, it's the ability to react to variations in the red and far-red spectrum that's of interest in the case of the experiments carried out in this thesis.

The photoreceptors responsible for this are called phytochromes and absorb light with a wavelength from 620 to 850 nm, covering both visible red and far red light (Ophilia & Eros. 2017). Phytochrome can morph between two forms depending on what kind of light it absorbs, with red light inducing transformation into the Pfr form, and subsequent far-red light reverting the phytochrome complex back into its Pr form. Interesting to note is that the two forms also absorb different wavelengths, with the Pr form absorbing red light and the Pfr form far-red light, meaning the two forms are mutually exclusive sensory organs (Taiz et al. 2018). As mentioned earlier the function of light sensing proteins is to convert light ratios into signals usable by the plant. In the case of phytochrome this takes the shape of the Pfr form of the protein being physiologically active, meaning it works as a signal transmitter in and of itself (Morgan & Smith. 1978). The main reaction to heightened levels of the Pfr form of phytochrome in a growing plant is to initiate shade avoidance reactions such as stem elongation to make the growing plant more competitive in the race upward for access to sunlight

(Ballare & Pierik. 2017). In other words, a high Pfr ratio will result in a plant changing its morphology and grow taller than one in which the Pr form of phytochrome is dominant. Regarding the formation of tipburn in 'Frillice', it has been speculated that taller plants have a lower chance of developing tipburn bad enough to warrant not being able to sell the product (Knoop. 2019).

2.4 Abiotic stress factors

Abiotic stress factors refer to non-living factors of chemical or physical properties, such as light and temperature and the availability of water, carbon dioxide, oxygen and nutrients in the area the plant is growing in (Taiz et al. 2018). As plants are unable to move to find more advantageous growing areas, they have instead developed several mechanisms by which to adopt to a changing local environment. Different plants are also able to survive in different biomes based on their ability to either live with little sunlight (shade adapted plants) or in the case of CAM plants, intense heat and copious amounts of direct light intensity.

While other stress factors such as CO^2 and temperature variations can impact the growth of plants in various ways, the focus for this thesis is on variations in light intensity and photoperiod and their impact on the growth and emergence of tipburn in lettuce. As such the growing conditions regarding variables such as carbon dioxide, temperature and nutrient availability, were set to and held at what is considered to be an optimal level for growth in *Lactuca Sativa L*.

Lettuce, being a shade adapted plant, deals well with lower light intensities. And as mentioned earlier, growing in such an environment lessens the chance of tipburn forming on a plant (Murdoch et al, 2000) since it results in a slower growth rate due to a limited amount of light usable for photosynthesis being available to the plant. When shade adapted plants are exposed to excess high intensity light, such as is possible in a greenhouse environment, the amount of light absorbed by the plants enhanced numbers of PSII reaction centres overwhelms the systems ability to turn harvested light energy into sugars via the Calvin-Benson cycle (Taiz et al. 2018). This again results in the plant having to find ways of dumping the excess energy to avoid lasting harm to the delicate photosynthetic pathways and mechanisms. One such method is to divert the surplus electrons generated by the PS systems to atmospheric oxygen in the leaf. This gets rid of the excess charge but leads to the formation of excessive amounts of reactive oxygen species (ROS) that can be of further danger to the plant in question.

ROS species are highly reactive forms of atmospheric oxygen with at least one unpaired electron in their outer orbital making them, as the name implies, extremely prone to reacting with their environment. These interactions are uncontrolled, and as ROS species can interact with DNA, RNA and even the lips forming the cell wall to break them down, avoiding any excess of such species is of paramount importance. As ROS is formed as an unavoidable by-product of aerobic metabolism (present in all plants and animals) the cells have developed pathways to remove or otherwise incapacitate ROS species as they form (Choudhury et al. 2016). However, when a shade adapted plant gets subjected to intense light, the amount of ROS formed is too high for these systems to overcome, and the concentration of ROS can reach high enough levels to interfere with normal operation in the leaves, and in extreme cases enough irreversible damage to be fatal to the plant.

2.5 Normalized Difference Vegetation Index (NDVI)

Plants can use large parts of the visible spectrum for energy absorption through photosynthesis, and in addition several wavelengths bordering the visible spectrum are used for signalling purposes both in germination and during vegetative growth (Taiz et al. 2018). While UV-A, UV-B and far-red light (Near Infra-Red) waves can be sensed by a plant using specialized phytochromes, they are not absorbed by either PSI or PSII, meaning they either pass through green leaves or are reflected. This means that when white light hits a plant, some wavelengths (the visible spectrum) will be absorbed as best the plant is able to, while others such as NIR will be reflected or just pass straight through.

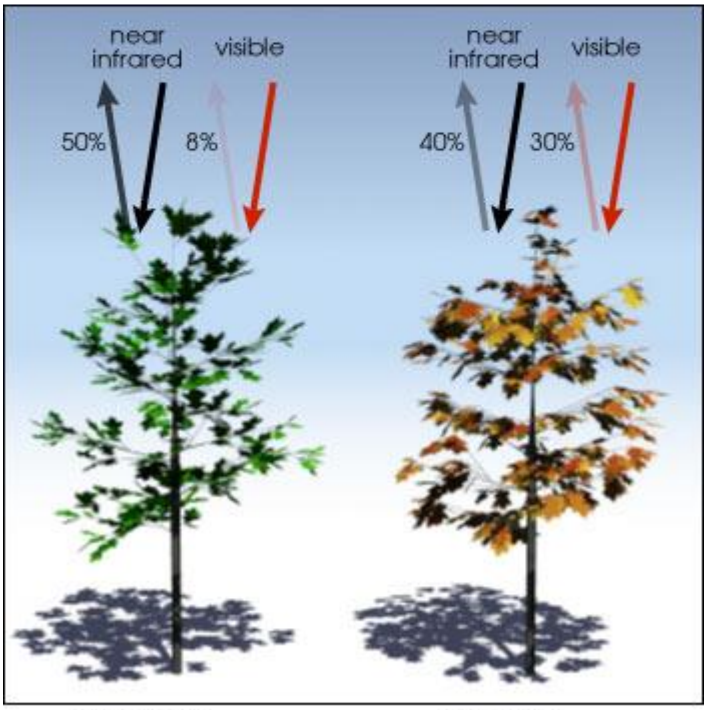
The amount of visible light absorbed by a plant has been shown to correspond to how healthy the plant is (Rouse et al.1974) with lower ability to absorb visible light, meaning it inversely reflects more light in the visible spectrum, also coinciding with being less able to reflect NIR light. This relationship between absorption rates can be used to determine if a plant, or a large area of vegetation, is healthy or currently facing stress factors impacting their overall health. By comparing absorbed red light (part of the normally absorbed visible light) to the amount of NIR light that’s deflected the NDVI can be used to assess the health of the plants in question following a simple formula:

$$NDVI = \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + \mathrm{RED}}$$

The use of the NDVI computational formula also eliminates many variables for the reflection of NIR light, such as bare soil, height differences and viewing angles (Balaghi et al. 2008).

As can be seen in figure 2, the ratio of reflected visible light (red included) to the reflected NIR light is used with the NDVI formula to produce a ratio of red to NIR light. This ratio, combined with either aerial or space photography can be used to survey enormous tracts of land or forests effectively (Remmel & Perera, 2001), meaning interventions to prevent loss of habitat or monitor the general productivity of an agricultural area can be done very efficiently (Weier & Herring, 2002).

The NDVI formula can also be used more locally to monitor the development of plants in greenhouses. This requires access to a NIR capable camera and software to measure the ratios of red to far red light but has large potential for in the field crop monitoring.



$$\frac{(0.50 - 0.08)}{(0.50 + 0.08)} = 0.72 \qquad \frac{(0.4 - 0.30)}{(0.4 + 0.30)} = 0.14$$

Figure 2: Comparison between the NDVI ratio found in a healthy plant compared to one that's under stress

3 Main objectives of the study

1: To identify the impact of different lighting schedules and light quality on the formation of tipburn and general growth in *Lactuca Sativa L* 'Frillice'.

2: To investigate if the use of images taken with NIR capable cameras can be used to detect the formation of tipburn before it becomes visible to the naked eye.

4 Materials and methods

4. 1 Cultivation of plants for the experiment:

Each batch of plants to be used in experiments had to first be grown to an appropriate size (5 leaf stage) as to yield data on tipburn formation during their time in the growth chambers. 'Frillice' (*Lactuca Sativa L.)* seeds supplied by Norgro A.S (Norway) were planted in biodegradable pots filled with peat of the type Degernes Torv (Degernes Torvstrøfabrikk AS, Norway). The pots were put into trays and left to germinate in a dark chamber with a temperature of 15°C and a relative humidity (RH) of 60 %.

After germination the plants then spent another three weeks in a greenhouse growing to reach the five-leaf stage mentioned earlier. During this stage the plants were watered once a day with a fertilizer solution of 1.5 EC, with additional watering being administered if needed. The lighting in the greenhouse was provided by 400 Watts HPS (High Pressure Sodium, Osram NAVT 400W, Munich, Germany) lamps producing a photon flux density of 150 µmol/m^2/s that was on for 18 hours a day. The temperature of the greenhouse was kept to 20 °C with an RH of 60 % day and night, controlled by a Priva climate computer (Priva, De Lier, The Netherlands).

In all four batches of plants were grown for the experiments, the first being grown in late august 2019 and the last being grown in January 2020, with the other two batches dispersed in between as each experiment ended.

4.2 Growth chamber setup:

The two growth chambers used in the experiments were set up to be mechanically identical before variables were introduced to the various batches of lettuce for the different experiments. Each chamber consisted of three growing racks containing cutouts for seven plants each, for a total of 21 plants per growing chamber per experiment (Fig 3). These racks were fed from a box of growth medium on the floor by use of a pump connected to an electrical timer set to go off for one minute every other hour around the clock. The racks were all placed on a slight downward angle so any leftover growth medium could drain by gravity into two collection boxes at the end of the chamber. The distance from the racks to the lighting armatures above were set to be approximately 1,5 meters in both chambers (minor variations due to different materials used to create the downward angle for the racks in each chamber)

Figure 3: Newly moved lettuces in their growing racks, the watering lines can be seen in the back of the picture, and underneath is the reservoir of growth medium (black box). Photo: Herman Bang

4.2.1 Nutrient solution:

The nutrient solution used was mixed on site from two stock solutions to
reach an electrical conductivity (EC) of 2.0 (measured using a ScanGrow Conductivity Meter) and a pH of 5. Runoff solution from the racks was not recycled in any of the experiments.

Table 1: Stock solutions used to create the growth medium

Stock Solution 1:	Stock Solution 2:
Calcium Nitrate ($Ca(NO_3)_2$) – 100 kg	Pioner Basis Agurk – 125 g
Potassium Nitrate (KNO_3) – 25 kg	Pioner iron chelate 6 % EDDHA – 1 kg
Calsium Chloride – 6 kg	

Table 2: Content (ppm) of macro and micronutrients used in the nutrient solution for the experiments.

Cations ppm	NH4	NH4-N	K	Na	Ca	Mg	
(mg/l)	1.8	1.4	282	32	148	29	
Anions ppm	No3	NO3-N	Cl	S	HCO3	P	
(mg/l)	750	169	64	48	6.1	37	
Micronutrients	Fe	Mn	Zn	B	Cu	Mo	Si(mg/l)
ppb (µg/l)	1843	483	275	292	133	86	2.8

4.2.2 Lighting systems:

Both growth chambers were lit using LED lights installed by Evolys, with experiment three and four having more far-red LED diode rigs installed in chamber two to reach the desired ratio in red/far-red light and the other chamber remaining identical to experiment one and two.

During all experiments the Photosynthetic Photon Flux Density (PPFD) in the growing chambers were measured using a quantum meter (Li250A light meter, Li-Cor, USA). With the doors closed, the PPFD in each chamber for light between 400 and 700 nm were measured to make sure they were within the parameters for the experiment in question. For experiment 3 and 4 the ratio of red to far red light also had to be measured to be within the right parameters and for this a Red/Far-Red sensor from Skye UK was used, using the same technique of having the doors closed as the measurements were done.

Figure 4: a) Li250A light meter measuring the PPFD in a growth chamber. Photo: Martin Knoop

b) Red/Far-Red sensor from Skye (UK). Photo: www.skyeinstruments.com

During the third and fourth experiment the red to far red light ratio was modified in growth chamber 10 by adding extra far red emitting diodes to chamber 10, figure 5 shows how the light spectra in chamber 10 consequently has a spike around 715-750 nm to account for the extra far red light introduced. The spectra were recorded using a SpectraPen Mini (Photon Systems Instruments (PSI), Brno, Czech Republic) in both chambers.

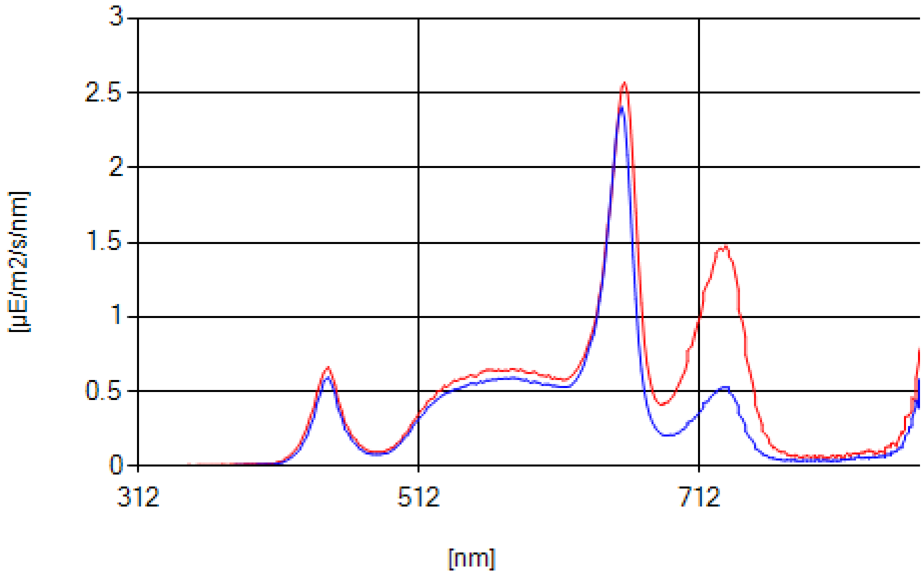


Figure 5: The light curve for the standard LED rigs (chamber 12, blue) compared to the extra far-red diodes added to chamber 10 (red) for experiment 3 and 4

4.3 Experiment 1: Same total irradiance but different photoperiods between treatments

In experiment number one the two chambers each received the same total sum of light in a 24-hour period, but in one of the chambers there was a night cycle of 12-hours while the other had 24-hours of light. Apart from this all other conditions were identical, with the plants receiving the same watering schedule and using the same growth medium. After approximately a month a random selection of 10 plants from each treatment were assessed for tipburn and had their fresh weight measured. For the methodology used for the assessment of tipburn see 4.7.

4.4 Experiment 2: Same light schedule and intensity with lower nutrient EC

The second experiment was run with the same parameters as for experiment 1, but with the electrical conductivity (EC) of the nutrient solution diluted down to 1,2 instead of the 2 used before. The lighting remained on a 12 and 24-hour cycle respectively, and the total intensity of the light remained the same for the two chambers as in the previous experiment. The experiment ran for four weeks with a bi daily inspection for tipburn formation, with images taken at each inspection. Two images were taken per chamber for each inspection, with one being a general shot of the plants growing and one being a close-up of a plant selected at the start of the experiment and followed throughout. Ten plants from each treatment were then assessed for tipburn damage and their fresh weight measured at the end of the experiment.

4.5 Experiment 3: Impact of higher red/far-red ratios with NIR photography analysis

The third experiment focused on if a higher ratio of red to far red light would have a discernible impact on the formation of tipburn on the lettuce. One growth chamber was set up with an average (for greenhouses) ratio of 0.8 while the other chamber had extra far red diodes installed by Evolys A.S to reach a ratio of 2.8. The experiment ran for four weeks, with normal inspection every other day and photography following the pattern of the last experiment. Ten plants from each treatment were then assessed for tipburn damage and their fresh weight measured.

4.6 Experiment 4: Impact of higher red/far-red ratios with NIR photography analysis

The final experiment closely emulated the third, with some minor corrections to light cycles and intensity (insert here). The main draw behind this experiment was to take pictures with a modified camera (insert make here) able to take images that include the far-red spectrum. These images were then later analysed using python as described in section X.X.

The growth chambers were inspected, and images were taken daily during this experiment, with the far-red camera being used until visible tipburn could be observed in both chambers around week 2. After this point the inspections were bi-daily until a month had passed, at which point assessment of tipburn and weighing of fresh weight was done identically to earlier experiments.

Table 3: Summary of setups for variables in the experiments.

		Temp (°C)	Air Humidity (%)	Daylength (hours)	Irradiance (µmol $m^{-2}s^{-1}$)	Daily light sum (mol×m^{-2} ×d^{-1})	R/RF ratio	EC/Ph
Exp 1	Chamber 10	20	65	12	360	15.55	0.8	2/6
	Chamber 12	20	65	24	180	15.55	0.8	2/6
Exp 2	Chamber 10	20	65	12	360	15.55	0.8	1.2/6

	Chamber 12	20	65	24	180	15.55	0.8	1.2/6
Exp 3	Chamber 10	20	65	18	180	11.66	2.83	2/6
	Chamber 12	20	65	18	180	11.66	0.8	2/6
Exp 4	Chamber 10	20	65	18	220	14.25	2.83	2/6
	Chamber 12	20	65	18	220	14.25	0.8	2/6

4.7 Assessment of tipburn:

Apart from continuous bi-daily observation and photography of the experimental plants as they grew to log the emergence of tipburn under the differing treatments, ten plants from each growth chamber were also closely assessed at the end of each experiment by use of a pre made form that ranged from 0-5 (Fig 6). The assessment of tipburn found on a leaf can be determined to a specific number with the following guide:

0 = No observable tipburn

1 = Tiny brown dots on the tips of some leaves

2 = Most tips of leaves have brown dots or discolouration

3 = Whole ends of leaves brown and/or soggy

4 = Most ends on the leaf are totally brown or soggy

5 = Large parts of the leaf is damaged and brown

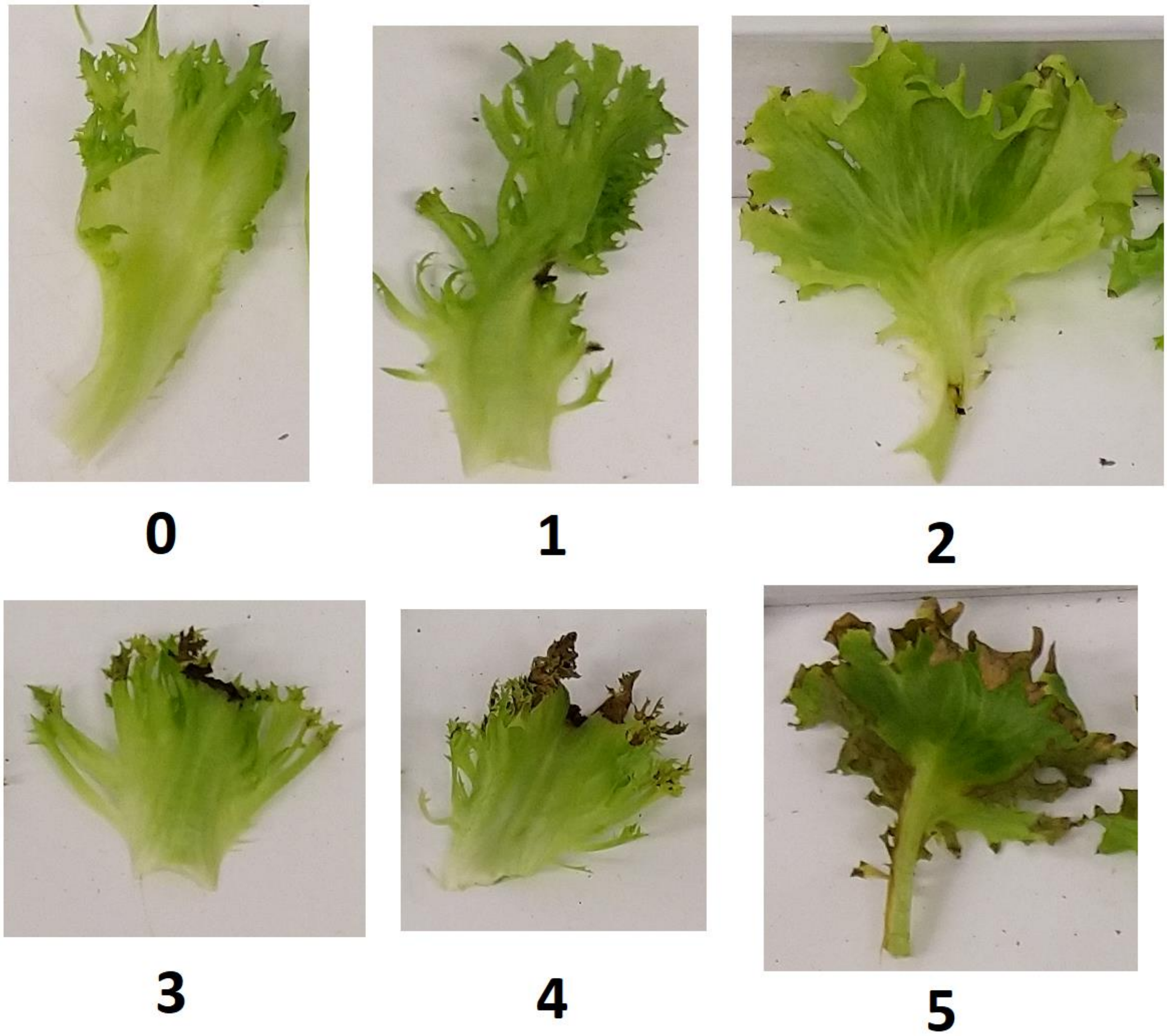


Figure 6: Examples of leaves corresponding to 0 to 5 values, taken from experimental plants. Dead leaves, meaning ones that are fully discoloured, are not considered for further analysis and discarded.

Each plant was cut at the stem and then dismantled to separate each leaf for assessment. The damage to each leaf and its number, 1 being the outermost leaf of the plant after the cotyledon with consecutive numbers indicating leaves closer to the core of the plant, were then registered in a form and stored. Leaves were only assessed if they were longer than 1 cm, meaning the newest leaves in the core of the plant were not part of the assessment, nor the fresh weight of the plant.

Figure 7: Each lettuce was picked apart and the leaves displayed from outermost (top left) to innermost (bottom right), with dead leaves and leaves under 1 cm in length not being registered. Photo: Herman Bang

4.8 Wet and dry weight measurements:

The entirety of each plant (minus the root system and central core) was also weighted with a fine scale, with an accuracy of 0,01 g. The weight used was zeroed using empty collection bags to avoid their weight being added to the total, before said bags were filled with the dismantled plants, then weighed, and logged. After this the bags were put in a dryer for up to several weeks before being weighed and logged again with the same zeroed weight, using the same type of bags for zeroing.

4.9 Far red image analysis:

Images were taken with the Canon PowerShot SX280 HS camera and then analysed using an academic extension for Python 3.7 called Spyder. Images were taken in each growth chamber on a day by day basis until visible tip burn was observed in both chambers. All images were taken from 0,5 meters in as close to a 90-degree angle from above possible, with a grey cardboard background in an effort to stop the background from reflecting enough light to impact the later calculations. The

Spyder software then creates a red to far red ratio on a pixel by pixel basing, based on averages from a 3x3 pixel grid for each individual pixel in the grid (Fig 8).

By highlighting the reflection of far red light by comparing it to the reflection of standard red light it is theorized that it should be possible to assess if the spread of far red reflection gives any indication of the spread of early stages of tipburn formation in the lettuce imaged. By then comparing the red/far-red ratios in the composite image created digitally to the actual observed spread once it is visible with the naked eye this thesis aimed to test this theory. The specific code used can be found in appendix 1.

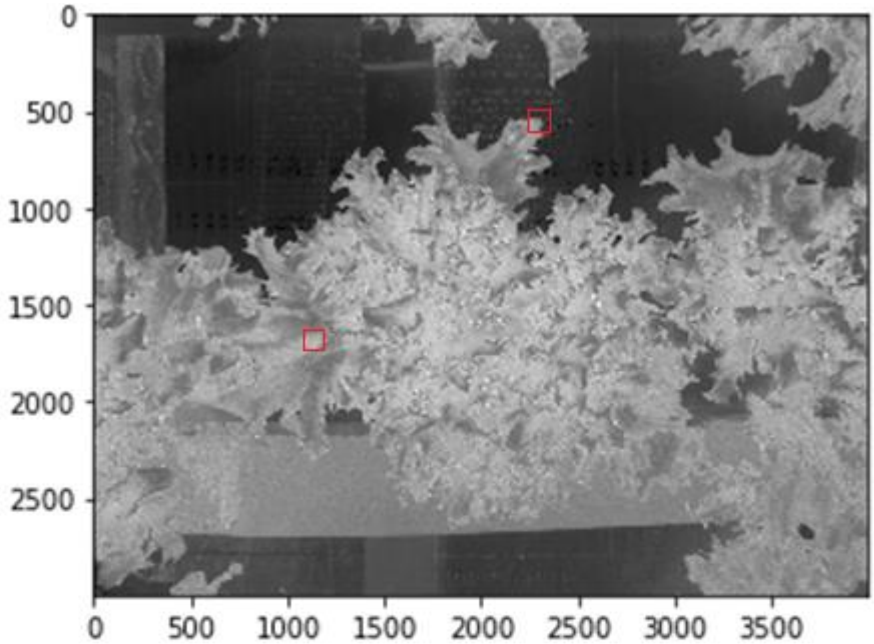


Figure 8: Example of an image (taken from chamber 12 during experiment 3) ran thorugh the Spyder code to yield a composite of red/far red reflected light. The red boxes shows what would be considered an outer and inner measuring point for red/far red ratios. Photo: Herman Bang

Statistical Analysis:

Statistical analysis was conducted using analysis of variance (One-way ANOVA) in Minitab (Version 16, Minitab Inc., PA, USA). Means were separated using Tukey's test at the 5% level of significance.

A nonparametric Mann-Whitney test was used to test effects of light quality on the severity of observed tipburn using a p value of 0.05 for the null hypothesis ($H_0 : N_1 - N_2 = 0$). This nonparametric test was used to avoid assumptions on data distribution.

5 Results

5.1 Experiment 1:

The goal of experiment 1 was to assess the impact of photoperiods on the formation of tipburn. As such the two growth chambers received the same amount of total light over the course of 24 hours, but chamber 10 only had a light period of 12 hours (with a PPFD/µmol $m^{-2}s^{-1}$ of 360) whereas chamber 12 had continuous light 24 hours a day (with a PPFD/µmol $m^{-2}s^{-1}$ of 180), resulting in a more intense light regiment for the plants in chamber 10 and no rest period for the plants in chamber 12. Apart from the light regimen the two growth chambers were set up identically, sharing the same light systems, a shared relative humidity of 65 % and temperature of 20-degree Celsius, within a +-10 % margin. 10 plants were chosen from each chamber, resulting in an N value of 10 for the statistical work seen in all the experiments. Table 3 shows one-way ANOVA analysis comparing different aspects of the plants grown in chamber 10 and 12, including freshweight (FW), dry weight (DW), number of leaves longer than 1 cm found on the plants and the length of the longest leaf.

As can be seen from the data there are statistically significant differences between the average dry weight (with there being a 23 % difference) of the plants growing under 12 hour light compared to the ones growing under 24 hour light. The average longest leaf found in the two treatments also varied by a significant 2.23 cm, with p values under 0.05 and having different letters assigned via Tukey test. The two other parameters, average fresh weight and the average number of leaves over 1 cm show no statistically significant variation, both sharing letters from the Tukey test and having p-values well over 0.05. The Mann-Whitney test for the average tipburn severity showed no statistically relevant difference between the 12 and 24 hour light treatments.

Table 4: Growth analysis from experiment 1. Results of the one-way ANOVA testing comparing the two sets of lettuce growing at a photoperiod of 12 and 24 hours with similar daily light sum. including Tukey comparisons of the means. N = 10, SD = Standard Deviation. Different letters in the Tukey test indicates significant differences in the mean at 5 % level.

	Dry weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	9.30	0.89	B	0.001
24 Hour period	12.05	1.10	A	
	Fresh weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value

12 Hour period	152.54	19.57	A	0.054
24 Hour period	176.72	31.50	A	
	Number of leaves			
Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	25.40	1.83	A	0.120
24 Hour period	27.40	3.41	A	
	Length of the longest leaf (cm)			
Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	14.49	0.55	B	0.001
24 Hour period	16.72	1.05	A	
	Mann-Whitney (Tipburn severity)			
P-Value	0.121			

In addition, there was also a qualitative analysis done on the final tipburn at the end of the experiment. Each of the 10 plants from each treatment was dissected and the tipburn for each leaf longer than 1 cm was catalogued as being between 0 for no tipburn and 5 for severe tipburn. The average tipburn for each leaf can be seen in the following graph, comparing the two treatments.

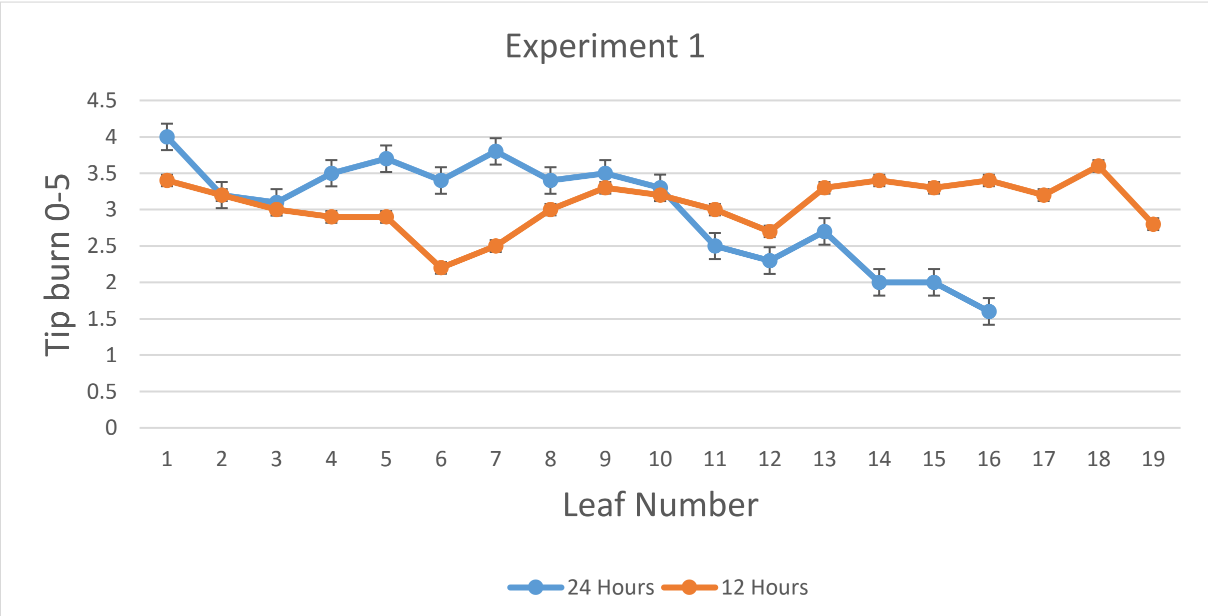


Figure 9: The average tipburn on each leaf, with leaf 1 being the outermost leaf of the lettuce plant after the cotyledon. Standard error bars have been added.

As can be seen from the graphs, the variation in tipburn between the two treatments varies quite a lot, with the plants having the more intense 12 hour lighting schedule having on average more tipburn over the entire plant, while the plants growing under 24 hour light show a tendency to have less tipburn on the newer leaves close to the centre of the plant. However, the Mann-Whitney test done to test the average tipburn score for the whole plant (all leaves included) did not show significant differences between the treatments (P=0.121, Table 4)

5.2 Experiment 2:

The second experiment was an evolution of the first, with the difference between the two being a lower EC in the nutrient solution fed to the plants, to see if this could have a positive effect on the formation of tipburn i.e. there being less of it or it taking longer to develop. The light regimens of 12- and 24-hour light schedules with the same total light sum remaining the same as well. The shared variables between the two chambers, such as temperature and humidity was kept the same as in experiment 1.

As can be seen in table 5, there is significant variance found in three of the four categories this time, with only the fresh weight of the two treatments remaining statistically identical to each other. The dry weight of the two treatments vary by 32 % between the 12- and 24-hour chambers, with the 24-hour treatment also resulting in on average 5.5 additional leaves with a length surpassing 1 cm. In addition, the 24-hour light cycle added an average of 1.67 cm to the longest leaf found compared to the 12-hour cycle grown plants. The Mann-Whitney analysis gave a p-value of 0.001, meaning that there is a significant difference in the severity of the average tipburn found in plants from the two differing treatments (table 5).

Table 5: Growth analysis from experiment 2. Results of the one-way ANOVA testing comparing the two sets of lettuce growing at a photoperiod of 12 and 24 hours with similar daily light sum. including Tukey comparisons of the means. N = 10, SD = Standard Deviation. Different letters in the Tukey test indicates significant differences in the mean at 5 % level.

	Dry weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	5.03	0.73	B	0.001
24 Hour period	7.39	1.33	A	
	Fresh weight (g)			

Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	62.56	10.56	A	0.164
24 Hour period	72.36	18.59	A	
	Number of leaves			
Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	21.90	2.13	B	0.001
24 Hour period	27.40	3.44	A	
	Length of the longest leaf (cm)			
Light conditions	Mean	SD	Tukey Test	P-Value
12 Hour period	9.79	0.784	B	0.001
24 Hour period	11.46	1.05	A	
	Mann-Whitney (Tipburn severity)			
P-Value	0.001			

The qualitative analysis of the tipburn found on the plants followed the same pattern as in experiment one, with the results presented in the following graph

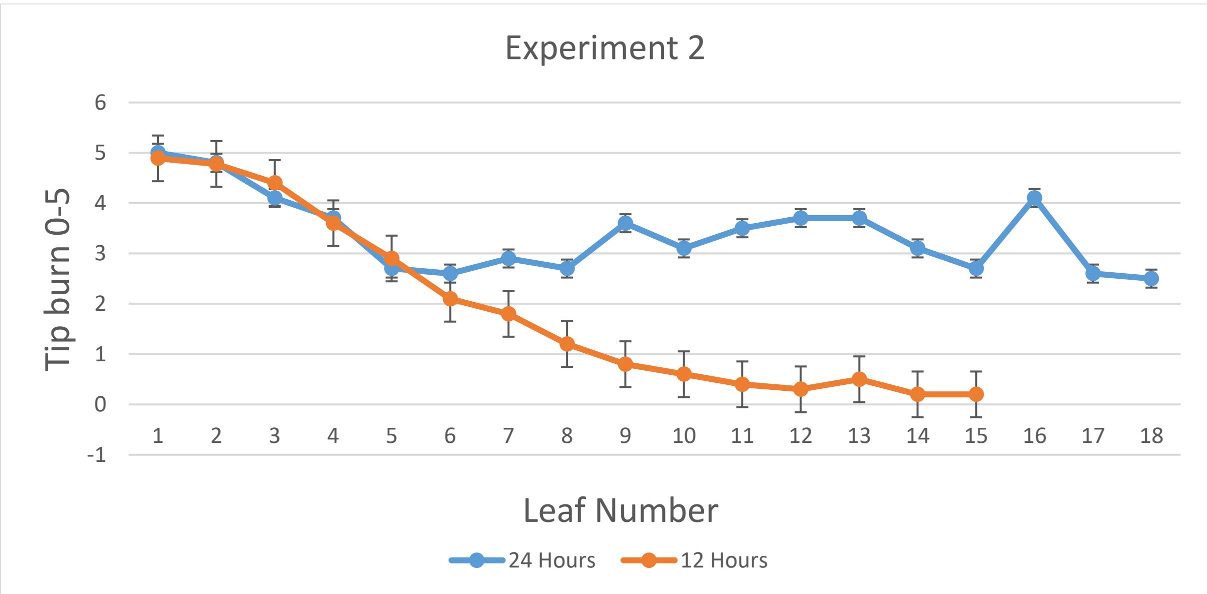


Figure 10: The average tipburn on each leaf, with leaf 1 being the outermost leaf of the lettuce plant after the cotyledon. Standard error bars have been added.

In experiment 2 the trends from the first experiment seems to have been reversed, with the plants subjected to high intensity light (Chamber 10) having less tipburn on the inner leaves of the plant, while more closely sharing the damage found on earlier leaves with the plants in chamber 12. The plants from the 24-hour treatment on the other hand, have similar heavy tipburn damage found on all leaves, while in the last experiment this was not the case. The Mann-Whitney test, with a p-value of 0.001 (table 5), indicates a statistically significant differences between the average tipburn in the plants from the two different treatments, with the plants growing under 24-hour light exhibiting on a worse intensity of tipburn on average.

5.3 Experiment 3:

The aim of this experiment was to investigate if adding additional far-red light to the top light and to test if this would have an impact on the formation of tipburn, and if so to see if this impact was positive or negative. The red to far-red ratio of light in the chambers were thus modified, as can be seen in table 3, with the other variables in the growing regimen remaining unmodified from earlier.

As can be seen in table 6 underneath the wet and dry weight of the two sets of plants do not differ in a statistically relevant way, with their p values being well over the cut-off of 0.05 and their assigned Tukey letters matching. However, there is a statistically relevant difference to be found in both the average numbers of leaves over 1 cm found on each plant (with the standard light regimen producing 7.5 extra leaves) and the longest leaf found with the Far-Red regimen resulting in an average length increase of 2.4 cm compared to the standard regimen. A p-value of 0.427 for the Mann-Whitney analysis means there is no discernible statistical difference between the two treatment forms in regard to the average tipburn seen on the plants.

Table 6: Growth analysis from experiment 3. Results of the one-way ANOVA testing comparing the two sets of lettuce growing with different ratios of red to far red light. including Tukey comparisons of the means. N = 10, SD = Standard Deviation. Different letters in the Tukey test indicates significant differences in the mean at 5 % level.

	Dry weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	10.34	1.86	A	0.433
Standard	9.80	0.97	A	
	Fresh weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	110.17	25.46	A	0.380
Standard	118.67	15.59	A	
	Number of leaves			

Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	22.80	2.70	A	0.001
Standard	30.30	2.49	B	
	Length of the longest leaf (cm)			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	17.14	1.05	A	0.001
Standard	14.74	1.30	B	
	Mann-Whitney (Tipburn severity)			
P-Value	0.427			

The same qualitative analysis of the observed tipburn on plants from the two differing treatments as done in experiment 1 and 2 was performed in experiment 3 as well, the data is presented underneath

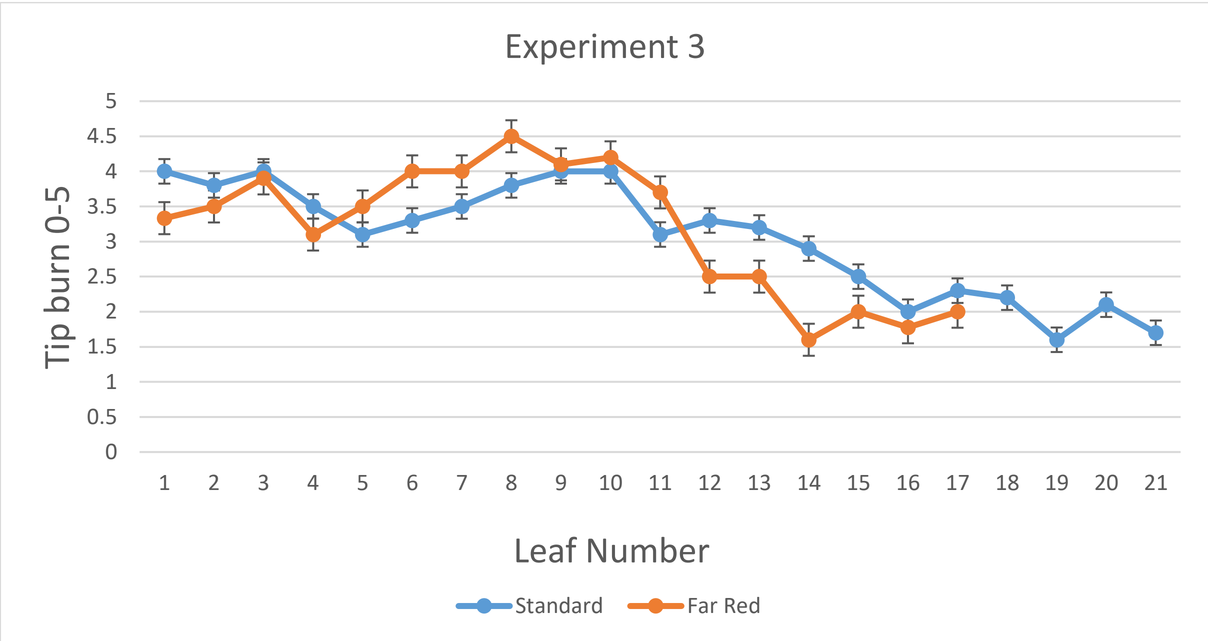


Figure 11: The Average tipburn on each leaf, with leaf 1 being the outermost leaf of the lettuce plant after the cotyledon. Standard error (SE) bars have been added. N=10.

This graph, compared to the ones from the earlier experiments, show a tighter relationship between the two treatments regarding tipburn formation found at the end of the experiment. The averages of the two treatments cross each other several times coming closer to the newest leaves found in the core of the lettuce plant, showing a more uniform spread of tipburn between the two batches.

The Mann-Whitney test ran for the average tipburn found on plants from the two treatments show no statistically relevant differences between the two, with P=0.427 (Table 6).

Finally, the other main purpose of experiment 3 was to use a NIR-capable camera and Python analysis to tentatively research if tipburn can be spotted in the NIR-spectra before being visible to the naked eye. As described in the materials and methods, images were taken each day, and each image analysed via the Spyder extension for Python. The comparison between the Red/NIR ratio found at the edge of the leaf, as compared to a point further in on the leaf, and the relationship between the two treatments, can be seen in the graph underneath:

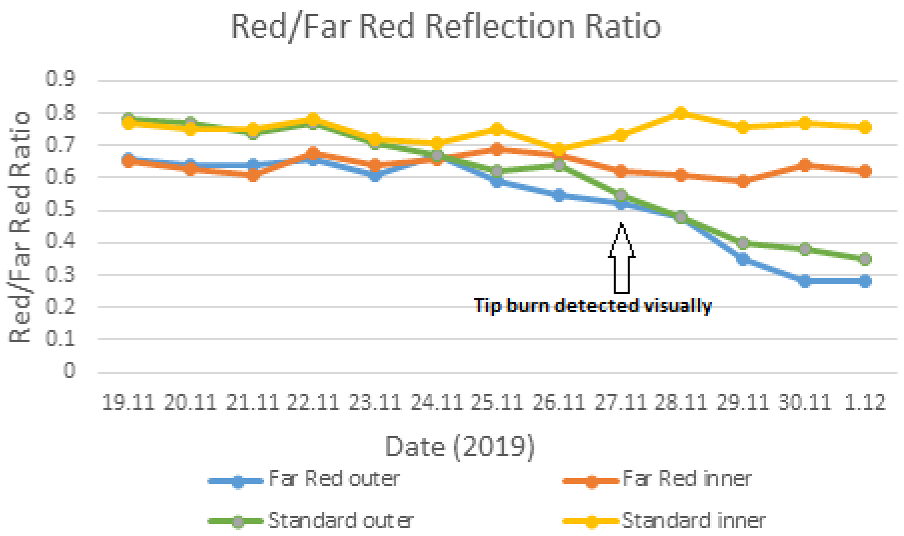


Figure 12: The ratio of red to far red light reflected off two selected points on a lettuce leaf, one on the inner part of the leaf and the other along the edge of the leaf (see figure 8). The two growth chamber treatments are included in the same graph for comparison purposes. N=1.

The graph shows a decrease in reflected red light, as the ratio between red and far red decreases towards 0, in the tips of the leaves in both treatments. However, this increase in the ratio towards more red light being reflected also coincides with the visible formation of outer tipburn in the subject plants, as can be seen from the image underneath. This raises the question that, with this setup, it might not be possible to actually see any precursors to tipburn using far-red photography analysis.

Figure 13: Cropped part of image taken of lettuce plant in chamber 12 on the 27.11.2019, showing visible tipburn formation in the areas used to measure red/far red reflection ratio. Photo: Herman Bang

5.4 Experiment 4:

The last experiment saw the PPFD raised to 220 µmol $m^{-2}s^{-1}$ for both chambers, this due to a malfunction in the growing light requiring the PPFD to be raised for it to be equal in both growing chambers, while keeping the other variables such as temperature and relative humidity and light periods consistent with experiment 3. Table 7 summarizes the ANOVA comparison between the two batches.

As compared to experiment 3, there was just one variable between the two batches that yielded any kind of statistically relevant variation: the comparison between the mean of the longest leaves for each batch, with the far-red regimen grown plants having, on average, their longest leaf being 1.86 cm longer than the plants grown under standard light. The other three categories all have p values well over 0.05 and Tukey tests place them under the same letter, meaning they don't vary from each other enough to be statistically relevant. The Mann-Whitney test shows a p value under the cut-off of 0.05, meaning there is a relevant difference in the average amount of tipburn found on plants from the two differing treatments, with the far-red treatment having the lowest average value with a marginal difference.

Table 7: Growth analysis from experiment 3. Results of the one-way ANOVA testing comparing the two sets of lettuce growing with different ratios of red to far red light. including Tukey comparisons of the means. N = 10, SD = Standard Deviation. Different letters in the Tukey test indicates significant differences in the mean at 5 % level.

	Dry weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	9.35	1.15	A	0.566
Standard	9.09	0.76	A	
	Fresh weight (g)			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	157.90	29.96	A	0.735
Standard	162.49	29.63	A	
	Number of leaves			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	31.5	3.50	A	0.340
Standard	33.10	3.78	A	
	Length of the longest leaf (cm)			
Light conditions	Mean	SD	Tukey Test	P-Value
Far-Red	15.68	1.30	A	0.003
Standard	13.82	1.06	B	
	Mann-Whitney (Tipburn severity)			
P-Value	0.16			

Experiment 4 also had the same qualitative observation and analysis of the final tipburn found on the plants from each treatment, collated in the graph below.

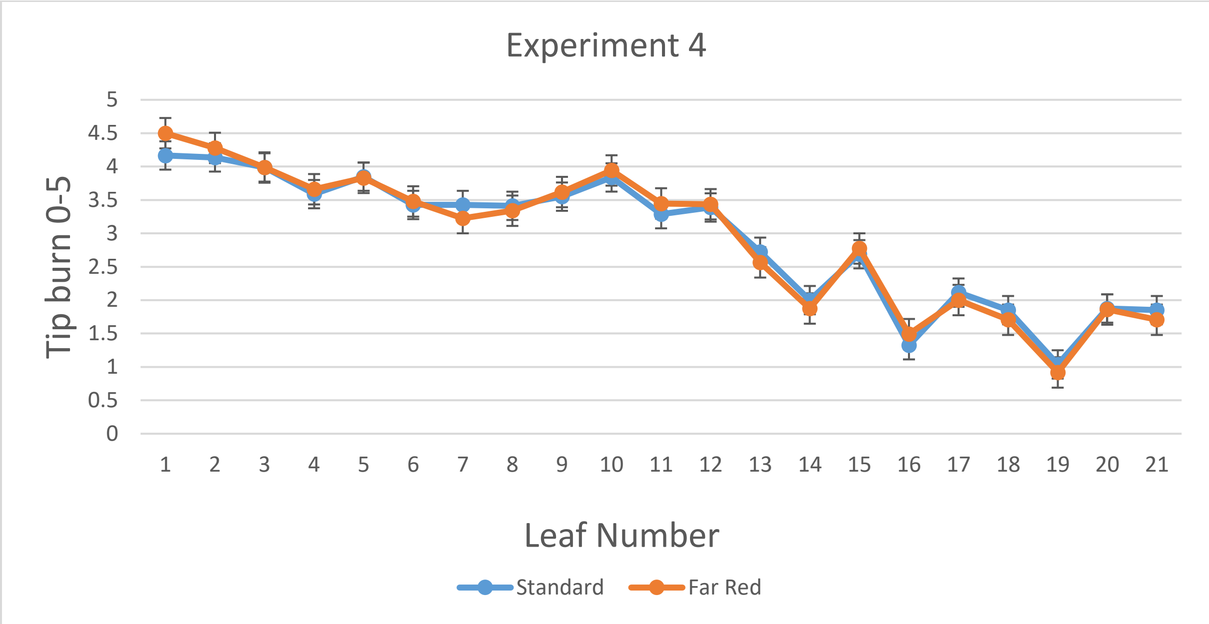


Figure 14: The Average tipburn on each leaf, with leaf 1 being the outermost leaf of the lettuce plant after the cotyledon. Standard error (SE) bars have been added. N=10.

In this case, the graphs for the two treatments follow each other very closely, and both treatments had over 21 leaves that could be analysed (in comparison to the earlier experiments, in which the leaf number varied greatly between treatments). It is by far the cleanest graph of the four experiments and shows a gradual decline of tipburn not seen in the earlier graphs.

The same analysis of images taken during the growth period as used in experiment 3 was used in experiment 4 as well, to collate the presented graph below

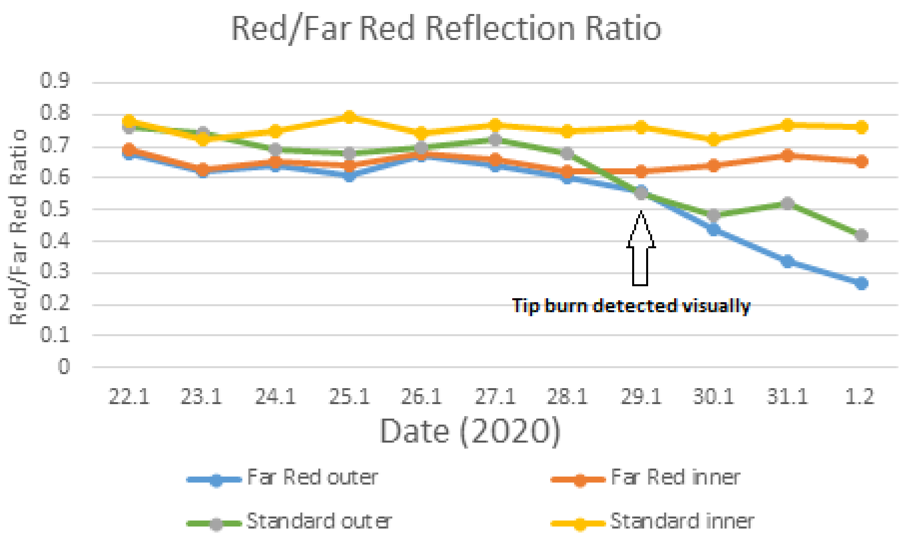


Figure 15: The ratio of red to far red light reflected off two selected points on a lettuce leaf, one on the inner part of the leaf and the other along the edge of the leaf. The two growth chambers are included in the same graph for comparison purposes. N=1.

The same trends as in experiment 3 are seen in experiment 4, with the more abrupt fall in the red to far red ratio coinciding with the formation of visible tipburn in the plants in question, again especially in the plants growing under the far-red treatment.

The Mann-Whitney statistical analysis showed no significant differences between the average tipburn found on plants from the two treatments (P=0.16, Table 7).

6 Discussion

As mentioned in 2.4 several abiotic factors such as temperature, relative humidity, CO_2 concentration and nutrient availability can impact the health and subsequently growth of most plants (Taiz et al. 2018). In the experiments ran in this thesis all of these remained as constant as possible, leaving only the light intensity, photoperiod and light quality as variables for testing for impact on the formation of tipburn. All of these three variables of a light regimen in a greenhouse setting have previously been shown to impact the growth, morphology and damage to leaves in lettuce (Sago. 2016) but testing for outer tipburn in the 'Frillice' cultivar as a reaction to light conditions is more of an unexplored territory.

In Norway a large part of the greenhouse grown produce produced in a year consists of 'Frillice' and other leafy greens and lettuces, and as such there is both a scientific and economical interest in mapping out what the optimal growing environment for the cultivar would entail, and what variables have the largest impact on the production value of the plants as of today almost 20 % of the crop is lost to diseases and the formation of tipburn bad enough to warrant not selling the crop (Knoop. 2019). The results for this thesis however showed a high incidence of tipburn in all experiments indicating that the spectral distribution of the LED lighting used in the experiments and the high light sum used in the set-up cannot be used in a commercial production setting. While LED's have several attractive attributes for inhouse plant production, such as a high efficiency per µmol/W and a lower heat production, the average LED diode also produces light spectra with increased amounts of red and blue light as compared to natural light (Knoop. 2019). This in turn can lead to light stress on plants growing under unmodified LED growing lights and necessitates specialized LED banks to avoid stress that can potentially impact the production value of the growing crop.

6.1 High light intensity treatments with varying photoperiods

Figure 9 shows that in experiment 1 the plants growing under higher intensity light (12 hour photoperiod with a PPFD of 360) on average had higher average tipburn on all of the leaves above 1 cm in length, while experiment 2 (fig 10) showing the lower intensity (24 hour photoperiod, 180 PPFD) grown plants as having worse average tip burn, especially on the innermost leaves, as compared to the almost pristine leaves found on the plants grown under higher intensity light. If this is linked to the lowering of the EC of the nutrient solution during experiment 2 is difficult to conclude. However, nutrient uptake is usually connected to transpiration. Transpiration rate was not measured in these experiments, but high light intensity is known to increase transpiration (ref). It is possible that an EC=2.0 leads to a higher uptake of nutrients during the 12-hour with high light and accumulates high levels of nutrients in the outer tips compared to an EC of 1.2. High salt concentration, usually of calcium, is seen as one of the prime reasons for the formation of tipburn (Mattson. 2015).

This varying reaction to adverse lighting conditions in greenhouses isn't entirely unheard of, as Cox and McKee (1975) found that cultivars of lettuce usually grown outside in adverse light conditions can react negatively when grown in a more controlled greenhouse environment, with tipburn developing even when grown under functionally identical light regimens to what would be found in the field. Its still interesting to see that the two experiments resulted in such disparaging results, with only the EC of the nutrient solution being changed between the experiments. In conclusion, the effect of light regime on tipburn incidence is dependent on the concentration of nutrients.

6.2 Heightened red to far-red light ratio

As mentioned earlier in the theory part of this thesis (2.3) the ratio of red to far-red light in a plants environment can have large impacts on how the plant develops throughout its lifecycle. With the ability to pick up on this ratio by the way of phytochromes, plants can react to changing light conditions by way of the light dependant Pr-Prf forms of phytochrome (Taiz et al. 2018) and activate shade-adaptations to try and get an edge on competitors growing in the same environment (Ballare & Pierik. 2017). This usually takes the form of additional vertical growth at the expense of other important developments such as the number of new leaves, creation of new photosynthetic machinery and storage organs (Franklin & Whitelam. 2005), and as such is of interest in the case of whether this focus on vertical growth impacts tipburn formation in a meaningful manner.

Both experiment 3 and 4 had functionally identical setups (with the exception of a slightly higher PPFD for experiment 4 due to mechanical issues) and as such had results that echoed each other closely, with a few exceptions. Both experiments showed an obvious physiological difference between the plants grown under a higher R/RF ratio when compared to the ones grown in a standard setting, with the former having their longest leaves being almost 2 cm longer than the latter. Both experiments also showed that the plants grown under high R/RF ratio exhibited a steep decline in the amount of leaves longer than 1cm found (with the average for the standard treatment being 5-8 extra leaves compared to the high R/RF grown ones), which could point to the expenditure of vertical growth impacting the amount of new leaves formed (Neff et al. 2000).

The qualitative data for the tipburn present at the end of each experiment (fig 11 and 14) shows a much closer relationship than in experiment 1 and 2, with the graph for experiment 4 (fig 14) having the scale of the tipburn observed almost follow each other identically. This means that, at least in these experiments, the only impact the heightened R/RF ratio had on the 'Frillice' crop was an increase in the average length of the longest leaf found and fewer leaves on average than the plants grown under standard conditions. The R/RF ratio does not seem to impact the severity of the tipburn found on harvest during the experiments in a meaningful manner with the methods employed here to analyse the growth of the plants, which coincides with the findings for Knoop (2019) for plants grown under LED lighting.

6.3 Detecting tipburn formation with NIR capable photography and analysis

During experiment 3 and 4 the both treatments had plants photographed with a NIR capable camera (see section 4.9) to check for the ratio of reflectance found for red and far-red light. As described in section 2.5 this ratio can be used to say something about whether or not a plant is healthy or stressed, and it is theorized that this can be used to detect the precursors of tipburn before its visible to the naked eye, thus aiding growers in reacting to the formation of tipburn before it even forms.

However the data derived from the images taken with the R/RF ratio measured both on the outside rim and the inner part of the leaf (as seen if fig 8) wasn't able to predict the occurrence of tipburn before it was visible with the naked eye. As can be seen in figure 12 and 15 the R/RF ratio only drops quickly once the point where the tipburn is visible (see fig 13) has started, meaning with the equipment used for this thesis it does not seem like its possible to use the R/RF ratio to predict the active occurrence of tipburn in 'Frillice', however this may not be the case once more specialized instruments are developed (Lara et al. 2016).

7 Conclusion

Experiment 1 and 2:

High light sum, both given as 12-hour and 24-hour with LED (peak in blue and red) induced severe tipburn in 'Frillice'. However, the response to photoperiod seems to be dependent on EC level. When the EC was 2.0, no significant difference in average tipburn score was found but 12-hour lighting induced more tipburn on the young developing leaves compared to 24-hour lighting. On the other hand, when the EC was reduced to 1.2, an opposite response was seen and plants exposed to 12-hour lighting developed sinificantly less tipburn, and almost no damage on young leaves, compared to the 24-hour lighting.

Experiment 3 and 4:

The experiments with heightened R/RF ratio for one test chamber show the expected morphological differences for growing under additional red light, such as increased leaf length (Taiz et al. 2018), but no statistically relevant connection between the tipburn formation in the two treatments could be found, meaning in these experiments additional R/RF ratios does not seem to impact the formation or severity of observed tipburn.

NDVI analysis

With the results gathered from experiment 3 and 4 it does not seem that, with the setup used in this thesis, it is possible to use NDVI analysis of NIR images to predict the formation of tipburn before it becomes visible to the naked eye in growing 'Frillice'.

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Image used for Figure X: https://luv2garden.com/hydroponic_nft.jpg

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Appendix 1

Code used in Spyder (Python) for NDVI Analysis: (The IMG.JPG is a placeholder and needs to be switched to the relevant image for each analysis).

```
import matplotlib.image as mpimg
import matplotlib.pyplot as plt

# Read Images
img = mpimg.imread('IMG.JPG')
#img = img.astype(float)
# Output Images
plt.imshow(img)

img_nir = img[:,:,0].astype(float)
img_green = img[:,:,1].astype(float)
img_red = img[:,:,2].astype(float)

plt.figure()
plt.imshow(img_nir,cmap='gray')

plt.figure()
plt.imshow(img_green,cmap='gray')

plt.figure()
plt.imshow(img_red,cmap='gray')

plt.figure()
plt.hist(img_red.ravel(),256, [0,256], color='black'); plt.show()

ndvi_img = (img_nir-img_red)/(img_nir+img_red)
plt.figure()
plt.imshow(ndvi_img,vmin=0, vmax=1.,cmap='gray')
```

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