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Growth, nutrient content and tipburn in lettuce (*Lactuca sativa* L. 'Frillice') in response to light quality and aerial environment.

Vekst, næringsinnhold og bladrandskade hos salat (*Lactuca sativa* L. 'Frillice') dyrket under ulik lyskvalitet og luftsammensetning.

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Plant Sciences – Plant production systems

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Summary

The incidence of tipburn in the vegetable cultivation industry has been a major challenge to the Norwegian growers particularly to the growers of Frillice lettuce (*Lactuca sativa L.* 'Frillice'). In periods, Norwegian growers can have up to 20% loss due to tipburn in greenhouse production of 'frillice'. This thesis aim is to investigate how climate factors, including light, relative air humidity and CO_2 are influencing tipburn occurrence and severity in lettuce (*Lactuca stiva L.* 'Frillice') and the role of nutrient especially calcium in this disorder to further broaden the understand to already existing studies done. It focuses on the role played by climate and to identify better cultivation practise that can help to control the tipburn incidence.

Common assumptions by a number of researchers has attributed this condition to various abiotic stress factors including high light intensity, elevated relative air humidity and temperature as well as nutrient deficiency like calcium (Ca). The Ca deficiency is coupled with transpiration limitation as a major contributor to this physiological disorder. Tipburn in lettuce has been described as a necrotic condition occurring in the outer margins.

To better understand this condition, several manipulations of climate condition were tested in controlled growth chambers to assess the level to which each factor would influence tipburn occurrence in 'Frillice' lettuce. Two lamp types: white LEDs (light emitting diodes) and HPS (High pressure sodium) were used and effects of light intensity, from low (150µmol$m^{-2}s^{-1}$) to high (300µmol$m^{-2}s^{-1}$) and light quality (additional far-red), elevated relative air humidity (RH) at night and elevated CO_2 was investigated.

Day temperature of 18℃- and 20℃-night temperature was kept constant throughout all experiments. Elevated RH during night (90% compared with 70%) and elevated CO_2 (400ppm – 1000ppm) were tested.

Nutrient analyses were conducted for plants exposed to 150µmol$m^{-2}s^{-1}$ on both source (outer) and sink leaves (inner) it was measured N, C, Ca, Mg, and K to assess if a relationship exist between nutrients, especially Ca, and tipburn. An analysis of antioxidant capacity (FRAP) was also performed on source and sink leaves and roots to assess the level of antioxidants in plants grown at high RH during night to verify if there exist any relationship between FRAP and tipburn occurrence.

High light intensity generally increased the severity of tipburn under all climate conditions. In general, white LED increased outer tipburn under moderate light compared with HPS but under

high light intensity HPS induced more tipburn than white LED. Additional far-red light did not strongly influence on the incidence of tipburn. Treatments with elevated RH during night resulted in the strongest reduction in severity under both moderate and high light conditions, with HPS and LED as light source. Elevated CO_2 reduced tipburn severity in both LED and HPS, but the effect was strongest in HPS.

Lower calcium content was found in the sink leaves compared with source leaves in all experiments, but no correlation was seen between calcium and tipburn. Antioxidant capacity measured in leaves and roots from the experiment with elevated RH during nigh did not show any correlation with tipburn incidence and is not a good indicator for tipburn

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Abbreviations

FW = Fresh weight

DW = Dry weight

Ca = Calcium

Mg = Magnesium

K = Potassium

N = Nitrogen

C = Carbon

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

UV = Ultraviolet (light)

EC = Electric conductivity

C = Celcius

1 INTRODUCTION

The consumption of leafy vegetables are common food items in a well-balanced diet. Lettuce *(Lactuca sativa L.)*, a C3 plant, is considered one of major food crops cultivated and consumed within the European Union. A FAOSTAT report stated (FAO Statistics Division, 2011) , the total production quantity of lettuce and chicory in the European Union was 3 023 174 tons in 2010 (Gonzalez, 2016).The main producing countries were Italy and Spain producing 843 344 tons and 809 200 tons, respectively.

A report on a survey conducted on green lettuce in Scandinavian food shops, lettuce was listed as the third most popular vegetable in Sweden, fourth most popular in Finland and Denmark, and sixth most popular in Norway (Johnson M.et al., 2016).

In Norway, lettuce *(Lactuca sativa L.)* is produced in the field during summer and in greenhouses all year-around. Greenhouse production, controlled environment like growth chamber cultivation and vertical farming have been on the rise worldwide, especially in the Western part of the world. Popular to the Norwegian market is the greenhouse grown cultivar, 'Frillice' lettuce (*Lactuca Sativa L.*) which is faced with the major physiological disorder, tipburn. Tipburn is believed to be both an environmental and a genetic issue (Hume, 1964, Bangerth, 1979) with some association with growth rate (Collier and Tibbits, 1982; Saure, 1998) and calcium deficiency (Shear,1975, Wissemeier, 1996)

Collier and Tibbits, (1982) describe tipburn as a physiological disorder occurring as a necrotic tissue at the margin and/or apex of leaf which is believed to be associated with low local concentration of calcium (Ca) (Thibodeau and Minotti, 1969; Collier and Tibbits, 1984). Olson et al. (1967) suggested this necrosis may result from rupturing of laticifer cells. The impact from climate like high irradiance and high relative air humidity (RH) is however not exempted (Bangerth 1979; Collier and Tibbits, 1984). This deficiency in calcium is not just about the quantities available but also impacted by interruption with the uptake, transport and translocation which could be influenced by un-optimal climate.

Saure (1998) believed it could be a stress related disorder and not only a Ca related disorder. However, it is still not clear if tipburn is related to Ca deficiency, stress, or both. Cultivation in controlled environment like greenhouses allows optimization of all environmental conditions to help achieve the full genetic potential of crops. However, the climate will vary depending on season and time of the day. Some climate factors are also difficult to control like RH and light climate.

Frantz J. et al. (2004) commented on an increasing rate of tipburn found under high irradiance and claimed that plants with a higher growth rate requires more Ca.

Considering the role environment plays in tipburn occurrence, this thesis seeks to manipulate various climate factors including irradiance and light quality, relative air humidity, and CO_2 concentration during growth to investigate which climate practices could best control or eliminate tipburn problems. Furthermore, since Ca is believed to have an important role in tipburn development, this thesis sheds light on how different environmental factors affects Ca content in addition to other cations in young sink leaves and old source leaves and assess if there exist any correlation with the development of tipburn. In commercial greenhouse production of lettuce, the use of high-pressure sodium lamps (HPS) are common, but growers show increased interest in novel light technology like light emitting diodes (LEDs). Hence, all experiments in this thesis was performed with both HPS and LED as a light source to study the interaction between light quality and other climate factors.

1.1 Objectives

- To assess how far-red light, elevated CO_2 and elevated air humidity at night will influence on growth and severity of tipburn under moderate and high light intensity and to investigate the role of calcium, potassium, and magnesium content of lettuce.
- To study the same climate variables under two important light sources: the traditional high-pressure sodium (HPS) lamps and light emitting diodes (LEDs) to understand if the light source is important for the response to climate.
- To assess the role of antioxidant power in the occurrence of tipburn

2 THEORY

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

Lettuce is described as an annual crop from the daisy family, Asteraceae, lettuce (*Lactuca sativa L.*) , and is a leafy vegetable most often used as fresh salad (Mou, 2008) and is sometimes cultivated for its stem and seeds (Fischer, 2018). Originally farmed by ancient Egyptians,

lettuce was considered a weed, whose seeds were used to create oil. It was transformed into an important food crop raised for its succulent leaves and oil-rich seeds (Katz and Weaver, 2003). Leaf lettuce is among the four botanical varieties of lettuce *(Lactuca Sativa L.)*,(encyclopaedia britanica, 2020; Weaver, 1997) that is cultivated including: (a) celtuce, or asparagus lettuce (variety *augustana*), with narrow leaves and a thick, succulent, edible stem; (b) head, or cabbage, lettuce (variety *capitata*), with the leaves folded into a compact head; (c) leaf, or curled, lettuce (variety *crispa*), with a rosette of leaves that are curled, finely cut, smooth-edged; and (d) romaine, lettuce (variety *longifolia*), with smooth leaves that form a tall, oblong, loose head. There are two classes of head lettuce: the butterhead types, such as Bibb lettuce, and crispyhead types, such as iceberg lettuce (Bradley et al., 2010). Several distinct cultivars with over 65 varieties of lettuce (*Lactuca Sativa L.*) has since been documented from the late 19th century.

Advancement of breeding and domestication over the years have resulted in several positive changes in lettuce such as delayed bolting, larger seeds, larger leaves and heads, better taste and texture, a lower latex content, and different leaf shapes and colours.

Frillice, the cultivar used in the present study is a cross between iceberg lettuce and curly endive, with thick green leaves and crispy like iceberg but with a crinkled top like curly endive (Weaver, 1997). It has a flavour range between quite neutral, mild, and slightly bitter taste. Lettuce (*Lactuca Sativa L.*) is a cool-season crop that grows well in the spring and fall in most regions and will even tolerate a light frost. Seed germination is best at 12°C-18°C emerging in about 5 to 10 days (Bradley et al., 2010).

2.2 Nutritional wealth of lettuce

Most lettuce varieties are eaten fresh and are commonly served as the base of green salads. Lettuce is generally a rich source of vitamin K and A and a moderate source of folate and iron, though the nutritional quality varies, depending on the variety. It is considered a refreshing choice during hot weather due to it high water content, however, it is low in fibre. In addition to this, it also provides calcium, potassium, magnesium, and some amount of vitamin C. It is known to be low in calories, sugar, and fat (Gebhardt et al. 2012, USDA). A 100 g of fresh, raw-lettuce provides up to 247% daily values of vitamin-A, and 4,443 µg of β-carotene (Carotenes convert into vitamin-A in the body; 2 µg of carotene is considered equivalent to 1 IU of vitamin-A). It is also known for its antioxidant properties.

2.3 Greenhouse production

Hydroponic system is the commonly adopted method of production in most greenhouses cultivation of lettuce both in Norway and other parts of the world. It is commonly produced either by using the NFT (nutrient film technique) or the floating raft method, both as closed systems. The ability to provide satisfactory warmth in winter months and either shading or chilling for water in the summer months is a major requirement in hydroponic production (Kaiser and Ernst, 2016). This thesis focuses on NFT which is the method adapted. Seedlings are placed through holes either along a plastic pipe, tube or enclosed trough which allows only the roots to extend inside. Cultivation begins without spacing between seedlings but with time they are spaced out by shifting individual gutters or tubes or pipe apart about 15cm.

The nutrient solution with an electric conductivity(E.C) between 1.5 – 2.0 (pers. com. Espedal, 2019) is initially stored in a reservoir, pumped out into channels/ tubes at a sloped angle, drained down to a catchment system, then filtered or aerated and cycled back to the reservoir for reuse (Parkell et al., 2018). A shallow stream of supplement continually flows over the bare roots inside the tubes. A flow rate between 0.26 - 0.53 gallons per minute is suggested (Kaiser & Ernst, 2016). To ensure proper delivery of nutrient solution to all plants in the gutters, the gutters/channels are put on a slight decrease (1-3%), for the most part at bench height. The supplement is dispensed at the elevated side of the tubes where it flows by gravity to the lower end. In most commercial production, the surplus is collected and redistributed.

Nutrient mixture can either be formulated by growers based on a standard or their own modified formula meeting their target of production or buy ready – to – mix products.

Kaiser & Ernst, (2016) commented that the pH of the nutrient solution can change during production since Hydroponic nutrient solution come up short on the buffering limit of soil, as such, monitoring is quite significant.

2.4 Tipburn

Tipburn is simply a necrosis on the margins of leaves occurring in greenhouse mainly during spring and summer but can also occur during the whole year. It is generally described as a physiological disorder relating to localized calcium deficiency (Uno et al.,2016; Brata and Tibbits, 2000) due to reduced calcium levels supplied to rapidly developing leaves (Saure, 1998).

Tipburn injury occurs as leaves at the growing points gets enclosed leading to reduced levels of calcium concentrations. This type of tipburn is called inner tipburn. The enclosure lessens transpiration and, in this way, decreases Ca transport (Marschner, 1995; Collier and Tibbits, 1982) as Ca transport in plants happens for the most part in the xylem by mass flow. Factors including transpiration, root pressure, and diurnal changes in water stress are responsible for this mass flow (Marschner, 1983). Outer tipburn occurs on the tips of outer source leaves. Tipburn is developing during production in the greenhouse and lettuce with inner tipburn is usually discarded. Lettuce with outer tipburn can be packed if the outer leaves are removed .Packed lettuce with tipburn will continue to develop the injury and reduce the shelf life. Many packaging organizations dismiss whole fields of lettuce with a tipburn occurrence more noteworthy than 5% (Jenni and Hayes, 2010).

2.5 The role of Calcium in plants

Calcium plays a major role in strengthening plant cell wall. It is required by plants for cellular signalling response and membrane integrity (Tonetto de Freitas et al.,2014). The largest pull of calcium in the plant cell is in the vacuole. The chloroplast, endoplasmic reticulum and the mitochondrial are all sites for the storage of Ca^{2+} in the cell organelles.

Research have shown that the high or low levels of calcium in the growth and development of plants contributes to a series of physiological disorders mostly attributed to other biotic and abiotic factors. This limitations has brought about what is described as calcium deficiency disorders spiking series of research as to how different methods could be adapted to either predict or bring down these deficiencies in crop plants (Saure, 2005; Ho and White, 2005).

To have a better understanding of how these deficiencies come about, it is important to be aware and understand the role of calcium at cell level in the life process of a plant. The role of Calcium is significant at the cellular level of a plant. Marschner (1995) commented on the impact calcium had on the strength and structure of the cell wall and membrane because it has the ability to enhance ion dehydration that help to bind to a number of anionic substance due to it large ion radius (Batistic & Kudla, 2010). Furthermore, it plays a major role in signal transduction. Willey 2016 and Batistic & Kudla, (2010) stresses on the major role played by calcium in cell response to a series of biotic and abiotic factors in cytosolic signal transduction pathway. High concentration of Ca^{2+} could be described as been toxic and can result in cell death. This is because of precipitation with other ionic substance and competition for binging sites with other cations that are needed for activation enzyme and efficient cell

metabolism (Willey 2016 and Batistic & Kudla, 2010). This require cytosolic Ca^{2+} to be under strict physiological and biochemical control. Calcium is required at high concentrations inside cell organelles, so it is accessible to be stacked into the cytosol during signal reactions, furthermore, as a counterion to inorganic and organic anions in the vacuole (Marschner, 1995).

2.6 Calcium deficiency disorders in leafy vegetables

According to Taylor & Locascio, (2004), the inward flow of water through matured leaves is exclusively through the xylem while that of young (low transpiring) leaves takes place through both the xylem and the phloem. Nutrients in the growing media are transported to the plant through water uptake (Ho & White, 2005). Calcium is known to be only mobile through the xylem whose rate of sap flow is controlled mainly by transpiration and growth rate. For this reason, older and mature leaves have a much higher Ca^{2+} accumulation than the low transpiring, young and enclosed leaves (Saure, 1998). Deficiency in calcium levels could be related to water shortage or uneven soil moisture affecting the transport of calcium through the plant. It could also be related to too much nitrogen in the soil (Mallikan et al. 1969). Calcium and Magnesium are restricted inside the plant cells and have antagonistic associations. Accordingly, a homeostatic harmony among Ca^{2+} and Mg^{2+} inside the plant is vital for ideal development and optimal turn of events(Tang Ren-Jie & Luan Sheng, 2017). The accumulation of calcium is higher at the base and lower at the tips of leaves (Barta & Tibbitts, 2000). Generally matured leaves are rarely affected by calcium deficiency unlike the new growth and rapidly growing tissues of the plant (Simon, 1978). In leafy vegetables the symptoms of Ca^{2+} deficiency is commonly seen at the tips of low-transpiring and enclosed leaves. Environmental condition is believed to influence Ca^{2+} availability. Aside low transpiration, conditions of high humidity and cold may result in Ca^{2+} deficiency.

Calcium deficiency symptoms are seen initially as localized tissue necrosis leading to stunted plant growth, necrotic leaf margins on young leaves or curling of the leaves, and eventual death of terminal buds and root tips (Saure, 1998 and De Freitas, 2016). Even though tipburn is commonly viewed as a calcium inadequacy issue, side effects can happen notwithstanding abundant supplies of calcium in most vegetable developing soils. The problem rather is, moving adequate calcium to the quickly developing internal leaves. According to (Kuronuma et al. 2019; Kuronuma et al. 2020), tipburn is mostly brought about by the failure of the plant

to translocate satisfactory measures of Ca^{2+} to the tips of the upper leaves, which is related with an expansion in the circulation of Ca^{2+} in the roots.

2.6 Abiotic Stress - impact of climate

Plant in general are both affected by both biotic and abiotic factors. These are factors that may compromise the expected growth and reproductive potential of a plant resulting in stress. Stress as described by Taiz & Zeiger, (2015) is any environmental condition that hinders the plant from achieving its full potential. With emphasizes on abiotic stress factors including environmental parameters such as humidity, drought, light, soil pH, temperature, oxygen, CO_2, and several others tend to suppress the full potential of a plant.

The environment in which plant live has a major influence on their survival. Plants may have to either adapt or acclimate to their changing surrounding since they are sessile. In greenhouses and growth chambers, climate is monitored and controlled by climate computer systems to assist plants to possibly achieve their full potential. A better understanding of the physiological and developmental processes of lettuce 'Frillice' is important to control the occurrence of tipburn.

2.6.1 Relative humidity

Transpiration is essential for nutrient uptake by plants. According to Willey, (2016), transpiration is greatly influenced by relative air humidity.
Humidity levels fluctuate with changes in air temperature both inside and outside greenhouses and growth chambers, and plants are constantly adding water to the air through transpiration. According to (Collier and Wurr, 1981; Collier and Tibbitts, 1984) low humidity in the day can cause a rise in tipburn since more water is unevenly transpired through exposed leaves while the interior meristem is in high humidity. This may result in increased occurrence of tipburn as transpiration is slower in the meristem leading to reduced flow of Ca^{2+} to young leaves (Frantz et al., 2004). On the other hand, a low night humidity may cause a decline in plant turgor potential and bring about less Ca^{2+} been directed to tips and meristem by guttation (Frantz et al., 2004).

A rise in temperature results in an increase in its water holding capacity (British Colombia, 1994) and as such decreasing relative air humidity (Collier and Wurr, 1981; Collier and Tibbitts, 1984).

Humidity can be considered as one of the most difficult and challenging factors to be controlled in the greenhouse. Any drop in temperature may affect the air humidity and therefore understanding its dynamics is essential to the grower. Not only does it affect transpiration but also plant root pressure and stomata aperture which will indirectly affect nutrient uptake and photosynthesis. A British Colombia factsheet (1994) on relative humidity commented that adjustment in the leaf stomata is the main plant mechanism that helps in coping with humidity. The closure and opening of stomata are a response to vapour pressure deficit. An increase in humidity results in a wider opening and vice versa. Garber and Cullen, (1971) stated that the increase in growth caused by increase humidity could be due to reduced stomatal resistance. However, when humidity levels are extremely high, the total uptake of minerals is reduced since plants are unable to evaporate enough water. Water vapour will always move from an area of high concentration (such as inside the leaf cavities) to an area of lower concentration (the greenhouse air). This is the principle behind evaporative transpiration.

2.6.2. CO_2

Willey (2016) commented that the primary production of plant biomass is based on the assimilation of carbon (carbon fixation) and that plants grown under optimum conditions, but different CO_2 levels produce significantly different amount of biomass. Taiz & Zeiger (2015) also stated that carbon represent almost half of plant dry matter. This makes it evident how significant carbon is when it comes to plant production. Elevated CO_2 (>400 ppm) has the potential to enhance the rate of photosynthesis and can lead to partly closure of the stomata which can reduce the amount of water loss during transpiration (Sirtautas et al. 2014; Ainsworth & rogers, 2007). This suggest a better water use efficiency (WUE) under elevated CO_2 but may also affect the amount of nutrient uptake. Supplemental CO_2 has the potential to cause changes in antioxidant activity as a secondary effect (Wang et al. 2003).

Willits and Peet, (1989) stated that CO_2 supplementation is most beneficial when ventilation system is closed during autumn, spring, and winter. Both et al. (1998); Mortensen, (1989) reported a 30% increase in photosynthesis assimilation and growth of lettuce (*Lactuca sativa L.*) which resulted from CO_2 supplement in the greenhouse. CO_2 level of 400-600 µmol mol^{-1} has been suggested as suitable for hydroponic lettuce production (Both et al. 1998). Different type of plant responds differently to rise in CO_2 concentration. Lettuce is a C3 plant. A study with a single leaf under elevated CO_2, showed several rises in temperature which is considered

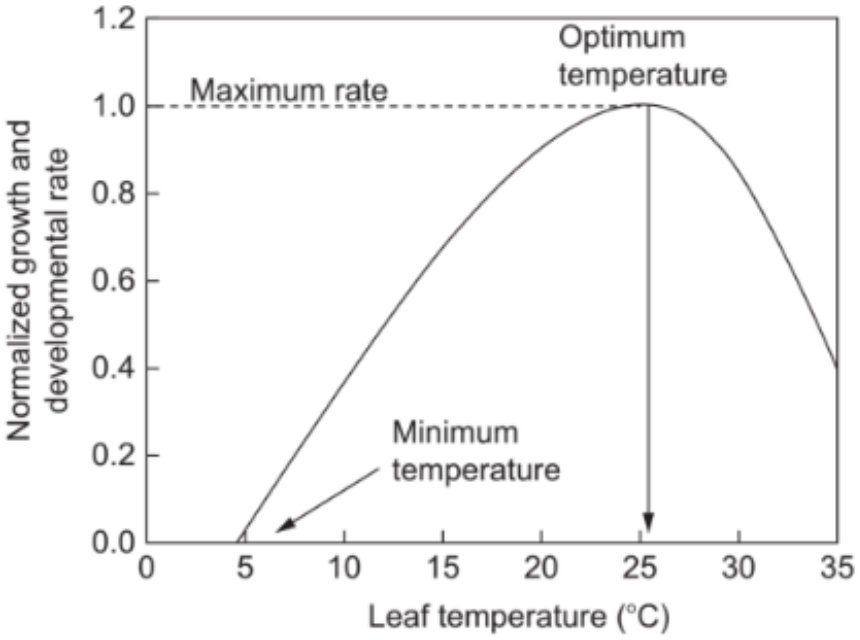
as optimum for photosynthesis in C3 species (Long, 1991) but also likely threat to tipburn occurrence.

CO_2 supplementation has been reported to cause either an increase or decrease in the leaf chlorophyll content of leaves (Zhao et al. 2010; Li and Gupta, 1993) and can also cause about 19% decrease in the nitrate level of leafy vegetables (Li and Gupta, 1993).

2.6.3 Temperature

Photosynthesis, growth, and development of plants are temperature dependent (Kozai et al. 2018; Taiz & Zeiger, 2015). A linear rise is observed in the growth and development of a plant as temperature rises for most plants. Stanghellini, (2019) stated that the main determining factor for development rate of lettuce was temperature. Different plant species and cultivar respond to different temperature optimum for various plant processes. Taiz & Zeiger, (2015) stated that plant grown under different temperatures show a photosynthetic thermal optimum that correlate with the temperature in the environment in which they grew. According to (Willey, 2016) a direct impact of changes is observed in organisms because of changes in temperature since it can alter the physical properties of molecules and their interaction.

Balancing temperature with other climate factors is especially important in the production of lettuce (*Lactuca sativa L.*) in the greenhouse and growth chambers. It is therefore important to best determine the right temperature. The difference between the leaf and air temperature is influence by day and night period as affected by the light irradiance. During the day higher leaf temperature is mostly recorded as compared to the air temperature and the vice visa at night (Choi et al. 2000). According to (Jie & Kong 1997), lettuce (*Lactuca sativa L*) is typically grown under low temperatures of 20-25℃ in the day. With Frillice production, 15℃ is set for seed germination while 18-20℃ set for growth.



Cooling systems, shading, humidification, heating systems (heating pumps and pipes) are better strategies for regulating temperatures in greenhouses.

2.6.4 Light – irradiance and photoperiod

An essential factor in growing plants in the greenhouse and growth chambers is light. All plants need sunlight as a source of energy for the basic process of photosynthesis. According to Stanghellini et al., (2019) wavelength from approximately 300nm – 2800nm is the amount of radiation coming from the sun unto the earth surface. Of this radiation spectrum, 400 -700nm is considered the photosynthetic active radiation (PAR) used by plant for photosynthesis. PAR measures the intensity of light directly affecting photosynthesis (Goldammer, 2019).

Not only do plants require light for photosynthesis but also for growth and development. Stanghellini et al. (2019) stated a rule of thumb which suggest that for every 1% rise in light results in 1% rise in yield. The amount of light received by a plant is necessary in determining the growth rate and length of time it remains active.

Intensity of light can vary per time of the day, weather, geographical location, and season. In the case of greenhouses and growth chambers, other climate factors and time plays major role to change. Light intensity or quantity is described as the total amount of light that plants receive (Taiz and Zeiger,2015; Goldammer, 2019)

The length of day has great impact on the development of some crops. Photoperiod is described as the period plant is exposed to light within 24 hours (Taiz & Zeiger, 2015; Goldammer, 2019). Season and latitude are the determiners of the duration of daylight and that not the length of light period but rather the uninterrupted period of darkness controls plant response to day length (Goldammer, 2019). Knowledge on how to manipulate the photoperiod in the greenhouse is essential in scheduling plants to meet some desired phenological stage and to reduce production time. The common practices in greenhouses for lettuce is mostly 6 hours of darkness with the 24-hour period.

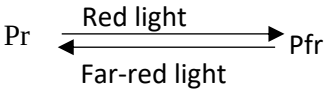
2.6.4.1 Light quality

Light quality is said to affect both morphology and photosynthesis. Goldammer, (2019) describes it as the light composition as to wavelength (color specifics typically expressed in nanometer) that are effective for photosynthesis and other plant growth processes. Specific plant functions are performed at different wavelength and are absorbed in varying amounts. For plants to be able to make effective use of these wavelength, they must have receptors capable of sensing (Taiz & Zeiger, 2015). Red and blue light are by far the two most important in the process of photosynthesis. A wavelength of 430 and 450nm of the blue light is considered the most important that promote vegetative and leaf growth, a part of the spectrum called "cool light" (Goldammer, 2019). Blue light also results in thickened and compacted leaves. Red light is the longer wavelength (600-700nm) implying less energy usage. With far red not considered as photosynthetically active, it has influence on growth. This wavelength (700 – 800nm) on plants result in shade – avoidance response. Plants under canopy and lower leaves receive more of this far red than red light resulting in elongation (Goldammer, 2019).

2.6.4.2 Phytochromes

Phytochrome is a plant growth regulating photoreceptor protein that absorbs primarily red and far-red light but also capable of absorbing blue light (Taiz & Zeiger, 2015). Morphology of plant is affected by the ratio between red and far-red light. Research has shown that the subsequent irradiation of far-red light (710-850nm) could cause a reverse on the effect of red light (620-700nm) with the first seen in the germination of lettuce seed. Further works have shown this reversibility in the stem and leaf growth (Taiz & Zeiger, 2015). There are 2 forms of phytochrome: the active form (Pfr) and the inactive form (Pr). These 2 forms can undergo a process called photoreversibility. Phytochrome is present in the red light-absorbing form (Pr) in etiolated seedlings which can be converted in far-red light-absorbing form (Pfr) by the introduction of red light. In darkness, Pfr can be reverted to Pr by introducing far-red light. This

is rather a slow process. A light source with high R/FR ratio (> 1) will lead to suppressed stem elongation compared to low R/FR ratio (< 1).



2.6.4.3 Lamp type

2.6.4.3.1 HPS – High pressure sodium lamp

Common to the Norwegian greenhouse commercial production is the use of HPS (high pressure sodium lamps) as supplementary light. HPS lamps are known to produce light mainly in the yellow and red end of the light spectrum. According to (Goldammer, 2019) HPS fixtures can provide full spectrum light having a heavier representation of middle wavelengths thus green, yellow, and red/far red light. This can lead to plant etiolation due to lack of blue light even though its usage can result in good quality plants (Goldammer, 2019). The outer presentation of HPS to the eye is seen as yellow. The lamps generate infra-red radiation and the leaf temperature is often 1-2°C higher than the air temperature under HPS lighting.

2.6.4.3.2 LED – Light emitting diode

Novel lighting technique like light emitting diode (LEDs) is of interest for greenhouse production. It is a new norm which is slowly but gradually getting recognition in the market of greenhouse production. They could be manufactured to meet one's specification; thus, to emit photon colors matching the absorbance peaks of essential pigments such as red and blue peaks of leaf photosynthetic action spectra (Goldammer, 2019). These LEDs has been noted for its energy saving ability – the feel cool to touch with no heat been produce/felt and even if they do at fixture level, it is easily dissipated. Approximately 32℃ is considered a standard operating temperature (Goldammer, 2019).

2.7 Oxidative stress

According to Kasote et al. (2015), two main powerhouses and sites identified for generation of ROS (reactive oxidative species) within a plant cell are the mitochondria and chloroplasts. Under different types of environmental stresses, accumulation of ROS occurs in the cell which are detoxified by specialised enzymes called antioxidants (Taiz & Zeiger, 2015; Laxa et at. 2019). ROS is produced during series of biochemical reactions within the cell and organelles of organism. The oxidative reaction resulting from environmental stresses leads to the production of free radicals. Reduction of molecular oxygen produces superoxide which acting

as a precursor of most ROS (Turrens, 2003). Slippage of electrons from the chloroplast and mitochondrial react with molecular oxygen to produce these radicals (Gill & Tuteja, 2010). They have the potential to cause significant damage to plant cell when in excess. ROS can trigger autocatalytic process of membrane oxidation leading to degradation of organelles and cell death (Taiz & Zeiger, 2015). Antioxidant systems helps to keep these ROS in balance since it has the potential to inhibit oxidation, and slow down or prevent cell damage resulting from the production of these free radicals. It can maintain a fine balance between energy linked roles and the control of ROS production (Gill and Tuteja, 2010). Antioxidants can delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate (Kasote et al.,2015)

Examples of ROS in plant cell include superoxides, singlet oxygen, hydrogen peroxide and hydroxyl radical. Common antioxidant system in plants includes the enzymatic and the non-enzymatic. Consisting of a low molecular weight, the nonenzymatic includes ascorbic acid, flavonoids, proline, carotenoids, phenolic acids etc. (Kasote et al., 2015).

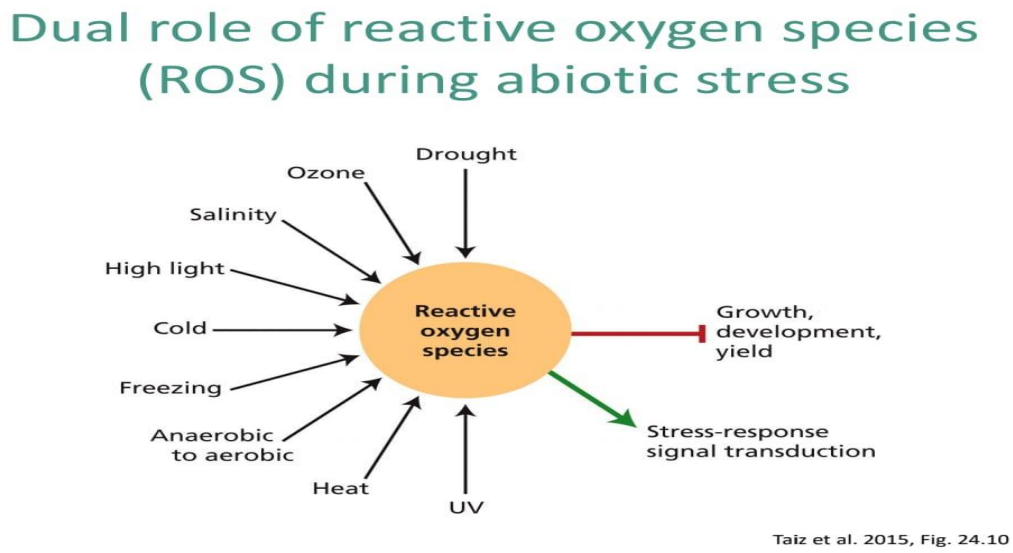


Figure 1: dual role of reactive oxygen species (ROS) during abiotic stress

3. MATERIALS AND METHODS

3.1 Pre-cultivation

Seeds of lettuce (*Lactuca sativa L.* 'Frillice') were obtained from Norgro (Norway) and seeded in peat soil of the type "Degernes torv" supplied by Degernes Torvstrøfabrikk AS (Norway).

Seeds were sown at about 4mm depth in 0.08liters degradable pots and kept in a dark room for 4 days until emergence (Fig. 2). The temperature was 15℃ and relative air humidity (RH) was kept 60% throughout the period. Seeds were watered with tap water only and the pots covered with 'agryl' to maintain the humid condition during the germination. The germinated seeds were transferred to the greenhouse after the 4^{th} day in darkness. The seedlings were grown for approximately 3 weeks until they have reached 4-5 true leaf stadia under a temperature of 20℃, RH 60% during day and night. Supplementary light for 18 hours was provided by high pressure sodium lamps (HPS) with a photon flux density (PFD) of 150 $\mu molm^{-2}s^{-1}$. Unlike the dark room, seedlings in the greenhouse were watered with a nutrient solution (EC= 1.5) once a day. The climate was controlled by a Priva climate computer (Priva, Zijweg, The Netherlands). To maintain humid conditions in the greenhouse, sprinklers were installed in the roof and sprinkled water automatically when the air was dry (<57%). The temperature set-point for ventilation was >20℃, and the HPS lamps were turned on when the outside irradiance was lower than 300 watts/m^2. The plants were grown in the greenhouse for approx. 3 weeks, and when they had developed 4-5 true leaves, they were moved to growth chambers (Fig. 2).

Fig. 2: Pre-cultivated seeds displayed in both dark room (left) and greenhouse (right). Picture: Ellen Kusi

3.2 Growth chamber Setup

While in wait for plants in the greenhouse to get ready for transfer, the growth chamber, was setup (Fig. 3) to the right climate conditions for receival of the seedlings when due. The growing system adapted in the chambers was the hydroponic nutrient film technique (NFT). 4 rows of gutters each with a capacity of 10 pot holdings were laid in each chamber, resulting in a total of 40 pot holdings per chamber. With two ends of the gutters, one enclosed and the other opened, hoses were connected to the enclosed side of the gutters to allow supply of nutrient solution to the plants at specific times. These hoses were connected to a black container serving

as a reservoir for the nutrient solution placed under the stands of the gutters. The dispensation of the solution was regulated by a timer (mueller SC 28 11 pro, Germany) that controls the number of times per day and the amount of nutrient solution going out at each time. The opened end of the gutter allowed flow of solution out of the gutter to avoid soaking. This was enhanced by elevating the enclosed side of the gutter creating a tilt. 2 transparent plastics boxes were placed beneath the side of the opened end to receive the surplus solution that came out after delivery. Transparent boxes were chosen to make it easy in assessing that the plants were getting equal amount of nutrient solution. To mimic the commercial system of lettuce production in Norway and promote uniform distribution of climate requirement, gutters of 1.5m long and 10cm wide were spaced about 25cm apart. The distance between holes were about 15cm

Fig. 3: A picture showing the setup of growth chamber. Picture: Ellen Kusi You need to refer to the figure in the text above, like I did for fig 1

Fig. 4: Newly transferred seedlings from greenhouse to the growth chamber. Picture: Ellen Kusi

A box with sensors for temperature and air humidity where placed above the plant canopy and connected to the PRIVA climate computer (Priva, De Lier, The Netherlands).

3.3 Light sources in the chamber experiments

Two light sources: HPS and LED were used in the chamber experiment; HPS lamps (400 watts, Gavita, Norway) and white light emitting diodes (LEDs) with additional far-red (185 watts, Evolys,Norway).

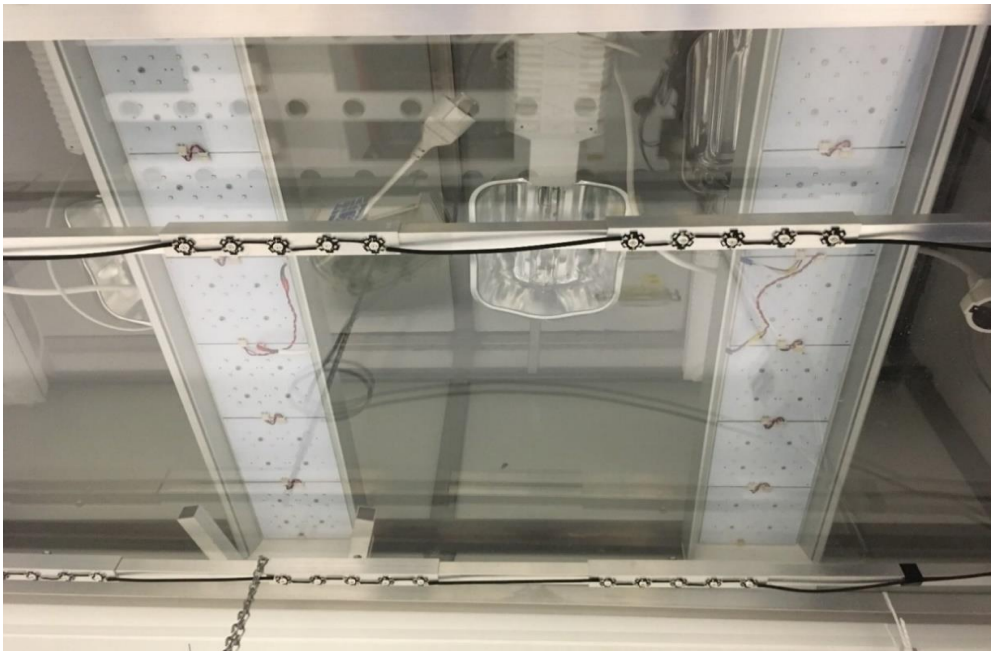


Fig 5: combined lamps HPS (lying vertical with 400W) and LED (lying vertical with 185W each) together with two dimmable far-red (lying horizontal, 80W). picture by Martin Knoop

3.3.1 PPFD (Photosynthetic Photon Flux Density) Adjustment

To regulate the light intensity for accuracy in each chamber, nets were used to block some of the rays coming from the lamps in the case of HP while that of LED were adjusted manually with a dimmer. The LED's already have fixed regulators attached to the monitor which are used to adjust the photon flux. . A quantum meter (Li-250A light meter, Li-Cor, USA) was used to measure the photosynthetic active radiation Measurement of the photon flux was done with the doors of the chambers closed to avoid external light influence. Variation were observed at different sides of the chamber where quantum meter was positioned and was +/- 10%.

Fig 6: optronic model 756 spectroradiometer for measuring spectral composition of light and Quantum meter used in measuring light irradiance: Picture: Martin Knoop

3.3.2 Spectral composition in chamber

To measure 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) an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, FL, USA), was used. The process was explained in (Suthaparan et al., 2018).

3.3.2 Red/far-red ratio

The red/far-red sensor (Skye red/far-red sensor, The UK), a 660nm and 730nm wavelengths was used to adjust the R/FR at 1.1 in the chambers that required addition of far-red as part of their setup. This was only measured in the LED treatments where additional lighting was given.

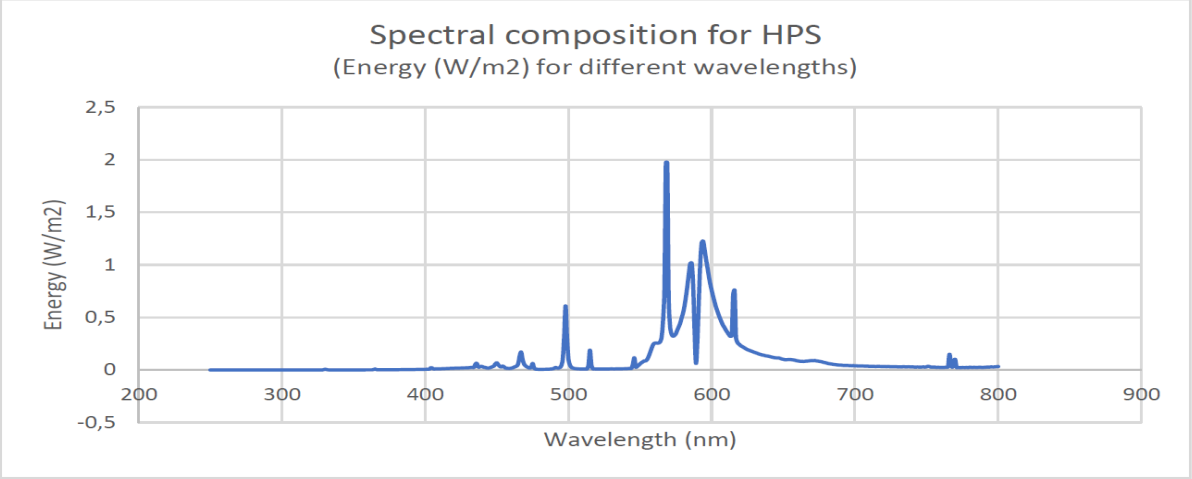


Fig 7: Spectral composition for 400 W HPS, (Gavita Norway). Used in the greenhouse compartment and the growth chambers in experiment 1,2,and 3.

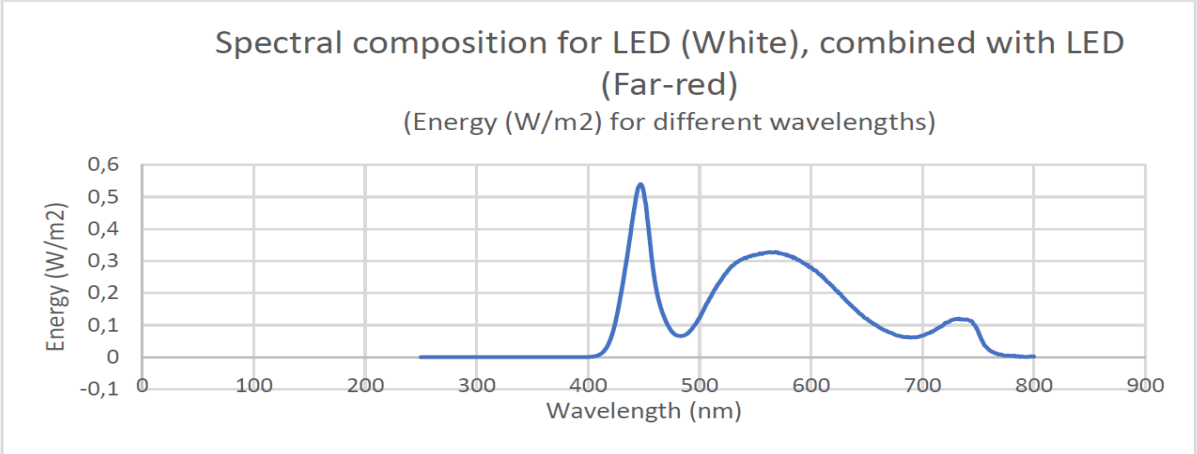


Fig 8: Spectral composition for 185 W LED (White), (Evolys Norway) in combination with 80 W (dimmable far-red) LED, (Evolys Norway). Used in the growth chambers in experiment 1,2,and 3.

3.4 Experimental design for experiment 1, 2 and 3.

Three experiments were designed to test effects of (1) light/far-red (Table 1), (2) elevated RH during night (Table 2), and elevated CO2 (table 3) on growth and incidence of tipburn (TB) under moderate light conditions. The experiment lasted for three weeks in moderate irradiance (150 µmolm^{-2}s^{-1}). This was then increased from 150 µmolm^{-2}s^{-1} to 300 µmolm^{-2}s^{-1} for a period of 1 week to study the resistance of the plants to increased irradiance.

3.4.1 Experiment 1: Effect of far-red light and high light on growth and tipburn severity

After pre-cultivation of lettuce for about 19 days as described previously, plants were transferred into the growth chambers under 2 different light sources: HPS and LED. For the

first 3 weeks in all 4 chambers, plants were exposed to a PPFD of 150 µmol m^{-2} s^{-1} with (1,1) or without far-red during the dark period of 6 hours. A total of 6hours of far-red was given when required. After the 3 weeks, the PPFD was elevated to 300 µmol m^{-2} s^{-1} in all the chambers lasting for 1 week after which the experiment ended. The RH was always kept at 70%. The other climate factors were kept the same in all 4 chambers (Table 1). Growth and tipburn sampling were done after 3 weeks of growing and after 1 week of high irradiance, as described below.

Table 1: Experimental climate set-up for experiment 1 with far-red light day extension.

Treatment	Lamp type	PAR, hrs	Day extension with FR	R/FR during day	Darkness, hrs	Temp, day	Temp, night	RH (%)
LED + FR	LED	18	YES, 6hrs	1.1	0	20°C	18°C	70
LED - FR	LED	18	NO	-	6	20°C	18°C	70
HPS + FR	HPS	18	YES, 6hrs	3.7	0	20°C	18°C	70
HPS - FR	HPS	18	NO	3.7	6	20°C	18°C	70

3.4.2 Experiment 2. Effect of high RH during night on growth and tipburn severity

After pre- cultivation, plants were transferred to the four chambers. Two of the chambers were given HPS as their light source and the remaining two haven LED. One chamber each from either light source received a rise in RH to 90% during the night period while the remaining 2 received the normal RH of 70% throughout the day. Plants in the LED were given external far-red with an R/FR of 1.1. Like the first experiment, plants are grown under 150µmol m^{-2} s^{-1} light for 3 weeks after which they were raised to 300µmol m^{-2} s^{-1} for 1 week. Samples were taken for assessment before and after increasing the light photon.

Table 2: experiment 2 treatment specification with emphasis on the difference in RH – relative humidity.

Treatment	Lamp type	PAR, hrs	Darkness	R/FR	Temp, day	Temp, night	RH during night (%)	RH during day (%)
LED/IRH	LED	18	6hrs	1.1	20°C	18°C	90	70
LED/NRH	LED	18	6hrs	1.1	20°C	18°C	70	70

HPS/IRH	HPS	18	6hrs	3.7	20℃	18℃	90	70
HPS/NRH	HPS	18	6hrs	3.7	20℃	18℃	70	70

3.4.3 Experiment 3, Effect of elevated CO_2 on growth and tipburn severity

After almost 3 weeks of pre-cultivation, lettuces were transferred to 4 chambers with different light sources and CO_2 concentrations (Table 3).

Table 3: details for treatment in experiment 3 with emphasis on CO_2 elevation in either one from the different light source.

Treatment	**Lamp type**	**PAR, hrs**	**R/FR**	**Dark hour**	**Temp, day**	**Temp, night**	**RH (%)**	**CO_2 (ppm)**
LED /ICO$_2$	LED	18	1.1	6	20℃	18℃	70	1000
LED/NCO$_2$	LED	18	1.1	6	20℃	18℃	70	400
HPS/ICO$_2$	HPS	18	3.6	6	20℃	18℃	70	1000
HPS/NCO$_2$	HPS	18	3.6	6	20℃	18℃	70	400

3.5 Nutrient Solution Mixture and Watering

To prepare the nutrient solution we used calcium nitrate (Yara, Norway), potassium nitrate, calcium chloride, pioneer basic cucumber and pioneer iron chelate, 6% EDDHA were used. The mixture is as shown below (Table x).

2 different stock solution were prepared to be used in the final solution in two different tanks. The tanks were filled each with 50 liters tap water with different measurements of the compounds added to the water. This mixture was thoroughly mixed for uniformity. Dilution mixture was done in a third tank filled to about 70 liters of tap water. From each of the stock, 50ml was taken and diluted into the third tank. This was done repeatedly until the E.C of the final mixture was 2.0. (Measured with an E.C meter (ScanGrow Conductivity meter, Denmark).

From the black reservoirs of nutrient solution kept inside the chamber, nutrient and water were delivered to the plants through an electrically aided pumps through the hose during the photoperiod. Lettuces received a total fertilizer solution of 110 – 130ml for 1minute every second hour during the photoperiod.

Fig. 9A: EC sensor meter for measuring nutrient solution electrical conductivity. Picture: Martin Knoop

Fig. 9B: Tank with diluted nutrient stock. Picture: Ellen Kusi

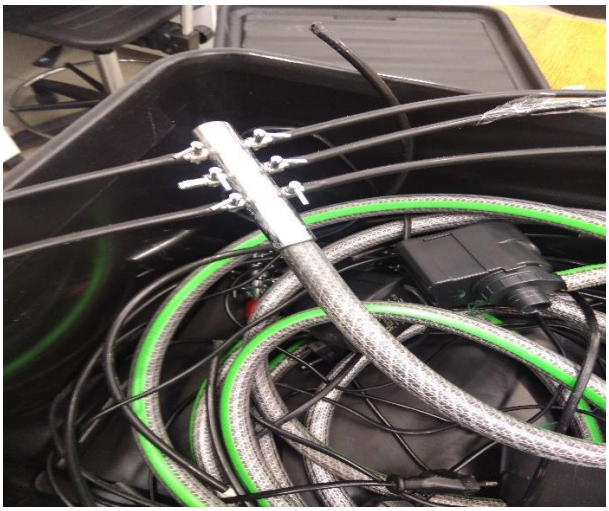


Fig 10C: Hose used in delivering nutrient solution. Picture: Ellen Kusi

Table 4: Recipe for the two nutrient stock solutions. The fertilizers were mixed in 50 L tap-water.

Stock A		**Stock B**	
	Amount		Amount
Calcium nitrate	2.5kg	Pioneer basic cucumber	3.125kg
Potassium nitrate	0.625kg	Pioneer Iron chelate, 6% EDDHA	0.025kg
Calcium chloride	0.15kg		

Sample of the final nutrient solution was taken for testing of nutrient content at Eurofins Agro Testing Norway AS. Components are as follows.

Table 5: Final actual nutrient solution content given to lettuce

		Cations ppm (mg/l)						
pH 5	EC (mS/cm 25°C 2.1	NH4 1.8	NH4-N 1.4	K 282	Na 32	Ca 148	Mg 29	
		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

3.6 Growth and tipburn Registration

Registrations were done after 3 weeks in moderate irradiance and after one week exposed to increased irradiance from 150µmol$m^{-2}s^{-1}$ to 300µmol$m^{-2}s^{-1}$. 10 lettuce plants that were randomly selected. Leaves were separated from the bunch and displayed on a table for better observation. This was done after the fresh weight (FW) had been weighed and recorded. Scale for levels of severity of tipburn range from 1 (less severe) to 5 (most severe) excluding the cotyledon and leaves <1cm (Appendix 2). All leaves were assessed based on the scale developed by the NLR (Norwegian Extension Service). Other morphological assessment on growth was also made included: number of leaves, length of the longest leaf, fresh and dry weight, water content and % water content.

3.7 Measurement of fresh weight (FW) and dry weight (DW)

The lettuces were harvested without the rooting part. The fresh and dry weight were measured on a balance (type). To measure the fresh weight (FW), excluding the root, the remaining shoot was placed on an electronic weighing balance and readings recorded (Fig. 11). This weight was marked as the fresh weight (FW). After assessment for tipburn, leaves were kept in a labelled envelope and dried in an oven at 62℃ for 7 days (Fig. 12A). An empty envelope just of the same size was also kept in the oven. The samples were then removed after a week, and dry weight (DW) measured. To do this, the dried empty envelope was first placed on the balance to check it weight then the scale was tarred. The dried samples were then placed on the scale and the weight recorded excluding the weight of the envelope. To determine the water content,

subtract the dry weight from the fresh weight then the % water content also calculated by dividing the water content value by the fresh weight then multiply by 100.

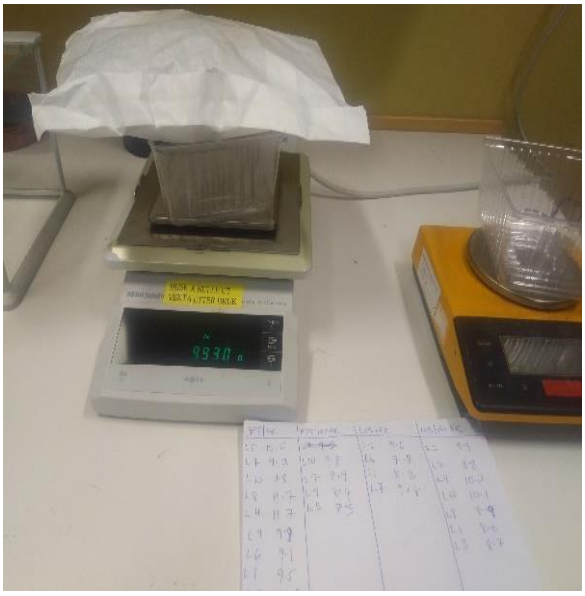


Fig. 11: Image showing how samples were weighed with accuracy of 0.01g. Picture: Ellen Kusi

Fig. 12A: Samples dried in oven under 62 ℃ for dry weight. Picture: Ellen Kusi

Fig. 12B: Samples dried in oven under 40 ℃ for nutrient analysis. Picture: Ellen Kusi

3.8 Nutrient Analysis Test

5 randomly selected lettuces were chosen from each treatment for nutrient analysis in experiment 1 and 3. In the case of experiment 2, 10 samples were taken. Leaves were selected from both inner and outer layer on all samples. These were kept in two different envelopes for drying. All samples taken for nutrient analysis was from moderate irradiance (150 µmolm-2s-1).

Per the arrangement of the leaves of the lettuce, 5 older leaves were taken from each selected sampled. The first 4 leaves were excluded due to some level of damage. Beginning from the 5th leaf to the 9th leaf number, these were selected as the 'source' leaf. From the innermost part of the lettuce, another 5 leaves were selected from the youngest leave above 1cm as the 'sink' leaves. All sampled leaves were placed in a well-labelled envelope that helps to differentiate the various treatment. The leaves were dried at 40°C between 7 – 10 days (Fig. 12B). Based on the size of the samples, a grinder (CYCLOTEC 1093 Sample mill by tecator) (Fig. 13) was used to ground the "source" leaves while a mortar and pistil was used ground the "sink" leaves (see fig). The ground samples were collected into a 50ml and 10ml labelled tubes then send to the LabTek laboratory (BioSci, NMBU) for test on nutrient elements, C, N, Ca, K and Mg (Fig 14). These measurements were done with ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) method (Greenfield, 1983)

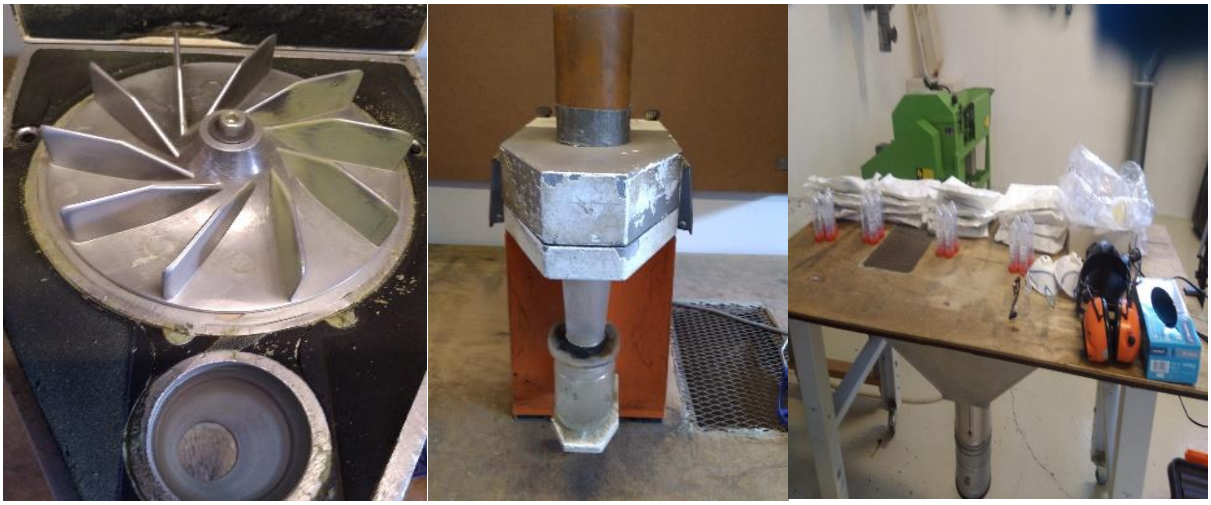


Fig. 13: Display of grinder used in grinding outer leaves samples. Picture: Ellen Kusi

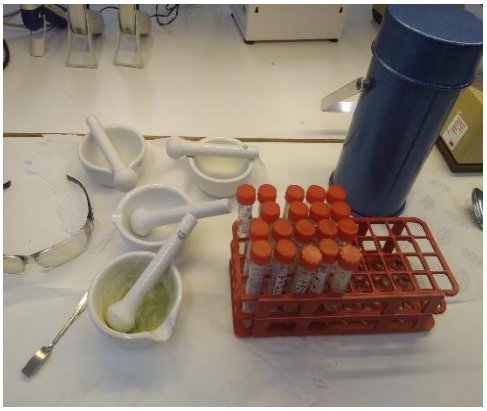


Fig. 14: Mortar and pistil used in grinding inn leaves samples

3.9 Antioxidant capacity – FRAP (Ferric Reducing Antioxidant Power)

3.9.1 Selection of samples

Three different parts of the lettuce plant were taken from each of the samples selected for analysis; source leaves, sink leaves and roots. In all treatments, 5 lettuce plant were randomly selected for sampling. To select for the "source" parts, the sixth fully expanded leaf from each 5 selected plants was taken. For "sink", 5 inner leaves from above 1cm were selected and part of the roots of each sample was also sampled. These selected parts were kept in a well labelled 50ml tube and immediately flash froze in liquid nitrogen to keep the freshness before storage in -80℃ freezer until usage.

3.9.2 Sample preparation method

After series of studies analysing antioxidant capacity through different methods including (DPPH)2,2 – diphenyl-1-picrylhydraoxyl and FRAP, a significant correlation has been established between these procedures and decision of a single method been enough was confirmed by (Clarke et al. 2013). Antioxidant power in whole leaflets was determined using an only the OxiSelect Ferric Reducing Antioxidant Power (FRAP) Asssay Kit (Cell Biolabs, Inc., CA, USA). Samples were ground in liquid nitrogen and 10mg weighed out into labelled Eppendorf tubes, then homogenised in 1 mL cold 1X assay buffer. The samples were then centrifuged at 12000rpm for 15minutes at 4℃ then the supernatant was collected into a new labelled Eppendorf tube. The samples were either tested immediately or stored at -80℃ for later use. The absorbance values of the reaction mixtures were measured unto a microplate reader (Biochrom Asys UVM 340 with KIM, UK) using 540nm as the primary wavelength. Each standard, sample and control were assayed in triplicate. Samples were measured against iron(II) standards. Results were converted to relative amounts.

3.10 Statistical data analysis

All results were documented and statistically analysed. The excel spread sheet was used to collate the raw data after which Minitab 19 windows version was used as statistical tool to statistically analyse the results, respectively. In Minitab 19, ANOVA one-way analysis was done on the various treatment followed by the Tukey's HSD *post hoc* test to separate the significantly different treatments. $p < 0.05$ were considered significantly different for these analyses. The variance analyses were performed on the morphological factors in all treatments to assess the impact and differences that were possibly exciting among them. Tipburn assessment was analysed with Minitab one-way analysis.

4 Results

4.1 Experiment 1

Experiment 1 was aimed at testing if far-red could influence the incidence and severity of tipburn under low irradiance and graduation to high irradiance under two different light sources while keeping temperature and relative air humidity from 18 – 20℃ and 70% respectively. Other plant physical growth parameters were also assessed.

Table 6A and B, displays the results for growth parameters from experiment 1. The data showed no significant differences between LED+FR and LED-FR treatments in all growth parameters in any of the irradiance levels (150 and 300 µmolm^{-2}s^{-1}) except for number of leaves at 300 µmolm^{-2}s^{-1} where the trend was that +FR increased the leaf number (p= 0.052, Table 6A). Plants exposed to LED+FR developed in average almost two more leaves than plants exposed to LED-FR. Table 1B, shows the data from experiment with HPS. A significantly higher differences (21.2% and 23.1%) was found for DW in HPS+FR under both moderate and high irradiance respectively compared to the other treatment. Under high irradiance, FW was found to be significantly higher for sink leaves (17.5% with p-value = 0.004). All other factors were higher but not significantly different under HPS+FR treatment.

Table 6A: Effect of far red (FR) light under low and high irradiance provided by LED on growth parameters of lettuce 'Frillice'. Data shows mean and standard deviation, for each parameter and treatment under the light source; LED at both 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1} irradiance with their p-values obtained from the ANOVA test. N = 10 in each treatment.

Treatment		Number of Leaves		Fresh weight, g		Dry weight, g		Water content (%)	
		150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}
LED+FR	Mean	14.30	20.70	92.54	160.42	9.50	9.06	89.58	94.33
	SD	0.95	1.57	10.83	16.82	0.97	0.928	1.76	0.51
LED-FR	Mean	14.50	19.00	91.54	175.03	9.73	9.25	89.34	94.65
	SD	0.85	2.05	10.96	27.33	0.77	0.78	1.60	0.52
P - value		0.626	0.052	0.840	0.167	0.764	0.626	0.756	0.188

Table 6B: Effect of far red (FR) light under low and high irradiance provided by HPS on growth parameters of lettuce 'Frillice'. Data displays the mean and standard deviation, for each parameters and treatment used under light source; HPS at both 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1} irradiance with their p-values obtained from the ANOVA test. N = 10 in each treatment.

Treatment		Number of Leaves		Fresh weight, g		Dry weight, g		Water content (%)	
		150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}
HPS+FR	Mean	15.20	22.00	96.49	194.76	11.15	10.76	88.29	94.46
	SD	0.63	1.70	12.42	22.46	1.31	1.23	1.76	0.48
HPS-FR	Mean	14.30	20.50	85.87	165.70	9.20	8.74	88.99	94.72
	SD	1.25	1.90	14.09	16.38	1.05	0.94	2.34	0.28
P - value		0.057	0.079	0.091	0.004	0.002	<0.001	0.463	0.153

Tipburn assessment

Severity score of 2 or less is considered not severe, score 3 is considered severe and that of 4 and 5 is very severe.

Fig 15 A and B, 16 A and B represent the tipburn assessment. The line graphs (Fig. 15A) give assessment of average individual leaf number in response to tipburn incidence and severity. Under moderate light intensity, treatments with additional far-red resulted in lower severity score of tipburn in outer /older leaves compared to those without. An almost zero(0 or <1) incidence and severity of tipburn was found in inner /younger leaves in all treatments. Under high irradiance, there appear to be a uniform respond to tipburn incidence and severity in all treatments (Figure 15B).

The barplot depicts tipburn assessment per averages of whole plant (all leaves) under both moderate and high irradiance. Within individual light irradiance, no significant differences exited among the treatments but between the two different light intensity (irradiance), there was found significant differences. HPS+FR and LED+FR recorded a significantly lower severity score.

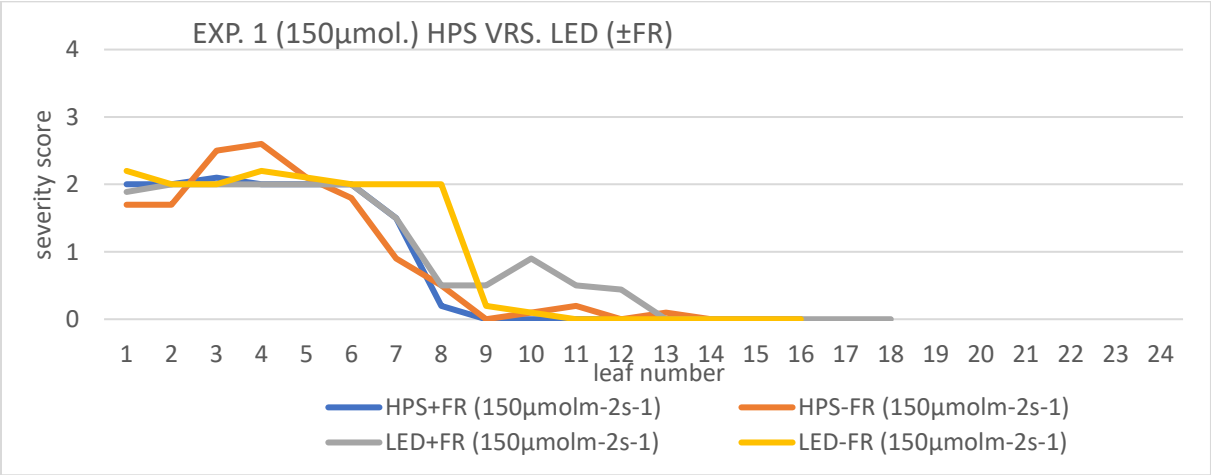


Fig 15A : Average tipburn severity score (0-5) on each leaf, where leaf 1 is the first outer leaf, in response to light source (LED and HPS) and FR light under moderate light intensity N=10

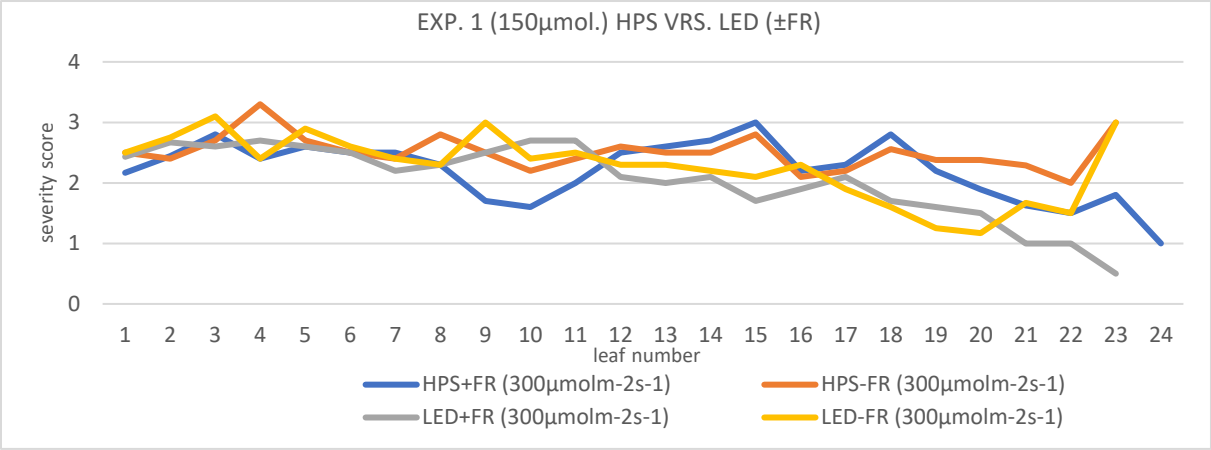


Fig 15B: Average tipburn severity score (0-5) on each leaf, where leaf 1 is the first outer leaf, in response to light source (LED and HPS) and FR light under high light intensity N=10.

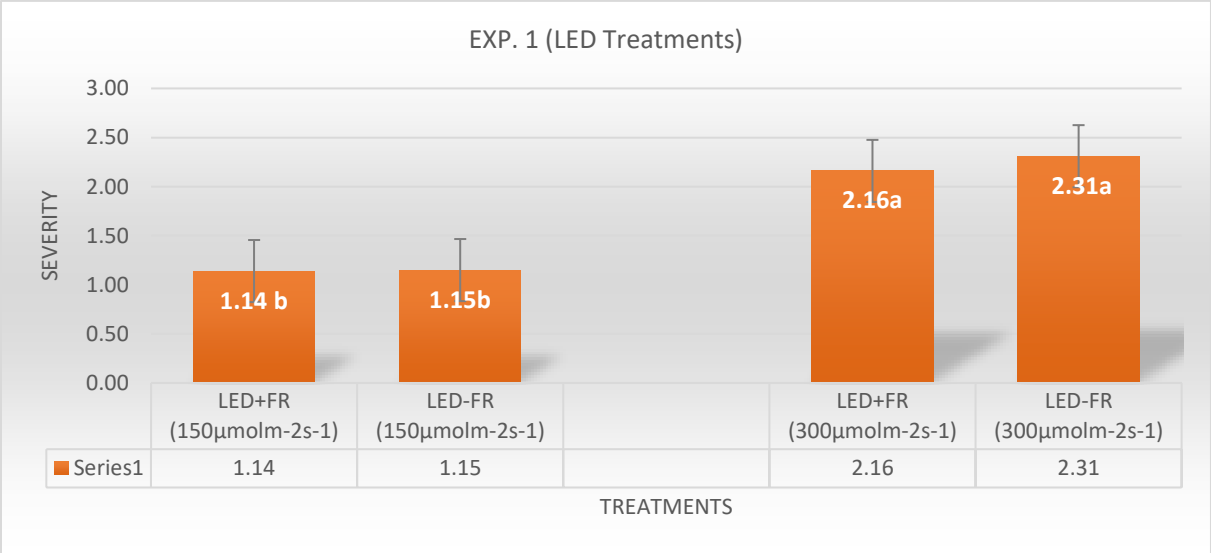


Fig 16A: A barplot of average severity score for tipburn for experiment 1, LED+FR and LED-FR treatment displaying both light irradiance of 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1}. A severity score of 2 or less is not severe, score 3 is severe and score 4 and 5 is very severe. N = 10 in each treatment.

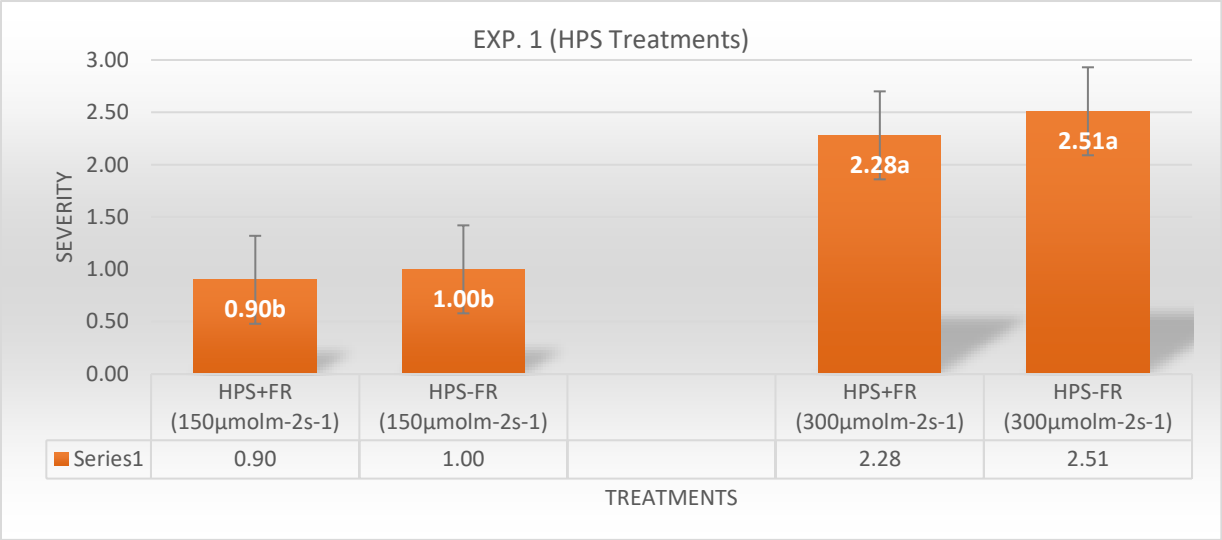


Fig 16B: A barplot of severity score for tipburn for experiment 1, HPS treatment displaying both light irradiance at 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1}. A severity score of 2 or less is not severe, score 3 is severe and score 4 and 5 is very severe. N = 10 in each treatment.

4.2 Experiment 2

The aim of this experiment was to assess how daily variation in RH by giving elevated relative air humidity (90%) during the dark period and moderate RH during day (70%) could influence on both tipburn occurrence and some physiological growth parameters of lettuce compared to constant RH (70%). Table 7A and 7B show the effects under LED and HPS at low and high irradiance

The level of RH under which plants were grown either had significant difference or not on both growth factors and tipburn incidence. No significant differences were found between number of leaves or dry weight for plants grown with LED/NRH and LED/IRH (Table 7A). Increased RH from 70% to 90% during the dark period at 18°C resulted in a significant increase in FW and % water content under high irradiances for FW and both moderate and high irradiance for % water content for LED treatments. A 15% increase in FW was recorded for plants under IRH treatment with a p-value 0.025 while % water content under IRH resulted 0.44% and 0.92% increase under both moderate and high irradiance respectively (Table 7A).

In the experiment with HPS, (Table 7B), small differences were found between NRH and IRH, except for DW at moderate irradiance that recorded a significant difference, and 20% higher DW for plants exposed to IRH was found compared to NRH.

Fig 17A and B, 18A and B give assessment on tipburn under elevated relative air humidity (IRH) and normal RH (NRH) under both light sources and light intensity. The line graph (Fig. 17A and B) provide detailed response of individual leaf number towards tipburn severity while (Fig. 18A and B) display for the average of the whole sample plant. A higher incidence of tipburn was found under NRH.

Table 7A: Effect of elevated RH (IRH) during the dark period compared to constant moderate RH (NRH) on growth parameters of lettuce 'Frillice' growing under low and high irradiance with LED as light source. ANOVA results showing the means and standard deviations with their p-value for growth parameters for the 2 treatments under LED source of light under both high and moderate irradiance. N = 10 in each treatment.

Treatment		Number of Leaves		Fresh weight, g		Dry weight, g		Water content (%)	
		150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}
LED/NRH	Mean	18.00	23.50	113.22	190.09	4.92	12.57	95.66	93.36
	SD	1.33	1.43	13.68	26.70	0.65	1.72	0.20	0.62
LED/IRH	Mean	17.90	24.00	118.78	218.84	4.66	12.56	96.08	94.22
	SD	1.287	1.70	19.65	26.06	0.92	1.14	0.42	0.58
P - value		0.866	0.486	0.472	0.025	0.476	0.988	0.011	0.005

Table 7B: Effect of elevated RH during night (IRH) compared to constant moderate RH (NRH) on growth parameters of lettuce 'Frillice' growing under low and high irradiance with HPS as light source. ANOVA results showing the means and standard deviations with their p-value for growth parameters for the 2 treatments under HPS source of light under both high and moderate irradiance. N = 10 in each treatment.

Treatment		Number of Leaves		Fresh weight, g		Dry weight, g		Water content (%)	
		150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}
HPS/NRH	Mean	20.10	26.80	89.73	178.50	4.58	12.28	94.85	92.99
	SD	1.29	1.32	15.45	37.90	0.78	1.81	0.65	0.87
HPS/IRH	Mean	20.20	26.40	102.96	164.20	5.53	12.16	94.61	92.33
	SD	1.08	1.77	13.08	40.00	0.81	1.93	054	1.68
P - value		0.461	0.574	0.054	0.421	0.015	0.887	0.388	0.282

Tipburn assessment

All treatments without elevation in RH under both LED and HPS resulted in higher severity score for tipburn. Increase of RH from 70% to 90% at 18°C had a positive impact in reducing the severity of tipburn. At moderate irradiance (150µmolm^{-2}s^{-1}), individual leaf numbers from the line graph (Fig 17A and B) showed almost zero incidence and severity of tipburn and even if it occurred it was considered not severe since it scored <1 under IRH for inner leaves. With older leaves however, even though incidence and severity existed in all treatments under both LED and HPS, treatments with IRH is on the low. High irradiance showed an increase in tipburn in incidence and severity. Even under high irradiance, LED treatment with IRH still recorded zero incidence for inner leaves. The bar plots (Fig. 18A and B) showed the level of significance within individual irradiance and between both irradiance under RH treatments.

Under moderate irradiance for both LED and HPS, there exist no significant difference for both treatments at 'normal RH and elevated RH'. Significant difference was however recorded for both LED and HPS at high irradiance for RH. A % decrease of 37.6% and 16.2% in severity under LED and HPS, respectively.

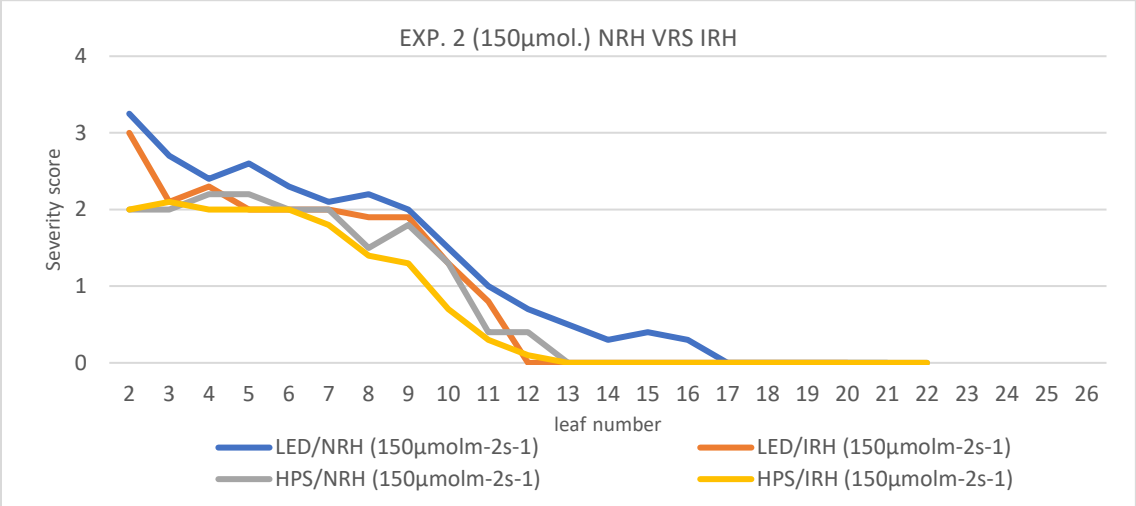


Fig 17A: Average tipburn severity score (0-5) on each leaf, where leaf 1 is the first outer leaf, in response to light source (LED and HPS) and RH under moderate light intensity N=10.

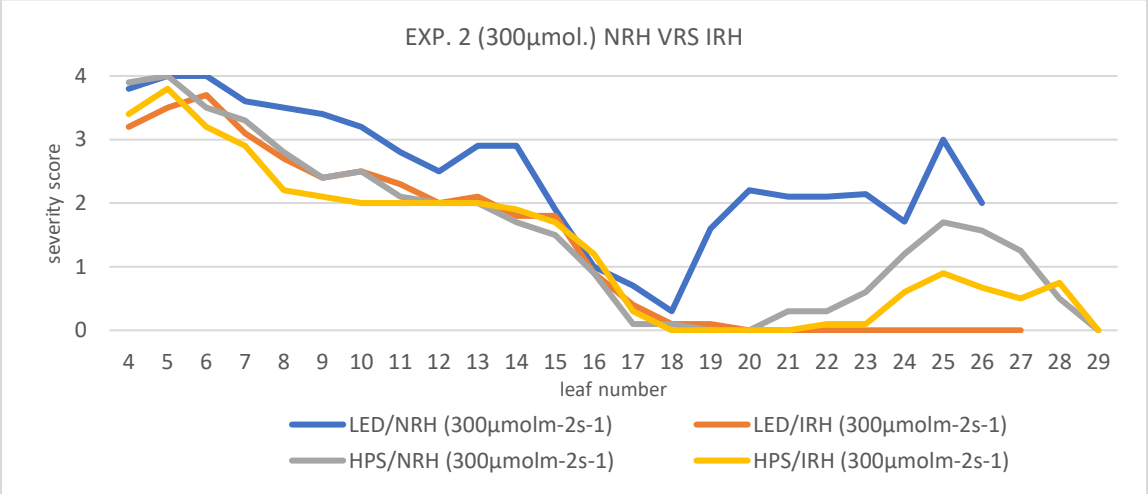


Fig 17B: Average tipburn severity score (0-5) on each leaf, where leaf 1 is the first outer leaf, in response to light source (LED and HPS) and RH under high light intensity N=10.

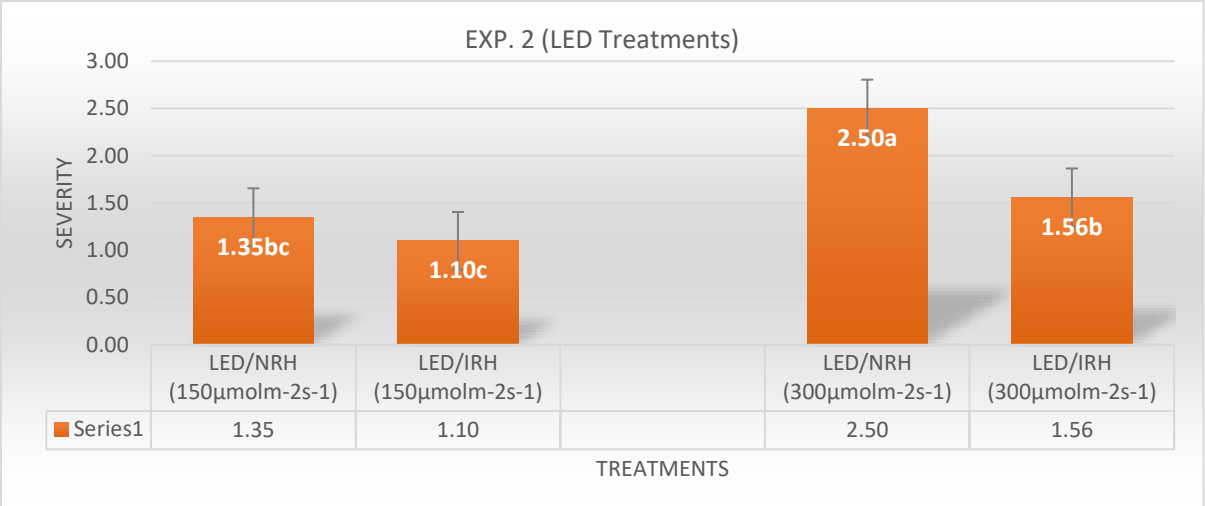


FIG 18A: A barplot of average severity score for tipburn for experiment 2, LED treatment displaying both light irradiance of 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1}. A severity score of 2 or less is not severe, score 3 is severe and score 4 and 5 is very severe. N = 10 in each treatment.

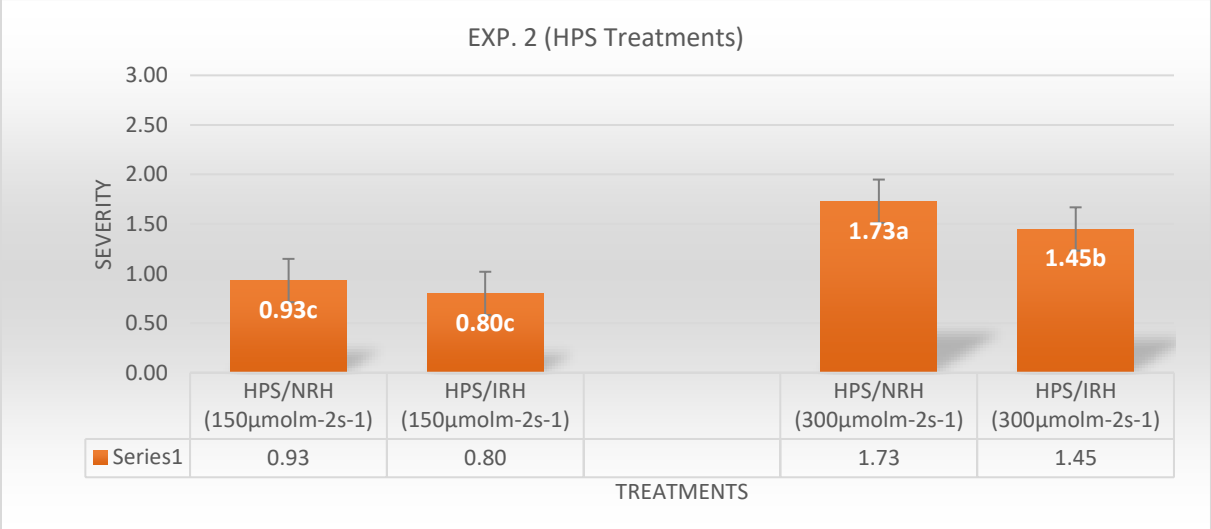


FIG 18B: A barplot of severity score for tipburn for experiment 2, HPS treatment displaying both light irradiance of 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1}. A severity score of 2 or less is not severe, score 3 is severe and score 4 and 5 is very severe. N = 10 in each treatment.

4.3 Experiment 3: Effect of elevated CO_2

The aim of this experiment was to assess how elevated CO_2 would impact on the occurrence of tipburn and influence on growth factors using an ambient CO_2 (400ppm) and elevated CO_2 (1000 ppm) of CO_2.

Table 3A and B show results for growth factors as influenced by elevated CO_2. In table 8A, while there were no significant differences between ambient and elevated CO_2 in all growth factors for treatments of LED under both moderate and high irradiance, all growth factors under HPS treatments were significantly different from each other except for FW at high

irradiance(Table: 8B). Elevated CO_2 in LED treatments resulted lower values in growth factors per their mean value except for DW while HPS treatments resulted an increase in FW and DW with decrease in number of leaves and % water content in elevated CO_2 compared to ambient.

Number of leaves were higher in HPS/NCO_2 6.8% (at p-value =0.012 for moderate irradiance) and 6.9% (at p-value =0.018 for high irradiance) compared to HPS/ICO_2 under both light intensity (Table: 8B), but the other parameters showed opposite effects. FW and DW increased by (14.0% and 5.8%) and (29.2% and 20.3%) under moderate and high intensity in response to elevated CO_2, respectively.

Fig 19A and B, 20A and B represent results for tipburn severity under both ambient/normal CO_2 (400ppm) and elevated CO_2 (1000ppm)under both light source at moderate and high intensity. Line graph gives details on individual leaf response to tipburn and the barplot concerns with whole plant averages.

Table 8A: Effect of elevated CO_2 (ICO_2) compared to constant moderate CO_2 (NCO_2) on growth parameters of lettuce 'Frillice' growing under low and high irradiance with LED as light source ....ANOVA results showing the means and standard deviations with their p-value for growth parameters for the 2 treatments under LED source of light under both high and moderate irradiance. N = 10 in each treatment.

Treatment		Number of Leaves		Fresh weight, g		Dry weight, g		Water content (%)	
		150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}
LED/NCO_2	Mean	25.00	29.50	186.92	278.7	7.74	14.37	95.86	94.83
	SD	1.63	1.84	28.13	31.90	1.24	1.36	0.24	0.25
LED/ICO_2	Mean	21.10	29.30	183.51	261.7	7.90	15.11	95.69	94.13
	SD	1.79	1.70	18.44	36.6	0.95	1.85	0.26	1.129
P - value		0.256	0.804	0.752	0.281	0.749	0.322	0.176	0.072

Table 8B: Effect of elevated CO_2 (ICO_2) compared to constant moderate CO_2 (NCO_2) on growth parameters of lettuce 'Frillice' growing under low and high irradiance with HPS as light source ....ANOVA results showing the means and standard deviations with their p-value for growth parameters for the 2 treatments under HPS source of light under both high and moderate irradiance. N = 10 in each treatment.

Treatment		Number of Leaves		Fresh weight, g		Dry weight, g		Water content (%)	
		150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}	150µmolm^{-2}s^{-1}	300µmolm^{-2}s^{-1}
HPS/NCO_2	Mean	26.60	33.70	156.03	255.4	5.93	13.08	96.20	94.85
	SD	1.65	2.00	13.64	34.10	0.62	1.84	0.16	0.61
HPS/ICO_2	Mean	24.90	31.50	177.94	270.30	7.66	15.73	95.69	94.17
	SD	0.99	1.78	20.01	33.20	0.95	2.37	0.25	0.53
P - value		0.012	0.018	0.010	0.333	<0.001	0.012	<0.001	0.016

Tipburn assessment

In both line graph, (fig 19A and B), treatments with elevated CO_2 showed reduced tipburn severity in older leaves. The opposite could be said for inner leaves. At moderate irradiance, elevated CO_2 caused an increase in LED/ICO$_2$ while the both treatments under elevated CO_2 at high intensity also had an increase. The barplots under LED treatments (Fig 20A) resulted no significance within moderate light intensity for CO_2 but within treatments under high intensity there was significance. A significantly lower severity of 24% for LED/ICO$_2$ was recorded under high irradiance. A p- value <0.001 was recorded between moderate and high intensity for CO_2 for tipburn severity. In HPS treatments, no significance was recorded under either moderate or high light intensity independently for CO_2 however between the two different light intensity, significant level of p-value <0.001 was attained.

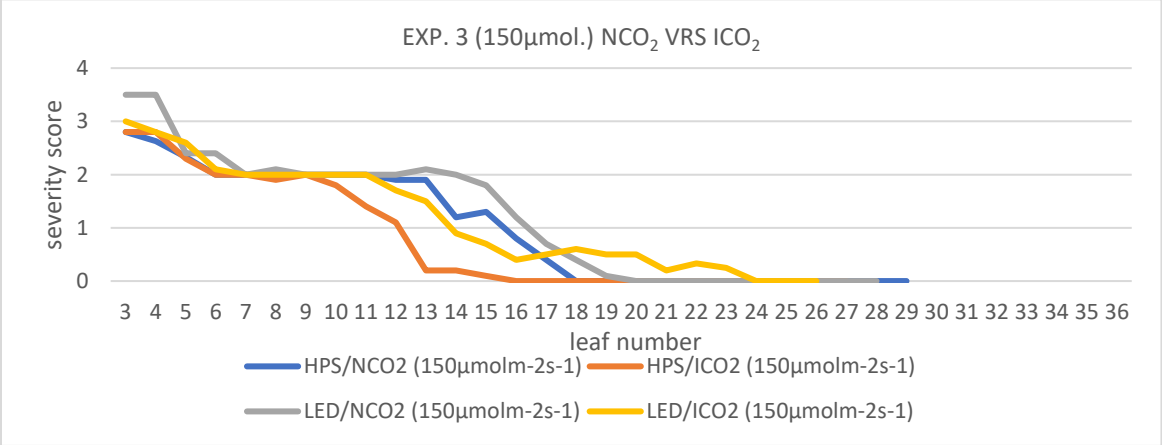


Fig 19A: Average tipburn severity score (0-5) on each leaf, where leaf 1 is the first outer leaf, in response to light source (LED and HPS) and CO_2 under moderate light intensity N=10

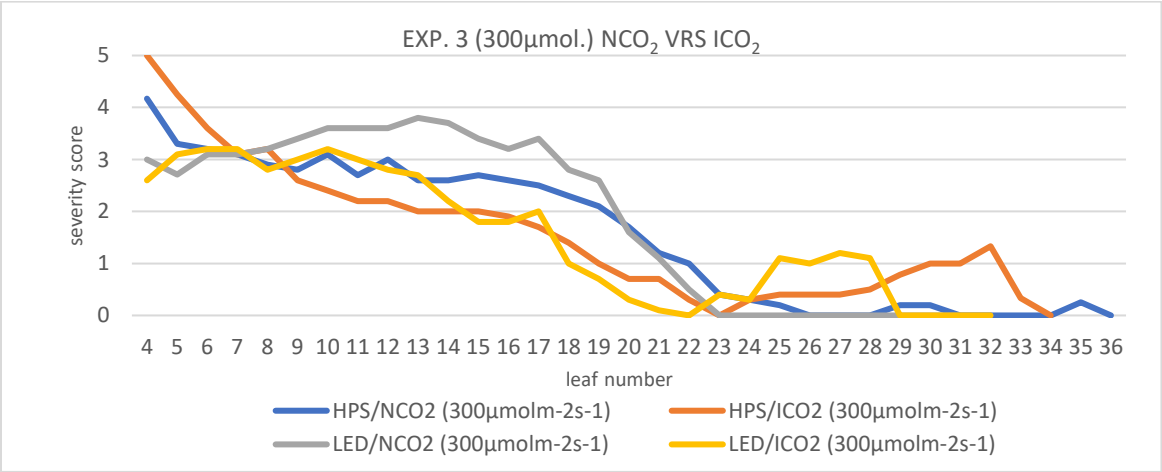


Fig 19B: Average tipburn severity score (0-5) on each leaf, where leaf 1 is the first outer leaf, in response to light source (LED and HPS) and CO_2 under HIGH light intensity N=10

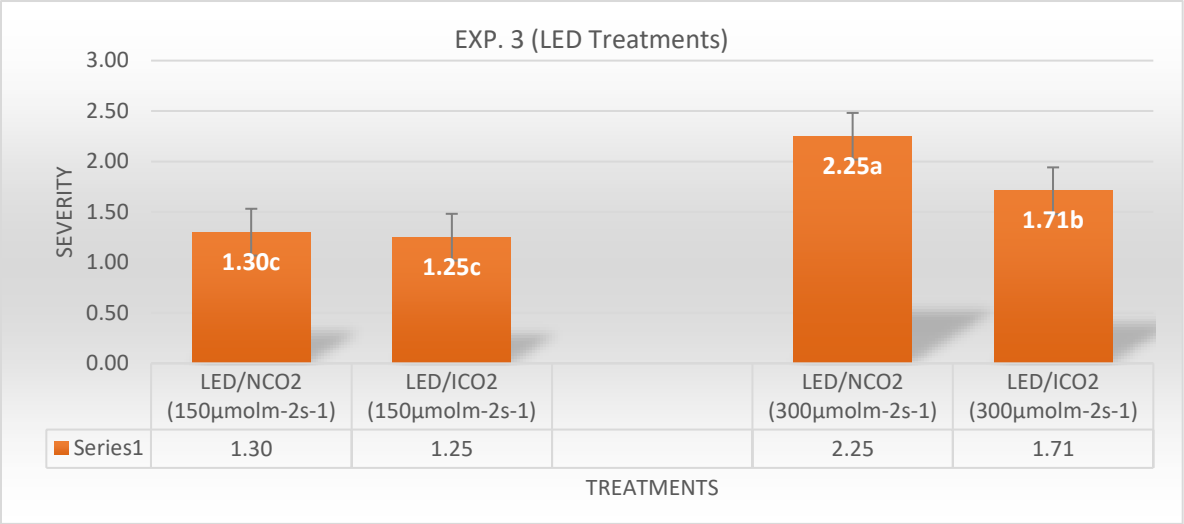


Fig 20A: A barplot of severity score for tipburn for experiment 3, LED treatment displaying both light irradiance of 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1}. A severity score of 2 or less is not severe, score 3 is severe and score 4 and 5 is very severe.

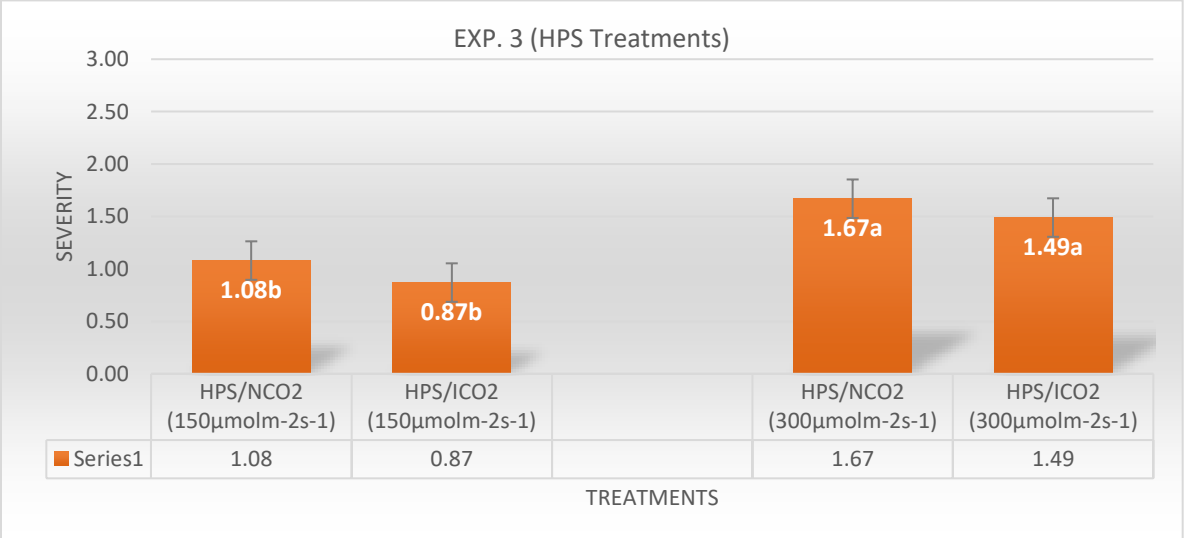


Fig 20B: A barplot of severity score for tipburn for experiment 3, HPS treatment displaying both light irradiance of 150µmolm^{-2}s^{-1} and 300µmolm^{-2}s^{-1}. A severity score of 2 or less is not severe, score 3 is severe and score 4 and 5 is very severe.

4.4 Nutrient Analysis Result

All three experiments at moderate irradiance were analysed for nutrients like nitrogen (N), carbon (C), calcium (Ca), potassium (P) and magnesium (Mg) in both source and sink leaves. The reason why only moderate irradiance plants were analysed was that this is the common irradiance to use in a commercial greenhouse during wintertime.

In table 9A and B, which correspond to EXP 1, there was a significant difference seen in the level of nitrogen for both source and sink leaves in HPS treatments. A significant lower content

of N was found in sink (11.9% with p-value <0.001) and source (8.7% with p-value = 0.004) leaves of plants exposed to HPS+FR compared to the other treatment. In source leaves a significantly higher K (6.5% with p-value= 0.014) was found in plants exposed to HPS+FR. Calcium content in both source and sink leaves of HPS+FR was seen to be only significantly higher than plants exposed to HPS-FR and not significantly different. The remaining elements under HPS treatment were all not significant. A significantly lower K and significantly higher C was found in sink leaves of plants exposed to LED+FR compared to the others.

For source leaves, the lowest N content was also found in plants exposed to HPS+FR but it was only significantly lower than plants exposed to HPS-FR, and not significantly difference from any of the LED treatments. For Mg while LED+FR recorded higher, HPS+FR recorded lower in response to addition of far-red.

In table 10A and B, corresponding to EXP 2 (elevated RH), A significant higher content of N and Mg was found in sink and source leaves of plants exposed to LED+FR (table 4A)compared to the other treatment. Ca and K in source leaves were also found to be significantly higher (p-value 0.027 and 0.013respectively) in LED/IRH compared to the other treatment but C was significantly lower (2.5% with p-value=0.009) in the sink leaves compared to the others. In HPS treatments, a significantly higher (15.4% and 11.4%)content of Mg was found in both source and sink leaves, respectively(table 10). There was no significance found in K between the 2 treatments. In source leaves, Ca was found to be significantly higher (8.7%) in HPS/IRH but C was rather significantly lower (4.1%) in the same treatment. N was found to be significantly higher in the sink leaves in HPS/IRH compared to the others.

In table 11A and B, representing EXP 3 (Elevated CO_2), C, Ca, and Mg were all found to be significantly different in both source and sink under LED treatments. A significant higher content of C was found in sink(4.9%) and source(5.1%) leaves of plants exposed to LED/ICO_2 compared to the other treatment(table xx). Ca and Mg were also found to be significantly lower in both source(16.9% and 16.5%) and sink(13.5% and 14.2%) leaves, respectively. K and N were however not significantly different. In HPS treatments, all elements recorded no significant differences except for N and Mg that was found to be significantly different in sink leaves compared to the other treatment. Both were significantly lower for HPS/ICO_2.

Table 9A : Effect of far red (FR) light under low irradiance on nutrient content of Lactuca sativa L under LED lamp. ANOVA results showing the mean, standard deviation and p – values for calcium (Ca), potassium (K),magnesium (Mg), nitrogen (N) and carbon (C) levels at 150µmolm^{-2}s^{-1} in both source and sink leaves for all treatment at dry weight at 95% confidence level. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		N%		C%		Ca mg/g		K mg/g		Mg mg/g	
		Source	Sink	Source	Sink	Source	Sink	Source	Sink	Source	Sink
LED+FR	Mean	4.46 A	5.76 A	31.72 A	37.08 A	9.81 A	3.14 A	123.41 A	78.56 B	3.35 A	2.22 A
	SD	0.24	0.30	0.65	0.37	0.98	0.44	7.67	5.07	0.45	0.19
LED-FR	Mean	4.38 A	5.72 A	32.32 A	36.18 B	9.49 A	3.23 A	119.33 A	85.99 A	3.27 A	2.22 A
	SD	0.28	0.57	0.64	0.19	0.19	0.29	9.44	1.13	0.18	0.13
P - value		0.648	0.894	0.176	0.001	0.501	0.454	0.474	0.013	0.721	0.843

Table 9B: Effect of far red (FR) light under low irradiance on nutrient content of Lactuca sativa L under HPS lamp. ANOVA results showing the mean, standard deviation and p – values for calcium (Ca), potassium (K),magnesium (Mg), nitrogen (N) and carbon (C) levels at 150µmolm^{-2}s^{-1} in both source and sink leaves for all treatment at dry weight at 95% confidence level. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		N%		C%		Ca mg/g		K mg/g		Mg mg/g	
		Source	Sink	Source	Sink	Source	Sink	Source	Sink	Source	Sink
HPS+FR	Mean	4.29 B	4.99 B	31.30 A	36.34 A	9.84 A	3.50 A	128.61 A	81.39 A	3.45 A	2.29 A
	SD	0.09	0.24	0.45	0.49	0.45	0.35	2.96	4.22	0.08	0.07
HPS-FR	Mean	4.70 A	5.67 A	31.58 A	36.27 A	9.38 A	3.29 A	120.67 B	86.77 A	3.61 A	2.42 A
	SD	0.21	0.08	0.77	0.58	0.85	0.55	4.87	4.82	0.31	0.18
P - value		0.004	<0.001	0.508	0.843	0.322	0.502	0.014	0.097	0.310	0.161

Table 10A: Effect of elevated relative air humidity under low irradiance on nutrient content of Lactuca sativa L. under LED lamp. ANOVA results showing the mean, standard deviation and p – values for calcium (Ca), potassium (K),magnesium (Mg), nitrogen (N) and carbon (C) levels at 150µmolm^{-2}s^{-1} in both source and sink leaves for all treatment at dry weight at 95% confidence level. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		N%		C%		Ca mg/g		K mg/g		Mg mg/g	
		Source	Sink	Source	Sink	Source	Sink	Source	Sink	Source	Sink
LED/NRH	Mean	4.93 B	5.05 B	30.87 A	35.68 A	10.92 B	3.14 A	140.31 B	90.77 A	3.65 B	2.26 B
	SD	0.29	0.30	1.48	0.68	1.68	0.34	10.84	7.00	0.57	0.17
LED/IRH	Mean	5.31 A	5.34 A	29.64 B	34.78 B	12.30 A	3.41 A	153.17 A	96.82 A	4.23 A	2.51 A
	SD	0.30	0.28	1.24	0.71	0.67	0.47	9.96	8.30	0.41	0.26
P - value		0.010	0.041	0.060	0.009	0.027	0.151	0.013	0.095	0.018	0.019

Table 10B: Effect of elevated relative air humidity under low irradiance on nutrient content of Lactuca sativa L. under HPS lamp. ANOVA results showing the mean, standard deviation and p – values for calcium (Ca), potassium (K),magnesium (Mg), nitrogen (N) and carbon (C) levels at 150µmolm^{-2}s^{-1} in both source and sink leaves for all treatment at dry weight at 95% confidence level. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		N%		C%		Ca mg/g		K mg/g		Mg mg/g	
		Source	Sink	Source	Sink	Source	Sink	Source	Sink	Source	Sink
HPS/NRH	Mean	4.57 A	5.03 B	32.99 A	38.46 A	11.48 B	3.61 A	132.73 A	70.33 B	3.88 B	2.29 B
	SD	0.33	0.27	1.48	0.69	0.71	0.32	8.78	10.04	0.32	0.19
HPS/IRH	Mean	4.67 A	5.35 A	31.64 B	38.37 A	12.48 A	3.77 A	137.35 A	64.73 B	4.48 A	2.55 A
	SD	0.29	0.31	1.21	0.65	0.98	0.48	8.95	8.55	0.33	0.27
P - value		0.460	0.023	0.025	0.784	0.018	0.387	0.259	0.197	0.001	0.025

Table 11A: Effect of elevated CO_2 light under low irradiance on nutrient content of Lactuca sativa L. under LED lamp. ANOVA results showing the mean, standard deviation and p – values for calcium (Ca), potassium (K),magnesium (Mg), nitrogen (N) and carbon (C) levels at 150µmolm^{-2}s^{-1} in both source and sink leaves for all treatment at dry weight at 95% confidence level. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		N %		C %		Ca mg/g		K mg/g		Mg mg/g	
		Source	Sink	Source	Sink	Source	Sink	Source	Sink	Source	Sink
LED/NCO_2	Mean	3.99 A	4.89 A	30.20 B	36.22 B	14.74 A	3.11 A	146.67 A	86.73 A	4.79 A	2.25 A
	SD	0.37	0.17	0.82	1.13	1.28	0.25	8.66	13.73	0.27	0.17
LED/ICO_2	Mean	3.76 A	4.89 A	31.74 A	37.98 A	12.25 B	2.69 B	137.07 A	76.93 A	4.00 B	1.93 B
	SD	0.58	0.18	1.21	1.19	1.85	0.24	7.29	7.00	0.70	0.11
P - value		0.481	0.986	0.046	0.043	0.039	0.031	0.094	0.193	0.045	0.007

Table 11B: Effect of elevated CO_2 light under low irradiance on nutrient content of Lactuca sativa L. under HPS lamp. ANOVA results showing the mean, standard deviation and p – values for calcium (Ca), potassium (K),magnesium (Mg), nitrogen (N) and carbon (C) levels at 150µmolm^{-2}s^{-1} in both source and sink leaves for all treatment at dry weight at 95% confidence level. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		N %		C %		Ca mg/g		K mg/g		Mg mg/g	
		Source	Sink	Source	Sink	Source	Sink	Source	Sink	Source	Sink
HPS/NCO_2	Mean	3.86 A	5.17 A	31.49 A	36.34 A	13.22 A	3.54 A	142.32 A	94.15 A	4.57 A	2.49 A
	SD	0.47	0.46	1.49	1.36	0.75	0.69	11.95	15.33	0.36	0.36
HPS/ICO_2	Mean	3.36 A	4.69 A	31.38 A	37.14 A	12.65 A	3.17 A	144.10 A	79.20 A	4.50 A	2.06 B
	SD	0.79	0.28	2.03	1.85	0.83	0.71	15.81	17.06	0.19	0.18
P - value		0.253	0.084	0.922	0.459	0.285	0.428	0.846	0.183	0.692	0.044

4.5 Antioxidant capacity (FRAP) results

The FRAP analysis was done only for experiment 2 (factor: relative air humidity) at 150µmolm^{-2}s^{-1} since these treatments showed the largest difference in tipburn (Fig X and Table X). The results showed significant difference in antioxidant levels only in the sink leaves among the treatments exposed to LED (Table 12A) and HPS (Table 12B). A higher antioxidant capacity was found in sink leaves exposed to IRH compared to NRH under LED. When exposed to HPS, an opposite effect was observed in sink leaves and the leaves exposed to NRH had significantly

higher FRAP values than IRH (Table 12B). Antioxidant level in source leaves or roots were not significantly affected by RH. However, the FRAP values in source leaves was in general higher compared to sink leaves (Table 12A and 12B).

Table 12A: Effect of elevated relative air humidity (IRH) compared with constant RH (NRH) under low irradiance (150 µmol m-2 s-1) on antioxidant capacity (FRAP) of Lactuca sativa L. under LED lamp. ANOVA results showing the mean, standard deviation, and p-value for level of antioxidant in source and sink leaves and the roots of lettuce at 150µmol$m^{-2}s^{-1}$. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		Source leaf, µM	Sink leaf, µM	Root, µM
LED/NRH	Mean	9.66 A	2.21 B	5.28 A
	SD	6.21	0.82	3.54
LED/IRH	Mean	6.75 A	5.09 A	9.54 A
	SD	3.03	1.99	8.17
P - value		0.374	0.018	0.317

Table 12B: Effect of elevated relative air humidity (IRH) compared with constant RH (NRH) under low irradiance (150 µmol m-2 s-1) on antioxidant capacity (FRAP) of Lactuca sativa L. under HPS lamp. ANOVA results showing the mean, standard deviation, and p-value for level of antioxidant in source and sink leaves and the roots of lettuce at 150µmol$m^{-2}s^{-1}$. A turkey test was used to separate the means values (means in the columns that do not share a letter are significantly different). N = 10 in each treatment.

Treatment		Source leaf, µM	Sink leaf, µM	Root, µM
HPS/NRH	Mean	11.27 A	7.44 A	15.19 A
	SD	6.33	3.09	6.48
HPS/IRH	Mean	4.93 A	2.70 B	13.88 A
	SD	1.85	2.25	9.16
P - value		0.064	0.024	0.800

5.0 Discussion

5.1 Light intensity

High light intensity is an especially important climate factor to initiate tipburn (Saure, 1998). More tipburn was observed in all experiments under high irradiance compared to moderate irradiance. Sago, (2016) observed a significantly linear increase for both FW and DW as light intensity increased. The same was found in the present experiments (Table 6A and B). The linear increase in FW and number of leaves could be attributed to the increase in photosynthetic rate caused by high light intensity. Tani et al., (2014) gave a general comment on the fact that

lettuce leaves exhibit a linear increase of net photosynthetic rate at a low PPFD (<200µmolm^{-2}s^{-1}) then rises concurrently until saturated PPFD level ≈500µmolm^{-2}s^{-1}. From most researchers (Collier and Tibbits, 1982; Wissemeier,1996), a proportional increase in growth rate and tipburn with respect to increase in light intensity was observed, which complement the findings of this experiment. Damage to the photosynthetic apparatus due to high light energy results in accumulation of ROS mostly leading to cellular injury or damage (Carassay et al. 2012). Irrespective of the treatment given, tipburn occurred in all experiments but under moderate light tipburn was concentrated mostly on the first 8 outer leaves with the highest severity mean score of <2 which is considered not severe in commercial production. Even though severity and incidence increased with increased PPFD in both light qualities, at moderate PPFD, LED treatments showed significantly higher score for tipburn compared to HPS treatments. However, under increased light intensity, plants exposed to HPS showed a higher severity score compared to LED. The spectral distribution of the white LED used in the experiment is more like natural sunlight compared to HPS. Hence, it is possible that lettuce plants tolerate higher irradiance in a more natural light specter like white LED. During summer in Norway, when the natural solar radiation is high, less tipburn is normally found compared to production in winter (pers. com. Espedal Norwegian grower). A comparison between field and greenhouse grown lettuce showed a highly significant correlation in the field trial between tipburn and total radiation received throughout the entire growing period (Wissemeier, 1996). There is earlier and higher sensitivity (intensity and occurrence) to tipburn than that on the field although only part of the total amount of radiation is receive in the greenhouse with almost absence of UV (Cox & McKee, 1976), (Barta & Tibbitts, 1991a). Clearly high light exposure does not generally result in tipburn injury and low light intensity does not ensure lack of tipburn. It could be said that other factors aside light intensity contributes to the occurrence of tipburn in greenhouse production. However, more inner tipburn seems to be more common in high light intensity but outer tipburn occurs in both moderate and high light intensity.

5.2 Additional far-red

The addition of far-red generally resulted in a slight reduction but not significantly different severity score in LED and HPS treatments under the two different light intensity (Fig 16A and B). Hence, Far-red light seems not to have a strong effect on tipburn in this study but a trend against a lower severity score was seen under both LED and HPS treatment compared to treatments without far-red (Fig 16A and B). This could possibly be related to a better transpiration in plants exposed to additional far-red leading to the slight increase in their

calcium levels in source leaves but not in the case of the sink leaves under LED+FR. Sink leaves in LED+FR from the line graph (Fig 15A) appear to have had some incidence under moderate light where sample were taken for nutrient analysis which could explain the low level of calcium compared to LED-FR. Comparatively, between LED and HPS treatments, LED+FR recorded a higher (26.7%) severity in tipburn than in HPS+FR under moderate irradiance(Fig 16A and B). However, under high irradiance, the opposite is seen where rather HPS with far-red increased in tipburn incidence and severity compared to LED+FR. This finding is in line with findings by (Knoop, 2019). Plants exposed to far-red light are known to better elongate allowing proper absorption of light even to inner leaves. Sarlikioti et al. (2011) estimated a rise in total light absorption upon internode length increase. The full benefit of the far-red may have been restricted by small experimental compartments where some light may have been lost to the side walls.

The impact of far-red on plant growth factors is mostly seen increase in leaf size, improvement in the absorption of light and a potential increase in growth. The DW, FW clearly significantly increased with additional far-red in HPS treatment (Table 6B) except for FW under moderate irradiance which had an increase but not significant. This can be related to increase in the number of leaves and increase leaf area as influence by a lower R/FR. Factors under LED treatment showed no significant differences but rather a linear increase in number of leaves and FW with different irradiance. The increase in these growth factors can as well be attributed to increase in photosynthesis under high light intensity.

5.3 Elevated relative air humidity during night

Several research papers have suggested a positive correlation between the incidence of tipburn and high ambient humidity in lettuce (Bottenberg and Tibbits 1968; Barta and Tibbits, 1986; Saure, 1998). In a screening experiment for susceptibility of new cultivars, Nagata and Stratton combined high temperature and high RH to induce tipburn, (Nagata and Stratton, 1994). In general, high RH is suggested to affect tipburn incidence because high RH can cause potential increase in growth. Tibbits and Bottenberg, (1976) recorded a drastic increase in growth rate of lettuce grown under high RH of 85% compared to that of 50% RH. Comparative discussions have been found with respect of the impact of high RH during the night. However, High RH and reduced transpiration during the night constrained tipburn frequency in cabbage and lettuce (Palzkill et al. 1976; Collier & Tibbitts, 1984).

However, in experiment 2, in the present study, no significant difference in growth (leaf number, FW and DW) was found at moderate light intensity (150 $\mu molm^{-2}s^{-1}$) provided by LED (Table 7A). At increased light intensity a significant effect was found in FW. However, water content (%) increased significantly in both moderate and high irradiance when the RH was high during the dark period. This shows that high RH during night has a neglectable effect on growth but more important for water content. On the other hand, when HPS was used as the light source an effect was found on DW at moderate irradiance indicating that the light source is important for the growth response to high night RH (Table: 7B). Hence, a change in growth rate influenced by high RH as recorded by (Tibbits and Bottenberg, 1976), was only seen with HPS as light source. It is important to point out that the increased RH is the present experiment lasted only for 6 hr. In the work of Tibbits and Bottenberg (1976) the increased RH was given throughout the day.

In the case of tipburn, plants that received a raise in RH from 70% to 90% in the dark period (6 hours) showed significant reduced incidence of tipburn compared to those that had constant amount of RH 70% under both light intensity. From the line graph, a total absence of inner tipburn was observed at moderate light intensity especially for those treatments with increased RH (Fig 17A). Even though a rise in severity was observed in older (outer) leaves tipburn per rise in light intensity, treatments with raised RH showed reduced severity (Fig 17A and B). Increased RH during night is believed to increase root pressure and hence calcium uptake in plants (Collier & Tibbits, 1984). Furthermore, older mature leaves have a better chance to accumulate more calcium due to their ability to transpire more while younger inner leaves have lower calcium content since transpiration is almost absent (Saure 1998) which was confirmed in the nutrient analysis test (Table 10A and B). This makes inner or younger leaves more susceptibility to tipburn incidence than in outer and older leaves (Koike and Smith, 2010). However, Ca accumulation is normally higher in the leaf blade rather than the tip (Barta and Tibbits, 2000), making the tip more susceptible to calcium deficiency symptoms. For these reasons, one would expect to see more tipburn incidence in the inner leaves rather than the outer but that was not the case for this experiment. Rather, an almost zero incidence of inner tipburn was recorded under both high and moderate irradiance if the RH was increased during night. The nutrient analysis showed much higher calcium content in outer leaves compared to inner leaves under both LED and HPS (Table 10A and B). Furthermore, more calcium was found in outer and inner leaves exposed to increased RH during night and with LED as a lamp source. However, Since the nutrient analysis was done on whole leaf and not just the edge/tips of leaf,

there could possibly have been more Ca at the tips in leaves exposed to high RH during night due to rise in root pressure. According to Vanhassel et al. (2016) and De Swaef et al. (2012), humid conditions during night will enhance root pressure which may cause the flow of calcium towards low-transpiring plant parts like the young leaves and tips. The almost absence of tipburn in the low transpiring inner leaves in this study may have experience some translocation of calcium caused by rise in root pressure from elevated nightly air humidity. Even though older leaves recorded high calcium content from the nutrient analysis in all treatments, significant level of tipburn severity was observed. It could be stated that high level of calcium content is not enough to prevent tipburn but possibly contributes to a higher tolerance. Elevated air humidity seems to have a greater impact on tipburn than far red light (Exp 1) and elevated CO_2 (Exp 3). This is in line with comments made by Saure (1998) and Vanhassel et al. (2016) that the overall incidence of tipburn is based on the level of external stress influenced by dynamics in the climate conditions.

5.3 Elevated CO_2

Supplementation of CO_2 in the greenhouse or growth chamber is expected to cause an increase in growth rate due to expected increased photosynthesis (Becker, 2016). Increased photosynthetic assimilation comes by supplemented CO_2. C3 plants response to photosynthetic assimilation is specific and only positive to a certain concentration (Gillig et al. 2020). Increased CO_2 (>1000ppm) will not cause any increase to net photosynthesis in some species when plants reach saturation point (Stanciel et al. 2000). All growth parameters (FW, DW, Number of leaves and % water content) generally exhibited a linear increase with light intensity (table 3A and B) similar as described in Tani et al. (2014) and Sago (2016). Even though an increase was seen, no significant difference between ambient/normal (400ppm) and elevated CO_2 (1000ppm) in plants exposed to LED was observed (Table 3A). However, from table: 3A, there was a better performance in growth for treatments under normal CO_2 level rather than under elevated CO_2 . This was not expected because under elevated CO_2, one expects an increase in growth (Mastalerz, 1977). As stated by (Hunt et al., 1984) a 30% increase in yield is possible during autumn, winter, and spring. It is expected an increase in potential net photosynthesis in C3 plant under CO_2 enrichment (Drake et al., 1997) which eventually can cause increase in yield. It is also common in commercial production of lettuce to add additional CO_2 in the production to increase yield.

When plants were exposed to HPS as a light source, all other growth factors, except FW at 300 $\mu molm^{-2}s^{-1}$, were significantly increased in plants exposed to elevated CO_2 compared with ambient CO_2 (Table 8B). This showed a direct opposite of treatments in LED. FW and DW was higher under elevated CO_2 compared to the normal (400ppm) under both light intensity: an indication of a positive influence on lettuce growth. On the contrary, the number of leaves and % water content recorded lower values under elevated CO_2. The reason why plants responded differently to CO2 under LED and HPS is not clear. However, it can be related to water relations. Elevated CO_2 normally induces stomata closure, but this is dependent on light quality. The light quality in LED contained more blue light (20%) compared to HPS (5%). Blue light is an opening signal for stomata, and this might be important for the response to elevated CO_2 (Zeiger, 1984; Briggs, 2005; Taiz and Zeiger, 2015).

In the assessment of tipburn incidence and severity, all treatments experienced different levels of incidence and severity. Comparison between normal (ambient) and elevated CO_2 showed lower incidence and severity of tipburn in older leaves in favour of elevated CO_2 treatments under both moderate and high light intensity (Fig 19A and B) even though treatments with ambient CO_2 recorded high calcium content. With respect to inner/sink leaves, observation was that an increased calcium content under ambient CO_2 resulted in decreased severity (Fig 19A and B). The lower calcium content found in plants under elevated CO_2 could be associated with the lower transpiration rate that comes with it (Gilliham et al. 2011). This reduction in transpiration is caused by reduced stomatal conductance which is caused by elevated CO_2 . The general account on tipburn from the bar graph (Fig 20A and B) showed a reduced but not significant severity in elevated CO_2 treatments for both LED and HPS under moderate light.

From Fig 19A and B) it could be said that, older leaves responded better to tipburn incidence and severity under elevated CO_2 than inner or sink leaves. Since elevated CO2 affects stomata opening and transpiration it could have improved the water balance and hence reduced tipburn.

Small differences were found in nutrient content between ambient and elevated CO2 as well as between LED and HPS

5.4 Nutrient content (The role of Ca and other minerals or cations in tipburn severity)

The incidence of tipburn has mostly been associated with deficiency in calcium. Barta and tibbits, (1986) found a reduced concentration of calcium in leaves affected by tipburn. Saure (1998) related rapid growth rate to increase in tipburn incidence. Others also has report that tipburn mostly occurr in rapidly expanding leaves since most of the calcium will be directed

for cell expansion (Collier and Tibbits, 1982; Wissemeier, 1996). Young leaves of rapidly growing plants tend to have exceptionally low Ca^{2+} content mostly at the margins (Saure, 1998).

Results from the present study did not show a clear relationship between calcium and incidence of tipburn. While experiment under elevated RH at night, reduced tipburn severity (Fig. 18A and B) and increased Ca content (Table 10A and B), experiment with elevated CO2 reduced tipburn severity (20A and B) with a reduced Ca content (Table: 11A and B). The opposite response makes it difficult to conclude on tipburn and Ca. Furthermore, the calcium measurements were only performed on plants grown at moderate irradiance not high irradiance. Hence, tipburn development under moderate irradiance is probably not related to calcium deficiency. Source leaves resulted in higher content of calcium compared to the sink leaves in all experiments. While additional far-red and elevated RH favoured calcium accumulation in their treatments, elevated CO_2 rather resulted in reduction comparative to the other treatments. Considering the individual factors in each experiment, the calcium content recorded was highest under treatments with CO_2 in older leaves especially for ambient CO_2. This however did not have any significant effect on tipburn severity. The antagonistic relation that exist between Ca, Mg, and K, where a decrease in calcium level will cause an increase in Mg and K (Levine and Coburn, 1984) was not seen in this experiment. A rather proportional relation where an increase in one cause an increase in another was observed.

5.5 Antioxidant (FRAP) content

Since plants exposed to elevated relative air humidity at night showed the largest variation in tipburn, the antioxidant test was done to see if there existed any correlation between tipburn incidence and the level of antioxidant produced by the lettuce with respect to source-, sink-leaves and roots. A trend observed in the results depicted a decreased antioxidant level in response to high RH at night in source leaves which is in line with results obtained in the study done by (Innes et al. 2019) . This was noted in both HPS and LED. Due to large variation the data it was not statistically significant in both source and roots. However significant differences were found in the sink leaves. (Table 12A and B) in both LED and HPS treatments at a p-value of 0.018 and 0.024, respectively. However, sink leaves developed in HPS/IRH had significantly lower and leaves developed in LED/IRH had significantly higher FRAP values compared with NRH. A possible reason for the difference may be due to the different light conditions used. HPS lamps have low amount of blue light (Innes et al. 2019). Blue light is said to increase accumulation of antioxidants (Siipola et al. 2015),phenolics and flavonoids in tomato and

lettuce (Kim et al. 2013; Ouzounis et al. 2015). However, sink leaves developed in HPS showed higher FRAP values than LEDs indicating that other factors than light quality is more important to explain FRAP values. Knowing the potential of antioxidant power as an indicator for a sample (plant leaves) being capable of scavenging excess ROS, a potential cause of oxidative damage, the aim was to see if the levels of these antioxidant had any correlation with the incidence and severity of tipburn in Exp 2, however, this was not the case. Treatments with increased RH rather had lower incidence and severity of outer tipburn in both LED and HPS. (Fig:17A and B) and (Fig 18A and B). From the FRAP assay, treatments under increased RH should have shown a higher susceptibility to tipburn incidence and severity due to their low production of antioxidant: an indicator of susceptibility of oxidative damage but rather the opposite was observed. Hence, the FRAP method is not a good indicator for tipburn incidence.

6. Conclusions

The occurrence of tipburn cannot be attributed to the effect of one single external factor. Doing this may be risky because the impact of external factors might be conflicting, depending upon their duration, intensity, and timing. Hence, to study effects of one external factors as an independent or single factor is difficult. However, in a greenhouse several climate factor can vary at the same time. Therefore, to study and understand interactions between different climate factors in controlled environment can contribute to important practical information useful for growers. In the present study the main conclusions are:

- High irradiance induced more outer and inner tipburn in all climate regimes tested.
- A higher growth rate (leaf number and FW) was found in high irradiance compared to moderate irradiance.
- Comparing the two far-red treatments response to tipburn, LED with far-red increased the incidence and severity of tipburn under moderate light intensity, however under high light intensity, HPS with far-red increased in tipburn severity and incidence.
- Outer tipburn occurred in all treatments, but was severe under high light intensity
- The strongest reduction in tipburn was found in plants exposed to elevated RH during night compared to constant RH and the strongest reduction was found at high irradiance
- HPS combined with elevated RH had a stronger reduction in the incidence and severity of tipburn compared with LED treatments and elevated RH

- HPS and LED treatments under elevated CO_2 reduced tipburn severity but HPS reduced more of the tipburn compared to LED. In general, HPS was the lamp type that induced less tipburn
- Inner sink leaves in all experiments contained lower Ca, K, and Mg levels compared to the source leaves
- No correlation was seen between calcium content and the occurrence of tipburn
- Antioxidant capacity was lower in sink leaves compared with source leaves but was not a good indicator of tipburn incidence

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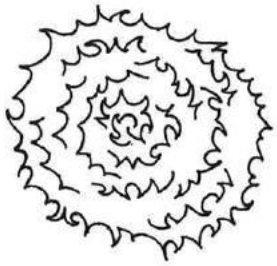
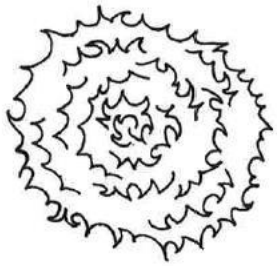
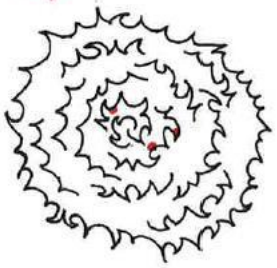
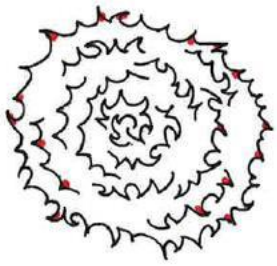
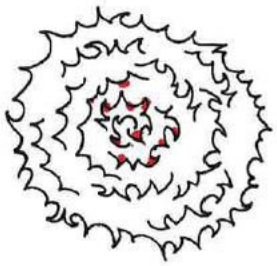
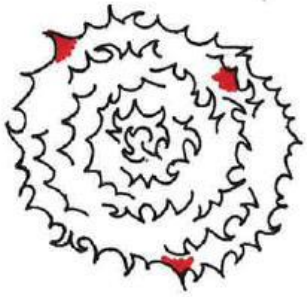
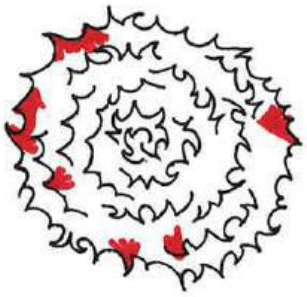
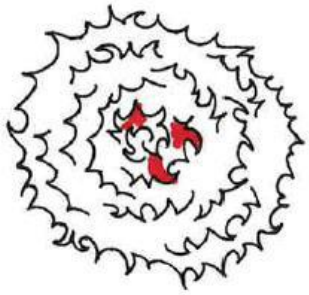
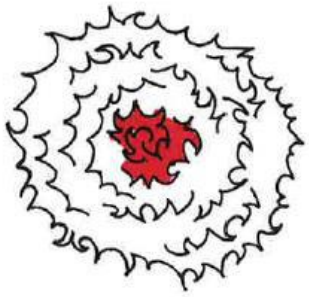
8. Appendix 1,

NLR registration form for inner and outer 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		

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		

Appendix 2, images of some inner and outer tipburn assessment.

The first two images, Fig 1 and 2 are displaying severe instance of outer tipburn under high irradiance

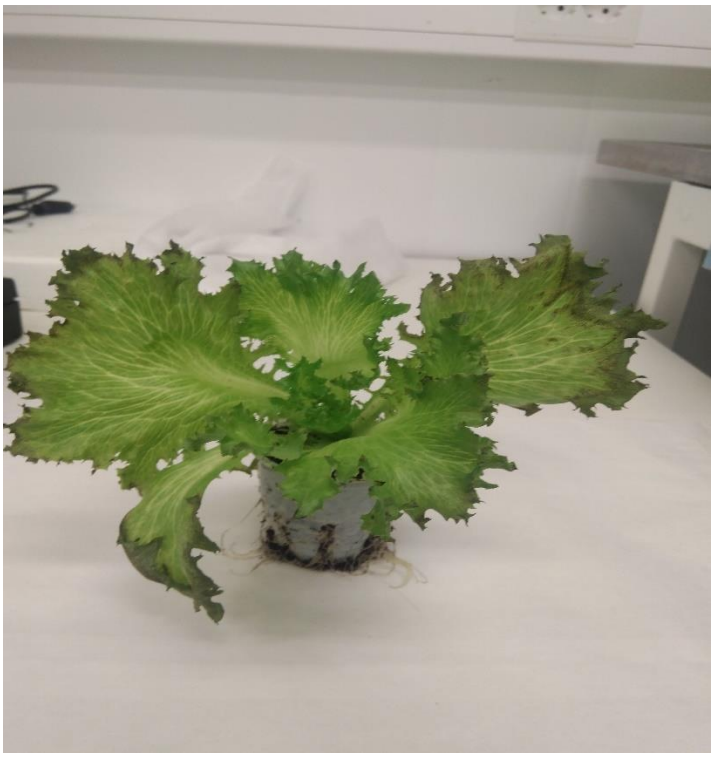


Fig 1: severe outer tipburn on entire plant. Picture: Ellen Kusi

Fig 2: Entire plant tipburn both inner and outer leaves

All four images below are various instances of severe inner tipburn after 5days of being exposed to high irradiance

Fig 3: images showing severe inner tipburn. Severity score = 5

First 10 days of lettuce being transferred into growth chamber. Outer tipburn

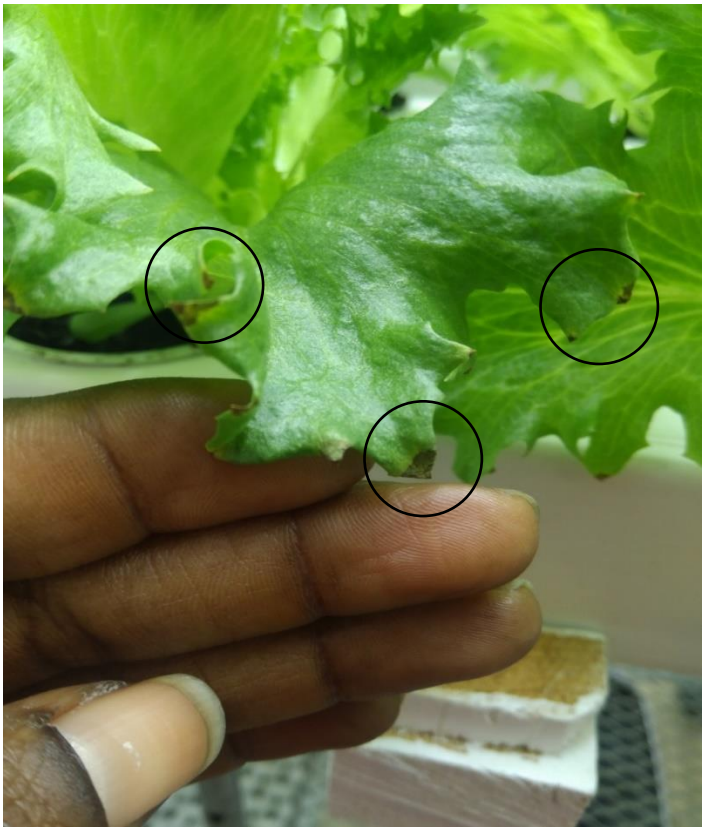


Fig 4: close view of outer tipburn. Picture: Ellen Kusi

Fig 5: close view of innermost tipburn. Picture: Ellen Kusi

Fig 6: sample of experimental plant grown with or without far-red treatment

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