Effects of tropospheric ozone on clover species in a high latitude perspective.

By assessment of visible injury, growth, stomatal conductance and chlorophyll content of plants grown under Nordic conditions.

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Abstract

Tropospheric ozone is a highly reactive secondary air pollutant which causes severe damage on human health and vegetation. Tropospheric ozone concentrations have been increasing since the industrial revolution and will continue to rise with increased emission of nitrogen oxides and volatile organic compounds.

Ozone enters the plant mainly through the stomata where the high oxidizing potential causes production of reactive oxygen species (ROS) which can lead to necrosis and foliar injury, biomass reduction and increased leaf senescence. Plants in northern regions have been shown to display a higher degree of injury than plants in lower latitudes despite lower ozone concentrations. Suggested causes include increased ozone fluxes, shorter nights or the lack of dark periods and increased ozone sensitivity in plants under longer photoperiods. A dry deposition model has been developed for European scale mapping and modelling of ozone fluxes. However, the model may not be suited to Nordic conditions due to the increased ozone injuries without increased ozone concentrations. This study shows that there is a shift in response under Nordic conditions, with a long photoperiod, that the DO3SE model needs to include in order to more accurately predict ozone fluxes in higher latitudes.

Results in this study indicate that ozone-response relationships are more meaningful if plant physiology and response mechanisms are accounted for. Most physiological parameters examined showed some change when exposed to ozone and visible foliar injury was present in all species and cultivars examined. Photoperiod had a significant effect when studying damage levels over time for *Trifolium subterraneum* and *Trifolium repens* cv. Norstar, whereas the estimated ozone dry deposition showed no difference with fixed climatic conditions except photoperiod indicating that the model is insufficient in high latitudes and in need of modification.

Acknowledgements

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Thank you for your inspiration, guidance and faith.

I would also like to thank the staff at the Phytotron for being so nice, helpful and full of knowledge.

Thank you to my friends and family for supporting me through the good times and the challenges during this period. Massive thanks go to my husband to be Olav Bjerke, without you this would not be possible. Thank you for allowing me to submerge myself in this project, and for bringing out the best in me.

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1 Introduction

1.1 Background

Clean air is a basic need for both human health and the environment. Regardless many cities around the world exceed the recommended limit of air pollutants. Ozone (trioxygen, O_3) in the troposphere is one of the major secondary air pollutants globally, and extensive research through decades show that the present ambient concentrations are sufficiently elevated to have an impacts on human health, crop yields and natural ecosystems (Ainsworth, Yendrek, Sitch, Collins, & Emberson, 2012; M. Ashmore, Toet, & Emberson, 2006; M. R. Ashmore, 2005; Fowler et al., 2008). Damage on plants as a result of increased ozone levels is well documented and include reduced stomatal conductance, reduction of carbon fixation, injury on foliage and reduced seed production (C. M. Futsaether et al., 2009; H. Pleijel, Eriksen, Danielsson, Bondesson, & Selldén, 2006; A. V. Vollsnes et al., 2009). Ozone concentrations has been increasing dramatically since the industrial revolution and will continue to rise with increasing anthropogenic emissions. This has devastating effects on e.g. food production and results in severe economic loss across the world (M. R. Ashmore, 2005; Fowler et al., 2008; ICP Vegetation, 2017).

Tropospheric ozone levels are highest in Central Europe, Eastern China and the Eastern USA (Fowler et al., 2008), but increasing temperatures and precursors gases emitted from anthropogenic sources, such as shipping, can increase ozone levels in Nordic regions (Peters et al., 2011). Impacts of ozone on human health and vegetation has been well established, and increasing background levels are raising concerns about future implications (Fowler et al., 2008).

1.2 Purpose of study

Ecosystems and climate are parts in a coupled system. They interact on multiple aspects both regionally and globally and can be studied on both short timescales such as seasons, and longer ones, spanning millennia. The climatological impact on vegetation result in a vegetation feedback on the climate (Bonan, 2008). The purpose of this master thesis is to examine one climate-vegetation interaction on a regional scale, and evaluate whether it should be represented

in a model estimating ozone effects on vegetation. More specifically, the effects of a long photoperiod on plant responses to ozone exposure are studied. The work done in this master thesis may give indications on the importance of this factor for high latitude vegetation modelling.

2 Theory

2.1 Ozone in the atmosphere

Ozone is an endothermic, highly oxidizing molecule discovered by Friedrich Schönbein in 1839. It is an oxygen allotrope containing three oxygen atoms, and it is a dangerous toxicant in high concentrations. EU's air quality directive sets the information limit at 90 ppb and the warning limit at 120 ppb. The name ozone is derived from the Greek *ozein*, meaning to smell, as the substance possesses a strong odour (Aas, Fiebig, Solberg, & Yttri, 2018; Roshchina & Roshchina, 2003).

Ozone is found in trace amounts throughout the atmosphere but is primarily located in what is commonly known as the *ozone layer*; a well-defined layer at altitudes between about 15 and 30 km (Holloway & Wayne, 2010).

2.1.1 Ozone in the stratosphere

The ozone production in the stratosphere is a part of a cycle that starts and ends with molecular oxygen. Ozone is produced in a photochemical reaction through two steps. First by molecular oxygen (O_2) being broken down by solar radiation (*hv)* with a wavelength < 242 nm,

$$O_2 + hv \rightarrow 2O \quad (1)$$

then combining with molecular oxygen to form ozone (Holloway & Wayne, 2010).

$$O + O_2 \rightarrow O_3 \quad (2)$$

Ozone can be distinguished from oxygen by its different photochemical properties. It absorbs light at wavelengths shorter than 290 nm, which is the same range where DNA and proteins absorbs radiation. Therefore, the ozone layer is a major contributor to the protection of all living organisms against damaging high-energy radiation (Roshchina & Roshchina, 2003).

2.1.2 Ozone in the troposphere

By the time sunlight reach the troposphere, most of the radiation with wavelengths less than 290 nm have been absorbed. Without radiation less than 290 nm, reaction (1) will not take place.

There are therefore two primary sources of ozone in the troposphere. The ozone which is produced *in situ* and the transported ozone from the stratosphere. The net flux of ozone transported from the stratosphere to the troposphere is estimated to be ~540 Tg y^{-1} and the chemical production of tropospheric ozone is ~4500 Tg y^{-1} (See Figure 2.1) (Fowler et al., 2008).

Production of tropospheric ozone involves the contribution of other chemicals such as carbon monoxide (CO), volatile organic compounds (VOC) and nitrogen oxides (NOx), the two latter being the major contributors. These chemicals are naturally found in the atmosphere, but the amount is increasing due to anthropological emissions (Hough & Derwent, 1990; Levy et al., 1997; Menon et al., 2007).

Tropospheric ozone that is produced involving NOx gasses happens in much the same way as the photolysis in the stratosphere.

First NOx, such as NO_2 is photolyzed by radiation with shorter wavelengths than 410 nm.

$$NO_2 + h\nu \rightarrow NO + O \qquad (3)$$

The resulting oxygen atom combines with molecular oxygen to form ozone as shown in reaction 2. This reaction is also part of a larger cycle that returns to O_2 and NO_2, and thus will not result in a fixed elevation of tropospheric ozone (Roshchina & Roshchina, 2003).

Many definitions of the term volatile organic compound are in use today. The European Union defines VOC as organic compounds from anthropogenic and biogenic sources that are capable of producing photochemical oxidants by reactions with nitrogen oxides in the presence of sunlight (UNION, 2008).

VOCs are commonly divided into methane and non-methane VOCs (nmVOCs). Biologically generated VOCs are primarily emitted from different terrestrial plants with isoprene being the most important. Emission increasing factors are such as temperature and sunlight which explains the diurnal pattern with high concentrations during mid-day (Fowler et al., 2008).

Methane gas (CH_4) is the most abundant greenhouse gas in the troposphere after CO_2 and H_2O. It is produced as the end product of decomposition of organic matter and has natural regional emission differences due to temperature and amount of organic matter. Major contributors include swamps, lakes, thawing tundra, rain forests etc. It is further emitted from anthropogenic sources such as coal-mining, landfills, deposition of lakes and flooded soils, and waste- and biomass burning (Roshchina & Roshchina, 2003).

VOCs (including methane) acts as precursors for ozone production. The reactions are different with the different VOCs, but most include a reaction that produces unsaturated hydrocarbons and contribute to the production of radicals that induce the formation of ozone (Roshchina & Roshchina, 2003).

The average lifetime of ozone in the troposphere increases with altitude and ranges from 1-2 days to several weeks in the upper troposphere, with an increased lifetime during the winter. Vegetation is one of the major sinks of tropospheric ozone and one of the contributing factors of the decreasing altitudinal gradient. The lifetime of ozone allows it to be transported to more remote rural areas used for agriculture and forestry (Fowler et al., 2008; Krupa et al., 2001; Meul, Langematz, Kroger, Oberlander-Hayn, & Jockel, 2018; Stevenson et al., 2006).

Studies suggest that surface concentrations of ozone have more than doubled since the industrial revolution and is increasing about 1% per year throughout the upper regions of the troposphere (Ainsworth et al., 2012; Hough & Derwent, 1990; Staehelin & Schmid, 1991).

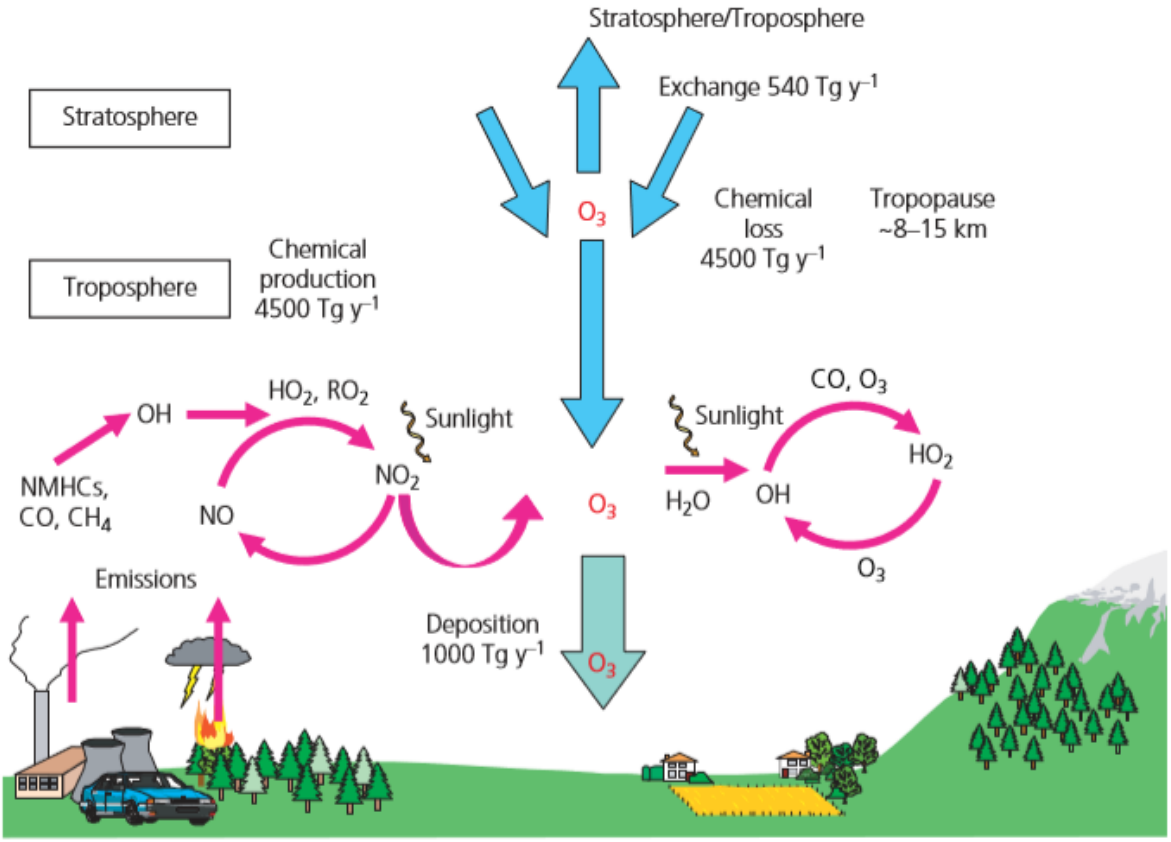


Figure 2.1 Sources and sinks of tropospheric ozone as published by Fowler in Ground-level ozone in the 21. Century: future trends, impacts and policy implications (2008). Data source: IPCC Fourth Assessment Report Working Group I Report "The Physical Science Basis"(Fowler et al., 2008; Menon et al., 2007).

2.1.3 Ozone toxicity and effects on plants

Ozone is an endothermic molecule, meaning that it stores energy as a result of how it is created in the reactions mentioned above. The O-O_2 bond and the O-O bond stores available energy in the O_3 molecule, and together with the high abundance of its precursors in the atmosphere this makes it one of the most important reactants in the troposphere (Holloway & Wayne, 2010). The toxicity of ozone has been extensively studied due to its abundance, because it is a component in photochemical smog, and for its potential impact on plants, humans and ecosystems (Krupa et al., 2001; Mehlman & Borek, 1987; Mustafa, 1990; Pryor, Squadrito, & Friedman, 1995).

Ozone is deposited into plants by diffusion through the stomata. Environmental factors that promotes stomatal opening such as sunlight, water availability, temperature, low internal CO_2 concentrations, increase the risk of ozone injury. Ozone induces oxidative stress in the plant

cells by forming reactive oxygen species (ROS) such as hydrogen peroxide through chemical reactions in exposed tissue. Ozone induced ROS can react with important cellular components such as fats, proteins, nucleic acids and carbohydrates (Pringle, Yu, Sachs, & Ellis, 2018). Plants that experience either acute or chronic exposure to ozone can show symptoms of foliar injury, decreased photosynthesis, reduced plant growth, reproductive capacity and can cause early senescence (Ainsworth et al., 2012; Krupa et al., 2001; Pell, Schlagnhaufer, & Arteca, 1997; A. V. Vollsnes et al., 2009; Wilkinson & Davies, 2010). The response mechanisms of the plant are dependent on the type of exposure and the response capability of the plant. The two different pathways are described in Figure 2.2 and 2.3.

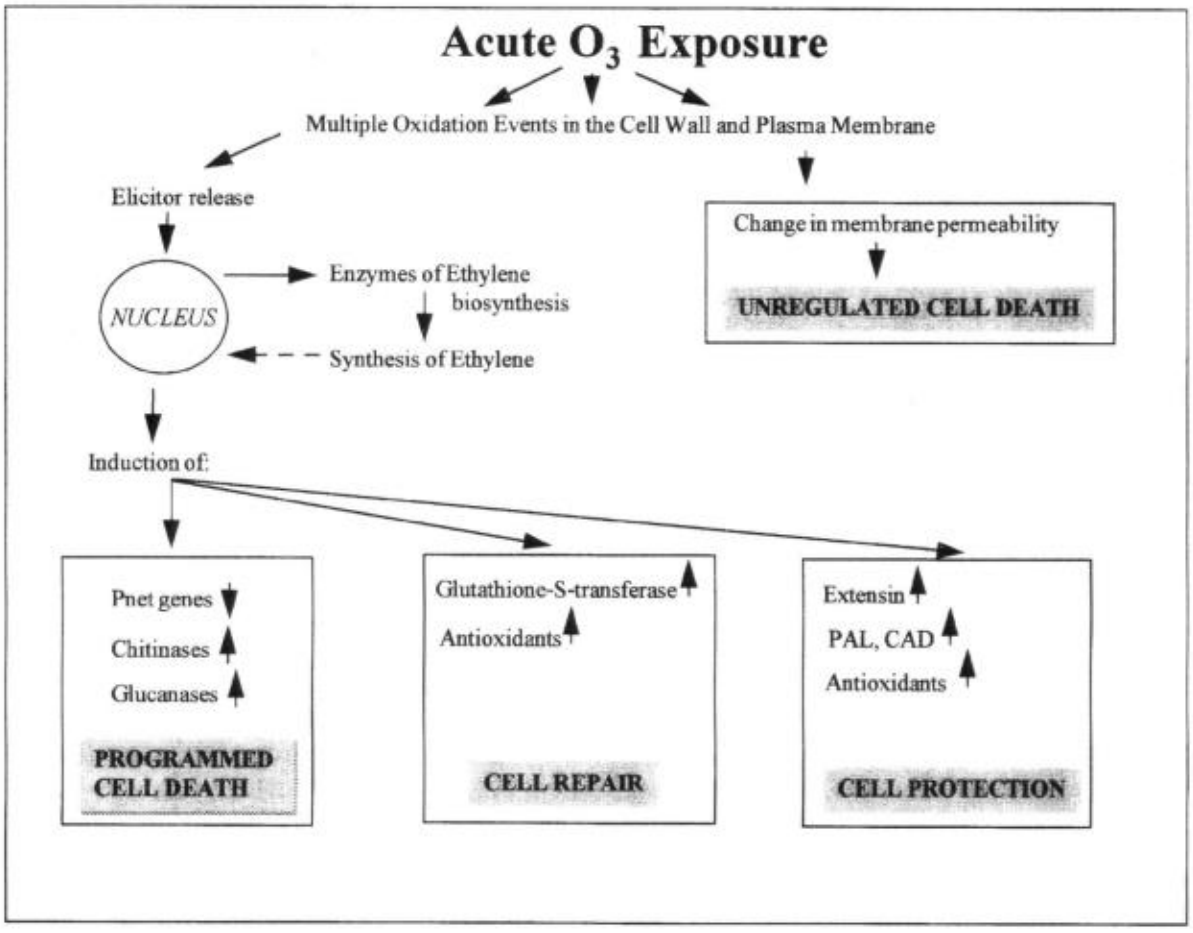


Figure 2.2 Potential mechanisms by which acute exposure to ozone can affect plant cells as presented in Pell et al. (1997). Pnet genes refer to genes encoding chlorophyll a/b protein (cab), glyseraldehyde-3-phosphate dehydrogenase (gap A sna gap B), and the small subunit of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco (rbcS). PAL and CAD refer to phenylalanine lyase and cinnamyl alcohol dehydrogenase, respectively.

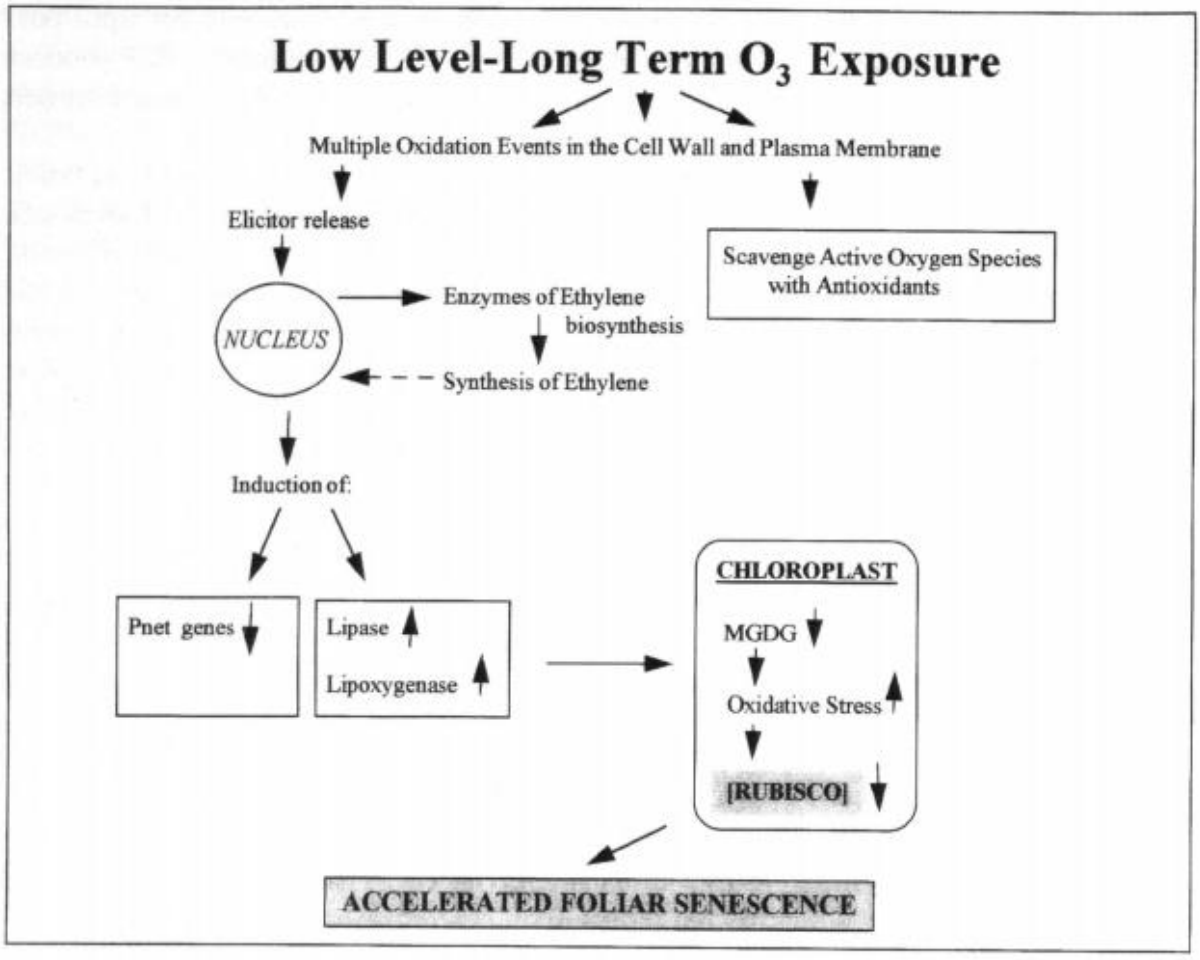


Figure 2.3 Potential mechanisms by which chronic exposure to ozone can affect plant cells and lead to accelerated foliar senescence as presented in Pell et al. (1997)

Visible foliar injury is used as a biomarker for ozone and can have different expressions in different species. The first cellular barrier ozone meets when entering a leaf is the stoma and cuticle. These structures thus function as the primary receptors in plants. When in the apoplast ozone degrades and reacts with important structures and organelles in the cells. It can then become distributed into the cells of the spongy parenchyma and the palisade parenchyma and causes the protoplast to be compressed and the cells to be destroyed. Ozone can cause four different visible foliar injuries: dotted pigment damage, bleaching, chlorosis and bilateral necrosis. The first three are caused by chronic exposure, while the latter is caused by an acute increase in ozone levels (Roshchina & Roshchina, 2003). An overview of the different symptoms of foliar injury is shown in Table 1 (Krupa et al., 2001).

Table1: Common symptoms of ozone-induced acute and chronic injury and the response of broadleaf and coniferous plants as presented in (Krupa et al., 2001).	
ACUTE INJURY	**CHRONIC INJURY**
Broad-leaved plants	
Bleaching: small unpigmented necrotic spots or more general upper surface bleaching. Palisade cells and, when injury is more severe, upper epidermal cells collapse and become bleached	*Pigmentation* (bronzing): leaves turn red-brown to brown as phenolic pigments accumulate.
Flecking: small necrotic areas due to death of palisade cells, metallic or brown, fading to tan, gray, or white	*Chlorosis*: may result from non-green pigmentation or may occur alone as chlorophyll breaks down.
Stippling: tiny punctate spots where a few palisade cells are dead or injured, may be white, black, red, or red-purple.	*Premature senescence*: early loss of leaves, flowers or fruit.
Bifacial necrosis: when entire tissue through the leaf is killed, bifacial, dead areas develop ranging in colour from white to dark orange-red. While small veins are usually killed along with the other tissue, larger veins frequently survive.	
Conifers	
Banding: clear bands of chlorotic tissue on semimature needle tissue following ozone episodes.	*Flecking and mottling*: flecking is the earliest symptom on the older needles of conifers. Mottling is generally associated with diffuse chlorotic areas interspersed with green tissue on first-year needles.
Tipburn: characterized by dying tips of young elongating needles. At first red-brown in colour, later turning brown, injury spreading from the tip downward.	*Premature senescence*: early loss of needles.

The effect of ozone on reduced plant growth is a result of ozone affecting growth factors such as nutrient uptake and CO_2 assimilation and are strongly linked to ozone affecting photosynthesis. Ozone cause stomatal closure, and reduce CO_2 assimilation directly, but also affects the photosynthesis by targeting different components of the cycle. The amount of rubisco can be reduced by ozone exposure either by direct oxidation or through suppression of mRNA production. Ozone can damage the light absorbing complexes in the chloroplasts, can interfere with the plant's electron transport, limit the amount of energy available to assimilate CO_2, and

affect carbon flow to roots and thus affect nutrient uptake (Krupa et al., 2001; Paoletti, Contran, Bernasconi, Günthardt-Goerg, & Vollenweider, 2010).

Ozone does not persist within the intercellular space but is decomposed to organic radicals and various reactive oxygen species (ROS) that damage proteins and membranes and lead to loss of physiological functions and cell death. The plant response to acute ozone exposure resembles the response to pathogen attack with an oxidative burst occurring. In ozone tolerant species and cultivars either the oxidative burst is suppressed, or the oxidative damage is localized to reduce damage (Vainonen & Kangasjärvi, 2015).

Plants respond to air pollutants as they would to other stress factors. Their strategies include avoidance, tolerance of ozone and compensation and repair after exposure. Ozone stress can be avoided by closure of the stomata. They can tolerate ozone stress by storing reactive oxygen species in organelles and tissues or through detoxification. Compensation as a form of adaptation occurs when plants are chronically exposed to ozone where their cells will adapt and become more resistant to later exposures (Heath & Taylor Jr., 1996). The repair mechanisms are driven by dark respiration. Plants recover from oxidative stress during the night, and this could explain why photoperiod is a factor when working with ozone damage. (De Temmerman, Vandermeiren, et al., 2002; A. V. Vollsnes et al., 2009).

2.2 Measuring critical levels for protection of vegetations

There are various methods used today to assess critical levels for ozone exposure to vegetation. In general, there are two approaches that uses the presence of ozone as a driving factor, concentration-based risk assessment or accumulative seasonal exposure-based risk assessment. The Tropospheric Ozone Assessment Report (TOAR) published by Mills et al. (2018) presents three metrics for measuring critical levels of ozone in vegetation where two are accumulative (AOT40 and W126) and one is concentration-based (PODy). AOT40 uses the accumulation of hourly mean ozone values above 40 ppb during daylight hours and is widely used in studies of ozone effects on vegetation. (Assis, Alonso, Meirelles, & Moraes, 2015; Fowler et al., 2008; Mills et al., 2018). W126 is a non-threshold metric described as *the sigmoidally weighted sum of all hourly ozone values observed during a specified daily and seasonal time window, where*

each hourly ozone value is given a weight that increases from zero to one with increasing value (Mills et al., 2018). Other thresholds are in use in other areas of study such as AOT60 used for measuring critical levels for human health by UNECE (Fowler et al., 2008). The concentration-based approach, M12 is based on the mean ozone concentrations during 08:00-19:59. Both M12 and W126 have an apparent drawback when applied to areas with more daylight hours in the higher latitudes.

This concentration-based metric of measuring critical levels of ozone has been used in multiple studies but has weaknesses that has resulted in development new approaches.

Flux based methods of measuring critical levels of ozone are in wide use today. The benefit of this approach is that it takes in to account factors that can affect stomatal conductance and the corresponding ozone deposition in vegetation (Assis et al., 2015). One such method are the Phytotoxic Ozone Dose above a threshold y (PODy) (Grünhage et al., 2012).

2.3 Methods for measuring ozone stress

There are various ways of measuring ozone stress to plants as there are several responses to ozone stress (see chapter 2.1.3.).

2.3.1 Visible injury

Various abiotic and biotic factors may cause foliar injury resembling those described for ozone exposure. They include other air pollutants, nutrient imbalance, weather extremes, insect damage and diseases caused by fungi, viruses and bacteria. To distinguish ozone injury from other causes, biotic and abiotic factors must be taken into consideration. Environmental conditions such as the concentration of ambient ozone, temperature, air movement, light, relative humidity and soil moisture are factors that either inhibits or promotes ozone injury. Biotic factors to consider are the number of plants and leaves affected, the location of symptoms on the plant and the known sensitivity of the plant to ozone (Flagler, 1998).

2.3.2 Biomass and carbon allocation

Information on how plants reallocate resources and alter growth patterns in response to ozone exposure is important in predicting and quantifying yield loss. Dry matter production is

primarily produced by carbon fixation and is therefore directly linked to the plants' ability to photosynthesize and the allocation of carbon compounds within the plant. Letchworth and Blum (1977) reported that *Trifolium repens* cultivar Ladino displayed both loss in above ground biomass and below ground biomass after being exposed to acute levels of ozone but varied with ozone concentration and age of plant at the time of exposure. In a review by Cooley and Manning (1987) labelled-carbon studies reported to show that ozone generally inhibits both CO_2 fixation and translocation in the primary leaf in bean plants (*Phaseolus vulgaris*) and that ozone suppresses the translocation of carbon to the roots.

2.3.3 Stomatal conductance

Ozone may directly decrease stomatal conductance (g_s). In acute exposure of *Arabidopsis* a rapid decrease in stomatal conductance occurred, accompanied by a burst of ROS in the guard cells which lead to a slower recovery back to initial states of stomatal conductance (Ainsworth et al., 2012). But studies presented in Wittig, Ainsworth, and Long (2007) suggest that decreased g_s is likely a symptom rather than a cause of declining light-saturated rate of leaf CO_2 uptake (A_{sat}) when exposed to ozone. Both g_s and A_{sat} are key parameters when studying the global and regional carbon cycle, and thus the response of g_s and A_{sat} to ozone are important to understand when looking at vegetation-climate interactions (Wittig et al., 2007).

2.3.4 Chlorophyll content

The study of ozone injury by determination of leaf chlorophyll content has been proposed to be a useful way of eliminating human bias in association with estimation of visible injury in leaves. The chlorophyll reduction is highly correlated with the amount of necrosis and chlorosis in leaves of *Phaseoulus vulgaris* L (Knudson, Tibbitts, & Edwards, 1977). Studies on soy bean show a linear decrease in both mean chlorophyll *a* and chlorophyll *b* concentrations as a function of ozone concentrations. When regarding the age of leaves, they showed an higher concentration of Chl *a* + *b* in younger leaves decreasing in older laves in all treatments regardless of plant age or ozone treatment (Reich, Schoettle, Raba, & Amundson, 1986). The same trends are shown in spring wheat (*Triticum aestivum*) in open top chamber treatments, in tobacco (*Nicotiana tabacum* L.) and spinach (*Spinacia oleracea* L.) (Saitanis, Riga-Karandinos, & Karandinos, 2001; Sakaki, Kondo, & Sugahara, 1983; Sandelius, Näslund, Carlsson, Pleijel, & Sellden, 1995).

2.4 Ozone modelling

The Deposition of Ozone for Stomatal Exchange (DO3SE) model has been used in many studies in the northern hemisphere (Assis et al., 2015; Calvete-Sogo, Gonzalez-Fernandez, et al., 2017; Cassimiro, Moura, Alonso, Meirelles, & Moraes, 2016; L. D. Emberson, Büker, & Ashmore, 2007; Sicard et al., 2016). The model is a dry deposition model designed to estimate the total stomatal flux of ozone. It has been developed to cover selected European land-cover types and selected plant species. It estimates the risk of ozone damage to vegetation and provides flux-model estimates according to UNECE long-range transboundary air pollution methodologies (L. Emberson, Ashmore, Cambridge, Simpson, & Tuovinen, 2000; L. Emberson, Wieser, & Ashmore, 2000; Stockholm Environment Institute, 2017a).

The newest interface (3.1.0) of the model can use either the older multiplicative algorithm for stomatal conductance or a new photosynthesis-based algorithm. This considers the influence of temperature (soil and air), vapor pressure deficit (VPD), photosynthetic active radiation (PAR), soil water content, and plant phenological stages on stomatal conductance (Assis et al., 2015; Stockholm Environment Institute, 2017b).

The model follows a standard resistance scheme (Figure 2.4) where the transfer of ozone from an atmospheric reference height to the sites of deposition is calculated from the resistance of the atmosphere, boundary layer and surface (R_{sur}). The surface resistance includes canopy resistance associated with stomatal resistance, the resistance of external plant parts, the underlying ground surface, and in-canopy resistance. Stomatal and external resistances to ozone deposition are defined at a needle or leaf level and are scaled up using leaf area indices or surface area indices (LAI and SAI). LAI scaling uses a canopy light extinction model to estimate the amount of sun available in the canopy. Because of this DO3SE is potentially capable of providing realistic estimates of whole canopy stomatal conductance (g_{sto}) (L. D. Emberson et al., 2007).

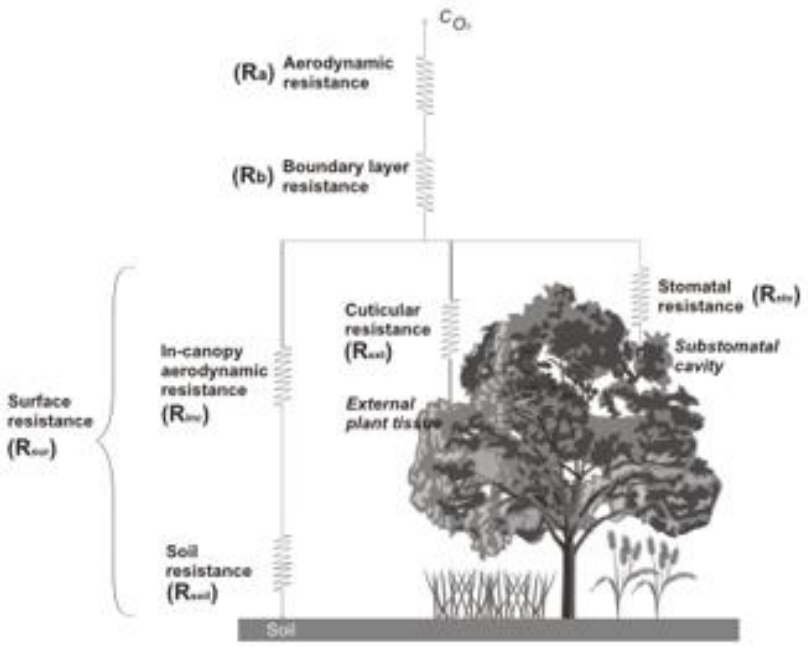


Figure 2.4 Illustration of DO3SE model including the different resistance components used in estimating stomatal conductance and ozone deposition (Stockholm Environment Institute, 2017a).

2.5 Ozone effects under Nordic conditions

All plants grow under specific environmental conditions determined by their region. Factors such as temperature, soil moisture, precipitation and light are conditions that affect the plants' sensitivity to stress, and therefore determines the plants' resistance to ozone induced injury (Roshchina & Roshchina, 2003). The amount of PAR available to the plant is a crucial factor for the plants' ability to fix carbon through photosynthesis. Plants, as well as other organisms, normally live under daily cycles of light and darkness, where the latitude and time of year determines the time ratio of light and darkness. From the equator with a 1-1 ratio of light and darkness, the daylight ratio increases towards the poles towards the summer and decrease towards winter. The extremes being no darkness during the summer months above the polar circle.

2.5.1 Ozone effects in Nordic regions

Ozone concentrations are determined by time of day and year and the amount of precursor pollutants available in the region. Mean ambient background concentration of ozone is considered to be approximately 40 ppb, mid-day during spring and summer months (Fowler et

al., 2008). The Intergovernmental Panel on Climate Change (IPCC) has presented a set of projections of how the climate can change given a different set of policy measures. The A2 storyline describes a heterogeneous world with high population growth, slow economic development and slow technological change. This scenario has an indication that ozone concentrations could rise 20-25% between 2015 and 2050 and increase by 40-60% by 2100 if current emission trends continue (Ainsworth et al., 2012). The biggest increases in ozone concentrations are projected to occur in the Northern Hemisphere because of increased precursors and favourable climatic conditions for formation of ozone (Wittig et al., 2007). RCP scenarios are more optimistic and project a decline in ozone concentrations under most scenarios, due to the reduction of anthropogenic emissions with the most significant reduction in Europe and North America (Kim et al., 2015).

De Temmerman, Karlsson, et al. (2002) showed in a European open top field study that *Solanum tuberosum* develop visible foliar injury at lower ozone concentration in Scandinavian sites in Sweden and Finland compared to sites in central Europe. Three hypotheses are suggested for this difference in visible injury compared to ambient ozone concentrations in northern latitudes. They are based on the difference in climatic summer conditions in northern and southern Europe with the northern growing season having favourable conditions for stomatal opening. One hypothesis suggests a larger ozone uptake (H Pleijel et al., 2000), the other suggests that the nights are too short to facilitate repair and recovery from oxidative stress (De Temmerman, Karlsson, et al., 2002; De Temmerman, Vandermeiren, et al., 2002). Studies done by A. V. Vollsnes et al. (2009) shows that long day conditions significantly increase the amount of visible foliar injury in *Trifolium subterraneum* compared to short day conditions in plants grown under the same environmental and climatic conditions besides from photoperiod, which supports the hypothesis presented by De Temmerman, but propose an alternative reason of plants being more sensitive to ozone under longer photoperiod and not being caused by the lack of repair. A study by Eriksen, Vollsnes, Futsaether, and Kruse (2012), exposed to the same ozone concentrations, displayed a difference in visible damage as a response to different photoperiods. Phytochrome stimulation was hypothesized to lead to signalled cell death in plants. Other explanations include increased oxidative stress triggered by salicylic acid accumulation (Dghim et al., 2013).

In Norway the ozone concentrations are generally considered below critical levels, but with anthropogenic emissions and transport from more industrialized areas ozone concentrations in

some regions of Norway are still within critical limit (Aas et al., 2018). The Norwegian Institute for Air Research (NILU) have stations across the country monitoring ozone concentrations as well as other climatic conditions. The north-south gradient in Norway can be represented using three stations that are shown in Figure 2.5:

- Svanvik in Sør-Varanger (69º 45'N, 30º 04'E)
- Hurdal in Akershus (60º 37'N, 11º 07'E)
- Birkenes observatory in Aust-Agder (58º 23'N, 8º 15'E)

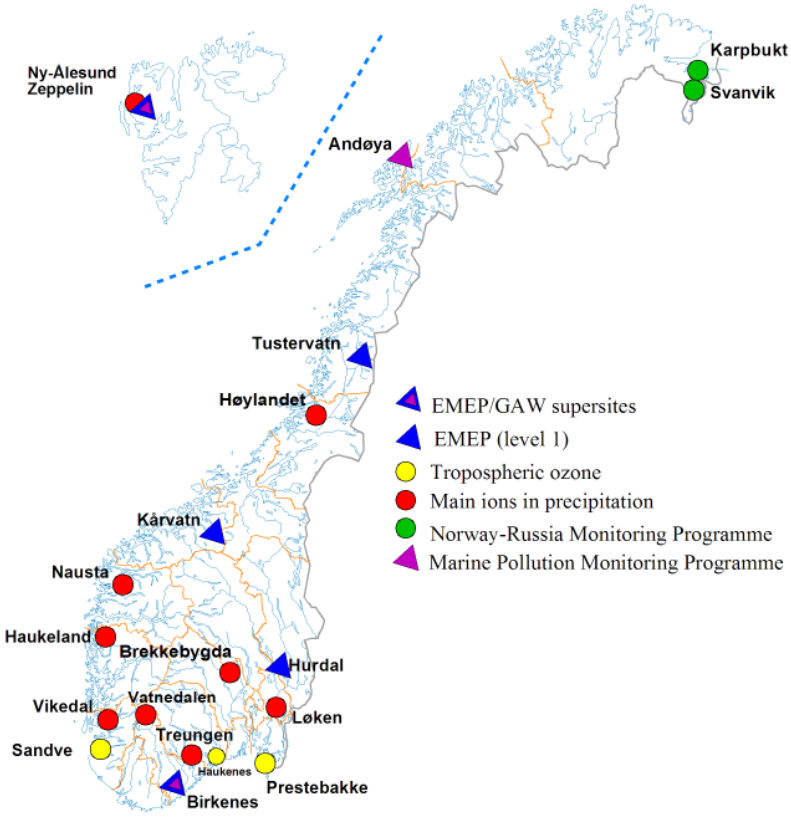


Figure 2.5 Research stations in operation by The Norwegian Institute for Air Research. The stations of interests are Svanvik, Hurdal and Birkenes located in Finnmark, Akershus and Aust-Agder (Norsk institutt for luftforskning, 2018).

The ozone concentrations in the period 01.05-30.06 are shown in Figure 2.6-8. The ozone concentrations are given in µg/m^3. The conversion to ppb is calculated by ppb =(24,45* µg/m^3)/3*atomic mass which is a conversion factor of ~2 (Boguski, 2006). Figure 2.6-8 show a mean of 38.5 ppb in Hurdal, 40.3 ppb in Birkenes and 34.2 ppb in Svanvik during the given two-month period. Missing data and data with less than 50% cover are excluded from the mean.

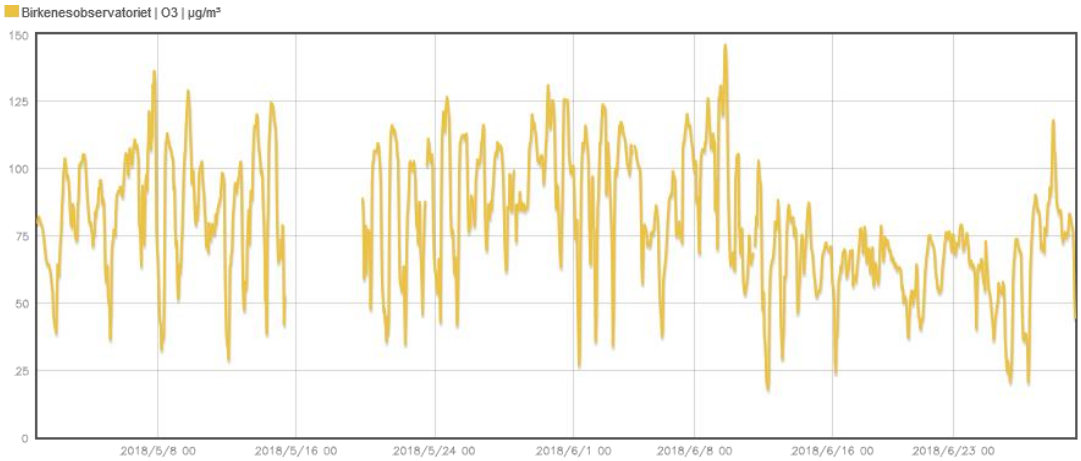


Figure 2.6 Ozone concentrations in µg/m^3 at Birkenes observatory 2018.05.01-2018.06.30. Gaps are caused by malfunctions of the recording machinery (Norsk institutt for luftforskning, Miljødirektoratet, & Statens vegvesen, 2018).

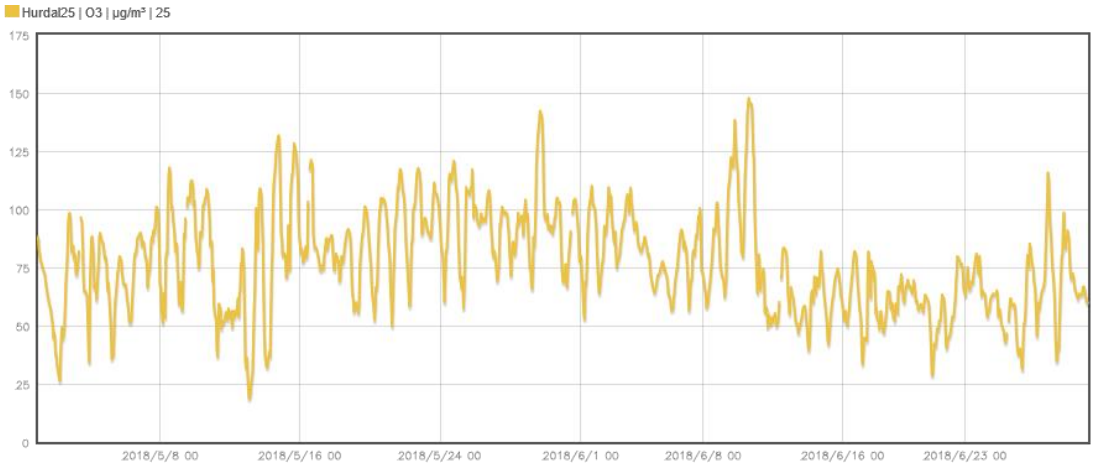


Figure 2.7 Ozone concentration in µg/m3 at Hurdal station 2018.05.01-2018.06.30 (Norsk institutt for luftforskning et al., 2018).

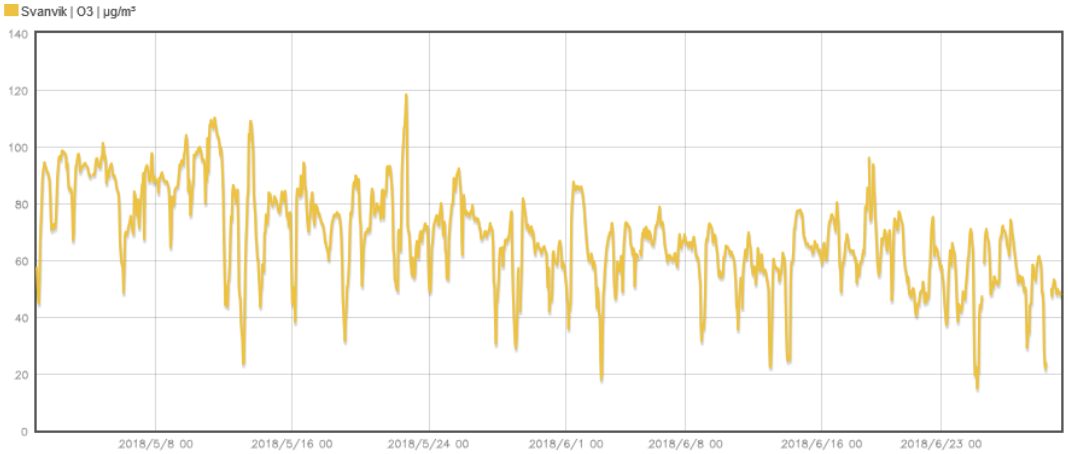


Figure 2.8 Ozone concentrations in µg/m3 at Svanvik station 2018.05.01-2018.06.30 (Norsk institutt for luftforskning et al., 2018).

2.5.2 Climate-Ozone interactions

The process of how the climate system affects tropospheric ozone levels and vegetation are complex and involve many interactions both well understood, and interactions not so well understood but which are emerging as important in the different interactions (Figure 2.9).

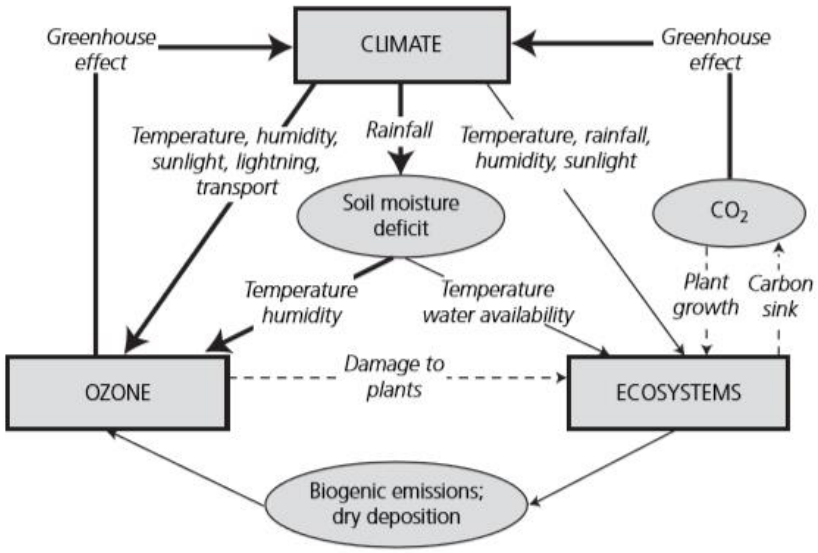


Figure 2.9 Interactions between climate, ecosystems and tropospheric ozone. Thick solid lines represent processes that are generally well understood, solid lines represent processes that are understood but uncertainties exist. Dashed lines represent important links but are generally not included in model projections (Fowler et al., 2008).

Different parameters control the production of tropospheric ozone. Higher temperatures increase the production rate of ozone when NOx gasses are available, especially during summer months. It also increases biogenic VOC emissions which leads to a higher concentration of ozone when sufficient NOx gases are present. The expected changes in atmospheric humidity can act as a negative feedback on tropospheric ozone over land but is dependent on shifts in major weather patterns and a change in precipitation patterns. Reduced precipitation and less clouds will have an impact on ozone concentrations through changes in carbon fixation and dry deposition (Fowler et al., 2008).

The transport of ozone through the altitudinal gradient in the atmosphere will also be affected by climate change by increasing the Brewer-Dobson circulation and increasing the influx of ozone from the stratosphere. The slower stratosphere is expected to cool and hence lower ozone destruction (Zeng & Pyle, 2003).

In a dryer climate the reduction of soil water availability is critical in determination on how vegetation will respond. Plants under water stress have a tendency of closing their stomata and thus decreasing the dry deposition of ozone and reduce CO_2 uptake. A decrease in stomatal conductance across forests can have an impact on regional climate by decreasing water transfer to the atmosphere and thus lowering precipitation and increasing surface temperature (Fowler et al., 2008; Wittig et al., 2007). Summer drying and change in precipitation distribution also increases the chance of forest fires which have an impact on ozone concentrations through emissions of NOx and VOC (Fowler et al., 2008).

The climate feedback on tropospheric ozone is a complex system, and there are many different suggestions on what the dominant interaction will be regarding effects on different spatial and time scales.

2.5.3 Ozone-Vegetation interactions

The ozone-vegetation interactions are important in regard to climate because vegetation influences both the sinks and sources of ozone (Fowler et al., 2008). One important vegetation-ozone interaction is natural VOC emissions.

VOC emissions from many plant species are sensitive to many environmental factors besides forest fires. Temperature and PAR are two major contributors and ozone concentrations are hence strongly controlled by regional and local climate. Temperature also affects emission rates of NO from soils and CH_4 from wetlands which again have an impact on both climate, ozone concentrations and vegetation (Fowler et al., 2008). Sanderson, Jones, Collins, Johnson, and Derwent (2003) showed a large increase in isoprene emissions and ozone concentrations from 1990 to 2090. In large areas they showed an estimated increase, which far exceeded the World Health Organizations limit of 60 ppbv. When including vegetation change, the ozone concentrations exceeded the limit in a much smaller area and was decreased in general which shows the important sours-sink relationship between ozone and vegetation.

2.6 Experimental objectives and hypothesis

The objectives of this study are to quantify the interaction between ozone exposure and photoperiod on several plant growth and physiology traits in controlled experiments. Further,

another objective is to model the ozone dose to the plants in the same experiments and evaluate whether a photoperiod effect should be included in future versions of the model, to better represent the results.

The hypotheses tested are:

H0: There is no difference between the groups in any treatment as described in Table 2.

H1: The visible foliar injury response to ozone exposure differs depending on photoperiod conditions, in *Trifolium subterraneum* and *Trifolium repens*.

H2: Ozone exposure leads to changed growth responses in plants depending on photoperiod.

H2a: The above ground biomass production differs when plants are subjected to the same daily ozone dose, but with different photoperiod conditions.

H2b: The below ground biomass production differs when plants are subjected to the same daily ozone dose, but with different photoperiod conditions.

H3: Ozone exposure affects different physiological responses of *Trifolium repens* cultivars depending on photoperiod.

H3a: Ozone exposure results in different stomatal conductance of *Trifolium repens* cultivars when plants are subjected to the same daily ozone dose, but with different photoperiod conditions.

H3b: Ozone exposure results in different chlorophyll content of *Trifolium repens* cultivars when plants are subjected to the same daily ozone dose, but with different photoperiod conditions.

The results will be used to discuss the physiological mechanisms included in the DO3SE model, and the relevance for plants growing under Nordic conditions with long photoperiod.

3 Materials and methods

3.1 Plant material

The genus *Trifolium* consists of about 300 species in the Fabaceae family. The genus has a cosmopolitan distribution and can be annual, biennial or perennial plants, and some species are commonly cultivated as fodder plants (Clark & Malte, 1913). Species of *Trifolium* are proven to be particularly ozone sensitive (Balls, Palmer-Brown, & Sanders, 1996; Benton et al., 2000; Karlsson et al., 1995; Mills, Hayes, et al., 2011).

3.1.1 *Trifolium subterraneum* L.

Subterranean clover, *Trifolium subterraneum,* L. (Figure 3.1), is an annual eudicot native to southern and western Europe, and can be found as far north as England, Netherland and south-east Hungary (Knight, Hagedorn, Watson, & Friesner, 1982). It has been used as a bioindicator for ozone in the ICP Crops program and is found to be more sensitive to ozone than *Trifolium repens* and *Trifolium pratense* (Karlsson et al., 1995).

Figure 3.1 Subterranean Clover, Trifolium subterraneum L. (Groom, 2012).

3.1.2 *Trifolium repens* L.

White Clover, *Trifolium repens*, (Figure 3.2), is a perennial herb geographical distributed throughout Europe, west Asia and north-west Africa. It is common in all of Norway up to an altitude of 1100 m a.s.l. and is considered a cosmopolite spread by humans (Lid, Lid, & Elven, 1994). Two cultivars were used Regal and Norstar. Norstar was used in experiment I is especially adapted to Norwegian climate and is considered a productive cultivar in the middle and northern part of Norway (Cecilia M Futsaether et al., 2015). Regal is a cultivar with one sensitive and one resistant clone used regularly in ozone experiment (Crous, Vandermeiren, & Ceulemans, 2006; Francini, Nali, Picchi, & Lorenzini, 2007).

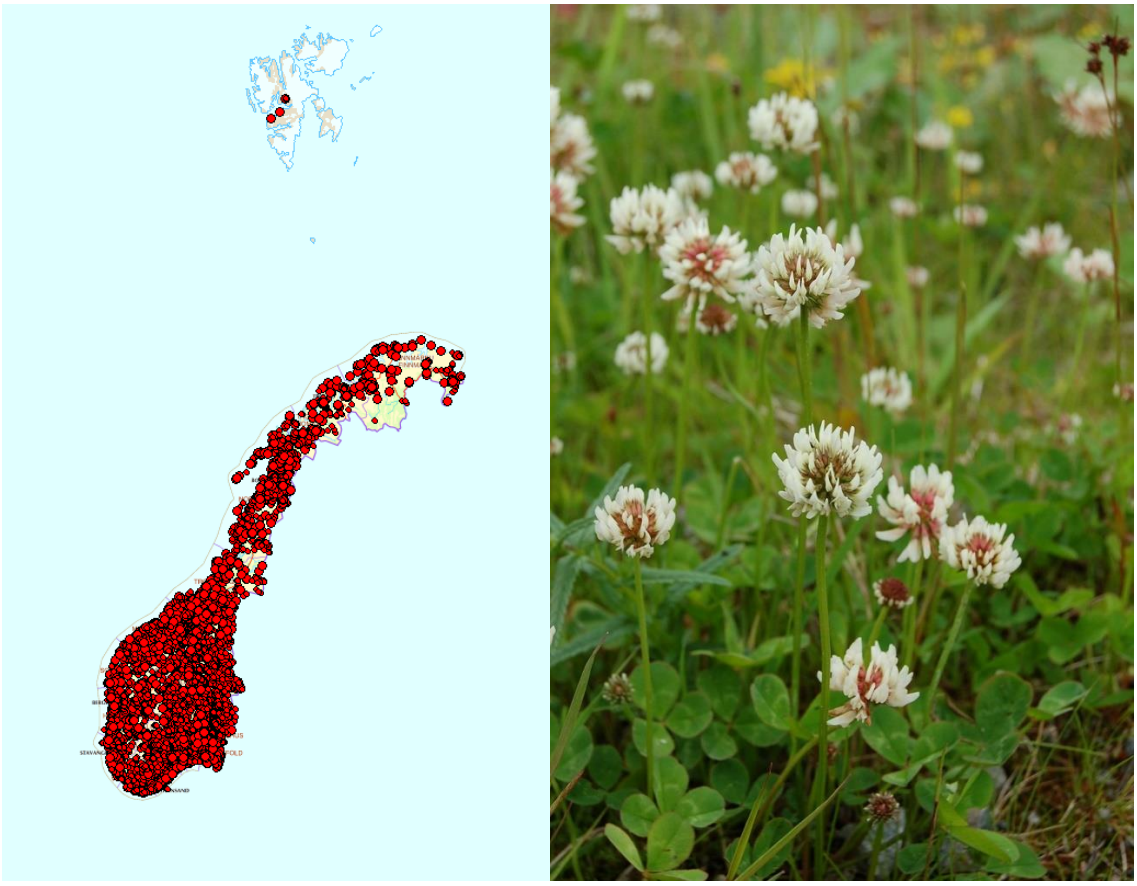


Figure.3.2 White Clover, Trifolium repens, and geographic distribution in Norway (Artsdatabanken, 2018).

3.2 Experimental design

To study ozone sensitivity in connection to prolonged daylight hours during growth season, three experiments were performed. The study design was a split-plot with ozone as the main plot factor and day length as the split plot factor. In each experiment the plants were divided in

to 4 treatments: Long day (LD) + charcoal filtered air (CFA), Short day (SH) + charcoal filtered air (CFA), Long day (LD) + charcoal filtered air enriched with ozone (O_3), Short day (SD) + charcoal filtered air enriched with ozone (O_3) (see Table 2).

Table 2: Experimental treatments applied in this study. A split-plot design with two factorial treatments, ozone treatment and photoperiod treatment.

Treatments	Long day (LD)	Short day (SD)
Charcoal filtered air	LD + CFA	SD + CFA
Ozone	LD + O_3	SD + O_3

In the experiments ozone was added for 6 hours between approximately 9.00 and 15.00. The exposure was done every other day for 6 days (3 days of exposure) in experiment I and II. Plants were randomly allocated to chambers 1-6, where 1-3 where exposed to ozone and 4-6 where controls. The different photoperiod treatments (LD/SD) were established in two different growth rooms with LD treatment being 12 hours of daylight and 12 hours of dim light from a fluorescent tube, and SD treatment being 12 hours of daylight and 12 hours of darkness. The response in visible injury were recorded the day after ozone exposure. In experiment III plants were exposed to ozone 4 days in a 14 days period with at least 2 days of photoperiod treatment between exposures. Otherwise, the same split-plot and daylength treatment were applied in experiment I and II. In experiment I *Trifolium subterraneum* L. were used, and in experiment II and III two different cultivars of *Trifolium repens* L. were used, the Norwegian cultivar Norstar and the American cultivar Regal, respectively. Experimental designs are illustrated in Table 3.

Table 3: Illustration of experimental design used in the three experiments. Ozone (O_3), Charcoal filtered air (CFA), Short day (SD), Long day (LD), Species 1 (SP 1): Trifolium subterraneum L. Species 2 (SP 2) Trifolium repens L.. Norstar cultivar (SP 2a), Trifolium repens L. cv Regal cultivars (NC-S and NC-R) SP2b). In experiment I and II there were one plant in each pot. In experiment III there were multiple plants per pot.

Treatments			Experiment I		Experiment II		Experiment III	
Ozone	Photo-period	Treatment combination	Species	Number of pots	Species	Number of pots	Species	Number of pots
O_3	LD	O_3 + LD	SP 1	9	SP 2a	9	SP 2b	6
CFA	LD	CFA + LD	SP 1	9	SP 2a	9	SP 2b	6
O_3	SD	O_3 + SD	SP 1	9	SP 2a	9	SP 2b	6
CFA	SD	CFA + SD	SP 1	9	SP 2a	9	SP 2b	6
Total number of pots (n)			36		36		24	

3.3 Ozone exposure system

Plants were exposed to ozone or charcoal filtered air in a closed exposure system consisting of six transparent Perspex chambers (inner dimensions: l*w*h; 445*415*795mm^3) in a controlled climate room (30 m^2). The ozone exposure system is shown in Figure 3.3.

All air entering the chambers were filtered (Dust filter and 8 charcoal filter cartridges from Camfil, Trosa, Sweden) to eliminate ambient ozone. Ozone were supplied to three of the six chambers from bottled oxygen (Praxair Norge AS), through an ozone generator (Anseros Ozomat COM 6060, Gärtringen, Germany), that produces ozone due to electrical discharge ($O_2 \rightarrow O$, $O + O_2 \rightarrow O_3$). The ozone levels were set to 70 ppb and controlled by a custom-made software and hardware controlling the ozone generator based on measurement values from the ozone monitor. Charcoal filtered air with or without ozone were transported into the exposure chambers. In all the cambers, the gas inlet and outlet were on opposite sides, and a low pressure inside the cambers prevented leakage to the exterior environment in the growth rooms. The chambers were divided into three sections with two perforated walls that increased the equal distribution of air through the chambers. Ozone concentrations were monitored and logged every minute using an ozone analyser (Photometric ozone analyzer Model 400. Advanced Pollution Instrumentation, San Diego, California, USA). Air was sampled in the middle of the chamber through Teflon tubes which were connected to the ozone analyser, the mean of the three chambers were recorded.

Figure 3.3 Ozone exposure system photographed during ozone exposure of Trifolium subterraneum. Left hand side chambers with CFA exposure, right hand side chambers with ozone exposure. Charcoal filters displayed in the middle. University of Oslo, 8. May 2018.

3.4 Climatic conditions during the experiment

3.4.1 Climatic conditions in the growth room before ozone exposure

Before ozone exposure all plants were cultivated under the same conditions with 16 hours of light and 8 hours of darkness. The light provided in the growth room came from metal halide Osram Powerstar HQI-BT 400 W lamp and is measured to ~200 µmol/m^2s. Relative humidity was set to above 60 % and temperature were set to 20 degrees Celsius (˚C) during daytime hours and 15˚C during night-time hours. Both temperature and humidity were logged by internal sensors in the growth chambers.

3.4.2 Climatic conditions in the growth rooms after ozone exposure

After ozone exposure plants were divided into two rooms with the same day-time conditions, but with differing night-time light conditions. Day-time PAR values were ~200 µmol/m^2s for

12H, but night-time PAR were either 0 µmol/m^2s short day or 0.9 - 1.5 µmol/m^2s long day which corresponds with levels established in previous photoperiod experiments (Otterholt, 2006). Temperature and relative humidity were continuously regulated and logged as described in 2.4.1.

Plants were only taken out of the controlled growth rooms during ozone exposure or to record response data.

3.4.3 Climatic conditions in the ozone exposure system

Microclimatic conditions in the ozone exposure system were monitored. Temperature were set to 20˚C, and both relative humidity and temperature were recorded by internal sensors in the system. Chambers were illuminated by metal halide lamps of 400 W (Osram Powerstar), positioned above the chambers and the amount of light available were recorded using (LiCor 250 with quantum sensor) the recordings are given in Lux. Previous tests done in the chambers establish the conversion factor between lux measurements and PAR measurements (µmol/m2s) in the phytotron to 0.016. All presented values of light quantities in this study are given in PAR.

3.5 Experiment I: Effects of ozone in relation to photoperiod on vegetative growth and visible injury of *Trifolium subterraneum*

Figure 3.4 Trifolium subterraneum cultivated in plant soil and perlite. Plants of uniform development was later selected. University of Oslo, 30. April 2018

3.5.1 Cultivation and growth conditions before ozone and daylength treatment

Seeds of *Trifolium subterraneum* were sown in trays containing plant soil (plantejord, Tjærbo torvfabrikk, Rakklestad, Norway) and perlite (Agra-perlite, Pull Rhenen, Rhenen The Netherlands) and placed in a controlled growth room (see Figure 3.4). The plants were grown under conditions as described in section 3.4.1. Values are given in appendix A1-1. After 14 days 36 uniform seedlings were moved in to 540 ml containers containing 500 ml of a solution containing macro- and micronutrients (pH: 4,74, PHM210, MeterLab, Radiometer Analytica S.A., France). The solution was mixed using Kristalon (9-5-25 (4,2-5,7) Mg+S+mikro) (Yara Vlaardingen B.V. The Netherlands) and Calcinit (Yara International ASA, by Yara Norge AS). The solution is described in Table 4. During the preparation of the solution the nutrients were introduced to distilled water under constant stir until solved. The solution was changed every 7-10 days and pH were measured after every solution change. The pH of the solution remained

relatively stable throughout the experiment due to the size of the containers. Values are given in appendix A1-4.

Table 4: Concentrations of macronutrients and micronutrients per L of distilled water used in experiment I and II.

Element	Kristalon, 2 ‰		Calcinit, 0,5 ‰		Total content	Total content
Tot N	9.00 %	180 mg N/L	15.50 %	77.5 mg N/L	257.5 mg N/L	18.39 mmol N/L
NO_3-N	8.00 %	160 mg N/L	14.40 %	72.0 mg N/L	232.0 mg N/L	16.57 mmol N/L
NH_4 - N	1.00 %	20 mg N/L	1.10 %	5.5 mg N/L	25.5 mg N/L	1.83 mmol N/L
P	4.80 %	96 mg P/L			96 mg P/L	1.55 mmol P/L
K	24.90 %	498 mg K/L			498 mg K/L	6.37 mmol K/L
Mg	4.20 %	84 mg Mg/ L			84 mg Mg/ L	3.47 mmol Mg/L
S	5.70 %	114 mg S/L			114 mg S/L	3.57 mmol S/L
Ca			19.00 %	380 mg Ca/L	380 mg Ca/L	9.48 mmol Ca /L
B	0.027 %	0.54 mg B/L			0.54 mg B/L	0.050 mmol B/L
Cu	0.004 %	0.08 mg Cu/L			0.08 mg Cu/L	1.250 μmol Cu/L
Fe	0.200 %	4.00 mg Fe/L			4.00 mg Fe/L	0.072 mmol Fe/L
Mn	0.060 %	1.20 mg Mn/L			1.20 mg Mn/L	0.022 mmol Mn/L
Mo	0.004 %	0.08 mg Mo/L			0.08 mg Mo/L	0.830 μmol Mo/L
	0.027 %	0.54 mg Zn/L			0.54 mg Zn/L	8.250 μmol Zn/L

The transparent containers were wrapped in aluminum foil to protect the root from direct light (Figure 3.5). The stems of the plants were fixed by rubber foam in a 12 mm diameter opening in the middle of the lid. Plants were kept in the growth room until they were 22 days old. Before start of ozone treatment, the length of the root system was measured.

Figure 3.5 Plants in the experimental unit. Seedlings were selected by their uniform development and transplanted to aluminum wrapped containers before exposure. University of Oslo, 12. May 2018.

3.5.2 Ozone exposure of plants

After 22 days six pots, each containing one seedling were put into each experimental chamber. The containers were marked with chamber number and daylight treatment but were otherwise placed in the chamber at random. Day one plants were contained in the experimental chambers for 6 hours then transferred to two different growth rooms with different daylight treatments as described in section 3.2. Day two the amount of visible injury was recorded. The same routine was repeated for day three and four, and five and six.

In half of the chambers ozone enriched air were added with a set amount of 70 ppb. 18 plants were exposed to ozone, and 18 were exposed to filtered air. Measured ozone concentrations with AOT40 and PODy are given in appendix A1-2 and D1-2.

3.5.3 Climate conditions during ozone exposure

During the ozone exposure the temperature inside the chambers was set to 20̊ C. Relative humidity were minimum of 60 % and the photosynthetic photosynthetically active radiation (PAR) is estimated to be between 68.35 and 190.07 µmol/m^2/s recorded as described in 2.4.3. Microclimatic conditions are given in appendix A1-2.

3.5.4 LD and SD treatment

To study the effect of photoperiod on ozone effects in plants, the plants were kept in either long-day or short-day conditions after the first day of ozone exposure and until the end of the experiment. The photoperiod of the two growth rooms are described in section 3.2. The climatic conditions in the growth rooms are given in appendix A1-3.

The pots were transferred from the growth rooms to the experimental chambers every other morning during exposure, and then moved back into the growth room after 6 hours of exposure. After the last exposure plants were cultivated for 3 days under long and short photoperiod before accumulated biomass were recorded.

3.5.5 Assessment of visible ozone-induced injury and biomass accumulation

At harvest only fully expanded trifoliate leaves were studied. The oldest trifoliate leaf was assigned leaf number one, the second oldest leaf number two, etc. To get a balanced study only leaf number one was studied at the first data recording, leaf one and two at the second data recording and leaf one, two and three at the third recording. The assessment followed the index presented by (University Corporation for Atmospheric Research, 2018), which divide the extent of ozone injury on the leaf surface into 6 categories, presented in Figure 3.7. To minimize human error damage was assessed on each leaflet instead of on each leaf as a whole, and each plant and leaf were examined without knowing which treatment the plant had gone through. The leaflets were numbered from left to right as shown in Figure 3.6 and the median was used for the entire leaf.

Figure 3.6 A trifoliate leaf divided into three leaflets. Damage score was set per leaflet and then estimated per leaf as the median of the three leaflets. University of Oslo. 20. December 2018.

To assess the amount of biomass accumulated during cultivation the length of the roots were measured before and after ozone treatment. Each plant was partitioned into above ground biomass and below ground biomass. The plants fresh weight was recorded, and the plants were dried for 48 hours at 105˚C. When constant weight was reached dry weight was recorded.

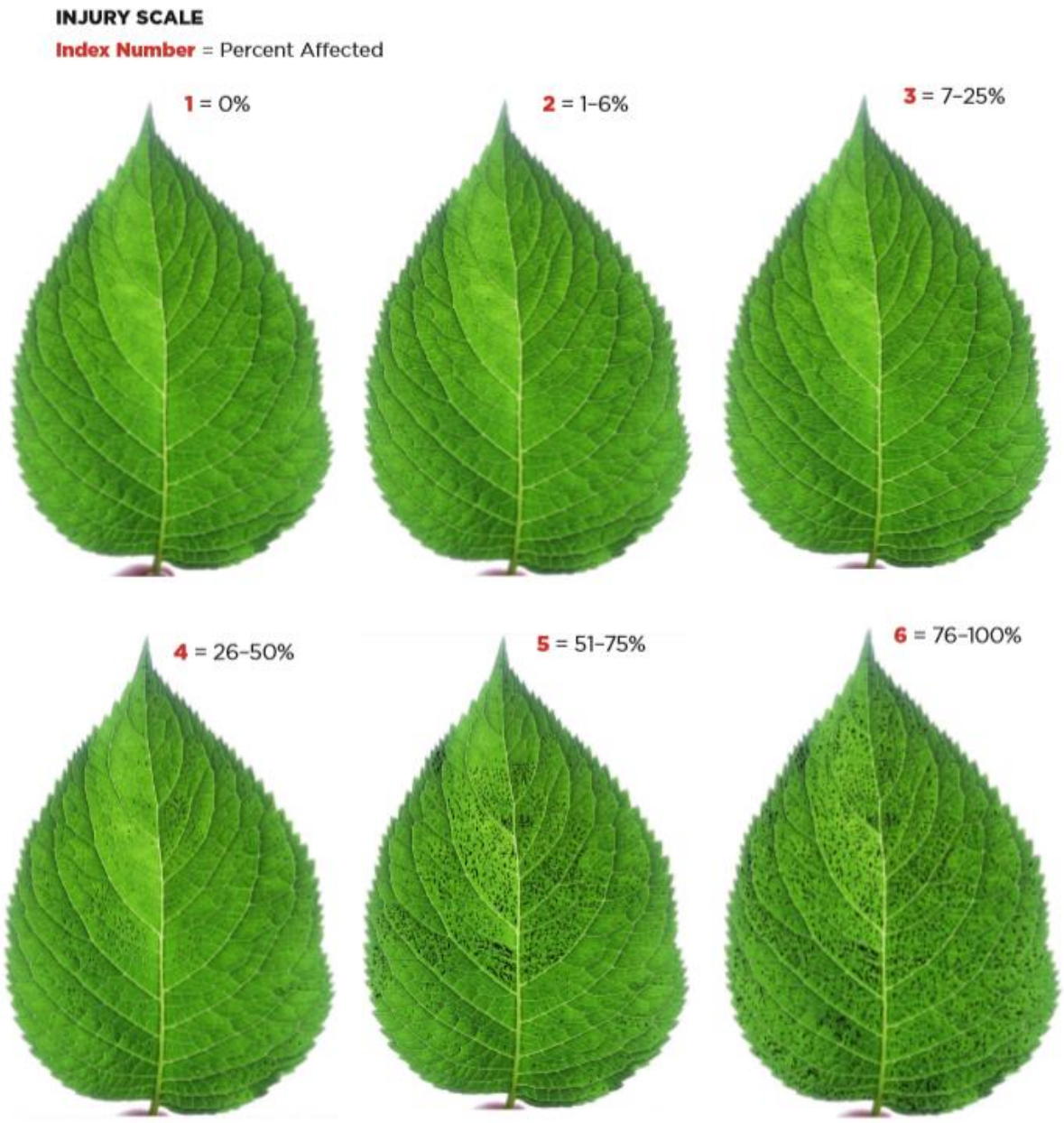


Figure 3.7 Ozone induced visible injury index used in ozone garden research presented by University Corporation for Atmospheric Research (2018).

3.6 Experiment II: Effects of ozone in relation to photoperiod on vegetative growth and visible injury of *Trifolium repens* L.

3.6.1 Cultivation and growth conditions before ozone and daylength treatment

Seeds of *Trifolium repens* cultivar Norstar (Strand unicorn A/S, Norway) were sown in trays containing soil and perlite and placed in a controlled growth room. The plants were grown under same conditions as in Experiment I. After 21 days 36 uniform seedlings were moved in to 540 ml containers containing 500 ml of a solution containing macro- and micronutrients (PHM210, MeterLab, Radiometer Analytica S.A., France). The solution is described in Table 4. Preparation of the solution and management of solution is described in section 3.5.1. Solution pH values are given in appendix A2-4.

The transparent containers were wrapped in aluminium foil to protect the root from direct light. The stems of the plants were fixed by rubber foam in a 12 mm diameter opening in the middle of the lid. Plants were kept in the growth room until they were 39 days old. Three plants did not survive the transfer from soil to solution, and the total number of plants in experiment II was therefore 33 plants, 16 plants were exposed to ozone and 17 plants were kept as controls.

3.6.2 Ozone exposure of plants

After 39 days six (or five) pots, each containing one seedling were put into each experimental chamber. The containers were marked with chamber number and daylight treatment but were otherwise placed in the chamber at random. Day one plants were contained in the experimental chambers for 6 hours then transferred to two different growth rooms with different daylength treatments as described in section 3.2. Day two the amount of visible injury was recorded. The same routine was repeated for day three and four, and five and seven. The difference from experiment I regarding time of ozone exposure was due to the heat experienced in Oslo during this period and the decision to not use energy and resources to cool down the Phytotron to conduct experiments while the temperature was at its highest. Therefore, the time from sowing

to the start of ozone was prolonged with 8 days, and the ozonation was conducted early in the morning in order to be done before the outdoor temperature reached its maximum.

As in experiment I half of the chambers had ozone enriched air added with a set amount of 70 ppb. 16 plants were exposed to ozone, and 17 were exposed to filtered air. Measured ozone concentrations with AOT40 and PODy are given in appendix A2-2 and D2-2.

3.6.3 Climate conditions during ozone exposure

During the ozone exposure the temperature inside the chambers was set to 20° C. Relative humidity were minimum of 60 % and the photosynthetic photosynthetically active radiation (PAR) is estimated to be between 81.3 and 238.3 µmol/m^2/s recorded as described in 2.4.3. Microclimatic conditions are given in appendix A2-2.

3.6.4 LD and SD treatment

Plants were kept in either long-day or short-day conditions after the first day of ozone exposure and until the end of the experiment. The photoperiod of the two growth rooms are described in section 3.2. The climatic conditions in the growth rooms are given in appendix A2-3.

The pots were transferred from the growth rooms to the experimental chambers for exposure to ozone or filtered air, and then moved back into the growth room after exposure. After the last exposure plants were cultivated for 3 days under long and short photoperiod before harvest.

3.6.5 Assessment of visible ozone-induced injury and biomass accumulation

Only fully expanded trifoliate leaves were included in the assessment. The assessment was done as in experiment I. Before the last data recording one leaflet was lost, and the total number of damage points was 593. The median of each trifoliate leaf was set as the index for the entire leaf.

The amount of biomass accumulated during the experiment was assessed as in Experiment I.

3.7 Experiment III: Effects of ozone in relation to photoperiod on visible injury, stomatal resistance and chlorophyll content on American clones of *Trifolium repens* L.

3.7.1 Cultivation and growth conditions before ozone and daylength treatment

Cuttings of *Trifolium repens* L. cv Regal sensitive- and resistant clone (NC-S and NC-R) sent from Kent Burkey, United States Department of Agriculture, Agricultural Research Service (USDA-ARS) in North Carolina was cultivated in 1 L pots with the same mix of soil and Perlite as in experiment I for 69 days. They were cultivated under the same climatic conditions as described in section 3.4.1. Plants was fertilized every 7 days using the same solution as was used as growth medium in experiment I and II, see Table 4 for concentrations. Weekly means of climatic conditions are given in appendix A3-1.

3.7.2 Ozone exposure of plants

70 days old plants were exposed to ozone 5 days during a 14-day period. They were exposed to 6 hours of ozone at 70 ppb on day one, five and eight. On day eight they were exposed to an ozone burst of about 100 ppb due to a malfunction with the gas container. The burst lasted for less than a minute and the values recorded increased normally after the episode. On day 13 and 14 the plant was exposed to half a day of ozone because of the heat in Oslo and the impossibility of conducting a full day of ozone exposure during day 13.

3.7.3 Climate conditions during ozone exposure

The climatic conditions were set to the same levels as in experiment I and II. Microclimatic conditions are given in appendix A3-2.

3.7.4 LD and SD treatment

As in experiment I and II plants were kept in either long-day or short-day conditions after the first day of ozone exposure and until the end of the experiment. The photoperiod of the two growth rooms are described in section 3.2. The climatic conditions in the growth rooms are given in appendix A3-3.

The pots were transferred from the growth rooms to the experimental chambers for exposure to ozone or filtered air, and then moved back into the growth room after 6 hours of exposure. After the last exposure plants were cultivated for 3 days under long and short photoperiod.

3.7.5 Assessment of visible ozone-induced injury and biomass accumulation

Due to the development of the cuttings, the second leaf of an axillary shoot was studied during the entire experiment. The second leaf was the second youngest leaf at the start of the experiment. Biomass accumulation was assessed by cutting the axillary shoot at the base of leaf number one, thus only harvesting biomass accumulated after ozone exposure.

The entire trifoliate leaf was studied and given a score of 1-6 as described in section 3.5.5. The youngest leaf was given the label Leaf one, the second youngest leaf two and so on. This study focused on leaf number two.

3.7.6 Assessment of physiological responses to ozone exposure and photoperiod.

The stomatal conductance was studies using a porometer (AP4, Delta-T Devices, Cambridge, UK). The starting levels was assessed by studying clones not included in the experiment during day one of exposure, this giving a baseline of future measurements. The stomatal conductance was measured two times during the experiment; day four and two days before harvesting.

After the second day of exposure the chlorophyll content of the leaves was assessed using a modulated fluorometer measuring chlorophyll content non-destructively (CCM-300, Opti-Sciences, Hudson, New Hampshire, USA). Since a calibration curve for the clover leaves has not been made, the values given are in arbitrary units.

3.8 Modelling ozone flux using DO3SE

To model ozone flux a minimum of environmental and climatic conditions must be known. The parameters used in this model is described in Table 5. Missing parameters was replaced by a conservative constant. A sensitivity test of the different parameters against total ozone flux is represented in appendix D4.

Two models have been used. A limited model only using input from the experiment when plants was in the exposure chambers, and a full model where parameters from the growth room are included. The input/output data is given in appendix D.

Table 5: A summary of necessary parameters to run the DO3SE model, and the origin of the parameters implemented. The actual input data is given in Appendix D.

Parameter	**Input**
Day of year and hour of day	Actual day and time of day of the experiment was used.
Temperature (Ts, C, Celsius)	Mean hourly temperature was estimated from data collected as described in section 3.4.3.
Vapour pressure deficit (VPD, kPa)	Was estimated using relative humidity and temperature described in section 3.4.3. Estimations was done using a VPD calculator provided by College of agriculture and life sciences (2018)
Meassured wind speed (uh, zR, m/s)	The windspeed in the exposure system has not been analysed. An estimate of 1 m/s is set as default on all estimations done in this study.
Precipitation (presip, mm)	The precipitation during the experiment is set to 0. During experiment I and II, the plants are kept in solutions and water availability is not a limiting factor.
Photosynthetically active radiation (PAR, µmol/m2/s)	Estimated PAR using lux data recorded as described in section 3.4.3. Lux data was recorded during day 1 of experiment I and day 8 of experiment III. Experiment I data was used for the entire modelling of experiment I, and experiment III data was used for modelling experiment II and III due

	to the similar weather conditions during that period. PAR estimations were done using a lux-par-conversion factor of 0.016 calculated from measurements done in the phytotron.
Preassure (P, kPa)	Standard atmospheric pressure was set as default.
Meassured O3 density (O3_zR, ppb)	Hourly mean was estimated from data collected during the experiment as described in 2.3.
PODy threshold	The PODy threshold for herbs and grass is set to 1 (Calvete-Sogo, González-Fernández, et al., 2017; Mills, Pleijel, et al., 2011).

3.9 Statistics

To study the effect of ozone and photoperiod on different plant responses both parametric and nonparametric tests are used to test the hypotheses stated in section 2.7.

All experiments and the associated statistical analyses has two binomial explanatory variables as described in Table 2; Ozone treatment and photoperiod. Treatment and placement was randomly assigned to the individual plants to ensure independence in the experimental design. Statistical calculations and graph production were done using R (Rx64 3.5.2 for windows) and Excel (Microsoft office 2010).

3.9.1 Parametric statistics

Parametric statistics relies on parameters that describe a population a sample is gathered from. A parametric test relies on some general assumptions including independence, homogeneity of variances, normality of error and linearity. In a parametric analysis the group means are tested. Examples of parametric tests are 1- and 2-sample t tests, ANOVA and Pearson Correlation test (Moore, McCabe, & Craig, 2014).

3.9.2 Nonparametric statistics

Nonparametric statistics do not rely on fixed parameters describing a population. A nonparametric test is used when the data don't meet the assumptions of a parametric test. This can be caused by a very small sample size, with ordinal or ranked data, with outliers that can't be removed, with highly skewed data, non-normal distribution etc. nonparametric tests compare medians rather than means. Examples of nonparametric tests are 1-sample Wilcoxon, Chi square, Fishers exact, Mann-Whitney test, Kruskal-Wallis test and Spearman Rank Correlation test (Moore et al., 2014; Pezzullo, 2013).

3.9.3 Growth parameters and physiological responses

Growth parameters and physiological responses gives continuous numerical responses and parametric tests are the preferred method given normally distributed data. A one-way ANOVA test was used and an p value of less than 0.05 is set as the significance level. A one-way ANOVA with only two groups gives the same output as a student t-test, but with a ANOVA interaction between variables can be included in the analysis. This means that a p value of less than, or equal to 0.05 reduces the chance of making a type I error to no more than 5 percent. This p value has become accepted as a reasonable level for significance, and is used widely today (Moore et al., 2014; Pezzullo, 2013).

To examine the connection between photoperiod and damage response in plants exposed to ozone and seek to establish a parameter that explains the hypothesized difference. To study this possible connection stomatal conductance was analysed in experiment III to look at gas exchange and the assumed difference due to photoperiod. A linear model is fitted to give a general explanation of the difference in photoperiod. R-squared values are used to give an approximation of how well fitted the model is.

3.9.4 Visible injury

Visible injury data are given in ranked categorical responses per leaflet. A median was estimated per leaf to reduce human error and due to the missing independence between leaflets from the same leaf. Because of the nature of the response data, it is possible to analyse the

categories as a categorical variable but also as a discreate variable. Multiple approaches can be made, but a nonparametric approach is the most appropriate approach.

In this study a Fishers exact test was used to study the effect of ozone and photoperiod separately on damage responses. A Fishers Exact Test analyses cross tabulated data and was invented by R.A. Fisher in the 1920 to give exact p values for tables with large or small cell counts (Pezzullo, 2013). The contingency tables analysed in this study have multiple cells with a cell count of zero due to the expected lack of visible damage in CFA exposed plants. A drawback of the Fishers exact test is that it is not designed to test between two ordinal categorical variables. This makes the Fishers Exact test insensitive to gradual trends across the ordinal categories (Pezzullo, 2013). Since only one variable in this dataset is ordinal, a Fishers Exact test will be used to study the stated hypotheses in section 2.7.

Given a significant result of the effect of ozone on visible damage on all data in the dataset the CFA exposed plants were excluded from the subsequent analysis of photoperiod and degree of damage.

To study the effect of time on the degree of damage between paired groups a Wilcoxon signed-rank test was used. This is a nonparametric test used to comparing matched pair in non-normally distributed data (Pezzullo, 2013). To study the effect of photoperiod on visible damage, damage scores from plants at a given time were paired within the same group at a later time, to study if the accumulated ozone dose had different effects in the two photoperiod groups.

4 Results

4.1 Experiment I

4.1.1 Growth parameters

Measured values of the growth parameters studied (root size and fresh and dry weight of above and below ground biomass) and calculated means and standard deviations for each treatment are given in appendix C1-1, C1-2 and C1-3.

To study the effect of ozone on the different growth parameters several analyses was performed. An analysis off all plants to investigate the total effect of ozone (n=36), then an analysis of ozone by photoperiod treatments separately (n=18). An analysis off all plants to investigate the total effect of photoperiod (n=36), then an analysis of photoperiod by ozone treatment separately (n=18). Finally, an analysis of the entire model with interaction between the parameters (n=36). The main results of the statistical analyses are given in Table 9.

Root size

Inspection of Q-Q-plots of recorded data given in appendix B1-1 revealed that change in root size (length in cm) was normally distributed. Therefore, a one-way ANOVA was run on the data with a 95% confidence interval (CI) for the mean difference. It was found that after the end of the experiment, root size change was significantly higher in the ozone exposed group (5.47 ± 0.77 cm, mean ± SD) than in the control group (3.80 ± 0.54 cm) (P=0.0363) the difference in mean being 1.67 ± 0.77 (95% CI, 0.11 to 3.23) cm. Photoperiod treatment did not show significance in root size change when all plants were included in the analysis (P =0.4613). Group means, quartiles and outliers are presented in Figure 4.1 and Table 6.

Ozone exposed plants grown under short-day photoperiod showed a significantly larger root size increase (2.42 ± 1.01 cm) compared to plants exposed to CFA (P = 0.029). No other significant effect was found when looking at the change in root size within different treatments. Statistics are given in Table 9.

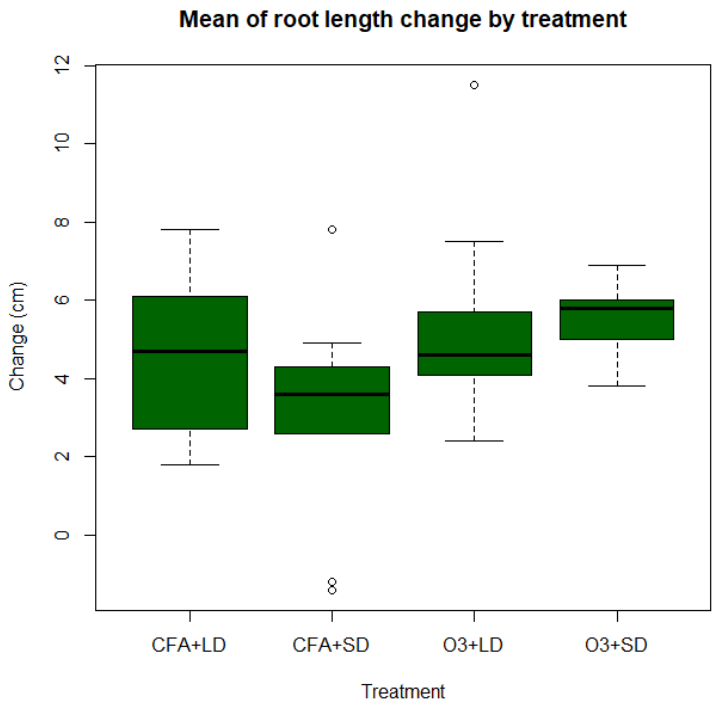


Figure 4.1 Boxplot of change in root size at harvest of Trifolium subterraneum, by treatments as described in Table 2 (n=36).

Table 6: Summary of quartiles and the mean of change in root size of *Trifolium subterraneum* (cm).						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	1.8	2.7	4.7	4.48	6.1	7.8
CFA+SD	-1.4	2.6	3.6	3.12	4.3	7.8
O3+LD	2.4	4.1	4.6	5.40	5.7	11.5
O3+SD	3.8	5.0	5.8	5.54	6	6.9

Above ground biomass

Recorded data is given in appendix B1-2. Total above ground biomass was not significantly affected by ozone or photoperiod treatment when all plants were included in the data analysis regardless of state of biomass (fresh or dry). P-values are given in Table 9. Group means, quartiles and outliers are presented in Figure 4.2 and Table 7. No significant difference was found between ozone exposed plants of different photoperiod treatments at fresh or dry state.

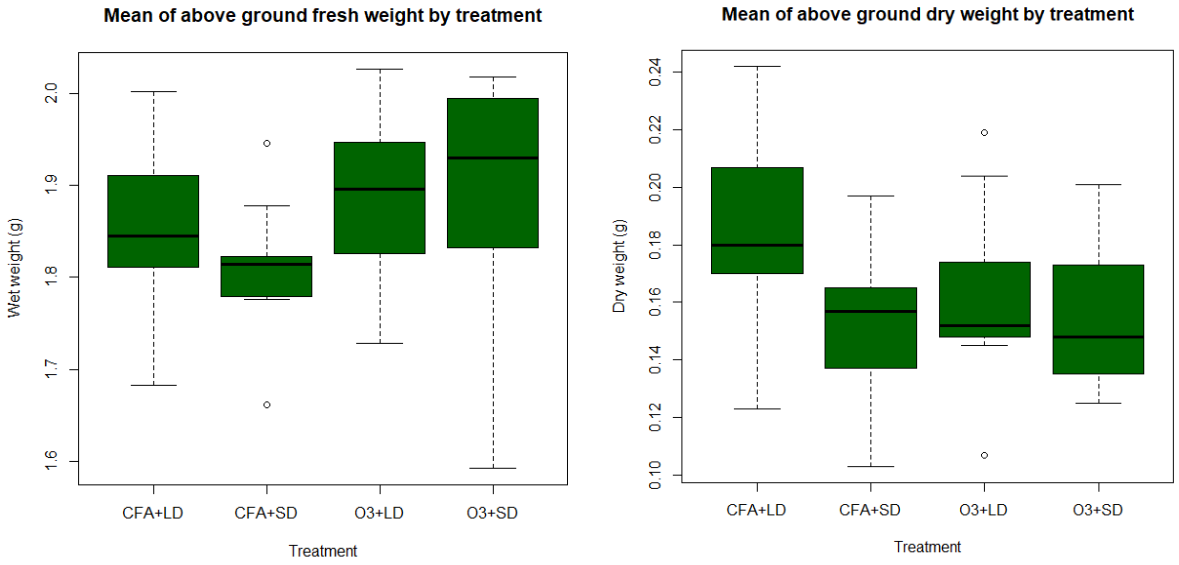


Figure 4.2 Boxplot of above ground biomass at harvest of Trifolium subterraneum, (dry and fresh), by treatments as described in Table 2 (n=36).

Table 7: Summary of quartiles and the mean of above ground fresh and dry mass of *Trifolium subterraneum* (g).						
Above ground fresh mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	1.682	1.811	1.845	1.847	1.911	2.002
CFA+SD	1.661	1.779	1.814	1.812	1.823	1.946
O3+LD	1.728	1.826	1.896	1.889	1.947	2.027
O3+SD	1.592	1.832	1.93	1.886	1.995	2.018
Above ground dry mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.123	0.170	0.180	0.1838	0.207	0.242
CFA+SD	0.103	0.137	0.157	0.1554	0.165	0.197
O3+LD	0.107	0.148	0.152	0.1611	0.174	0.219
O3+SD	0.125	0.135	0.148	0.1542	0.173	0.201

Below ground biomass

Recorded data is given in appendix B1-3. Total below ground biomass was not significantly affected by ozone or photoperiod treatment when all plants were included in the data analysis regardless of state of biomass (fresh or dry). P-values are given in Table 9. Group means, quartiles and outliers are presented in Figure 4.3 and Table 8. No significant difference was found between ozone exposed plants of different photoperiod treatments at fresh or dry state.

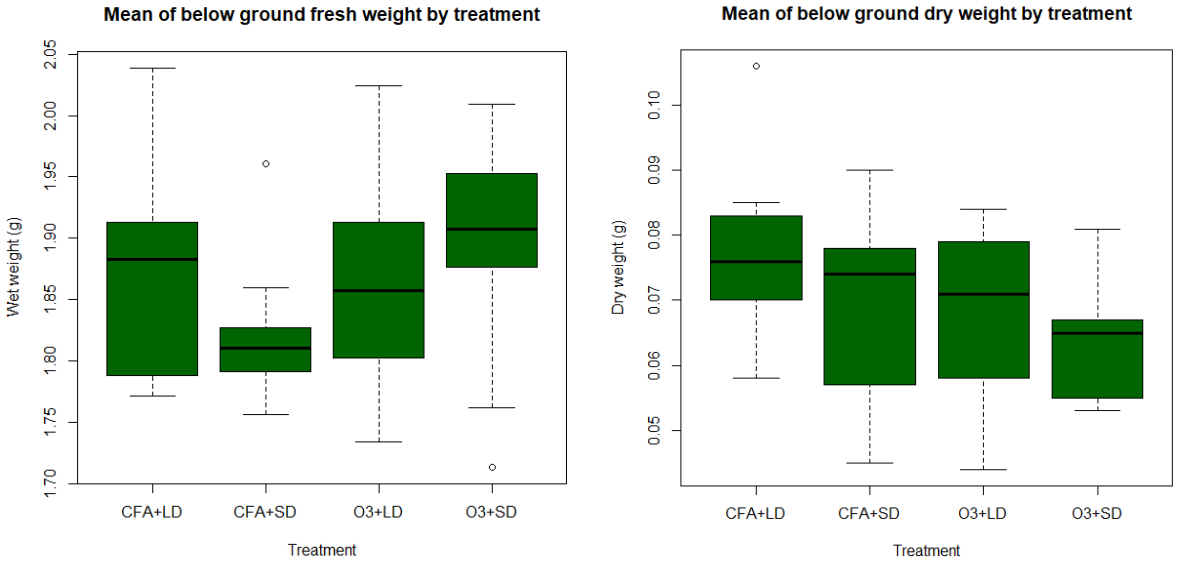


Figure 4.3 Boxplot of below ground biomass at harvest of Trifolium subterraneum, (dry and fresh), by treatments as described in Table 2 (n=36).

Table 8: Summary of quartiles and the mean of below ground fresh and dry mass of *Trifolium subterraneum* (g).						
Below ground fresh mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	1.771	1.788	1.883	1.874	1.913	2.039
CFA+SD	1.756	1.791	1.810	1.821	1.827	1.961
O3+LD	1.734	1.802	1.857	1.867	1.913	2.024
O3+SD	1.713	1.876	1.907	1.895	1.953	2.009
Below ground dry mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.058	0.070	0.076	0.07722	0.083	0.106
CFA+SD	0.045	0.057	0.074	0.070	0.078	0.090
O3+LD	0.044	0.058	0.071	0.06811	0.079	0.084
O3+SD	0.053	0.055	0.065	0.06444	0.067	0.081

Table 9: Statistical test (One-way ANOVA) conducted between treatments of ozone exposure and photoperiod. Significant differences are given in bold.

Parameter	Source	n	df	F value	Pr(>F)
Root size change	Ozone treatment	36	1	4.7486	**0.0364**
	Photoperiod	36	1	0.5553	0.4613
	Ozone | Short day	18	1	5.7196	**0.0294**
	Ozone | Long day	18	1	0.6250	0.4408
	Photoperiod | O3	18	1	0.0232	0.8808
	Photoperiod | CFA	18	1	1.2354	0.2828
Model with interactions	Photoperiod	36	1	0.6146	0.4388
	Ozone treatment	36	1	4.6868	**0.0380**
	Ozone treatment and Photoperiod	36	1	0.9428	0.3389
Above ground biomass Fresh Mass	Ozone treatment	36	1	2.7818	0.1045
	Photoperiod	36	1	0.2782	0.6013
	Ozone | Short day	18	1	1.8030	0.1981
	Ozone | Long day	18	1	0.8726	0.3641
	Photoperiod | O3	18	1	0.0032	0.9553
	Photoperiod | CFA	18	1	0.7329	0.4046
Model with interactions	Photoperiod	36	1	0.2852	0.5970
	Ozone treatment	36	1	2.6573	0.1129
	Ozone treatment and Photoperiod	36	1	0.1939	0.6627
Dry mass	Ozone treatment	36	1	1.2467	0.2720
	Photoperiod	36	1	2.8321	0.1016
	Ozone | Short day	18	1	1.8030	0.1981
	Ozone | Long day	18	1	1.9894	0.1775
	Photoperiod | O3	18	1	0.2378	0.6324
	Photoperiod | CFA	18	1	3.4352	0.0824
Model with interactions	Photoperiod	36	1	2.8636	0.1003
	Ozone treatment	36	1	1.3173	0.2596
	Ozone treatment and Photoperiod	36	1	1.0615	0.3106
Below ground biomass Fresh mass	Ozone treatment	36	1	1.2453	0.2723
	Photoperiod	36	1	0.1777	0.6760
	Ozone | Short day	18	1	3.5947	0.0762
	Ozone | Long day	18	1	0.0284	0.8682
	Photoperiod | O3	18	1	0.3639	0.5548
	Photoperiod | CFA	18	1	2.0814	0.1684
Model with interactions	Photoperiod	36	1	0.1836	0.6712
	Ozone treatment	36	1	1.2477	0.2723
	Ozone treatment and Photoperiod	36	1	1.8809	0.1798
Dry mass	Ozone treatment	36	1	2.8560	0.1002
	Photoperiod	36	1	1.5170	0.2265
	Ozone | Short day	18	1	0.8953	0.3581
	Ozone | Long day	18	1	2.0027	0.1762
	Photoperiod | O3	18	1	0.4236	0.5244
	Photoperiod | CFA	18	1	1.1804	0.2934

Model with interactions	Photoperiod	36	1	2.8636	0.1003
	Ozone treatment	36	1	1.3173	0.2596
	Ozone treatment and Photoperiod	36	1	1.0615	0.3106

4.1.2 Visible ozone-induced foliar injury

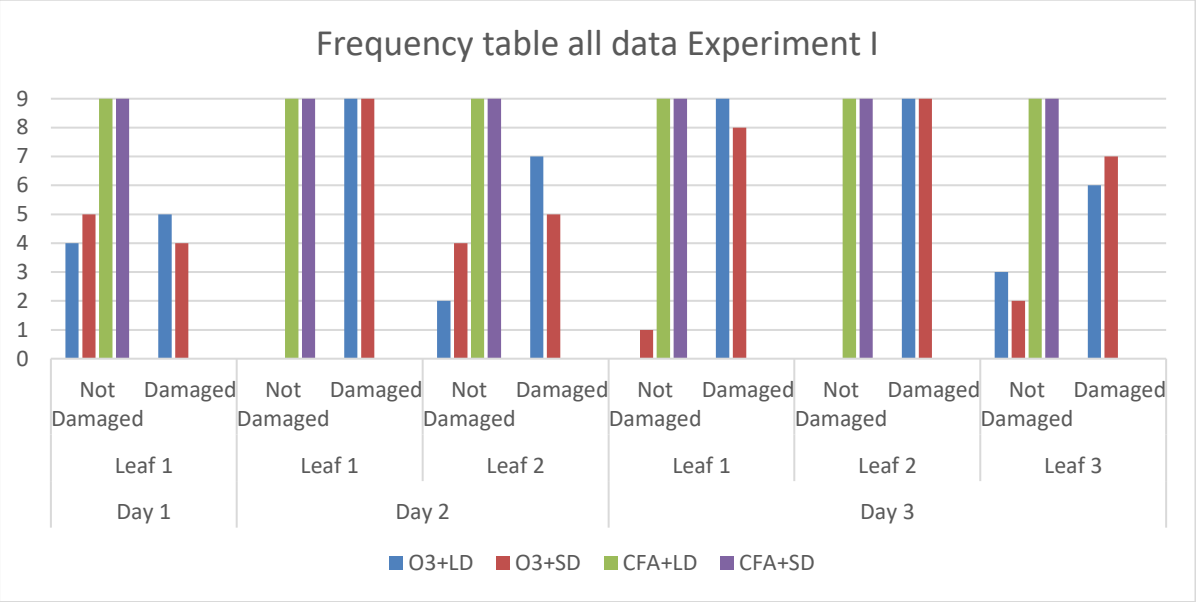


Figure 4.4 Damage frequencies by treatment as described in Table 2. Day 1-3 refers to day of recorded injury with an increase of ozone accumulation from day 1 till day 3. Leaf 1-leaf 3 refers to the age of the leaf examined with leaf one being the youngest fully developed trifoliate leaf at day one, and leaf 2 and 3 developing during the experiment with leaf 3 being the youngest leaf at day 3.

Trifolium subterraneum L. started to develop ozone induced damage symptoms after six hours of ozone exposure with a POD1 value of 0.0297 mmol/m^2 PLA. Signs of ozone damage included small grey and white spots. In experiment I, visible foliar injury occurred on 50% of *Trifolium subterraneum* after the first day of exposure, and after the second day 100% of leaf one samples showed symptoms of ozone damage. No visible symptoms were recorded on plants exposed to carbon filtered air. Recorded ozone-induced visible damage per plant per leaflet are given in appendix B1-4. Damage was more often seen on mature leaves compared to younger leaves which can be seen in Table 10 and Figure 4.4.

Table 10: Frequency table. Number of damaged samples per treatment group as described in Table 2 (n=36)						
			O3+LD	O3+SD	CFA+LD	CFA+SD
Day 1	Leaf 1	Not Damaged	5	4	9	9
		Damaged	4	5		
Day 2	Leaf 1	Not Damaged			9	9
		Damaged	9	9		
	Leaf 2	Not Damaged	2	4	9	9
		Damaged	7	5		
Day 3	Leaf 1	Not Damaged		1	9	9
		Damaged	9	8		
	Leaf 2	Not Damaged			9	9
		Damaged	9	9		
	Leaf 3	Not Damaged	3	2	9	9
		Damaged	6	7		

Table 11: Mean and standard deviation of recorded damage per treatment group as described in Table 2, with day and leaf as described in Figure 4.4.						
MEAN	Day1 Leaf1	Day2 Leaf1	Day2 Leaf2	Day3 Leaf1	Day3 Leaf2	Day3 Leaf3
O3+LD	2.00	3.37	3.48	3.93	4.52	2.78
O3+SD	2.17	2.96	2.78	3.93	4.15	2.37
CFA+LD	1.00	1.00	1.00	1.00	1.00	1.00
CFA+SD	1.00	1.00	1.00	1.00	1.00	1.00
STDEV	Day1 Leaf1	Day2 Leaf1	Day2 Leaf2	Day3 Leaf1	Day3 Leaf2	Day3 Leaf3
O3+LD	1.41	1.28	1.81	0.96	1.58	1.53
O3+SD	1.41	1.43	1.91	1.57	1.54	1.52
CFA+LD	0.00	0.00	0.00	0.00	0.00	0.00
CFA+SD	0.00	0.00	0.00	0.00	0.00	0.00

A Chi square test on all data, and Fishers exact tests on leaf and day specific data reports a significant difference between plants exposed to ozone and plants exposed to CFA regarding damage frequencies. The subsequent analysis of photoperiod treatment on damage showed no significant difference when studying each leaf per day separately (see appendix C1-4). When looking at the damage mean of each treatment the ozone exposed plants treated with a long day photoperiod displayed a higher degree of damage in general, though not significant (see Table 11).

When studying photoperiod within each treatment group separately with time as a factor, a significant difference was shown between most comparison under long day photoperiod, and

only one under short day photoperiod which indicates that there was a bigger change over time in the group exposed to long day photoperiod (see Table 12).

Statistics are presented in appendix C1-4.

Table 12: Test statistics done on damage frequencies and categorical damage in *Trifolium subterraneum*. Significant differences are given in bold.

Parameters	**n**	**Test statistics**	**P value**
Damage(frequency)~Ozone	all data (n=216)	Chi square	**1.11E-29**
Damage(frequency)~Photoperiod | Ozone	all ozonated plants (n=108)	Fishers Exact	0.2783
Damage(category)~Photoperiod | Ozone + LD	D1L1~D2L1 (n=18)	Wilcoxon	**0.0071**
Damage(category)~Photoperiod | Ozone + LD	D1L1~D3L1 (n=18)	Wilcoxon	**0.0136**
Damage(category)~Photoperiod | Ozone + LD	D2L1~D3L1 (n=18)	Wilcoxon	0.1198
Damage(category)~Photoperiod | Ozone + LD	D2L2~D3L2 (n=18)	Wilcoxon	**0.0179**
Damage(category)~Photoperiod | Ozone + SD	D1L1~D2L1 (n=18)	Wilcoxon	0.1138
Damage(category)~Photoperiod | Ozone + SD	D1L1~D3L2 (n=18)	Wilcoxon	0.0725
Damage(category)~Photoperiod | Ozone + SD	D2L1~D3L1 (n=18)	Wilcoxon	0.2021
Damage(category)~Photoperiod | Ozone + SD	D2L2~D3L2 (n=18)	Wilcoxon	**0.0199**

4.2 Experiment II

4.2.1 Growth parameters

Measured values of the growth parameters studied (root size and fresh and dry weight of above and below ground biomass) and calculated means and standard deviations for each treatment are given in appendix C2-1, C2-1 and C2-3.

As in experiment I, to study the effect of ozone on the different growth parameters several analyses were performed. An analysis off all plants to investigate the total effect of ozone (n=33), then an analysis of ozone by photoperiod treatments separately (n=16/17). An analysis off all plants to investigate the total effect of photoperiod (n=33), then an analysis of photoperiod by ozone treatment separately (n=16/17). Finally, an analysis of the entire model with interaction between the parameters (n=33). The main results of the statistical analyses are given in Table 16.

Root size

Inspection of Q-Q-plots of recorded data given in appendix B2-1 revealed that change in root size (length in cm) was normally distributed. Therefore, a one-way ANOVA was run on the data for the mean difference. It was found that after the end of the experiment, root size did not significantly change in the ozone exposed group (3.11 ± 1.02 cm) than in the control group (3.39 ± 0.71 cm) (P=0.7876) the difference in mean being -0.2757 ± 0.71 (95% CI, -2.35 to 1.79) cm. Photoperiod treatment did not show significance in root size change when all plants were included in the analysis (P = 0.5985). Group means, quartiles and outliers are presented in Figure 4.5 and Table 13.

No significant effect was found when looking at the change in root size within different treatments. The lack of significance may be caused by there not being a difference in elongation in the roots due to ozone exposure photoperiod, or because the dataset presented include a to small sample size. P-values of all analysis are given in Table 16.

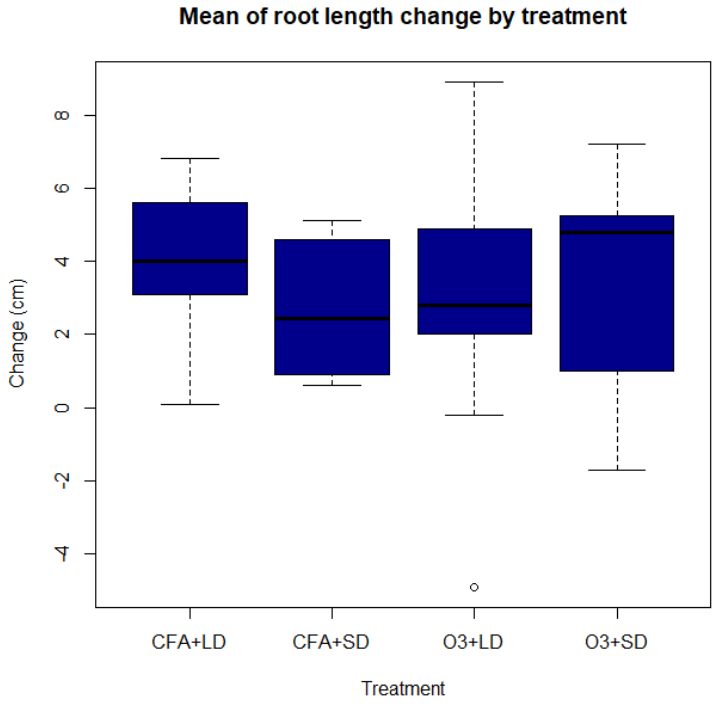


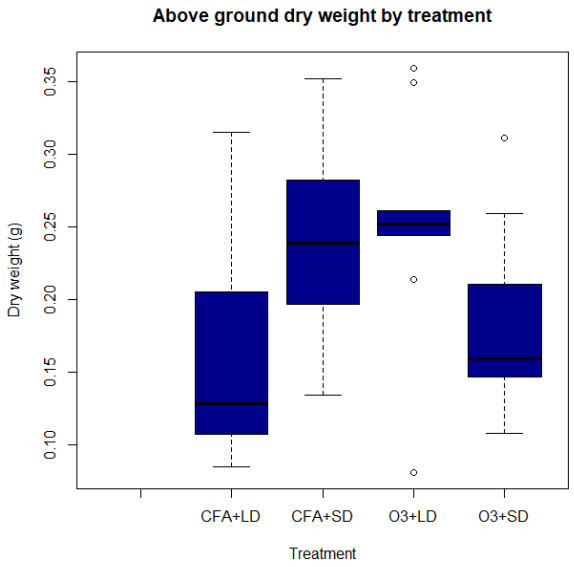
Figure 4.5 Boxplot of change in root size at harvest of Trifolium repens cv. Norstar, by treatments as described in Table 2 (n=33).

Table 13: Summary of quartiles and the mean of change in root size of *Trifolium repens* cv. Norstar (cm).						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.1	3.1	4.0	4.00	5.6	6.8
CFA+SD	0.6	1.0	2.5	2.70	4.6	5.1
O3+LD	-4.9	2.0	2.8	3.00	4.9	8.9
O3+SD	-1.7	1.0	4.8	3.26	5.3	7.2

Above ground biomass

Recorded data is given in appendix B2-2. Total above ground biomass was not significantly affected by ozone or photoperiod treatment when all plants were included in the data analysis regardless of state of biomass (fresh or dry). P-values are given in Table 16. Group means, quartiles and outliers are presented in Figure 4.6 and Table 14. A strong significant difference was found in response to ozone treatment in long day photoperiod plants when examining fresh biomass (p=0.008). The same significance can be seen in dry mass (p=0.02).

A significant effect in fresh mass was found for photoperiod in CFA exposed plants (p=0.03) which is not present in ozone exposed plants. This was also found in dry mass (p=0.03). When examining the linear regression with interactions a significant effect is found when including both ozone treatment and photoperiod in the model of fresh and dry mass (p=0.01, p=0.007).



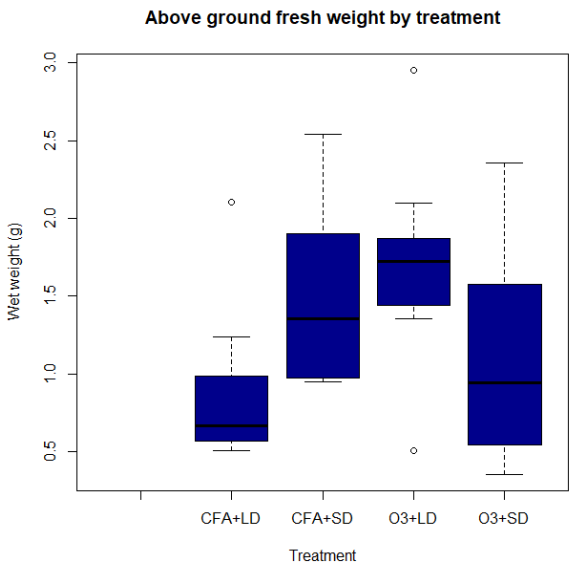


Figure 4.6 Boxplot of above ground biomass at harvest of Trifolium repens cv. Norstar, (dry and fresh), by treatments as described in Table 2 (n=33).

Table 14: Summary of quartiles and the mean of above ground fresh and dry mass of *Trifolium repens*(g).						
Above ground fresh mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.505	0.568	0.666	0.873	0.986	2.108
CFA+SD	0.949	0.98075	1.356	1.493875	1.84375	2.54
O3+LD	0.508	1.439	1.725	1.705778	1.871	2.952
O3+SD	0.356	0.5445	0.942	1.128857	1.5785	2.358
Above ground dry mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.085	0.107	0.128	0.159	0.205	0.315
CFA+SD	0.134	0.202	0.239	0.240	0.274	0.352
O3+LD	0.081	0.244	0.252	0.252	0.261	0.359
O3+SD	0.108	0.147	0.159	0.185	0.211	0.311

Below ground biomass

Recorded data is given in appendix B2-3. Total below ground biomass was not significantly affected by ozone or photoperiod treatment when all plants were included in the data analysis regardless of state of biomass (fresh or dry). P-values are given in Table 16. Group means, quartiles and outliers are presented in Figure 4.7 and Table 15. A significant effect (p=0.047) was found in dry mass of CFA exposed plants in response to photoperiod which did not occur in ozone exposed plants.

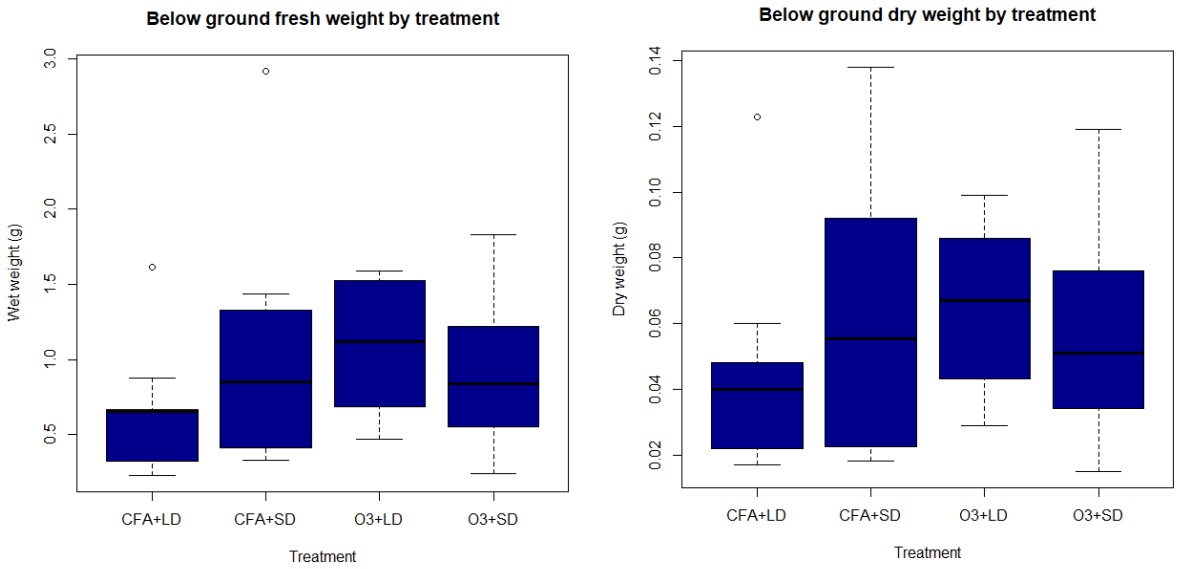


Figure 4.7 Boxplot of below ground biomass at harvest of Trifolium repens cv. Norstar, (dry and fresh), by treatments as described in Table 2 (n=33).

Table 15: Summary of quartiles and the mean of below ground fresh and dry mass of *Trifolium repens* cv. Norstar (g).						
Below ground fresh mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.231	0.323	0.657	0.645	0.669	1.614
CFA+SD	0.331	0.424	0.855	1.054	1.270	2.918
O3+LD	0.469	0.689	1.118	1.078	1.524	1.588
O3+SD	0.240	0.555	0.842	0.924	1.224	1.829
Below ground dry mass						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA+LD	0.017	0.022	0.040	0.0456	0.048	0.106
CFA+SD	0.018	0.024	0.056	0.062	0.084	0.090
O3+LD	0.029	0.043	0.067	0.066	0.086	0.084
O3+SD	0.015	0.034	0.051	0.058	0.076	0.081

Table 16: Statistical test (One-way ANOVA) conducted between treatments of ozone exposure and photoperiod. No significant differences were found.

Parameter	Source	n	df	F value	Pr(>F)
Root size change	Ozone treatment	33	1	0.0739	0.7876
	Photoperiod	33	1	0.2831	0.5985
	Ozone | Short day	15	1	0.1670	0.6895
	Ozone | Long day	18	1	0.4354	0.5188
	Photoperiod | O3	16	1	0.0191	0.8921
	Photoperiod | CFA	17	1	1.6605	0.2171
Model with interactions	Photoperiod	33	1	0.2803	0.6006
	Ozone treatment	33	1	0.0711	0.7916
	Ozone treatment and Photoperiod	33	1	0.5613	0.4598
Above ground biomass Fresh mass	Ozone treatment	33	1	1.4398	0.2393
	Photoperiod	33	1	0.0192	0.8907
	Ozone | Short day	15	1	1.0364	0.3272
	Ozone | Long day	18	1	8.9846	**0.0085**
	Photoperiod | O3	16	1	2.5277	0.1342
	Photoperiod | CFA	17	1	5.3962	**0.0347**
Model with interactions	Photoperiod	33	1	0.0385	0.8457
	Ozone treatment	33	1	1.6829	0.2048
	Ozone treatment and Photoperiod	33	1	7.1966	**0.0119**
Dry mass	Ozone treatment	33	1	0.8102	0.3750
	Photoperiod	33	1	0.0925	0.7630
	Ozone | Short day	15	1	2.3325	0.1507
	Ozone | Long day	18	1	6.4701	**0.0217**
	Photoperiod | O3	16	1	3.0262	0.1039
	Photoperiod | CFA	17	1	5.3861	**0.0348**
Model with interactions	Photoperiod	33	1	0.1372	0.7138
	Ozone treatment	33	1	0.9742	0.3318
	Ozone treatment and Photoperiod	33	1	8.1411	**0.0079**
Below ground biomass Fresh mass	Ozone treatment	33	1	0.7098	0.4060
	Photoperiod	33	1	0.4035	0.5299
	Ozone | Short day	15	1	0.1158	0.7391
	Ozone | Long day	18	1	4.6141	**0.0474**
	Photoperiod | O3	16	1	0.3727	0.5513
	Photoperiod | CFA	17	1	1.6367	0.2202
Model with interactions	Photoperiod	33	1	0.4491	0.5080
	Ozone treatment	33	1	0.7173	0.4040
	Ozone treatment and Photoperiod	33	1	1.8753	0.1814
Dry mass	Ozone treatment	33	1	0.5969	0.4456
	Photoperiod	33	1	0.1186	0.7329
	Ozone | Short day	15	1	0.1158	0.7391
	Ozone | Long day	18	1	2.3238	0.1469

	Photoperiod | O3	16	1	0.2833	0.6029
	Photoperiod | CFA	17	1	0.7904	0.3880
Model with interactions	Photoperiod	33	1	0.1353	0.7157
	Ozone treatment	33	1	0.5808	0.4521
	Ozone treatment and Photoperiod	33	1	1.0303	0.3185

4.2.2 Visible ozone-induced foliar injury

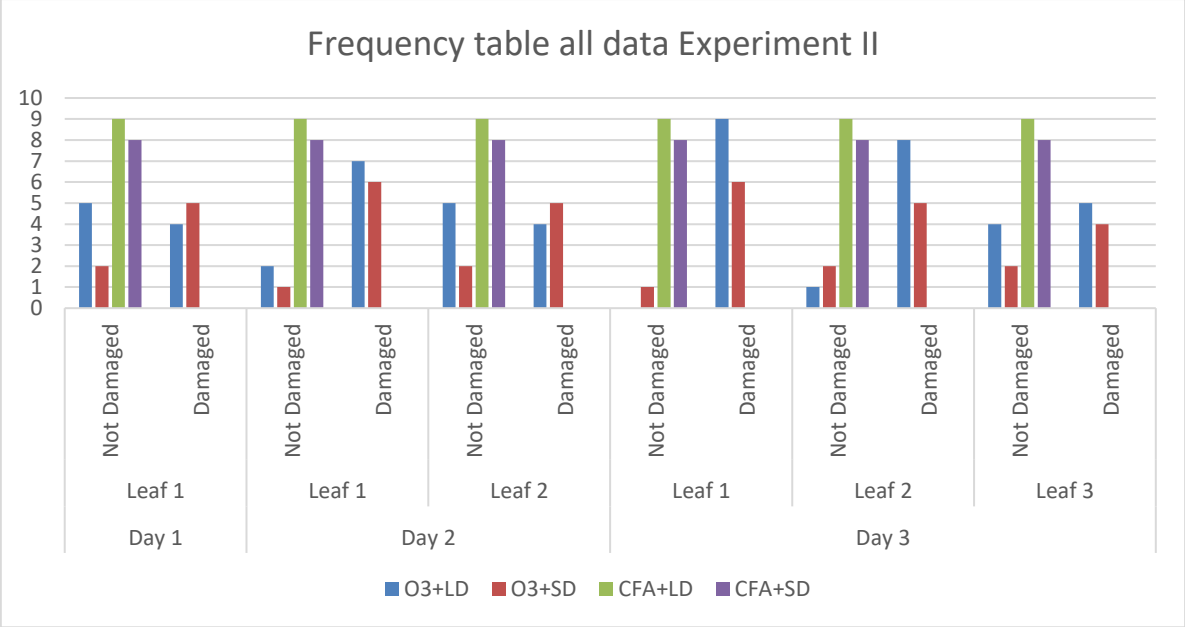


Figure 4.8 Damage frequencies by treatment as described in Table 2. Day 1-3 refers to day of recorded injury with an increase of ozone accumulation from day 1 till day 3. Leaf 1-leaf 3 refers to the age of the leaf examined with leaf one being the youngest fully developed trifoliate leaf at day one, and leaf 2 and 3 developing during the experiment with leaf 3 being the youngest leaf at day 3

T*rifolium repens* L. started to develop ozone induced damage symptoms after six hours of ozone exposure with a POD1 value of 0.0854 mmol/m^2 PLA. Signs of ozone damage included small grey and white spots and pleated edges on leaves as visible in Figure 4.9. After the second exposure all plants but one exposed to ozone showed signs of damage. No visible symptoms were recorded on plants exposed to carbon filtered air. Recorded ozone-induced visible damage per plant per leaflet are given in appendix B2-4.

Figure 4.9 Three examples of ozone-induced visible injury recorded as categorical damage 3, 4 and 5 respectively of Trifolium repens cv. Norstar after 3 days of exposure. University of Oslo, 9. June 2018

Damage was more often seen on mature leaves compared to than younger leaves which can be seen in Table 17 and Figure 4.8.

Table 17: Frequency table. Number of damaged samples per treatment group as described in Table 2 (n=33).

			O3+LD	O3+SD	CFA+LD	CFA+SD
Day 1	Leaf 1	Not Damaged	5	2	9	8
		Damaged	4	5		
Day 2	Leaf 1	Not Damaged	2	1	9	8
		Damaged	7	6		
	Leaf 2	Not Damaged	5	2	9	8
		Damaged	4	5		
Day 3	Leaf 1	Not Damaged		1	9	8
		Damaged	9	6		
	Leaf 2	Not Damaged	1	2	9	8
		Damaged	8	5		
	Leaf 3	Not Damaged	4	2	9	8
		Damaged	5	4		

Table 18: Mean and standard deviation of recorded damage per treatment group as described in Table 2, with day and leaf as described in Figure 4.4.

MEAN	D1L1	D2L1	D2L2	D3L1	D3L2	D3L3
O3+LD	1.96	2.70	1.78	3.52	2.63	2.15
O3+SD	2.00	2.48	3.62	3.62	3.15	3.52
CFA+LD	1.00	1.00	1.00	1.00	1.00	1.00
CFA+SD	1.00	1.00	1.00	1.00	1.00	1.00
STDEV	D1L1	D2L1	D2L2	D3L1	D3L2	D3L3
O3+LD	1.32	1.23	0.93	1.45	0.97	1.20
O3+SD	1.10	1.29	1.36	2.18	2.11	2.16
CFA+LD	0.00	0.00	0.00	0.00	0.00	0.00
CFA+SD	0.00	0.00	0.00	0.00	0.00	0.00

A Fishers exact test on all data and on leaf and day specific data reports a highly significant difference between plants exposed to ozone and plants exposed to CFA regarding damage frequencies ($p < 2.2e-16$). Leaf and day specific analysis also show significant difference in the two photoperiod treatments. The subsequent analysis of photoperiod treatment on damage showed no significant difference when studying each leaf per day separately (see appendix C2-4). When looking at the damage mean of each treatment the ozone exposed plants treated with a short day photoperiod displayed a higher degree of damage in general, though not significant (see Table 18).

When studying photoperiod within each group separately over time a Wilcoxon sum rank test was performed and showed a significant difference between leaf one on day one and leaf one on day three under long day photoperiod ($p= 0.031$), but no other comparison showed any significance (see Table 19).

Full statistics are presented in appendix C2-4.

Table 19: Test statistics done on damage frequencies and categorical damage in *Trifolium repens* cv. Norstar. Significant differences are given in bold.

Parameters	**n**	**Test statistics**	**P value**
Damage(frequency)~Ozone	all data (n=198)	Fishers Exact	**< 2.2e-16**
Damage(frequency)~Photoperiod | Ozone	all ozonated plants (n=96)	Fishers Exact	0.0556
Damage(category)~Photoperiod | Ozone + LD	D1L1~D2L1 (n=16)	Wilcoxon	0.1736
Damage(category)~Photoperiod | Ozone + LD	D1L1~D3L1 (n=16)	Wilcoxon	**0.0310**
Damage(category)~Photoperiod | Ozone + LD	D2L1~D3L1 (n=16)	Wilcoxon	0.0890
Damage(category)~Photoperiod | Ozone + LD	D2L2~D3L2 (n=16)	Wilcoxon	0.0975
Damage(category)~Photoperiod | Ozone + SD	D1L1~D2L1 (n=16)	Wilcoxon	0.0890
Damage(category)~Photoperiod | Ozone + SD	D1L1~D3L1 (n=16)	Wilcoxon	0.0579
Damage(category)~Photoperiod | Ozone + SD	D2L1~D3L1 (n=16)	Wilcoxon	0.1814
Damage(category)~Photoperiod | Ozone + SD	D2L2~D3L2 (n=16)	Wilcoxon	0.1736

4.3 Experiment III

Because one clone is ozone resistant and one is ozone sensitive, the analysis of the different clones has been conducted separately in order to withdraw as much information as possible resulting in a reduced sample size.

4.3.1 Growth parameters

Measured values of the growth parameters studied (Axillary shoot length and fresh and dry weight) and calculated means and standard deviations for each treatment and each cultivar are given in appendix B3-1,-3 and C3-1,-3.

As in experiment I and II, to study the effect of ozone on the different growth parameters several analyses were performed. An analysis off all plants of each cultivar to investigate the total effect of ozone (n=12), then an analysis of ozone by photoperiod treatments separately (n=6). An analysis off all plants to investigate the total effect of photoperiod (n=12), then an analysis of photoperiod by ozone treatment separately (n=6). Finally, an analysis of the entire model with interaction between the parameters (n=12). The main results of the statistical analyses are given in Table 23.

Axillary shoot

Inspection of Q-Q-plots showed a normal distribution of axillary shoot length. A one-way ANOVA was run on the data for the mean difference. It was found that after the end of the experiment, axillary shoot size did not significantly change in the ozone exposed group (NC-R: $p = 0.6079$, NC-S: $p= 0.8698$). Photoperiod treatment did not show significance in axillary shoot size when all plants were included in the analysis (NC-R: $p = 0.191$, NC-S: $p= 0.083$). Group means, quartiles and outliers are presented in Figure 4.10 and Table 20. The sensitive clone showed a higher degree of variation of axillary shoot length when exposed to ozone, than exposed to CFA which showed a similar growth pattern.

No significant effect was found when looking at the axillary shoot size within different treatments except axillary shoot size in NC-S response to photoperiod when exposed to CFA ($p= 0.00176$**)**. The lack of significance may be caused by there not being a difference in elongation in axillary shoot due to ozone exposure and photoperiod or because the dataset presented is too small in sample size. P-values of all analysis are given in Table 23.

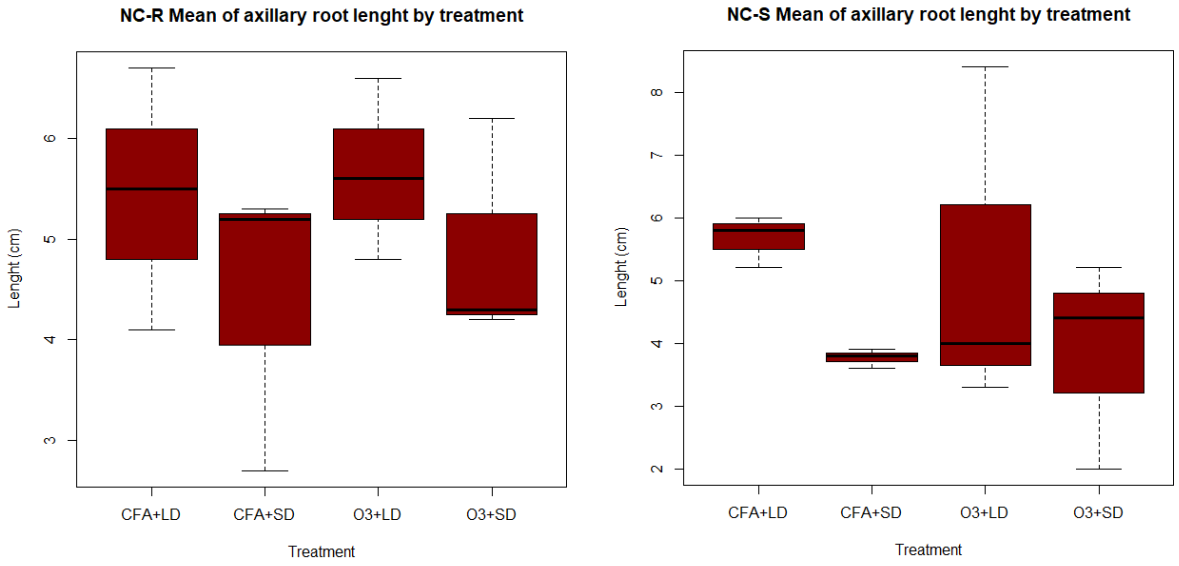


Figure 4.10 Boxplot of axillary shoot length at harvest of Trifolium repens cv. Regal, resistant (NC-R) and sensitive (NC-S) clone, by treatments as described in Table 2 (n=12).

Table 20: Summary of quartiles and the mean of axillary shoot length of *Trifolium repens* cv. Regal (cm).						
Axillary shoot NC-R						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA + LD	4.1	4.8	5.5	5.43	6.1	6.7
CFA + SD	2.7	3.9	5.2	4.40	5.3	5.3
O3 + LD	4.8	5.2	5.6	5.67	6.1	6.6
O3 + SD	4.2	4.3	4.3	4.90	5.3	6.2
Axillary shoot NC-S						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA + LD	5.2	5.5	5.8	5.67	5.9	6.0
CFA + SD	3.6	3.7	3.8	3.77	3.9	3.9
O3 + LD	3.3	3.7	4.0	5.23	6.2	8.4
O3 + SD	2.0	3.2	4.4	3.87	4.8	5.2

Accumulated fresh biomass

Total accumulated fresh biomass was not significantly affected by ozone or photoperiod treatment regardless of type of clone. P-values are given in Table 23. Group means, quartiles and outliers are presented in Figure 4.11 and Table 21. No significant difference was found

between ozone exposed plants of different photoperiod treatments at fresh state with NC-R or NC-S.

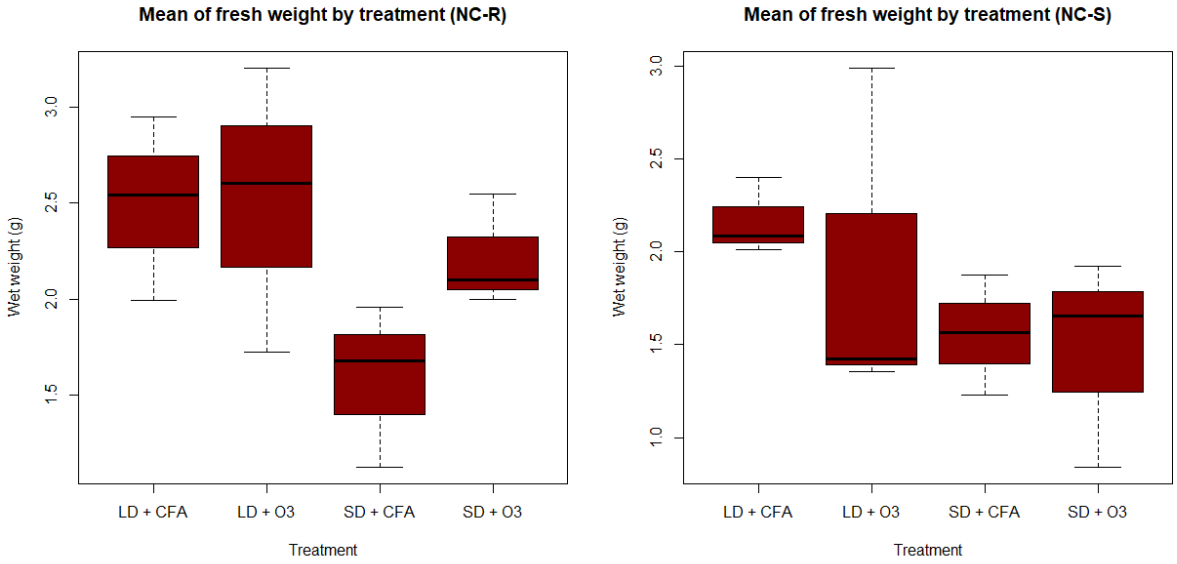


Figure 4.11 Boxplot of accumulated fresh biomass at harvest of Trifolium repens cv. Regal, resistant (NC-R) and sensitive (NC-S) clone, by treatments as described in Table 2 (n=12).

Table 21: Summary of quartiles and the mean of accumulated fresh biomass of *Trifolium repens* cv. Regal (g).						
Fresh mass NC-R						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA + LD	1.994	2.268	2.543	2.495	2.745	2.948
CFA + SD	1.125	1.401	1.678	1.586	1.817	1.561
O3 + LD	1.725	2.164	2.603	2.511	2.903	3.204
O3 + SD	2.001	2.050	2.099	2.216	2.323	2.548
Fresh mass NC-S						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA + LD	2.014	2.049	2.084	2.166	2.242	2.400
CFA + SD	1.232	1.398	1.565	1.558	1.271	1.877
O3 + LD	1.354	1.390	1.426	1.923	2.208	2.991
O3 + SD	0.840	1.247	1.655	1.472	1.788	1.922

Accumulated dry biomass

Total accumulated dry biomass was not significantly affected by ozone or photoperiod treatment regardless of type of clone. P-values are given in Table 23. Group means, quartiles and outliers are presented in Figure 4.12 and Table 22. No significant difference was found

between ozone exposed plants of different photoperiod treatments at fresh state with NC-R or NC-S. A significant difference was found in NC-S response to photoperiod when exposed to CFA (p= 0.0356).

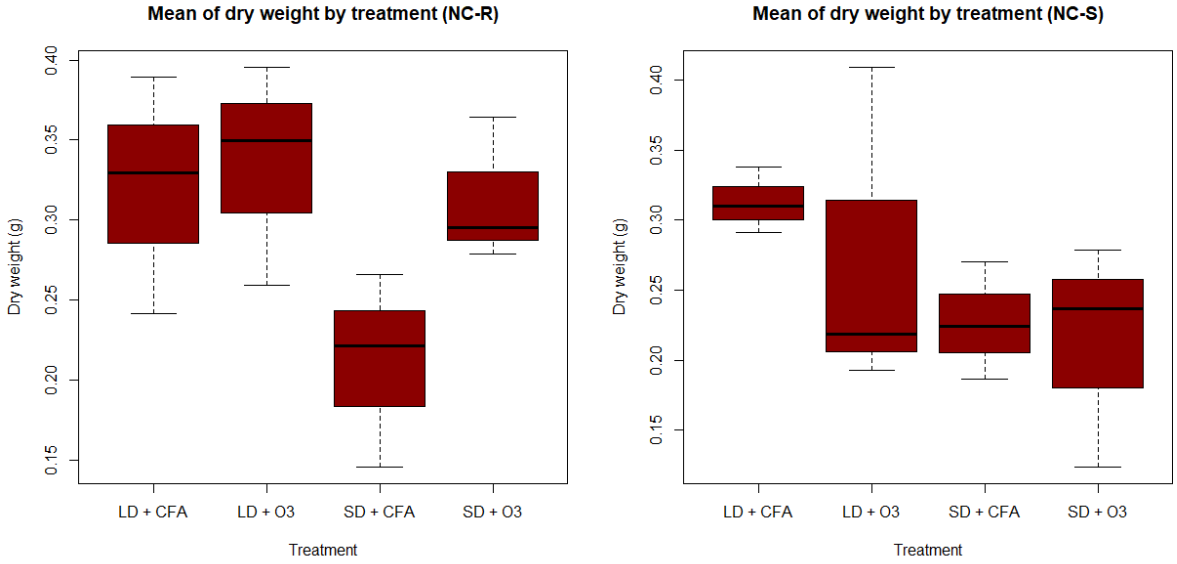


Figure 4.12 Boxplot of accumulated fry biomass at harvest of Trifolium repens cv. Regal, resistant (NC-R) and sensitive (NC-S) clone, by treatments as described in Table 2 (n=12).

Table 22: Summary of quartiles and the mean of accumulated fresh biomass of *Trifolium repens* cv. Regal (g).						
Dry mass NC-R						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA + LD	0.241	0.290	0.330	0.320	0.360	0.390
CFA + SD	0.146	0.183	0.221	0.211	0.243	0.266
O3 +LD	0.260	0.305	0.350	0.335	0.373	0.397
O3 + SD	0.279	0.287	0.295	0.313	0.330	0.364
Dry mass NC-S						
Treatment	Minimum.	1st Quartile.	Median	Mean	3rd Quartile	Maximum
CFA + LD	0.2913	0.301	0.310	0.313	0.324	0.338
CFA + SD	0.187	0.206	0.224	0.227	0.247	0.270
O3 + LD	0.193	0.206	0.219	0.274	0.314	0.409
O3 + SD	0.124	0.181	0.237	0.213	0.258	0.279

Table 23: Statistical test (One-way ANOVA) conducted between treatments of ozone exposure and photoperiod. Significant differences are given in bold.

				Resistant		Sensitive	
Parameter	**Source**	**n**	**df**	**F value**	**Pr(>F)**	**F value**	**Pr(>F)**
Axillary shoot length	Ozone treatment	12	1	0.2805	0.6079	0.0283	0.8698
	Photoperiod	12	1	1.9676	0.1910	3.7184	0.0827
	Ozone | Short day	6	1	0.2180	0.6648	0.0107	0.9225
	Ozone | Long day	6	1	0.0652	0.8111	0.0721	0.8016
	Photoperiod | O3	6	1	0.8464	0.4096	0.5379	0.5040
	Photoperiod | CFA	6	1	0.8292	0.4140	55.068	**0.0018**
Model with interactions	Ozone treatment	12	1	0.2713	0.6166	0.0314	0.8637
	Photoperiod	12	1	1.6345	0.2369	3.0163	0.1206
	Ozone treatment and Photoperiod	12	1	0.0359	0.8545	0.0804	0.7840
Accumulated biomass Fresh mass	Ozone treatment	12	1	0.9029	0.3644	0.2308	0.6413
	Photoperiod	12	1	4.0404	0.0722	3.0728	0.1102
	Ozone | Short day	6	1	4.4991	0.1012	0.0524	0.8302
	Ozone | Long day	6	1	0.0010	0.9766	0.1962	0.6807
	Photoperiod | O3	6	1	0.4087	0.5574	0.5209	0.5104
	Photoperiod | CFA	6	1	6.0624	0.0695	7.5745	0.0513
Model with interactions	Ozone treatment	12	1	1.1947	0.30621	0.2448	0.6341
	Photoperiod	12	1	4.1513	0.07597	2.5506	0.1489
	Ozone treatment and Photoperiod	12	1	1.0799	0.32911	0.0557	0.8193
Dry mass	Ozone treatment	12	1	2.0138	0.1863	0.3421	0.5716
	Photoperiod	12	1	2.6210	0.1319	3.3539	0.0977
	Ozone | Short day	6	1	5.4371	0.0801	0.0707	0.8035
	Ozone | Long day	6	1	0.0637	0.8132	0.3188	0.6025
	Photoperiod | O3	6	1	0.2145	0.6674	0.5385	0.5037
	Photoperiod | CFA	6	1	3.8765	0.1203	9.7207	**0.0356**
Model with interactions	Ozone treatment	12	1	2.5450	0.1493	0.3737	0.5580
	Photoperiod	12	1	3.2204	0.1105	2.8372	0.1306
	Ozone treatment and Photoperiod	12	1	1.4172	0.268	0.0855	0.7774

4.3.2 Visible ozone-induced foliar injury

Table 24: Frequency table. Number of damaged samples per treatment group as described in Table 2 (n=12)

Day		Resistant (NC-R)				Sensitive (NC-S)			
		O3+LD	O3+SD	CFA+LD	CFA+SD	O3+LD	O3+SD	CFA+LD	CFA+SD
1	Not Damaged	3	3	3	3	3	2	3	3
	Damaged						1		
2	Not Damaged	2	3	3	3	3	1	2	3
	Damaged	1					2	1	
3	Not Damaged	2	2	3	3		2	3	2
	Damaged	1	1			3	1		1
4	Not Damaged	2	3	3	3		2	2	3
	Damaged	1				3	1	1	

Only T*rifolium repens* L. NC-S clone showed some degree of ozone induced damage symptoms after six hours of ozone exposure with a POD1 value of 0.08488 mmol/m^2 PLA. After the last exposure only one plant of the resistant clone showed any sign of damage. 4 plants of the sensitive clone exposed to ozone showed damage symptoms, and one CFA exposed plant (see Figure 4.13 and Table 24). The visible symptoms recorded on plants exposed to carbon filtered air could be caused by other factors causing similar damage such as Thrips, a plant eating insect. Recorded ozone-induced visible damage per leaf per day are given in appendix B3-4. When looking at the damage mean of each treatment the ozone exposed resistant plants treated with a long day photoperiod displayed a higher degree of damage in general, though not significant. The sensitive clone shows a higher degree of damage in the short day photoperiod after the second day of ozone exposure but shows a higher level of damage in the long day photoperiod treated plants after the 3 day of ozone exposure (see Table 25).

Table 25: Mean and standard deviation of recorded damage per treatment group as described in Table 2, with day as described in Figure 4.13.

MEAN	Day1 res	Day1 sens	Day2 res	Day2 sens	Day3 res	Day3 sens	Day4 res	Day4 sens
O3+LD	1.00	1.00	1.33	1.00	1.33	2.67	1.33	4.33
O3+SD	1.00	1.33	1.00	1.67	1.33	2.00	1.00	2.33
CFA+LD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.33
CFA+SD	1.00	1.00	1.00	1.33	1.00	1.33	1.00	1.00
STDEV	Day1 res	Day1 sens	Day2 res	Day2 sens	Day3 res	Day3 sens	Day4 res	Day4 sens
O3+LD	0.00	0.00	0.58	0.00	0.58	1.15	0.58	1.53
O3+SD	0.00	0.58	0.00	0.58	0.58	1.73	0.00	2.31
CFA+LD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58
CFA+SD	0.00	0.00	0.00	0.58	0.00	0.58	0.00	0.00

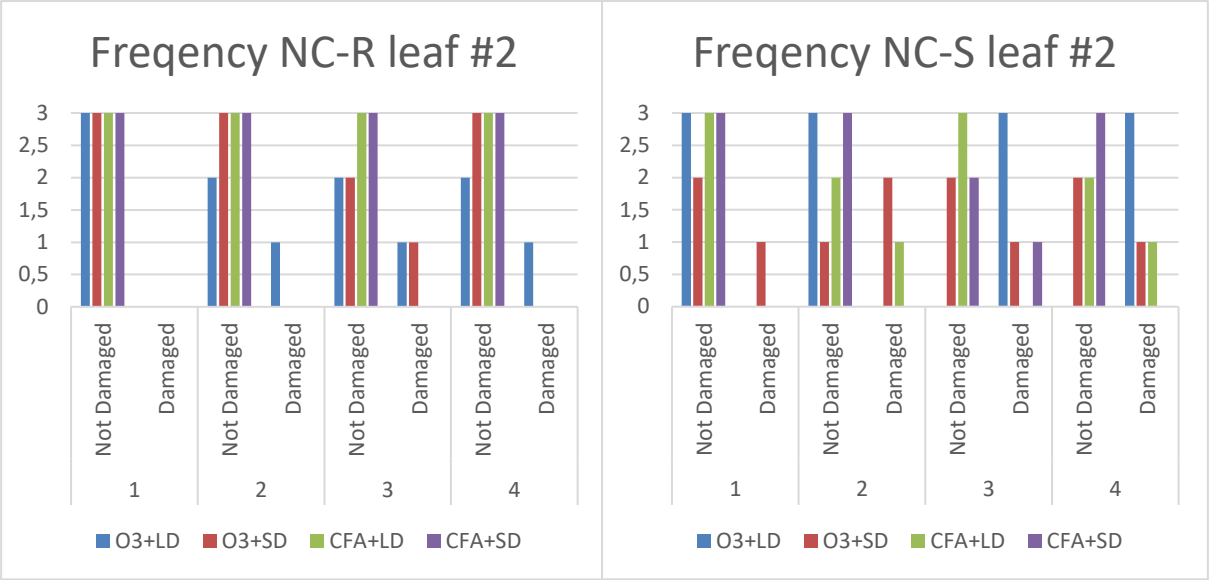


Figure 4.13 Damage frequencies by treatment as described in Table 2. Day 1-4 refers to day of recorded injury with an increase of ozone accumulation from day 1 till day 4.

Recorded damage have a higher frequency and is more severe in the sensitive clone (NC-S) compared to the resistant clone. But there is no significant difference between ozone treated and CFA treated plants in all treatment groups. Photoperiod have no significant impact on the difference between treatment groups. Due to the small sample size, analysis is highly susceptible to all recorded data points and multiple test statistics have been used in order to compensate for small samples and equal cell frequencies. The main results of the statistical analyses are given in Table 26 and all results and methods are given in appendix C3-3.

Table 26: Test statistics done on damage frequencies in *Trifolium repens* cv. Regal. No significant differences were found.

Parameters	**n**	**Test statistics**	**P-value res**	**P-value sens**
Damage(frequency)~Ozone	all data (n=48)	Fishers Exact	0.1090	0.0674
Damage(frequency)~Photoperiod | Ozone	all ozonated plants (n=24)	Fishers Exact	0.5901	1

4.3.3 Physiological responses

Agathokleous, Saitanis, Wang, Watanabe, and Koike (2016) presents stomatal conductance and photosynthesis as two of the primary variables affected by ozone exposure besides visible damage. These two factors are studied directly with stomatal conductance, or indirectly with chlorophyll content.

Stomatal conductance

One of the direct links between DO3SE estimations and the empirical data is the leaf stomatal conductance. The stomatal conductance was measured after 1 day of ozone exposure whit a POD1 value of 0.08488 mmol/m^2 PLA and after 4 days of exposure with a POD1 value of 0.33090.08488 mmol/m^2 PLA (see appendix B3-5, Figure 14 and Table 27). Test statistics are given in Table 29. With the sensitive clone NC-S, no significant difference was found when studying ozone exposed plants. A significance was found in CFA exposed plants in response to photoperiod (p=0.01654). With the resistant clone, a significant difference was found in plants exposed to ozone as a response to photoperiod with an increase in stomatal conductance in plants exposed to a long day photoperiod (p=0.00794). A fitted linear model shows a significant difference when including both ozone exposure and photoperiod treatment (p=0.0023). Due to the very small sample size and the lacking data in this group, the significance in this analysis is highly uncertain. Comparing paired measurements over time no significance was achieved.

In future discussion of stomatal conductance and repair mechanisms, the linear models presented in Table 28 is used.

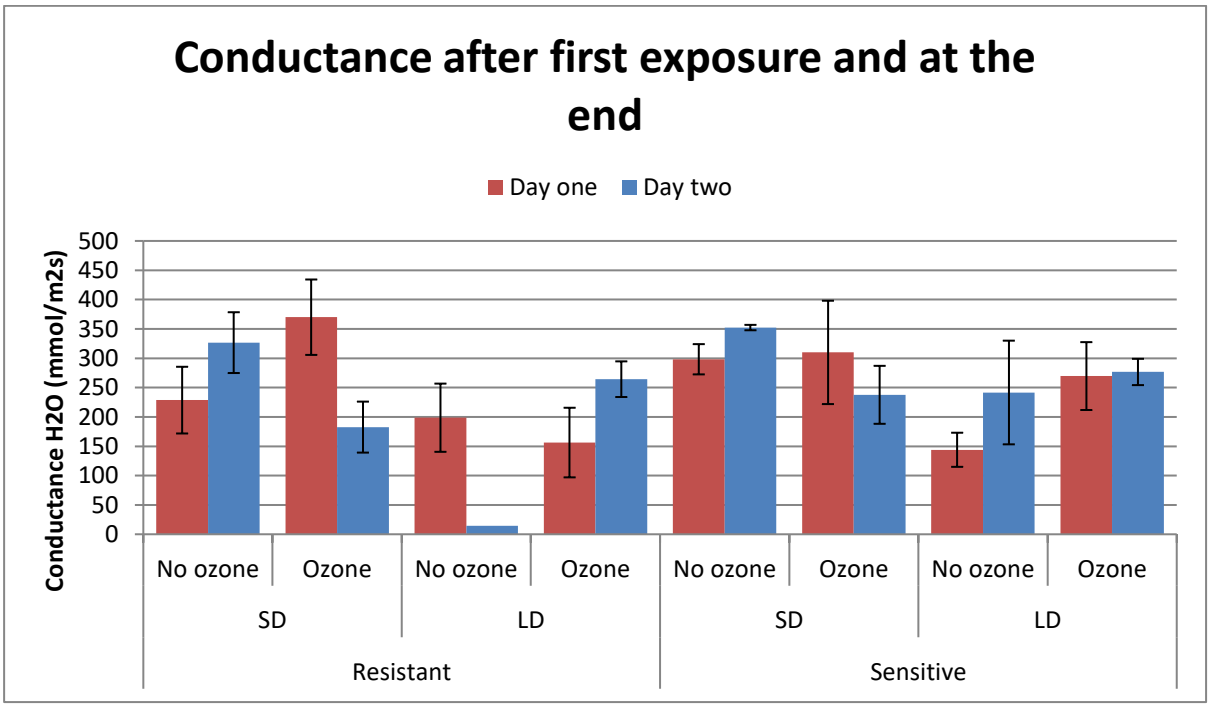


Figure 4.14: Stomatal conductance measured at two different times during the experiment. Day one is between the first and second ozone exposure, and day two is after the last day of ozone exposure. Study design as describes in Table 2.

Table 27: Stomatal conductance of *Trifolium repens* cv. Regal after first and last ozone treatment(mmol/m^{2}s)

Clone	Treatment		Mean cond after first exp.	SE cond after first exp.	Mean cond end	SE cond end
Resistant	SD	CFA	229	57	327	52
		O3	370	64	183	43
	LD	CFA	199	58	14	
		O3	156	59	264	30
Sensitive	SD	CFA	298	26	352	5
		O3	310	88	238	49
	LD	CFA	144	29	242	88
		O3	270	58	277	22

Table 28: Summary of linear regression model of stomatal conductance in ozone treated plants of both clones (n=12).				
Coefficients	Estimate (mmol/m2s)	Std. Error (mmol/m2s)	t value	p value
Intercept day one	213	47.82	4.454	0.00123
SD phototreatment	127	67.63	1.878	0.08982
Intercept day two	270.5	25.59	10.57	9.54E-07
SD phototreatment	-60.33	36.19	-1.667	0.126
	Multiple R-squared	Adjusted R-squared	F-statistics	p-value
Day one	0.2607	0.1868	3.527	0.08982
Day two	0.2175	0.1392	2.779	0.1265

Table 29: Test statistics done on stomatal conductance in *Trifolium repens* cv. Regal. Significant differences given in bold.

				Resistant		Sensitive	
Source	**Test statistics**	**n**	**df**	**F value**	**Pr(>F)**	**F value**	**Pr(>F)**
Day one							
Ozone treatment	ANOVA	12	1	0.4731	0.5072	1.2423	0.2911
Photoperiod	ANOVA	12	1	3.7672	0.0810	2.8538	0.1220
Ozone | Short day	ANOVA	6	1	2.7129	0.1713	0.0162	0.9050
Ozone | Long day	ANOVA	6	1	0.2599	0.6370	3.7662	0.1243
Photoperiod | O3	ANOVA	6	1	5.9677	0.0710	0.1465	0.7214
Photoperiod | CFA	ANOVA	6	1	0.1362	0.7308	15.762	**0.0165**
Ozone treatment	ANOVA	12	1	0.6874	0.4311	1.4949	0.2562
Photoperiod	ANOVA	12	1	4.1639	0.0756	3.0037	0.1213
Ozone treatment and Photoperiod	ANOVA	12	1	2.3658	0.1626	0.3398	0.3398
Day two							
Ozone treatment	ANOVA	12	1	0.0725	0.7938	0.1877	0.6741
Photoperiod	ANOVA	12	1	1.5439	0.2609	0.1513	0.7054
Ozone | Short day	ANOVA	6	1	4.5332	0.1003	0.4308	0.5474
Ozone | Long day	ANOVA	6	1	40.19	**0.007938**	0.1475	0.7205
Photoperiod | O3	ANOVA	6	1	2.373	0.1983	0.5165	0.5121
Photoperiod | CFA	ANOVA	6	1	21.699	**0.01867**	0.3413	0.5904
Ozone treatment	ANOVA	12	1	0.2731	0.6174	0.1635	0.6966
Photoperiod	ANOVA	12	1	4.9916	0.0606	0.1323	0.7255
Ozone treatment and Photoperiod	ANOVA	12	1	21.9181	**0.0023**	0.5769	0.4693
Day one ~ Day two	t.test	23/24			0.7625		0.7071
Day one ~ Day two | O3 + SD	t.test	6			0.0732		0.5134
Day one ~ Day two | CFA + SD	t.test	6			0.2716		0.7660

Day one ~ Day two | O3 + LD	t.test	6	0.1803	0.9156
Day one ~ Day two | CFA + LD	t.test	5/6	0.0915	0.3529

Chlorophyll content

Chlorophyll content was measured after he second day of exposure when the plants had been exposed to a POD1 value of 0.17057mmol/m^2 PLA (see appendix D3-2). Results are given in appendix B3-6, Figure 4.15 and Table 30. The data was analysed using a linear model and an ANOVA test. Results are given in Table 31. A significant difference was found in ozone exposed plants in response to photoperiod in the resistant clone, with long-day photoperiod plants having a significantly higher level of chlorophyll content compared to short day exposed plants (p=0.041). No other analysis achieved significance.

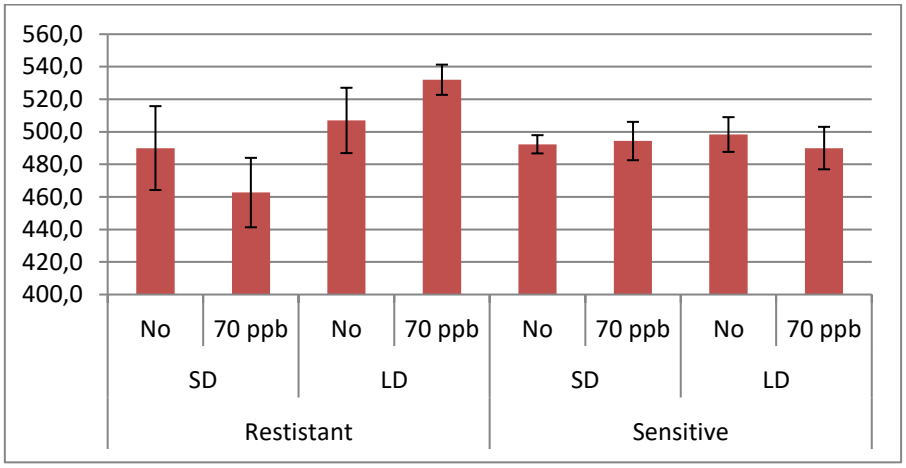


Figure 4.11 Chlorophyll content measured after the second ozone exposure. Study design as describes in Table 2.

Table 30: Chlorophyll content of Trifolium repens cv. Regal after the second ozone treatment (arbitrary units).

Cultivar	Treatment		Mean Chlorophyll content	SE Chlorophyll content
Resistant	SD	CFA	490	25.8
		O3	462.7	21.3
	LD	CFA	507	20.1
		O3	532	9.3
Sensitive	SD	CFA	492.3	5.6
		O3	494.3	11.8
	LD	CFA	498.3	10.7
		O3	490	13.1

Table 31: Test statistics done on chlorophyll content in *Trifolium repens* cv. Regal. Significant differences given in bold.

			Resistant		Sensitive	
Source	**n**	**df**	**F value**	**Pr(>F)**	**F value**	**Pr(>F)**
Ozone treatment	12	1	0.0024	0.9622	0.1071	0.7503
Photoperiod	12	1	4.7734	0.0538	0.0073	0.9334
Ozone | Short day	6	1	0.6674	0.4598	0.0235	0.8857
Ozone | Long day	6	1	1.2772	0.3216	0.2444	0.6469
Photoperiod | O3	6	1	8.8783	**0.0408**	0.0607	0.8175
Photoperiod | CFA	6	1	0.2708	0.6303	0.2479	0.6447
Ozone treatment	12	1	0.0034	0.9550	0.0882	0.7740
Photoperiod	12	1	4.6330	0.0635	0.0061	0.9396
Ozone treatment and Photoperiod	12	1	1.7024	0.2283	0.2348	0.6409

4.4 DO3SE estimations

A comparison between calculated PODy, total stomatal ozone flux and AOT40 values for the first 24 hours including ozone exposure and short day and long day treatment showed no difference between plants from different photoperiods. Thus, it is clear that the periods outside the ozone exposure hours does not influence the calculations of these values in the DO3SE model. As a consequence, a limited model, only using the ozone exposure hours in the calculations, was chosen.

Figure 4.16-4.18 show the estimated AOT40, POD0 and POD1 of experiment I to III. In the model the hourly mean from the different experiments was used to run the simulation. Climatic input data are presented in appendix D1-1, D2-1 and D3-1. The estimated output is given in appendix D1-2, D2-2 and D3-2.

The output can only be viewed as an estimation due to some of the input data being used multiple times as a standard across days and experiments. In order to be more precise, measurements of all input data must be used.

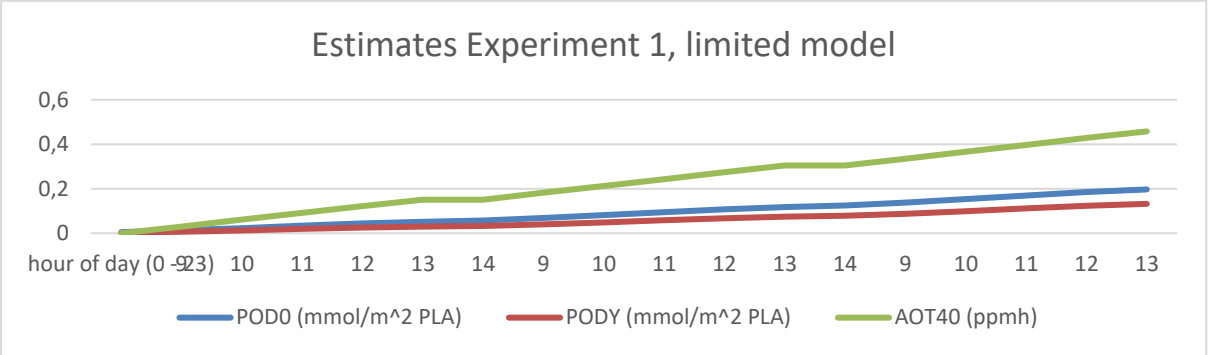


Figure 4.12 POD0, POD1 and AOT40 estimates for experiment I after 3x6 hours of exposures to ozone. Input and output data is given in Appendix D.

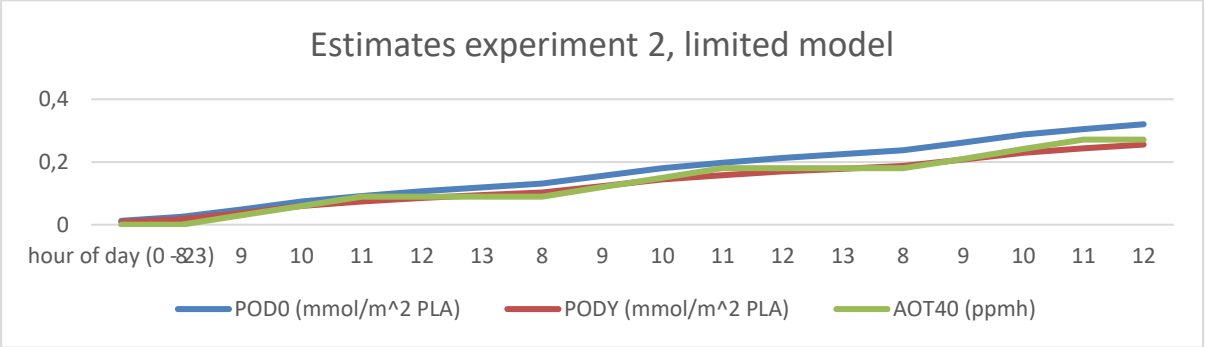


Figure 4.13 POD0, POD1 and AOT40 estimates for experiment II after 3x6 hours of exposures to ozone. Input and output data is given in Appendix D.

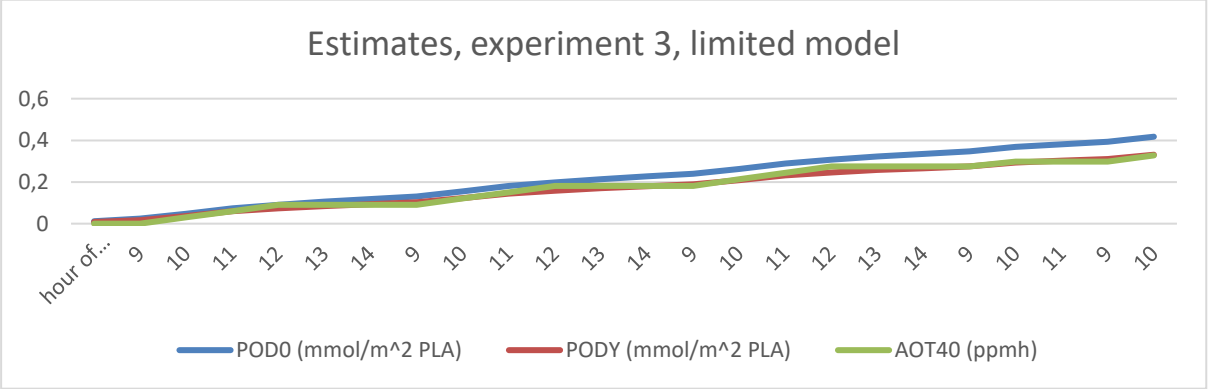


Figure 4.14 POD0, POD1 and AOT40 estimates for experiment III after 3x6 + 2x3 hours of exposures to ozone. Input and output data is given in Appendix D.

5 Discussion

5.1 Discussion of methods

5.1.1 Closed ozone exposure system

Balls et al. (1996), shows that microclimatic conditions have a strong influence on the extent of ozone injury in *Trifolium subterraneum* because of their ability to regulate stomatal conductance since ozone diffuses through the stomata. In the closed exposure system VPD, PAR and temperature were monitored during the ozone exposure period and was kept at relativly stable levels throghout the different experiments. The climatic conditions are easily manipulated and due to the influence of these microclimatic variables, and the benefit of not polluting the work environment, a closed ozone exposure system was chosen to study the effect of photoperiod on ozone induced injury. Temperature, VPD and wind are conditions that affect total estimated flux in the DO3SE model. Levels used in this study are concervative, meaning they are not being a driving factor of the estimated POD1 levels. Sensitivity graphs are given in appendix D4.

5.1.2 Simulated daylength treatment

The long day and short day photoperiod used was simulated in different growth rooms with equal set climatic conditions except night time PAR levels which were set as described in section 3.4.2. The use of environmentally controlled growth rooms to study photoperiod effect is a much-used solution which ensures controlled microclimatic conditions.

5.1.3 Nutrient solution vs soil as growth medium

In experiment I and II plants were transplanted from soil to solution before ozone exposure, whereas they were kept in soil during the entire experiment in experiment III. The benefits of transplanting seedling into solution was primarily to optimize nutrient and water availability. Stomatal conductance and ozone injury is strongly influenced by irrigation with a decrease in ozone injury symptoms when plants reduced stomatal conductance in response to a reduction in soil moisture (Bungener et al., 1999), therefore a constant availability of water is an important

factor. A perk of transplantation is that it makes the study of the root systems simpler. Plants in experiment III were watered daily and should not have been subjected to decreased irrigation.

5.1.4 Applied ozone concentrations

The northern hemisphere baseline ozone concentrations vary between 20 and 40 ppb and is usually highest during the spring. On top of this baseline level, episodes with increasing levels due to long-range transport are common during the summer (Aas et al., 2018). The level of 70 ppb of ozone used in these experiments is therefore set higher than the main baseline concentrations but is within the limit of actual ozone concentrations registered during the summer months (see section 2.5.1).

5.2 Discussion of results

5.2.1 Ozone effects and the effects in relation to photoperiod on visible injury

Previous studies of *Trifolium subterraneum* and *Trifolium repens* as well as other *Trifolium* species show an effect of photoperiod on the severity of visible ozone induced injury as well as on other physiological responses to ozone exposure (e.g. A. V. Vollsnes et al. (2009), C. M. Futsaether et al. (2009)), Crous et al. (2006)). This study attempted to assess these responses to ozone exposure and study the effect of photoperiod to understand how changes in ozone levels in northern regions can affect local plants and understand the severity of the possible consequences.

This result from experiment I confirms the high sensitivity of this clover species and the effectiveness of *Trifolium subterraneum* as a bioindicator for ozone (Karlsson et al., 1995). The same trends were seen in experiment II, but not all leaves showed injury symptoms after three days of exposure. This is probably a result of Norstar being less sensitive than *Trifolium suterraneum.* In experiment III the sensitive clone showed a higher degree of injury than the resistant clone which corresponds with results from Francini et al. (2007). In both experiment I and II symptoms were more severe and appeared earlier in more mature leaves which can be explained by the level of antioxidants in younger leaves, and their ability to increase the antioxidant reaction in response to oxidative stress (Heath & Taylor Jr., 1996).

None of the experiments showed a significant difference in visible damage in response to photoperiod. Experiment II was close with a p value of 0.0556. But when studying damage over time within each treatment group a significant increase in damage occurred in experiment I when comparing leaf one day one with day two and three, and with comparing leaf two day two with day three this indicates a larger change in the categorical response to ozone exposure when grown under long day photoperiod compared to short day photoperiod. Experiment II showed the same significance when comparing long day photoperiod leaf one day one with day three indicating that a larger POD1 levels are needed to significantly alter the group median between the paired groups. Experiment II had some draw backs with the small sample size having a large impact on the test statistics. When studying the contingency table of experiment III and level of damage, after day three and four of exposure the same trends are visible with more damage occurring in the long day ozonated group. The most profound difference between the present study and other similar studies are the time between ozone exposures. In most experiments exposures was done on consecutive days, and all the data collection was done at the end of the experiment. In the present study the plants may have had time to recover between exposures.

Experiment I-III showed a significant response to ozone exposure, but more variation in response when studying the different cultivars response to photoperiod. *Trifolium subterraneum* is commonly used as a bioindicator as previously mentioned, and the American *Trifolium repens* clones have been used in monitoring experiments across Europe (Ball et al., 1998; Karlsson et al., 1995; Mills, Hayes, et al., 2011). *Trifolium subterraneum* responded as predicted, and the sensitive clone of *Trifolium repens* responded in the same manner. The *Trifolium repens* cultivar Norstar is adapted to the northern climate, and thus should be more resistant to the combination of long photoperiod and air pollution and we don't find a clear trend of increased visible injury in long-day photoperiod. Hypothesis H1 which stated that visible foliar injury response to ozone exposure differ depending on photoperiod treatment in the different *Trifolium* species is only partly validated for 22 days old seedlings of *Trifolium subterraneum*, 39 days old seedlings of *Trifolium repens* Norstar and 70 days old seedlings of Regal clones.

When comparing these results with other studies such as C. M. Futsaether et al. (2009), a significant difference in response to photoperiod could have been expected. Such a difference can be caused by enhancement of injury or a reduction in repair processes under long

photoperiod treatment. Roshchina and Roshchina (2003) names ethylene as an influencing factor for ozone injury, and a study by Sinn, Schlagnhaufer, Arteca, and Pell (2004) found that ethylene production was correlated with the extent of foliar injury in S*olanum tuberosum*. The production of ethylene having a peak in production mid-day (Thain, Vandenbussche, Laarhoven, & Dowson-Day, 2004), and being enhanced by stress (Hopkins & Hüner, 2004) can contribute to the recorded increase in ozone induced damage in plants grown under long day photoperiod. Eriksen et al. (2012) showed that nocturnal light conditions and the stimulation of the phytochrome system can promote local lesions, a type of programmed cell death. The repair during darkness is also a factor promoting a higher degree of ozone induced damage in long photoperiod (De Temmerman, Karlsson, et al., 2002).

In this study *Trifolium subterraneum* was found to be the fastest reacting species to ozone exposure. *Trifolium repens* cv. Norstar was found to be more sensitive to ozone than cultivar Regal (both sensitive and resistant clone). Sensitivity to ozone depend on species, cultivar and even ecotype (Karlsson et al., 1995), and when comparing different species and cultivars this must be taken into consideration. A study done by Cecilia M Futsaether et al. (2015) ranked the sensitivity of the species in this study as *Trifolium subterraneum* > *Trifolium repens,* when exposed to 70 ppb of ozone which corresponds with the observed results. The results from that study indicated that the daylength-dependent response to ozone might not be restricted to cultivars and ecotypes but be a more general response.

To better understand the response more studies are needed with bigger and more robust sample sizes, as well as a test under different ozone levels both acute and chronic.

5.2.2 Ozone effects and the effects in relation to photoperiod on growth parameters

As with previous studies very few results in accumulated biomass resulted in a significant difference between groups of ozone treatment or photoperiod treatment. In experiment I a significant increase was found in root size change in length when exposed to ozone compared to CFA exposed plants. This was especially significant in plants grown under short photoperiod. The lack of significance in other comparisons could be due to there being no effect of ozone or photoperiod but could also be the result of the short time-span from start ozone exposure to harvest. As described in Ane V. Vollsnes, Kruse, Eriksen, Oxaal, and Futsaether (2010) the difference in growth parameters can take weeks to develop.

As described in section 2.2 ozone can affect photosynthesis and CO_2 accumulation, directly and decrease the accumulated biomass. An increase in repair respiration could result in the opposite effect and increase the accumulated above ground biomass at the expense of below ground biomass. No such trend was visible in this study and there is need for future study.

Hypothesis 2 which states that there is a difference in growth responses of plants exposed to ozone compared to plants not exposed to ozone remains valid for *Trifolium subterraneum* but can be rejected for *Trifolium repens* cultivars Norstar and Regal. Hypothesis H2a stating that above ground biomass differs under different photoperiod conditions can not be validated for *Trifolium subterraneum* and *Trifolium repens.* A difference was achieved for *Trifolium repens* plants not exposed to ozone in response to photoperiod, but this significance did not exist within the group being exposed to ozone. The corresponding below ground biomass hypothesis can not be validated for any of the *Trifolium* species and cultivars which agrees with most similar studies mentioned.

In experiment III significant difference in axillary shoot length and dry mass was found when studying photoperiod in CFA exposed NC-S plants. The fact that this significant difference was present in CFA exposed plants but not ozone exposed plants could be caused by a response to ozone that evens out the difference in photoperiod treatment but in all probability, it is caused by the small variation between the samples of CFA exposed plants.

5.2.3 Ozone effects and the effects in relation to photoperiod on stomatal conductance and chlorophyll

Few significant differences were achieved when studying stomatal conductance and chlorophyll content, and the significance found can to some degree be explained by machinery malfunction and a small sample size. In this experiment some significant difference was achieved when studying stomatal conductance in CFA exposed plants in response to photoperiod. This is very likely caused by machinery malfunction but could in theory also be a result of an ozone induced response that evens out the difference in stomatal conductance.

Other studies of *Trifolium repens* cultivar Regal, NC-S and NC-R, found a much higher stomatal conductance in the sensitive clone after ozone exposure (Crous et al., 2006). They found no physiological changes in the resistant clone, but most parameters changes in the sensitive clone as a result of ozone exposure. They concluded that the ability to reduce ozone

uptake is crucial and that genotypes that have the ability to control stomatal closure while maintaining a high level of photosynthesis is a key factor to the difference in ozone sensitivity. As described in section 2.4.3 and 2.4.4 ozone can reduce both stomatal conductance and chlorophyll content. Stomatal conductance can decrease directly because of ozone damaging the guard cells. It can also be a result of ozone induced decrease in photosynthesis. The reduction in chlorophyll levels are shown to be highly correlated with necrosis and early senescence. Damage of stomata can occur before the onset of visible foliar damage and is not in general linked to visible injury. Biomass can also be reduced or there can arise a shift in biomass allocation without visible injury. But only after onset of visible damage can development be monitored without using destructive methods. In this study we found no connection between stomatal conductance and the level of visible damage in either clone as shown in Figure 5.1 and 5.2. In the resistant clone injury score increased as stomatal conductance increased, and in the sensitive clone the opposite effect took place. No significant trend can be concluded with using these results and hypothesis H3, a and b can not be validated.

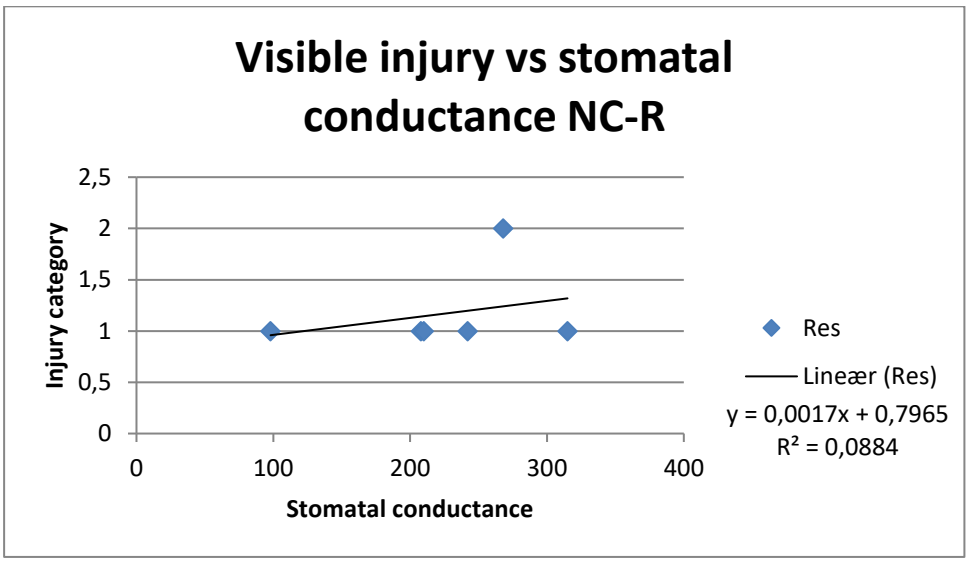


Figure 5.1 Visible injury vs stomatal conductance in Trifolium repens cv. Regal resistant clone. The linear model shows an increase in injury in response to increased stomatal conductance.

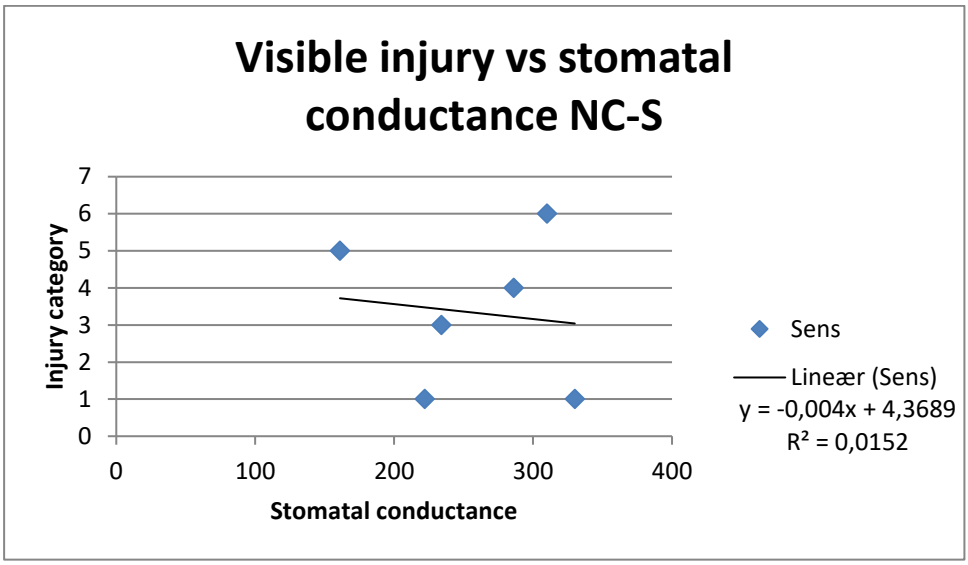


Figure 5.2 Visible injury vs stomatal conductance in Trifolium repens cv. Regal sensitive clone. The linear model shows a decrease in injury in response to increased stomatal conductance.

5.3 Using DO3SE to model ozone stress in Nordic conditions

The UNECE Convention on Long-Range Transported Air Pollution (LRTAP) use the DO3SE model to provide information to European policy makers on the interactions between tropospheric ozone, vegetation and climate. Making sure that the DO3SE model is suited to Nordic conditions is a prerequisite for informed decisions in order to reduce the consequences of this air pollutant. The fact that the accumulated flux-based methods do not differ when the location changes above the polar circle, and that the photoperiod and subsequent lack of repair time during the midnight sun is not a considered factor in the algorithm shows that the model can be improved to better suit the Nordic regions. Figure 5.3 and 5.4 shows visible ozone induced damage in one leaf over several days with the corresponding POD1 value. Experiment III clearly shows a difference in ozone damage as a result of clone and photoperiod treatment, with the sensitive clones displaying a higher level of damage, and the long day photoperiod clearly being more sensitive than the short day photoperiod treatment. This difference is not equally solid in experiment I and II but should be studied further to optimize the DO3SE algorithm.

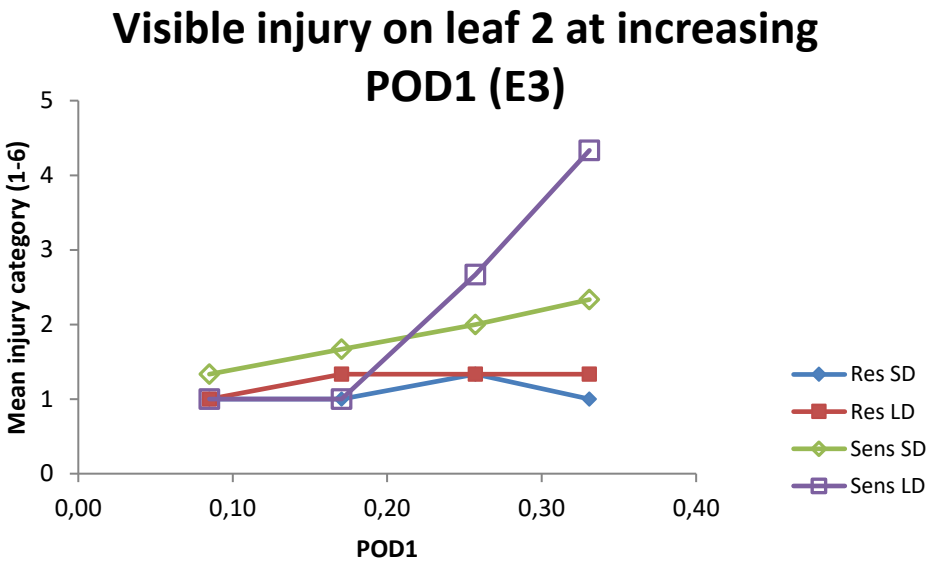


Figure 5.3 Visible injury vs POD1 values in Trifolium repens cv. Sensitive clone under long day photoperiod shows the biggest categorical damage at the resulting POD1 levels.

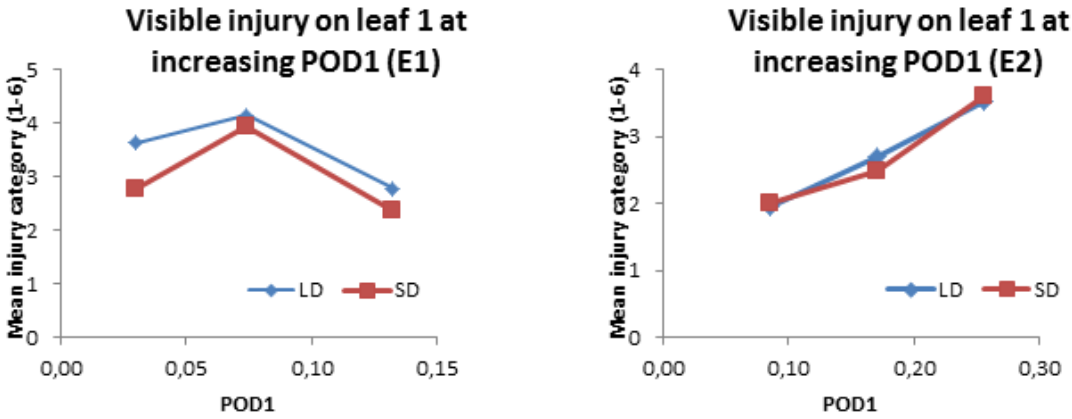


Figure 5.4 Visible injury vs POD1 values in Trifolium subeterraneum in experiment I, and Trifolium repens cv. Norstar in experiment II. Long day photoperiod shows a slightly higher categorical damage at different POD1 levels in experiment I. This difference is neglectable in experiment II.

Another approach for improving DO3SE is to look at the difference in stomatal conductance. This could in theory with more robust data be used as a link to calculate the hypothesized repair parameter and include this parameter to the DO3SE algorithm in the scenario where ozone induced foliar damage have a clear impact on the stomatal conductance. Reduced leaf area, through increased necrosis, could in theory have a linear inverse response to stomatal conductance. The estimated leaf stomatal conductance varied as shown in Figure 5.3 and 5.4,

but still differed from the estimated stomatal mean which varied between 70.42 ± 30.81 mmol/m^2s to 77.66 ± 26.54 mmol/m^2s (experiment III day 5 and day 3, se appendix B3-5 and D3-2). Stomatal conductance measured in Crous et al. (2006) gave conductance levels of 255 and 401 for sensitive and resistant clone which matches fairly well with results from the present study. Stomatal conductance is dependent on multiple climatic conditions such as light. There might be a difference between the measured levels in the exposure chambers, PAR levels during exposure and the values used in the model.

If in theory the results achieved in this study was close to the actual connection between stomatal conductance and the repair mechanism the input parameter for repair could possibly be calculated as the difference in group mean between groups of different photoperiods. In this study that would correspond within the range of the regression coefficients given in Table 28 as a measure of repair with the true value being somewhere within the range. This being highly hypothetical, much more research must be made to establish the type of response, if any. Stomatal conductance must also be measured on different species, cultivars and ecotypes in multiple photoperiod treatments in order to establish if the responses are general or species specific. It is fairly safe to validate that there are physiological mechanisms that DO3SE does not consider when estimating POD1 values for plants growing under long day photoperiod.

5.4 Ozone impacts on vegetation in a changing climate

The combination of increased air pollution and global warming can have a major impact in vegetation. In nature everything interacts. When a system changes due to elevated tropospheric ozone and its effects on plants, multiple responses are expected to occur. A shift in the source-sink balance of carbon in the Nordic regions due to elevated ozone concentrations can have cascade effects. Tropospheric ozone can reduce plant primary production and reduce crop yields, but the increased atmospheric carbon dioxide will in turn increase plants primary production. Sitch, Cox, Collins, and Huntingford (2007) propose that a simultaneous increase in CO_2 and O_3 will lead to stomatal closure and a reduction of gas uptake, and hence limit both the damaging effect of ozone and the fertilization effect of carbon. This can in turn lead to an increased accumulation of carbon in the atmosphere and a continuous closure of stomata and a change in the source sink balance of the ecosystem.

Carbon fixation in plants is in a large degree invested in below ground biomass, and an important potential effect of increased ozone levels are the reduced accumulation of carbon through reduced stomatal conductance and photosynthesis. None of the experiments in this study showed any effect on root biomass accumulation when exposed to ozone under different photoperiods, but many other studies have shown a connection (Agathokleous et al., 2016; Andersen, 2003; Ane V. Vollsnes et al., 2010).

5.5 Conclusion

Results in this study indicate that ozone-response relationships are more meaningful if they consider plant physiology and the response mechanisms. This study related accumulative ozone uptake and some physiological responses in *Trifolium subterraneum* and two *Trifolium repens* cultivars. Most physiological parameters examined showed some change when exposed to ozone. Visible foliar injury was present in all species and cultivars examined and photoperiod was significant when studying damage levels over time for *Trifolium subterraneum* and *Trifolium repens* cv. Norstar. The DO3SE estimated stomatal conductance levels were much lower than the actual levels measured which makes the DO3SE estimations and the empirical data difficult to relate. The aim of this study was to examine plant responses to elevated ozone concentrations in Nordic regions. It is difficult to establish a clear trend on these results presented in this study, but it is safe to conclude that photoperiod have an impact on many different aspects and that more research is needed in order to improve infrastructure such as the DO3SE model used to make future predictions and environmental policies.

It is hard to determine how physiological studies can relate to ecological events. But by better understanding single plants, the future studies of whole ecosystems can be better defined. Even though the data are collected from individual plants, and sometimes a single leaf within the individual plant, the data obtained from this and similar studies can hopefully help understand more complex systems.

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Appendix

Appendix A: Growth conditions

Appendix A1: Experiment I

Appendix table A1-1: Temperature and relative humidity in the growth room before ozone exposure (Room 14)

	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
16.04-17.04.	14.4	18.7	21.2	14.4	14.7	15
17.04-18.04	14.4	18.6	21.3	14.3	14.7	14.9
18.04-19-04	14.5	18.7	21.3	14.4	14.7	15
19.04-20.04	14.4	18.5	21.1	14.4	14.8	15
20.04-21.04	14.3	18.4	21.4	14.4	15.7	14.9
21.04-22.04	14.2	18.4	21.2	14.3	14.6	14.9
22.04-23.04	14.5	18.6	21.1	14.5	14.8	15
23.04-24.04	14.3	18.6	21.2	14.3	14.7	15.5
24.04-25.04	14.3	18.4	21.3	14.3	14.7	15.5
25.04-26.04	14.4	18.5	21.3	14.3	14.7	15
26.04-27.04	14.2	18.5	21.2	14.4	14.6	14.8
27.04-28.04	14.3	18.4	21.2	14.3	14.7	15.6
28.04-29.04	14.4	18.5	21.3	14.4	14.7	15.1
29.04-30.04	14.4	18.4	21.1	14.2	14.7	15.6
30.04-01.05	14.6	18.6	21.2	14.4	14.7	15.1
01.05-02.05	14.5	18.6	21.2	14.5	14.8	15
02.05-03.05	14.2	18.5	21.2	14.2	14.6	15.6
03.05-04.05	14.3	18.5	21.2	14.5	14.8	15.2
04.05-05.05	14.4	18.5	21.2	14.5	14.7	15
05.05-06.05	14.3	18.6	21.4	14.3	14.6	15.5
06.05-07.05	14.2	18.6	21.2	14.3	14.6	14.8
07.05-08.05	14.5	18.5	21.3	14.4	14.7	15

	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
16.04-17.04.	56.4	70.3	87.4	71.5	74.2	77.1
17.04-18.04	56.7	68.9	87.4	70.6	72.4	74.2
18.04-19-04	55	67.9	83	64.5	68.5	75.9
19.04-20.04	55	69.9	89.1	74.2	76	79.1
20.04-21.04	55.5	69.2	88.3	64	69.6	74.7
21.04-22.04	52.5	67.3	83.9	63.3	69.2	73.2
22.04-23.04	53.5	88.8	80.3	65	67.4	75.2
23.04-24.04	54.2	69.2	86.9	64.7	69.4	75.2
24.04-25.04	53	67.9	85.4	64.2	79.7	74.5
25.04-26.04	52.8	66.1	77.9	64	69.1	73
26.04-27.04	54.5	67	83.7	64.7	66.7	75.9
27.04-28.04	54.2	67.4	81	64.5	69	75.9
28.04-29.04	54.4	67.4	80.1	64.5	69.6	74
29.04-30.04	54.7	67.1	80.8	64.5	69.6	74.9
30.04-01.05	53.8	67.2	80.8	64.7	69.1	75.4
01.05-02.05	54	68.3	84.9	66.4	67.9	69.6
02.05-03.05	53.8	67.3	82	64.5	68.7	75.2
03.05-04.05	54	68.8	86.9	64.5	69.4	74.9
04.05-05.05	53.8	68.6	86.9	70.8	72.4	75.7
05.05-06.05	56.2	71.4	94.2	75.4	78.3	80.1
06.05-07.05	56.2	70.8	92.2	66.7	68.8	71.3
07.05-08.05	62.5	70.8	88.6	75.4	77.5	80.1

Appendix table A1-2: Climatic conditions in the experiment exposure chambers during experiment

Date	Time	Temp. mean	RH mean	VPD	PAR*	Ozone mean
8.5.2018	09:00-10:00	20.7	67.71	0.789	68.35	66.8
	10:00-11:00	20.6	67.92	0.779	135.51	70.5
	11:00-12:00	20.6	68.02	0.776	162.49	70.4
	12:00-13:00	20.8	66.85	0.815	190.07	70.1
	13:00-14:00	20.6	67.12	0.798	170.82	70.4
	14:00-15:00	20.6	68.09	0.775	118.25	70.0
10.5.2018	09:00-10:00	20.6	65.40	0.84	68.35	69.3
	10:00-11:00	20.7	65.42	0.844	135.51	70.5
	11:00-12:00	20.6	65.37	0.841	162.49	70.6
	12:00-13:00	20.6	64.48	0.862	190.07	70.6
	13:00-14:00	20.6	64.46	0.863	170.82	70.7
	14:00-15:00	20.7	64.98	0.855	118.25	70.5
12.5.2018	09:00-10:00	20.0	73.32	0.624	68.35	69.9
	10:00-11:00	20.0	73.27	0.625	135.51	70.7
	11:00-12:00	20.7	70.17	0.728	162.49	70.9
	12:00-13:00	20.6	69.53	0.74	190.07	70.9
	13:00-14:00	20.7	69.87	0.736	170.82	71.1
	14:00-15:00	20.6	68.81	0.757	118.25	69.9

Appendix table A1-3: Temperature and relative humidity in LD and SD photoperiod rooms after ozone exposure

Room 14 (SD)	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
08.05-09.04.	19.1	19.8	20.9	14.1	14.7	19.5
09.05-10.05	14.8	19.7	21.2	14.3	14.8	19.8
10.05-11.05	14.9	19.7	21.4	14.4	14.9	19.7
11.05-12.05	14.9	19.6	21.4	14.3	14.8	19.7
12.05-13.05	14.9	19.6	21.3	14.5	14.8	19.5
13.05-14.05	14.7	19.6	21.2	14.3	14.8	19.6
15.05-15.05	14.8	19.7	21.5	14.1	19.7	21.5
15.05-harvest	14.8	19.7	21.8			

Room 14 (SD)	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
08.05-09.04.	59.4	67.3	73.2	54.7	71.5	88.3
09.05-10.05	61.1	67.6	80.3	54.7	78.2	86.9
10.05-11.05	56.2	68.4	78.1	59.1	92.4	95.9
11.05-12.05	64.7	76.8	99.5	57.7	82.8	92.0
12.05-13.05	53.8	67.9	79.6	64.0	73.8	91.3
13.05-14.05	59.9	90.7	95.9	59.8	90.7	95.9
15.05-15.05	56.7	70.5	87.1	55.2	76.0	86.4
15.05-harvest	60.8	70.1	87.1			

Room 15 (LD)	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
08.05-09.04.	19.3	19.7	20.5	14.3	14.8	19.5
09.05-10.05	14.9	19.7	21.3	14.3	14.9	19.3
10.05-11.05	14.6	19.7	21.2	14.3	14.9	19.6
11.05-12.05	14.8	19.7	21.4	14.2	14.7	19.4
12.05-13.05	14.7	19.7	21.4	14.2	14.8	19.4
13.05-14.05	14.9	19.7	21.1	14.2	14.8	19.3
14.05-15.05	14.5	19.7	21.2	12.1	14.9	20.4
15.05-harvest	14.6	19.6	21.2			

Room 15 (LD)	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
08.05-09.04.	64.2	67.2	70.3	57.4	69.0	82.0
09.05-10.05	62.3	67.5	75.2	57.9	76.2	85.2
10.05-11.05	64.0	67.7	81.0	60.6	89.9	94.7
11.05-12.05	65.0	74.3	100.0	59.1	82.5	93.9
12.05-13.05	64.2	70.1	82.7	57.7	69.2	84.7
13.05-14.05	63.7	70.4	84.7	61.8	88.0	96.6
14.05-15.05	63.7	69.3	85.4	57.2	72.6	87.4
15.05-harvest	62.3	68.7	82.7			

Appendix table A1-4: Solution pH during experiment.

pH measured in 4 random pots	pH old solution	pH new solution
02.05.2018		4,74
09.05.2018	4,73-5.17	4.72

Appendix A2: Experiment II

Appendix table A2-1: Temperature and relative humidity in the growth room before ozone exposure

	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
27.04-28.04	14.3	18.4	21.2	14.3	14.7	15.6
28.04-29.04	14.4	18.5	21.3	14.4	14.7	15.1
29.04-30.04	14.4	18.4	21.1	14.2	14.7	15.6
30.04-01.05	14.6	18.6	21.2	14.4	14.7	15.1
01.05-02.05	14.5	18.6	21.2	14.5	14.8	15
02.05-03.05	14.2	18.5	21.2	14.2	14.6	15.6
03.05-04.05	14.3	18.5	21.2	14.5	14.8	15.2
04.05-05.05	14.4	18.5	21.2	14.5	14.7	15
05.05-06.05	14.3	18.6	21.4	14.3	14.6	15.5
06.05-07.05	14.2	18.6	21.2	14.3	14.6	14.8
07.05-08.05	14.5	18.5	21.3	14.4	14.7	15
08.05-09.04.	19.1	19.8	20.9	14.1	14.7	19.5
09.05-10.05	14.8	19.7	21.2	14.3	14.8	19.8
10.05-11.05	14.9	19.7	21.4	14.4	14.9	19.7
11.05-12.05	14.9	19.6	21.4	14.3	14.8	19.7
12.05-13.05	14.9	19.6	21.3	14.5	14.8	19.5
13.05-14.05	14.7	19.6	21.2	14.3	14.8	19.6
14.05-15.05	14.8	19.7	21.5	14.1	19.7	21.5
15.05-16.05	14.8	19.7	21.8	14.2	14.7	20.5
16.05-17.05	14.6	19.7	21.1	14.2	14.7	20.3
17.07-18.05	14.5	19.6	21.2	14.2	14.7	19.4
18.05-19.05	14.7	19.6	21.5	14.3	14.8	19.6
19.05-20.05	14.8	19.6	21.2	14.3	14.8	19.7
20.05-21.05	14.9	19.7	21.3	14.4	14.8	19.3
21.05-22.05	14.8	19.6	21.3	14.2	14.7	19.8
22.05-23.05	14.6	19.6	21.3	14.4	14.8	19.5
23.05-24.05	15.0	19.6	21.2	14.3	14.8	19.4
24.05-25.05	14.8	19.6	21.3	14.3	14.8	19.3
25.05-26.05	14.9	19.5	21.1	14.4	14.9	19.8
26.06-27.05	14.8	19.5	21.2	14.1	14.8	19.2
27.05-28.05	14.7	19.5	21.3	14.4	14.8	19.5
28.05-29.05	15	19.6	21.3	14.2	14.7	19.3
29.05-30.05	14.7	19.6	21.5	14.4	14.8	19.4
30.05-31.05	14.9	19.6	21.3	14.1	14.7	19.4
31.05-01.06	14.7	19.5	21.2	14.2	14.8	19.8
01.06-02.06	14.6	19.5	21.3	14.1	14.7	19.3
02.06-03.06	14.7	19.6	21.3	14.4	14.7	20.0
03.06-04.06	15.0	19.6	21.0	14.5	14.9	20.7

	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
27.04-28.04	54.2	67.4	81	64.5	69	75.9
28.04-29.04	54.4	67.4	80.1	64.5	69.6	74
29.04-30.04	54.7	67.1	80.8	64.5	69.6	74.9
30.04-01.05	53.8	67.2	80.8	64.7	69.1	75.4
01.05-02.05	54	68.3	84.9	66.4	67.9	69.6
02.05-03.05	53.8	67.3	82	64.5	68.7	75.2
03.05-04.05	54	68.8	86.9	64.5	69.4	74.9
04.05-05.05	53.8	68.6	86.9	70.8	72.4	75.7
05.05-06.05	56.2	71.4	94.2	75.4	78.3	80.1
06.05-07.05	56.2	70.8	92.2	66.7	68.8	71.3
07.05-08.05	62.5	70.8	88.6	75.4	77.5	80.1
08.05-09.04.	59.4	67.3	73.2	54.7	71.5	88.3
09.05-10.05	61.1	67.6	80.3	54.7	78.2	86.9
10.05-11.05	56.2	68.4	78.1	59.1	92.4	95.9
11.05-12.05	64.7	76.8	99.5	57.7	82.8	92
12.05-13.05	53.8	67.9	79.6	64	73.8	91.3
13.05-14.05	59.9	90.7	95.9	59.8	90.7	95.9
14.05-15.05	56.7	70.5	87.1	55.2	76	86.4
15.05-16.05	60.8	70.1	87.1	64.2	85.3	98.1
16.05-17.05	58.4	69.9	87.1	54.2	70.8	92
17.07-18.05	61.8	67.5	85.2	52.8	69.7	87.1
18.05-19.05	55	66.9	86.4	51.6	67.4	81.5
19.05-20.05	63	66.5	74.5	53.8	69.5	81.8
20.05-21.05	60.6	69	80.5	55.7	80.3	89.3
21.05-22.05	61.1	69.7	86.9	53.5	70.2	90.3
22.05-23.05	62.5	67.7	87.1	54.5	72.6	83.5
23.05-24.05	64.0	69.1	86.4	54.2	74.1	84.9
24.05-25.05	51.1	61.8	83.5	49.4	74.0	78.1
25.05-26.05	51.3	61.6	85.7	49.6	72.0	80.8
26.06-27.05	51.6	56.5	69.1	49.6	75.4	80.1
27.05-28.05	52.1	59.9	80.8	49.4	71.1	81.0
28.05-29.05	45.0	57.8	83.7	52.5	84.6	88.1
29.05-30.05	56.4	72.0	89.8	56.7	87.1	92.7
30.05-31.05	58.9	68.5	88.3	53.0	84.5	90.3
31.05-01.06	58.1	69.0	87.6	51.3	75.1	80.1
01.06-02.06	54.2	63.9	88.6	55.2	90.2	97.6
02.06-03.06	61.1	75.3	97.1	56.7	94.3	99.3
03.06-04.06	60.8	80.9	103.0	56.9	90.6	97.3

Appendix table A2-2: Climatic conditions in the experiment exposure chambers during experiment

Date	Time	Temp. mean	RH mean	VPD	PAR*	Ozone mean
4.6.2018	08:00-09:00	20.2	66.16	0.848	85.0136	68.4
	09:00-10:00	20.7	64.29	0.872	81.3432	70.0
	10:00-11:00	20.7	64.95	0.856	238.308533	69.8
	11:00-12:00	20.7	62.58	0.914	277.04	70.0
	12:00-13:00	20.7	63.73	0.886	136.111733	69.9
	13:00-14:00	20.6	65.27	0.843	109.449867	70.4
6.6.2018	08:00-09:00	17.8	59.13	0.833	85.0136	68.4
	09:00-10:00	18.8	60.77	0.851	81.3432	70.3
	10:00-11:00	19.8	57.97	0.971	238.308533	70.1
	11:00-12:00	20.7	56.20	1.070	277.04	69.9
	12:00-13:00	20.6	56.35	1.059	136.111733	70.7
	13:00-14:00	20.7	58.60	1.011	109.449867	70.7
8.6.2018	08:00-09:00	20.5	56.07	1.060	85.0136	69.0
	09:00-10:00	20.5	62.93	0.894	81.3432	70.5
	10:00-11:00	20.7	63.29	0.897	238.308533	69.8
	11:00-12:00	20.7	63.19	0.899	277.04	70.8
	12:00-13:00	20.6	62.61	0.908	136.111733	70.2
	13:00-14:00	20.7	65.09	0.853	109.449867	70.0

Appendix table A2-3: Temperature and relative humidity in LD and SD photoperiod rooms after ozone exposure

Room 14 (SD)	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
04.06-05.06.	14.9	19.6	21.2	14.4	14.8	19.7
05.06-06.06	14.6	19.5	21.2	14.3	14.8	19.6
06.06-07.06	14.8	19.6	21.1	14.4	14.8	19.6
07.06-08.06	15.1	19.6	21.3	14.4	14.8	20.1
08.06-09.06	14.7	19.6	21.3	14.4	14.8	19.9
09.06-10.06	14.8	19.6	21.3	14.3	14.7	20.1
10.06-11.06	14.7	19.6	21.3	14.3	14.8	20.1
11.06-harvest	14.5	19.6	21.3			

Room 14 (SD)	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
04.06-05.06.	47.9	58.6	81.3	45.7	55.5	63.0
05.06-06.06	38.7	48.7	80.3	41.3	54.7	56.7
06.06-07.06	41.1	51.0	73	50.6	80.1	85.2
07.06-08.06	55.2	68.0	87.1	53.5	76.6	85.9
08.06-09.06	51.6	60.2	84.9	52.8	84.8	93.4
09.06-10.06	63.5	76.3	94.9	54.2	90.0	95.9
10.06-11.06	64.2	74.7	97.3	56.7	93.4	97.8
11.06-harvest	64.5	72.1	94.7			

Room 15 (LD)	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
04.06-05.06.	15.1	20.1	21.8	13.6	15.3	20.6
05.06-06.06	16.4	20.1	22.0	13.7	15.2	21.1
06.06-07.06	15.5	19.9	21.8	13.7	15.1	19.9
07.06-08.06	16.4	19.9	21.9	14.0	14.9	18.8
08.06-09.06	15.0	19.8	21.5	13.8	14.9	19.5
09.06-10.06	15.0	19.9	22.0	14.1	14.9	18.8
10.06-11.06	14.5	20.0	22.1	14.1	14.8	19.3
11.06-harvest	14.9	19.9	21.9			

Room 15 (LD)	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
04.06-05.06.	41.6	55.5	79.1	42.6	51.1	61.1
05.06-06.06	34.0	43.0	70.6	36.0	49.3	55.7
06.06-07.06	36.0	48.8	76.6	49.1	76.5	87.1
07.06-08.06	52.1	64.5	83.2	55.0	72.9	87.8
08.06-09.06	46.7	56.9	77.9	49.6	80.0	90.8
09.06-10.06	53.8	72.4	91.0	55.7	85.8	95.9
10.06-11.06	52.8	72.6	95.6	56.4	89.3	97.8
11.06-harvest	52.8	72.5	95.6			

Appendix table A2-4: Solution pH during experiment.

pH measured in 4 random pots	pH old solution	pH new solution
18.05.2018		4,68
27.05.2018	4.42-4.61	4.72
06.05.2018	4.02-4.69	4.65

Appendix A3: experiment III

Appendix table A3-1: Temperature and relative humidity in the growth room before ozone exposure weekly mean day and night.

	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
13.04-19.04	14.7	19.6	21.3	14.3	14.8	20
20.04-26.04	14.6	19.6	21.4	14.2	14.7	19.5
27.04-03.05	14.5	19.6	21.3	14.2	14.8	19.6
04.05-10.05	14.6	19.6	21.5	14.1	14.8	20.1
11.05-17.05	14.5	19.7	22.5	14.1	14.8	20.5
18.05-24.05	14.6	19.6	21.5	14.2	14.8	19.8
25.05-31.05	14.7	19.6	21.5	14.1	14.8	19.8
01.06-07.06	14.6	19.6	21.3	14.1	14.8	20.7
08.06-14.06	14.5	19.6	21.3	14.3	14.8	20.1
15.06-22.06	14.7	19.6	21.2	14.1	14.7	20

	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
13.04-19.04	57.9	66.8	85.4	52.1	70.9	89.1
20.04-26.04	59.6	66.5	81.8	52.5	70.5	88.3
27.04-03.05	59.4	66.8	83.5	53.8	69.4	86.9
04.05-10.05	56.2	68.1	85.9	53.8	75.7	95.1
11.05-17.05	56.7	70.8	99.5	52.8	79.5	98.1
18.05-24.05	51.1	67.4	87.1	49.4	71.8	90.3
25.05-31.05	45	63.8	89.8	49.4	78.4	92.7
01.06-07.06	38.7	63.8	103	41.3	78.2	99.3
08.06-14.06	47.7	68	97.3	46.2	84	97.8
15.06-22.06	41.3	62.6	91	42.3	74.8	95.6

Appendix table A3-2: Climatic conditions in the experiment exposure chambers during experiment

Date	Time	Temp. mean	RH mean	VPD	PAR*	Ozone mean
22.6.2018	09:00-10:00	20.1	54.52	1.070	85.0136	66.1
	10:00-11:00	20.1	67.78	0.758	81.3432	69.9
	11:00-12:00	20.0	68.70	0.732	238.308533	70.0
	12:00-13:00	20.0	70.26	0.696	277.04	70.1
	13:00-14:00	20.0	71.35	0.670	136.111733	70.5
	14:00-15:00	20.0	70.96	0.697	109.449867	70.4
26.6.2018	09:00-10:00	20.6	59.98	0.971	85.0136	67.0
	10:00-11:00	20.6	63.05	0.897	81.3432	70.4
	11:00-12:00	20.7	63.14	0.900	238.308533	70.0
	12:00-13:00	20.6	64.66	0.858	277.04	70.2
	13:00-14:00	20.6	64.59	0.859	136.111733	71.0
	14:00-15:00	20.6	63.71	0.881	109.449867	70.8
29.6.2018	09:00-10:00	20.5	63.62	0.878	85.0136	69.6
	10:00-11:00	20.7	67.63	0.791	81.3432	70.7
	11:00-12:00	20.6	66.82	0.805	238.308533	71.0
	12:00-13:00	20.7	65.86	0.834	277.04	70.7
	13:00-14:00	20.7	64.31	0.872	136.111733	70.7
	14:00-15:00	20.7	64.28	0.872	109.449867	70.7
4.7.2018	09:00-10:00	20.8	62.90	0.912	85.0136	68.5
	10:00-11:00	20.7	65.75	0.836	81.3432	71.1
	11:00-12:00	20.6	67.71	0.784	238.308533	62.9
5.7.2018	09:00-10:00	20.7	71.28	0.701	277.04	69.6
	10:00-11:00	20.6	71.52	0.692	136.111733	69.4
	11:00-12:00	20.6	72.61	0.665	109.449867	70.5

Appendix table A3-3: Temperature and relative humidity in LD and SD photoperiod rooms after ozone exposure

Room 14 (SD)	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
22.06-23.06	15.1	19.6	21.1	14.1	14.7	19.3
23.06-24.06	14.7	19.6	21.2	14.3	14.7	19.5
24.06-25.06	14.9	19.6	21.3	13.8	14.8	19.4
25.06-26.06	14.7	19.8	21.4	14.4	14.8	19.5
26.06-27.06	14.8	19.8	21.5	14.3	14.8	20.2
27.06-28.06	14.8	19.7	21.4	14.4	14.8	19.5
28.06-29.06	14.9	19.7	21.4	14.2	14.7	20.4
29.06-30.06	14.7	19.6	21.3	14.3	14.7	19.8
30.06-01.07	14.7	19.7	21.2	14.2	14.7	19.5
01.07.02.07	14.5	19.6	21.4	14.3	14.7	19.4
02.07-03.07	14.8	19.7	21.3	14.1	14.7	19.3
03.07-04.07	14.6	19.7	21.5	14.1	14.8	20
04.07-05.07	14.7	19.7	21.4	14.3	14.9	19.8
05.07-06.07	14.7	19.6	21.3	14.5	14.9	20.5
06.07-07.07	14.9	19.7	21.4	14.4	14.8	19.9
07.07-08.07	15	19.7	21.4	14.3	14.8	20.2
08.07-harvest	14.9	19.7	21.4			

Room 14 (SD)	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
22.06-23.06	41.1	50.6	84.9	41.1	54.2	59.6
23.06-24.06	39.2	56.7	88.6	50.6	73.3	79.6
24.06-25.06	49.9	59.7	88.3	50.1	73.7	80.1
25.06-26.06	56.4	69.5	91.3	56.7	97.2	90.8
26.06-27.06	60.8	76.6	93.9	56.7	87.1	91.5
27.06-28.06	59.4	74	91.5	59.6	92.4	96.4
28.06-29.06	52.3	70	103	53.5	66.5	86.1
29.06-30.06	43.5	53	83	42.8	57.6	60.1
30.06-01.07	43.8	54.3	86.6	43.3	61.5	71
01.07.02.07	47.9	62	88.6	46.9	69.3	74.9
02.07-03.07	49.4	60.7	87.4	51.3	80.2	85.9
03.07-04.07	53.3	67	91.3	56.2	80.1	92
04.07-05.07	55.7	69.2	93.4	57.2	87.4	93.7
05.07-06.07	69.8	82.1	98.8	56	91.9	96.6
06.07-07.07	62	73	91	56	75.1	86.1
07.07-08.07	50.8	60.1	84.9	55.2	69.3	81.8
08.07-harvest	47.9	64.7	89.8			

Room 15 (LD)	Day			Night		
Temperature	Min	Mean	Max	Min	Mean	Max
22.06-23.06	15	20.1	22	13.5	15.2	19.1
23.06-24.06	14.2	20.1	22	13.7	15.2	19.5
24.06-25.06	16.4	20.1	22.2	13.8	15.2	20.4
25.06-26.06	14.3	20	22.1	13.8	15.2	20.3
26.06-27.06	16.7	20	22	13.8	15.2	19.1
27.06-28.06	16	20	22	14.1	14.8	19.2
28.06-29.06	15	19.9	22.5	14.1	14.9	18.9
29.06-30.06	15.5	19.9	21.9	13.9	14.9	19.4
30.06-01.07	14.8	19.8	22	14.1	14.8	20
01.07.02.07	14.9	19.8	21.4	14.1	15	19.5
02.07-03.07	15.2	19.8	22.2	14.1	14.8	19
03.07-04.07	15.8	19.8	21.6	14.2	14.8	20
04.07-05.07	14.8	19.7	21.6	14.5	14.9	19.3
05.07-06.07	15.1	19.7	21.6	14.1	14.7	19.4
06.07-07.07	14.8	19.8	21.6	14.1	14.8	20.7
07.07-08.07	15	19.8	22	14.1	14.9	19.9
08.07-harvest	14.9	19.9	22.5			

Room 15 (LD)	Day			Night		
Relative humidity	Min	Mean	Max	Min	Mean	Max
22.06-23.06	34.3	46.4	75.4	36.5	49.5	56.7
23.06-24.06	36	48.6	80.5	47.4	67.9	79.8
24.06-25.06	42.6	53.6	79.1	45	67.7	77.9
25.06-26.06	49.1	64	85.7	52.5	81.7	93.9
26.06-27.06	53.5	69.5	83.5	56.7	82.4	94.7
27.06-28.06	51.1	65.5	86.1	58.6	87.8	96.8
28.06-29.06	43.8	64.3	98.6	51.8	61.7	75.4
29.06-30.06	37.2	48.3	76.4	37.7	53.6	57.9
30.06-01.07	39.6	48.4	81	39.4	57.5	67.4
01.07.02.07	44.8	56.2	84.7	43	64.7	71.5
02.07-03.07	45	54.7	77.1	46.5	75.1	81.5
03.07-04.07	48.9	60.5	85.2	55.5	76.4	88.1
04.07-05.07	51.3	60.9	82.5	54.7	83.4	91.3
05.07-06.07	63.3	75.9	93	56.7	89.6	97.8
06.07-07.07	52.8	64.5	89.1	50.4	70.8	83.9
07.07-08.07	40.9	50	70.6	44.5	63	69.8
08.07-harvest	38.4	53.9	77.1			

Appendix B: Recorded values for each sample, treatment means, and standard deviations.

Appendix table B1: Experiment I

Appendix table B1-1: Root size

Plant_number	Ozone	Chamber	Daylenght	Root_size_start	Root_size_end	Change
1	1	1	LD	11.7	15.4	3.7
2	1	1	LD	10.2	14.8	4.6
3	1	1	LD	12	16.1	4.1
4	1	1	SD	12.5	17.5	5
5	1	1	SD	10	16	6
6	1	1	SD	12	15.8	3.8
7	1	2	LD	9.5	14.5	5
8	1	2	LD	12.5	20	7.5
9	1	2	LD	10.5	12.9	2.4
10	1	2	SD	13.5	18.4	4.9
11	1	2	SD	10.1	15.9	5.8
12	1	2	SD	9.4	15.9	6.5
13	1	3	LD	15.6	27.1	11.5
14	1	3	LD	9	14.7	5.7
15	1	3	LD	11.3	15.4	4.1
16	1	3	SD	13.6	18.8	5.2
17	1	3	SD	12	18.9	6.9
18	1	3	SD	10.1	15.9	5.8
19	0	4	LD	9.4	15.5	6.1
20	0	4	LD	8.6	13.3	4.7
21	0	4	LD	11.6	13.4	1.8
22	0	4	SD	13.3	17.2	3.9
23	0	4	SD	10.4	18.2	7.8
24	0	4	SD	15.3	17.9	2.6
25	0	5	LD	12.5	15.2	2.7
26	0	5	LD	12.8	19.5	6.7
27	0	5	LD	11.6	13.5	1.9
28	0	5	SD	17.7	16.3	-1.4
29	0	5	SD	17.1	15.9	-1.2
30	0	5	SD	15.4	19	3.6
31	0	6	LD	15.8	18.6	2.8
32	0	6	LD	11.1	18.9	7.8
33	0	6	LD	13.6	19.4	5.8
34	0	6	SD	12.6	16.2	3.6
35	0	6	SD	17.4	22.3	4.9
36	0	6	SD	10.6	14.9	4.3

Appendix table B1-2: Above ground biomass (Fresh and dry mass)

Plant_number	Ozone	Chamber	Daylenght	Fresh	Dry
1	1	1	LD	1.8	0.145
2	1	1	LD	1.947	0.204
3	1	1	LD	1.826	0.219
4	1	1	SD	2.018	0.18
5	1	1	SD	1.995	0.135
6	1	1	SD	1.592	0.125
7	1	2	LD	2.027	0.148
8	1	2	LD	1.728	0.149
9	1	2	LD	2.015	0.152
10	1	2	SD	2.004	0.141
11	1	2	SD	1.971	0.201
12	1	2	SD	1.832	0.128
13	1	3	LD	1.896	0.174
14	1	3	LD	1.856	0.107
15	1	3	LD	1.905	0.152
16	1	3	SD	1.722	0.148
17	1	3	SD	1.93	0.173
18	1	3	SD	1.906	0.157
19	0	4	LD	1.845	0.153
20	0	4	LD	1.848	0.123
21	0	4	LD	1.811	0.17
22	0	4	SD	1.811	0.128
23	0	4	SD	1.819	0.195
24	0	4	SD	1.661	0.157
25	0	5	LD	1.83	0.207
26	0	5	LD	1.911	0.207
27	0	5	LD	1.682	0.197
28	0	5	SD	1.814	0.197
29	0	5	SD	1.823	0.161
30	0	5	SD	1.779	0.103
31	0	6	LD	1.763	0.18
32	0	6	LD	2.002	0.242
33	0	6	LD	1.927	0.175
34	0	6	SD	1.946	0.156
35	0	6	SD	1.878	0.165
36	0	6	SD	1.776	0.137

Appendix table B1-3: Below ground biomass (Fresh and dry mass)

Plant_number	Ozone	Chamber	Daylenght	Fresh	Dry
1	1	1	LD	1.772	0.074
2	1	1	LD	2.024	0.083
3	1	1	LD	1.734	0.084
4	1	1	SD	1.907	0.081
5	1	1	SD	1.713	0.055
6	1	1	SD	1.953	0.066
7	1	2	LD	1.899	0.058
8	1	2	LD	1.802	0.054
9	1	2	LD	1.814	0.071
10	1	2	SD	2.009	0.065
11	1	2	SD	1.876	0.067
12	1	2	SD	1.893	0.055
13	1	3	LD	1.984	0.079
14	1	3	LD	1.913	0.044
15	1	3	LD	1.857	0.066
16	1	3	SD	1.993	0.061
17	1	3	SD	1.945	0.077
18	1	3	SD	1.762	0.053
19	0	4	LD	1.899	0.07
20	0	4	LD	1.975	0.058
21	0	4	LD	1.788	0.067
22	0	4	SD	1.794	0.057
23	0	4	SD	1.791	0.078
24	0	4	SD	1.815	0.07
25	0	5	LD	1.913	0.085
26	0	5	LD	1.815	0.083
27	0	5	LD	2.039	0.074
28	0	5	SD	1.756	0.09
29	0	5	SD	1.81	0.074
30	0	5	SD	1.827	0.045
31	0	6	LD	1.784	0.076
32	0	6	LD	1.771	0.106
33	0	6	LD	1.883	0.076
34	0	6	SD	1.86	0.075
35	0	6	SD	1.961	0.085
36	0	6	SD	1.772	0.056

Appendix table B1-4: Visible ozone induced damage

Plant_ number	Ozone	Chamber	LD/SD	D1L1.1	D1L1.2	D1L1.3	D2L1.1	D2L1.2	D2L1.3
1	1	1	LD	5	6	5	6	6	5
2	1	1	LD	1	1	1	2	3	2
3	1	1	LD	2	1	2	3	2	3
4	1	1	SD	1	1	1	2	1	2
5	1	1	SD	1	1	1	1	2	2
6	1	1	SD	5	3	5	5	3	5
7	1	2	LD	1	1	1	2	2	2
8	1	2	LD	3	2	2	4	4	4
9	1	2	LD	2	2	1	4	4	2
10	1	2	SD	1	1	1	1	3	3
11	1	2	SD	4	4	3	5	6	4
12	1	2	SD	3	4	4	2	2	2
13	1	3	LD	1	1	1	5	4	4
14	1	3	LD	3	3	3	4	4	4
15	1	3	LD	1	1	1	2	2	2
16	1	3	SD	3	2	2	3	3	3
17	1	3	SD	1	1	1	2	2	2
18	1	3	SD	2	1	1	4	5	5
19	0	4	LD	1	1	1	1	1	1
20	0	4	LD	1	1	1	1	1	1
21	0	4	LD	1	1	1	1	1	1
22	0	4	SD	1	1	1	1	1	1
23	0	4	SD	1	1	1	1	1	1
24	0	4	SD	1	1	1	1	1	1
25	0	5	LD	1	1	1	1	1	1
26	0	5	LD	1	1	1	1	1	1
27	0	5	LD	1	1	1	1	1	1
28	0	5	SD	1	1	1	1	1	1
29	0	5	SD	1	1	1	1	1	1
30	0	5	SD	1	1	1	1	1	1
31	0	6	LD	1	1	1	1	1	1
32	0	6	LD	1	1	1	1	1	1
33	0	6	LD	1	1	1	1	1	1
34	0	6	SD	1	1	1	1	1	1
35	0	6	SD	1	1	1	1	1	1
36	0	6	SD	1	1	1	1	1	1

D2L2.1	D2L2.2	D2L2.3	D3L1.1	D3L1.2	D3L1.3	D3L2.1	D3L2.2	D3L2.3	D3L3.1	D3L3.2	D3L3.3
6	6	6	5	5	5	6	6	6	6	5	5
1	1	1	3	3	3	1	2	2	1	1	1
4	4	4	3	2	3	4	4	4	1	1	2
1	1	1	3	1	2	2	2	2	3	2	2
2	3	2	4	3	4	3	4	3	1	1	1
5	5	5	5	4	4	6	6	6	2	2	2
1	1	1	3	3	3	2	3	2	1	1	1
4	5	4	5	5	5	6	6	6	4	4	3
5	4	4	4	4	3	6	4	5	2	2	2
3	3	2	3	4	4	3	4	3	2	2	1
6	6	6	5	6	4	6	6	6	6	6	6
1	1	1	1	1	1	3	2	3	1	1	1
5	5	6	5	5	5	6	6	6	4	2	3
4	3	4	4	5	4	5	4	5	3	4	4
1	2	2	3	4	4	5	5	5	4	4	4
1	1	2	6	5	6	3	4	3	3	2	2
1	1	1	5	5	5	5	4	5	2	4	2
5	4	5	5	5	5	6	6	6	1	3	3
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1

Appendix B2: Experiment II

Appendix table B2-1: Root size

Ozone	Chamber	Daylenght	Root_size_start	Root_size_end	Change
1	1	LD	21.8	28.6	6.8
1	1	LD	8.8	17.7	8.9
1	1	LD	28.4	28.2	-0.2
1	1	SD	11.8	13.8	2
1	1	SD	12.4	10.7	-1.7
1	1	SD	15.3	21	5.7
1	2	LD	23.1	18.2	-4.9
1	2	LD	14	18.9	4.9
1	2	LD	16.6	19.2	2.6
1	2	SD	11.2	18.4	7.2
1	2	SD	15.1	19.9	4.8
1	2	SD			
1	3	LD	13.4	15.4	2
1	3	LD	13.3	17.4	4.1
1	3	LD	16.8	19.6	2.8
1	3	SD	17.4	22.2	4.8
1	3	SD			
1	3	SD	13	13	0
0	4	LD	14	18.8	4.8
0	4	LD	18.4	21.5	3.1
0	4	LD	13.6	19.2	5.6
0	4	SD	19.8	24.9	5.1
0	4	SD	16.1	17.2	1.1
0	4	SD	13.2	14.4	1.2
0	5	LD	12.3	16.3	4
0	5	LD	13.4	16.6	3.2
0	5	LD	10.9	17.7	6.8
0	5	SD	21.4	22	0.6
0	5	SD			
0	5	SD	15.5	20.1	4.6
0	6	LD	17.5	19.5	2
0	6	LD	19.5	19.6	0.1
0	6	LD	20.5	26.9	6.4
0	6	SD	17.8	22.4	4.6
0	6	SD	14	17.7	3.7
0	6	SD	11.4	12.1	0.7

Appendix table B2-2: Above ground biomass (Fresh and dry mass)

Plant_number	Ozone	Chamber	Daylenght	Fresh	Dry
1	1	1	LD	2.100	0.259
2	1	1	LD	0.508	0.081
3	1	1	LD	1.74	0.359
4	1	1	SD	2.358	0.311
5	1	1	SD	0.356	0.156
6	1	1	SD	0.703	0.108
7	1	2	LD	1.725	0.214
8	1	2	LD	1.661	0.251
9	1	2	LD	1.439	0.261
10	1	2	SD	1.026	0.162
11	1	2	SD	0.942	0.159
12	1	2	SD		
13	1	3	LD	2.952	0.349
14	1	3	LD	1.356	0.244
15	1	3	LD	1.871	0.252
16	1	3	SD	2.131	0.259
17	1	3	SD		
18	1	3	SD	0.386	0.137
19	0	4	LD	0.522	0.09
20	0	4	LD	2.108	0.315
21	0	4	LD	0.685	0.128
22	0	4	SD	2.02	0.257
23	0	4	SD	1.785	0.352
24	0	4	SD	0.949	0.208
25	0	5	LD	0.666	0.108
26	0	5	LD	1.240	0.209
27	0	5	LD	0.986	0.18
28	0	5	SD	0.989	0.221
29	0	5	SD		
30	0	5	SD	2.54	0.299
31	0	6	LD	0.568	0.107
32	0	6	LD	0.505	0.205
33	0	6	LD	0.577	0.085
34	0	6	SD	0.956	0.134
35	0	6	SD	1.437	0.185
36	0	6	SD	1.275	0.265

Appendix table B2-3: Below ground biomass (Fresh and dry mass)

Plant_number	Ozone	Chamber	Daylenght	Fresh	Dry
1	1	1	LD	1.349	0.086
2	1	1	LD	0.469	0.029
3	1	1	LD	0.81	0.056
4	1	1	SD	1.829	0.119
5	1	1	SD	0.278	0.017
6	1	1	SD	0.832	0.051
7	1	2	LD	1.524	0.082
8	1	2	LD	1.118	0.067
9	1	2	LD	0.634	0.043
10	1	2	SD	1	0.057
11	1	2	SD	0.842	0.051
12	1	2	SD		
13	1	3	LD	1.588	0.099
14	1	3	LD	0.689	0.043
15	1	3	LD	1.524	0.091
16	1	3	SD	1.447	0.095
17	1	3	SD		
18	1	3	SD	0.24	0.015
19	0	4	LD	0.231	0.02
20	0	4	LD	1.614	0.123
21	0	4	LD	0.661	0.042
22	0	4	SD	1.438	0.108
23	0	4	SD	0.749	0.047
24	0	4	SD	0.386	0.019
25	0	5	LD	0.657	0.048
26	0	5	LD	0.876	0.06
27	0	5	LD	0.45	0.038
28	0	5	SD	0.331	0.018
29	0	5	SD		
30	0	5	SD	2.918	0.138
31	0	6	LD	0.32	0.022
32	0	6	LD	0.323	0.017
33	0	6	LD	0.669	0.04
34	0	6	SD	0.96	0.064
35	0	6	SD	1.214	0.076
36	0	6	SD	0.437	0.026

Appendix table B2-4: Visible ozone induced damage

Plant_number	Ozone	Chamber	LD/SD	D1L1.1	D1L1.2	D1L1.3	D2L1.1	D2L1.2	D2L1.3	D2L2.1	D2L2.2
1	1	1	LD	1	1	1	1	1	1	1	1
2	1	1	LD	4	2	3	4	2	3	1	1
3	1	1	LD	1	1	1	3	4	4	1	1
4	1	1	SD	4	1	4	5	1	5	4	2
5	1	1	SD	3	3	3	3	3	3	3	3
6	1	1	SD	2	2	1	2	2	1	2	2
7	1	2	LD	2	2	2	2	2	2	3	3
8	1	2	LD	3	3	3	3	3	3	3	3
9	1	2	LD	1	1	1	2	3	4	1	1
10	1	2	SD	1	1	1	1	1	1	1	1
11	1	2	SD	1	2	2	2	3	3	1	1
13	1	3	LD	1	1	1	1	1	1	1	1
14	1	3	LD	4	5	5	4	5	5	3	3
15	1	3	LD	1	1	1	3	3	3	2	2
16	1	3	SD	1	1	1	2	2	1	3	3
18	1	3	SD	2	4	2	3	4	4	5	5
19	0	4	LD	1	1	1	1	1	1	1	1
20	0	4	LD	1	1	1	1	1	1	1	1
21	0	4	LD	1	1	1	1	1	1	1	1
22	0	4	SD	1	1	1	1	1	1	1	1
23	0	4	SD	1	1	1	1	1	1	1	1
24	0	4	SD	1	1	1	1	1	1	1	1
25	0	5	LD	1	1	1	1	1	1	1	1
26	0	5	LD	1	1	1	1	1	1	1	1
27	0	5	LD	1	1	1	1	1	1	1	1
28	0	5	SD	1	1	1	1	1	1	1	1
30	0	5	SD	1	1	1	1	1	1	1	1
31	0	6	LD	1	1	1	1	1	1	1	1
32	0	6	LD	1	1	1	1	1	1	1	1
33	0	6	LD	1	1	1	1	1	1	1	1
34	0	6	SD	1	1	1	1	1	1	1	1
35	0	6	SD	1	1	1	1	1	1	1	1
36	0	6	SD	1	1	1	1	1	1	1	1

D2L2.3	D3L1.1	D3L1.2	D3L1.3	D3L2.1	D3L2.2	D3L2.3	D3L3.1	D3L3.2	D3L3.3
1	3	3	3	3	3	3	3	3	3
1	3	4	2	2	1	3	2	1	1
1	6	6	6	2	2	4	1	1	1
2	6	6	6	4	2	3	4	5	5
3	6	6	6	6	6	6	6	6	6
2	2	2	1	2	2	2	2	2	1
3	2	2	2	3	3	3	2	2	1
3	3	3	3	3	3	3	3	3	3
1	2	3	4	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	2	3	3	1	1	1	1	1	1
1	2	3	3	4	4	4	5	4	4
3	6	6	6	4	3	2	1	1	1
2	3	3	3	2	2	2	3	3	3
3	2	2	2	2	4		4	5	4
5	6	6	6	6	6	6	6	6	6
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1

Appendix B3: Experiment III

Appendix table B3-1: Axillary shoot

Cultivar	Ozone	LD/SD	Axillary shoot
Res	0	SD	5.2
Res	0	SD	2.7
Res	0	SD	5.3
Res	1	SD	4.3
Res	1	SD	6.2
Res	1	SD	4.2
Res	0	LD	5.5
Res	0	LD	4.1
Res	0	LD	6.7
Res	1	LD	5.6
Res	1	LD	6.6
Res	1	LD	4.8
Sens	0	SD	3.6
Sens	0	SD	3.9
Sens	0	SD	3.8
Sens	1	SD	4.4
Sens	1	SD	5.2
Sens	1	SD	2
Sens	0	LD	5.8
Sens	0	LD	5.2
Sens	0	LD	6
Sens	1	LD	4
Sens	1	LD	3.3
Sens	1	LD	8.4

Appendix table B3-2: Accumulated biomass for NC-R (Fresh and dry mass)

Cultivar	Ozone	LD/SD	Fresh weight	Dry weight	D/W
Res	0	SD	1.6779	0.2211	0.13177186
Res	0	SD	1.1247	0.1455	0.12936783
Res	0	SD	1.9561	0.2658	0.13588262
Res	1	SD	2.0007	0.279	0.13945119
Res	1	SD	2.5482	0.3643	0.14296366
Res	1	SD	2.0987	0.2953	0.14070615
Res	0	LD	2.5429	0.3297	0.12965512
Res	0	LD	1.9936	0.2413	0.12103732
Res	0	LD	2.9478	0.3895	0.13213244
Res	1	LD	2.6029	0.3498	0.13438857
Res	1	LD	3.2037	0.3957	0.12351344
Res	1	LD	1.7254	0.2595	0.15039991

Appendix table B3-3: Accumulated biomass for NC-S (Fresh and dry mass)

Cultivar	Ozone	LD/SD	Fresh weight	Dry weight	D/W
Sens	0	SD	1.232	0.1869	0.15170455
Sens	0	SD	1.8773	0.27	0.14382358
Sens	0	SD	1.5645	0.2241	0.14324065
Sens	1	SD	1.655	0.2371	0.14326284
Sens	1	SD	1.9215	0.2786	0.14499089
Sens	1	SD	0.8399	0.1237	0.14727944
Sens	0	LD	2.0136	0.2913	0.14466627
Sens	0	LD	2.3995	0.3378	0.14077933
Sens	0	LD	2.0843	0.3098	0.14863503
Sens	1	LD	1.4259	0.2187	0.15337681
Sens	1	LD	1.3537	0.1929	0.14249834
Sens	1	LD	2.9908	0.4094	0.13688645

Appendix table B3-4: Visible ozone induced damage

Ozone	Daylenght	D1 res	D1 sens	D2 res	D2 sens	D3 res	D3 sens	D4 res	D4 sens
1	SD	1	1	1	1	2	1	1	1
1	SD	1	2	1	2	1	4	1	5
1	SD	1	1	1	2	1	1	1	1
0	SD	1	1	1	1	1	1	1	1
0	SD	1	1	1	2	1	2	1	1
0	SD	1	1	1	1	1	1	1	1
1	LD	1	1	2	1	2	2	2	4
1	LD	1	1	1	1	1	4	1	6
1	LD	1	1	1	1	1	2	1	3
0	LD	1	1	1	1	1	1	1	2
0	LD	1	1	1	1	1	1	1	1
0	LD	1	1	1	1	1	1	1	1

Appendix table B3-5: Stomatal conductance

Cultivar	Chamber	LD/SD	ozone	Cond start	Cond end
Res	1	KD	1	350	218
Res	2	KD	1	490	93
Res	3	KD	1	270	242
Res	4	KD	0	116	410
Res	5	KD	0	272	300
Res	6	KD	0	298	280
Res	1	LD	1	230	324
Res	2	LD	1	39	151
Res	3	LD	1	200	330
Res	4	LD	0	310	270
Res	5	LD	0	114	670
Res	6	LD	0	172	102
Sens	1	KD	1	305	260
Sens	2	KD	1	465	35
Sens	3	KD	1	160	216
Sens	4	KD	0	256	1260
Sens	5	KD	0	294	9
Sens	6	KD	0	345	24
Sens	1	LD	1	220	29
Sens	2	LD	1	204	315
Sens	3	LD	1	385	214
Sens	4	LD	0	111	107
Sens	5	LD	0	202	420
Sens	6	LD	0	119	204

Appendix table B3-6: Chlorophyll content

Cultivar	plant number	LD/SD	Ozon	Chlorophyll content
Res	17	SD	0	541
Res	19	SD	0	471
Res	20	SD	0	458
Res	15	SD	1	484
Res	23	SD	1	484
Res	24	SD	1	420
Res	13	LD	0	490
Res	14	LD	0	547
Res	22	LD	0	484
Res	16	LD	1	515
Res	18	LD	1	534
Res	25	LD	1	547
Sen	6	SD	0	484
Sen	13	SD	0	503
Sen	16	SD	0	490
Sen	11	SD	1	509
Sen	14	SD	1	503
Sen	24	SD	1	471
Sen	8	LD	0	509
Sen	10	LD	0	509
Sen	21	LD	0	477
Sen	15	LD	1	515
Sen	19	LD	1	484
Sen	20	LD	1	471

Appendix C: Statistical details

Appendix C1: Experiment I

Appendix table C1-1: Mean and standard deviation of root size.

Root size	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	5.40	5.54	4.48	3.12
SD	2.69	0.93	2.24	2.89

Appendix table C1-2: Mean and standard deviation of above ground biomass (Fresh and dry mass)

Fresh	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	1.889	1.886	1.847	1.812
SD	0.098	0.145	0.094	0.750

Dry	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	0.161	0.154	0.184	0.155
SD	0.034	0.026	0.035	0.030

Appendix table C1-3: Mean and standard deviation of below ground biomass (Fresh and dry mass)

Fresh	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	1.867	1.895	1.874	1.821
SD	0.097	0.100	0.093	0.061

Dry	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	0.068	0.064	0.077	0.070
SD	0.014	0.010	0.014	0.015

Appendix table C1-4: Fishers exact, Chi Square and Wilcoxon signed rank test on visible ozone induced damage

Parameters	**n**	**Test statistics**	**P value**
Damage(frequency)~Ozone	all data (n=216)	Chi square	**1.11E-29**
Damage(frequency)~Ozone	D1L1 (n=36)	Fishers Exact	**0.001033**
Damage(frequency)~Ozone	D2L1 (n=36)	Fishers Exact	**2.20E-10**
Damage(frequency)~Ozone	D2L2 (n=36)	Fishers Exact	**2.97E-05**
Damage(frequency)~Ozone	D3L1 (n=36)	Fishers Exact	**4.19E-09**
Damage(frequency)~Ozone	D3L2 (n=36)	Fishers Exact	**2.20E-10**
Damage(frequency)~Ozone	D3L3 (n=36)	Fishers Exact	**7.42E-06**
Damage(frequency)~Photoperiod | Ozone	all ozonated plants (n=108)	Fishers Exact	0.2783
Damage(frequency)~Photoperiod | Ozone	D1L1 (n=18)	Fishers Exact	0.6130
Damage(frequency)~Photoperiod | Ozone	D2L1 (n=18)	Fishers Exact	0.0745
Damage(frequency)~Photoperiod | Ozone	D2L2 O3 (n=18)	Fishers Exact	0.3019
Damage(frequency)~Photoperiod | Ozone	D3L1 O3 (n=18)	Fishers Exact	0.3614
Damage(frequency)~Photoperiod | Ozone	D3L2 O3 (n=18)	Fishers Exact	0.1946
Damage(frequency)~Photoperiod | Ozone	D3L3 O3 (n=18)	Fishers Exact	0.1076
Damage(category)~Photoperiod | Ozone + LD	D1L1~D2L1 (n=18)	Wilcoxon	**0.007086**
Damage(category)~Photoperiod | Ozone + LD	D1L1~D3L2 (n=18)	Wilcoxon	**0.01356**
Damage(category)~Photoperiod | Ozone + LD	D2L1~D3L1 (n=18)	Wilcoxon	0.1198
Damage(category)~Photoperiod | Ozone + LD	D2L2~D3L2 (n=18)	Wilcoxon	**0.01788**
Damage(category)~Photoperiod | Ozone + SD	D1L1~D2L1 (n=18)	Wilcoxon	0.1138
Damage(category)~Photoperiod | Ozone + SD	D1L1~D3L2 (n=18)	Wilcoxon	0.07249
Damage(category)~Photoperiod | Ozone + SD	D2L1~D3L1 (n=18)	Wilcoxon	0.2021
Damage(category)~Photoperiod | Ozone + SD	D2L2~D3L2 (n=18)	Wilcoxon	**0.01991**

Appendix C2: Experiment II

Appendix table C2-1: Mean and standard deviation of root size.

	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	3.00	3.26	4.00	2.70
SD	4.00	3.24	2.16	1.97

Appendix table C2-2: Mean and standard deviation of above ground biomass (Fresh and dry mass)

Fresh	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	1.706	1.129	0.873	1.494
SD	0.649	0.805	0.523	0.579

Dry	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	0.252	0.185	0.159	0.240
SD	0.080	0.073	0.076	0.068

Appendix table C2-3: Mean and standard deviation of below ground biomass (Fresh and dry mass)

Fresh	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	1.078	0.924	0.645	1.054
SD	0.436	0.577	0.420	0.853

Dry	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	0.066	0.058	0.046	0.062
SD	0.025	0.038	0.032	0.044

Appendix table C2-4: Fishers exact and Wilcoxon signed rank test on visible ozone induced damage

Parameters	n	Test statistics	P value
Damage(frequency)~Ozone	all data (n=198)	Fishers Exact	**< 2.2e-16**
Damage(frequency)~Ozone	D1L1 (n=33)	Fishers Exact	**0.0002966**
Damage(frequency)~Ozone	D2L1 (n=33)	Fishers Exact	**9.77E-07**
Damage(frequency)~Ozone	D2L2 (n=33)	Fishers Exact	**2.97E-04**
Damage(frequency)~Ozone	D3L1 (n=33)	Fishers Exact	**1.54E-08**
Damage(frequency)~Ozone	D3L2 (n=33)	Fishers Exact	**9.77E-07**
Damage(frequency)~Ozone	D3L3 (n=33)	Fishers Exact	**8.65E-05**
Damage(frequency)~Photoperiod | Ozone	all ozonated plants (n=96)	Fishers Exact	0.0556
Damage(frequency)~Photoperiod | Ozone	D1L1 (n=16)	Fishers Exact	0.4236
Damage(frequency)~Photoperiod | Ozone	D2L1 (n=16)	Fishers Exact	0.9371
Damage(frequency)~Photoperiod | Ozone	D2L2 O3 (n=16)	Fishers Exact	0.6329
Damage(frequency)~Photoperiod | Ozone	D3L1 O3 (n=16)	Fishers Exact	0.1831
Damage(frequency)~Photoperiod | Ozone	D3L2 O3 (n=16)	Fishers Exact	0.4278
Damage(frequency)~Photoperiod | Ozone	D3L3 O3 (n=16)	Fishers Exact	0.2958
Damage(category)~Photoperiod | Ozone + LD	D1L1~D2L1 (n=16)	Wilcoxon	0.1736
Damage(category)~Photoperiod | Ozone + LD	D1L1~D3L2 (n=16)	Wilcoxon	**0.03103**
Damage(category)~Photoperiod | Ozone + LD	D2L1~D3L1 (n=16)	Wilcoxon	0.08897
Damage(category)~Photoperiod | Ozone + LD	D2L2~D3L2 (n=16)	Wilcoxon	0.09751
Damage(category)~Photoperiod | Ozone + SD	D1L1~D2L1 (n=16)	Wilcoxon	0.08897
Damage(category)~Photoperiod | Ozone + SD	D1L1~D3L2 (n=16)	Wilcoxon	0.05791
Damage(category)~Photoperiod | Ozone + SD	D2L1~D3L1 (n=16)	Wilcoxon	0.1814
Damage(category)~Photoperiod | Ozone + SD	D2L2~D3L2 (n=16)	Wilcoxon	0.1736

Appendix C3: Experiment III

Appendix table C3-1: Mean and standard deviation of axillary shoot.

Axillary shoot	Resistant				Sensitive			
	O3+LD	O3+SD	CFA+LD	CFA+SD	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	5.667	4.900	5.433	4.400	5.233	3.867	5.667	3.767
SD	0.902	1.127	1.301	1.473	2.765	1.665	0.416	0.153

Appendix table C3-2: Mean and standard deviation of accumulated biomass (Fresh and dry mass)

Fresh	Resistant				Sensitive			
	O3+LD	O3+SD	CFA+LD	CFA+SD	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	2.511	2.216	2.495	1.586	1.923	1.472	2.166	1.558
SD	0.743	0.292	0.479	0.423	0.925	0.564	0.205	0.323

Dry	Resistant				Sensitive			
	O3+LD	O3+SD	CFA+LD	CFA+SD	O3+LD	O3+SD	CFA+LD	CFA+SD
Mean	0.335	0.313	0.320	0.211	0.274	0.213	0.313	0.227
SD	0.069	0.045	0.075	0.061	0.118	0.080	0.023	0.042

Appendix table C3-3: Fishers exact, Chi square and Wilcoxon signed rank test on visible ozone induced damage

			Resistant	Sensitive
Parameters	**n**	**Test statistics**	**P value**	**P value**
Damage(frequency)~Ozone	all data (n=48)	Fishers Exact	0.109	0.06735
Damage(frequency)~Ozone	D1L2 (n=12)	Chi square	1	1
Damage(frequency)~Ozone	D2L2 (n=12)	Fishers Exact	1	1
Damage(frequency)~Ozone	D3L2 (n=12)	Fishers Exact	0.455	0.3182
Damage(frequency)~Ozone	D4L2 (n=12)	Fishers Exact	1	0.2424
Damage(frequency)~Photoperiod | Ozone	all ozonated plants (n=24)	Fishers Exact	0.0674	0.06735
Damage(frequency)~Photoperiod | Ozone	D1L2 (n=6)	Chi square	0.2231	1
Damage(frequency)~Photoperiod | Ozone	D2L2 (n=6)	Chi square	1	0.4
Damage(frequency)~Photoperiod | Ozone	D3L2 O3 (n=6)	Fishers Exact	1	0.6
Damage(frequency)~Photoperiod | Ozone	D4L2 O3 (n=6)	Fishers Exact	1	0.4
Damage(category)~Photoperiod | Ozone + LD	D1L2~D2L2 (n=6)	Wilcoxon	1	NA
Damage(category)~Photoperiod | Ozone + LD	D1L2~D3L2 (n=6)	Wilcoxon	1	0.1736
Damage(category)~Photoperiod | Ozone + LD	D1L2~D4L2 (n=6)	Wilcoxon	1	0.25
Damage(category)~Photoperiod | Ozone + LD	D2L2~D3L2 (n=6)	Wilcoxon	NA	0.1736
Damage(category)~Photoperiod | Ozone + LD	D2L2~D4L2 (n=6)	Wilcoxon	NA	0.25
Damage(category)~Photoperiod | Ozone + LD	D3L2~D4L2 (n=6)	Wilcoxon	NA	0.1736
Damage(category)~Photoperiod | Ozone + SD	D1L2~D2L2 (n=6)	Wilcoxon	NA	1
Damage(category)~Photoperiod | Ozone + SD	D1L2~D3L2 (n=6)	Wilcoxon	1	1
Damage(category)~Photoperiod | Ozone + SD	D1L2~D4L2 (n=6)	Wilcoxon	NA	1
Damage(category)~Photoperiod | Ozone + SD	D2L2~D3L2 (n=6)	Wilcoxon	1	1
Damage(category)~Photoperiod | Ozone + SD	D2L2~D4L2 (n=6)	Wilcoxon	NA	1
Damage(category)~Photoperiod | Ozone + SD	D3L2~D4L2 (n=6)	Wilcoxon	1	1

Appendix D: DO3SE estimations

Appendix D1: Experiment I

Appendix table D1-1: DO3SE input limited model

Day of year	hour of day (0 - 23)	Temperature (Ts, C, Celsius)	Vapour Pressure Deficit (VPD, kPa)	Meassured wind speed (uh, zR, m/s)	Precipitation (presip, mm)	Preassure (P, kPa)	Meassured O3 density (O3_zR, ppb)	PAR (umol/m^2/s)
128	9	20.7	0.789	1	0	101.325	66.8	60.2352
128	10	20.6	0.779	1	0	101.325	70.5	119.413
128	11	20.6	0.776	1	0	101.325	70.4	143.186
128	12	20.8	0.815	1	0	101.325	70.1	167.494
128	13	20.6	0.798	1	0	101.325	70.4	150.532
128	14	20.6	0.775	1	0	101.325	70	104.213
130	9	20.6	0.84	1	0	101.325	69.3	60.2352
130	10	20.7	0.844	1	0	101.325	70.5	119.413
130	11	20.6	0.841	1	0	101.325	70.6	143.186
130	12	20.6	0.862	1	0	101.325	70.6	167.494
130	13	20.6	0.863	1	0	101.325	70.7	150.532
130	14	20.7	0.855	1	0	101.325	70.5	104.213
132	9	20	0.624	1	0	101.325	69.9	60.2352
132	10	20	0.625	1	0	101.325	70.7	119.413
132	11	20.7	0.728	1	0	101.325	70.9	143.186
132	12	20.6	0.74	1	0	101.325	70.9	167.494
132	13	20.7	0.736	1	0	101.325	71.1	150.532
132	14	20.6	0.757	1	0	101.325	69.9	104.213

Appendix table D1-2: DO3SE output limited model including AOT40 and PODy values limited model

CO2 (ppm)	Gsto (mmol/m^2/s)	Gsto_l (mmol/m^2/s)	Fst (nmol/m^2/s)	E1 Ftot (nmol/m^2/s)	POD0 (mmol/m^2 PLA)	PODY (mmol/m^2 PLA)	AOT40 (ppm)
391	13.89856815	20.94312096	1.36102891	4.496376514	0.004899704	0.001299704	0
391	24.8080883	35.31375122	2.387772083	5.39257431	0.013495684	0.006295684	0.0305
391	28.55189133	39.79639053	2.67496562	5.603853703	0.023125559	0.01232556	0.0609
391	32.06702423	43.77931213	2.916492224	5.779675484	0.033624932	0.019224932	0.09100001
391	29.64605713	41.05716324	2.756222725	5.667620182	0.043547332	0.025547335	0.12140001
391	22.23005486	32.09376907	2.16167593	5.203757286	0.051329367	0.029729368	0.1514
391	16.25601959	26.92156982	1.804609299	4.812263012	0.05782596	0.032625962	0.1514
391	29.31592941	45.41233444	3.038619041	5.879560947	0.068764992	0.039964989	0.18189999
391	33.8602562	51.16679001	3.410100937	6.228033066	0.081041358	0.048641354	0.21249999
391	38.15717697	56.26868057	3.73123765	6.545221329	0.094473816	0.058473811	0.24309999
391	35.19768524	52.78778458	3.517461061	6.3359828	0.107136674	0.067536667	0.27379999
391	26.20491409	41.27154541	2.773009777	5.646885872	0.117119506	0.073919505	0.30429998
391	18.09287453	32.82710266	2.210790396	5.224798203	0.12507835	0.078278348	0.30429998
391	32.90488434	55.3631134	3.687209368	6.640045643	0.138352305	0.087952301	0.33499998
391	38.23561096	62.54949951	4.138274193	7.120310783	0.153250098	0.099250086	0.36589998
391	43.22014236	68.77282715	4.523935795	7.564249039	0.169536263	0.111936256	0.39679998
391	39.78546143	64.53110504	4.273112774	7.278530598	0.184919462	0.123719461	0.42789999
391	29.41862679	50.43306732	3.330299139	6.240641594	0.196908534	0.132108539	0.4578

Appendix D2: Experiment II

Appendix table D2-1: DO3SE input limited model

Day of year	hour of day (0 - 23)	Temperature(Ts, C, Celsius)	Vapour Pressure Deficit (VPD, kPa)	Meassured wind speed (uh, zR, m/s)	Precipitation (presip, mm)	Preassure (P, kPa)	Meassured O3 density (O3_zR, ppb)	PAR (umol/m^2/s)
155	8	20.2	0.848	1	0	101.325	68.4	74.9415
155	9	20.7	0.872	1	0	101.325	70	71.6844
155	10	20.7	0.856	1	0	101.325	69.8	210.0054
155	11	20.7	0.914	1	0	101.325	70	244.1415
155	12	20.7	0.886	1	0	101.325	69.9	119.9487
155	13	20.6	0.843	1	0	101.325	70.4	96.4581
157	8	17.8	0.833	1	0	101.325	68.4	74.9415
157	9	18.8	0.851	1	0	101.325	70.3	71.6844
157	10	19.8	0.971	1	0	101.325	70.1	210.0054
157	11	20.7	1.07	1	0	101.325	69.9	244.1415
157	12	20.6	1.059	1	0	101.325	70.7	119.9487
157	13	20.7	1.011	1	0	101.325	70.7	96.4581
159	8	20.5	1.06	1	0	101.325	69	74.9415
159	9	20.5	0.894	1	0	101.325	70.5	71.6844
159	10	20.7	0.897	1	0	101.325	69.8	210.0054
159	11	20.7	0.899	1	0	101.325	70.8	244.1415
159	12	20.6	0.908	1	0	101.325	70.2	119.9487
159	13	20.7	0.853	1	0	101.325	70	96.4581

Appendix table D2-2: DO3SE output limited model including AOT40 and PODy values

CO2 (ppm)	Gsto (mmol/m^2/s)	Gsto_l (mmol/m^2/s)	Fst (nmol/m^2/s)	E2: Ftot (nmol/m^2/s)	POD0 (mmol/m^2 PLA)	PODY (mmol/m^2 PLA)	AOT40 (ppm)
391	13.8939257	53.501873	3.45132804	8.25861073	0.01242478	0.00882478	0
391	13.3349142	51.7217636	3.41474962	8.30782223	0.02471788	0.01751788	0
391	35.7555847	105.993652	6.62150717	13.156497	0.04855531	0.03775531	0.0298
391	40.6829033	113.766396	7.07568693	14.1886883	0.07402778	0.05962778	0.0598
391	21.6193352	75.9192047	4.88785505	10.171505	0.09162405	0.07362406	0.08970001
391	17.6475945	65.0019684	4.26140165	9.35308838	0.10696509	0.08536511	0.08970001
391	13.5332947	52.113327	3.39415097	8.24427795	0.11918404	0.09398405	0.08970001
391	13.1611967	51.0481644	3.40902686	8.35693264	0.13145654	0.10265655	0.08970001
391	35.6212196	105.595734	6.64784288	13.2260151	0.15538877	0.12298878	0.1198
391	39.5815773	110.687119	6.89416885	13.9487553	0.18020777	0.14420779	0.1497
391	21.1218491	74.1726303	4.83989048	10.1799936	0.19763137	0.15803139	0.1804
391	17.5758915	64.7382431	4.26184702	9.37334251	0.21297403	0.16977404	0.1804
391	13.5863132	52.3176193	3.40505147	8.25225449	0.22523221	0.17843223	0.1804
391	13.3288307	51.6985245	3.44001579	8.37143898	0.23761627	0.18721628	0.1804
391	35.7553406	105.993652	6.62150717	13.1564484	0.26145369	0.20745371	0.2102
391	40.6825829	113.766396	7.15655231	14.3507814	0.28721729	0.2296173	0.241
391	21.6148605	75.9042587	4.90960741	10.2176418	0.30489188	0.24369189	0.2712
391	17.6508694	65.0147705	4.23652792	9.29752159	0.32014337	0.25534338	0.2712

Appendix D3: Experiment III

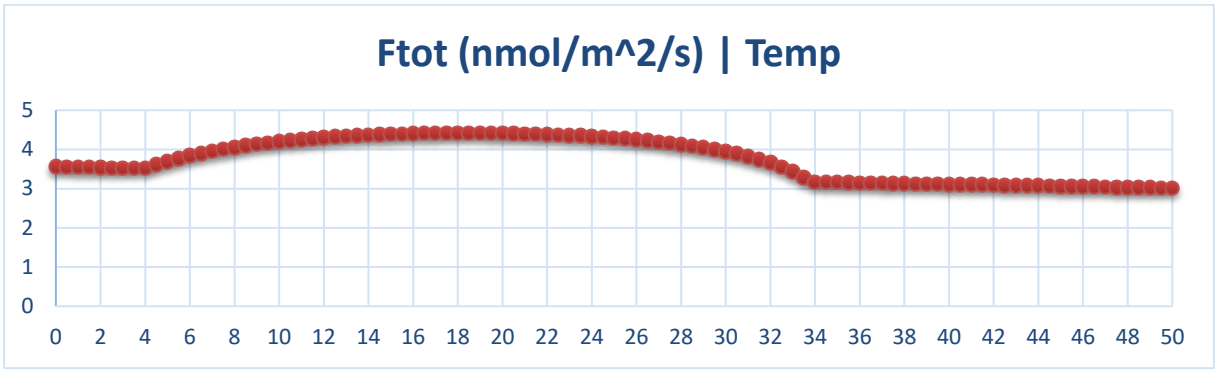
Appendix table D3-1: DO3SE input limited model

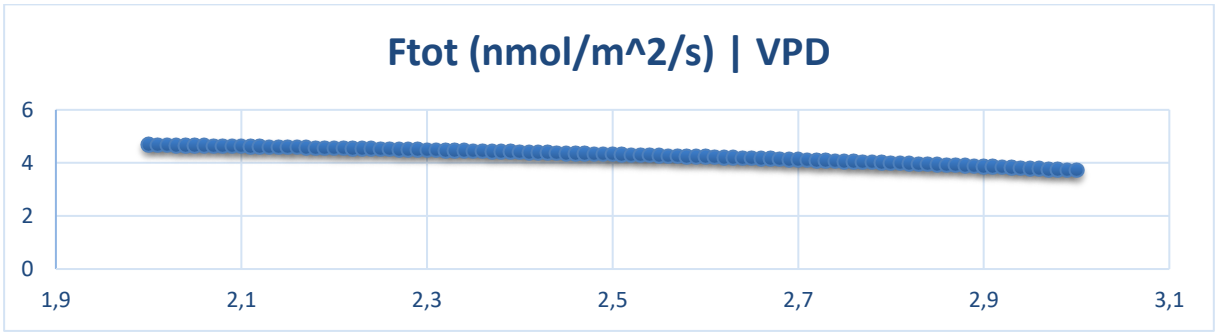
Day of year	hour of day (0 - 23)	Temp.(Ts, C, Celsius)	Vapour Pressure Deficit (VPD, kPa)	Meassured wind speed (uh, zR, m/s)	Precipitation (presip, mm)	Preassure (P, kPa)	Meassured O3 density (O3_zR, ppb)	PAR (umol/m^2/s)
173	9	20.1	1.07	1	0	101.325	66.1	74.9415
173	10	20.1	0.758	1	0	101.325	69.9	71.6844
173	11	20	0.732	1	0	101.325	70	210.005
173	12	20	0.696	1	0	101.325	70.1	244.142
173	13	20	0.67	1	0	101.325	70.5	119.949
173	14	20	0.697	1	0	101.325	70.4	96.4581
177	9	20.6	0.971	1	0	101.325	67	74.9415
177	10	20.6	0.897	1	0	101.325	70.4	71.6844
177	11	20.7	0.9	1	0	101.325	70	210.005
177	12	20.6	0.858	1	0	101.325	70.2	244.142
177	13	20.6	0.859	1	0	101.325	71	119.949
177	14	20.6	0.881	1	0	101.325	70.8	96.4581
180	9	20.5	0.878	1	0	101.325	69.6	74.9415
180	10	20.7	0.791	1	0	101.325	70.7	71.6844
180	11	20.6	0.805	1	0	101.325	71	210.005
180	12	20.7	0.834	1	0	101.325	70.7	244.142
180	13	20.7	0.872	1	0	101.325	70.7	119.949
180	14	20.7	0.872	1	0	101.325	70.7	96.4581
185	9	20.8	0.912	1	0	101.325	68.5	74.9415
185	10	20.7	0.836	1	0	101.325	71.1	71.6844
185	11	20.6	0.784	1	0	101.325	62.9	210.005
186	9	20.7	0.701	1	0	101.325	69.6	74.9415
186	10	20.6	0.692	1	0	101.325	69.4	71.6844
186	11	20.6	0.665	1	0	101.325	70.5	210.005

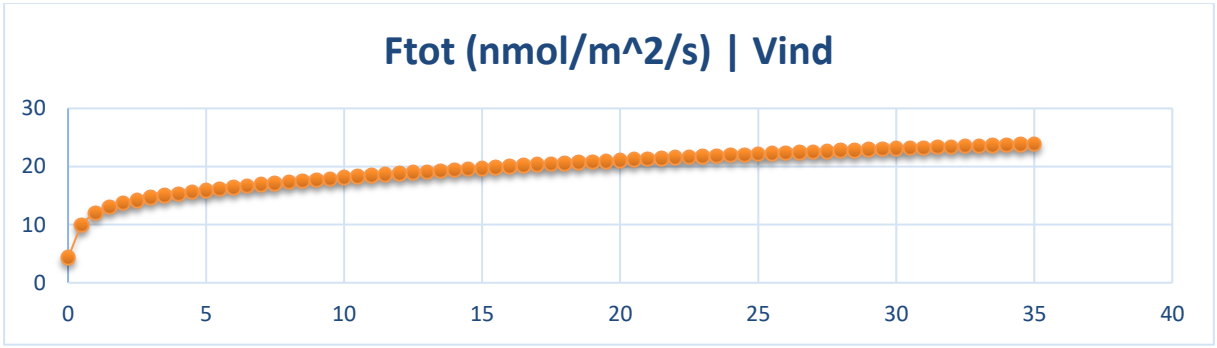
Appendix table D3-2: DO3SE output limited model including AOT40 and PODy values

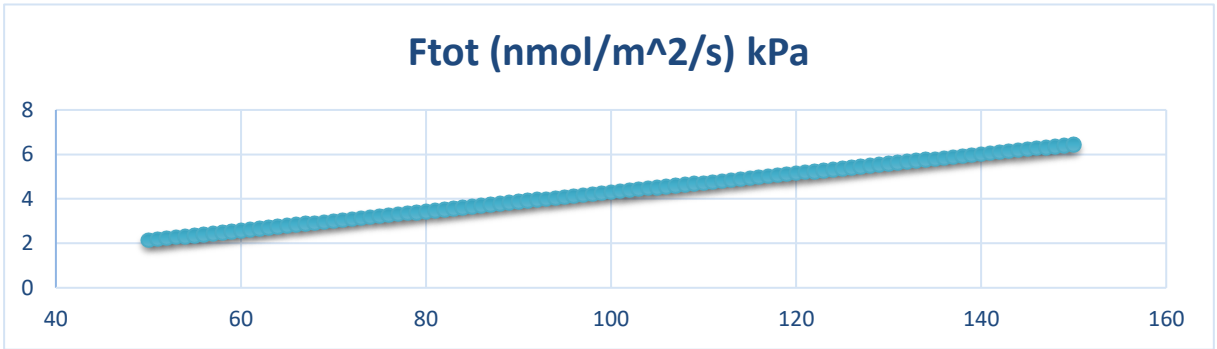
CO2 (ppm)	Gsto (mmol/m^2/s)	Gsto_l (mmol/m^2/s)	Fst (nmol/m^2/s)	E3 Ftot (nmol/m^2/s)	POD0 (mmol/m^2 PLA)	PODY (mmol/m^2 PLA)	AOT40 (ppm)
391	13.5075455	52.0291176	3.24930716	7.89891958	0.01169751	0.00809751	0
391	13.3046055	51.6177673	3.41032982	8.30588531	0.02397469	0.01677469	0
391	35.6588135	105.72467	6.64112473	13.2058516	0.04788274	0.03708274	0.03
391	40.5767059	113.477692	7.08660126	14.221653	0.07339451	0.05899451	0.0601
391	21.5651302	75.7265396	4.9299612	10.2711945	0.09114236	0.07314237	0.0906
391	17.6086273	64.8497772	4.26076031	9.3633337	0.1064811	0.08488111	0.0906
391	13.9086342	53.5737457	3.38037825	8.08181763	0.11865046	0.09345047	0.0906
391	13.3288555	51.7115822	3.43479013	8.35672474	0.1310157	0.10221571	0.0906
391	35.7496452	105.993652	6.64047909	13.1929798	0.15492143	0.12252144	0.1206
391	40.6719894	113.744003	7.09706974	14.2318916	0.18047088	0.14447089	0.15079999
391	21.6157379	75.9042587	4.96555758	10.33428	0.1983469	0.1587469	0.18179999
391	17.6499252	65.0019684	4.28561401	9.40676403	0.21377511	0.17057511	0.18179999
391	13.9052019	53.560215	3.51191235	8.39750957	0.226418	0.179618	0.18179999
391	13.3315487	51.7217636	3.44889688	8.39011097	0.23883404	0.18843403	0.18179999
391	35.7427444	105.972786	6.73644161	13.3845739	0.26308522	0.20908523	0.2128
391	40.6801109	113.766396	7.14644337	14.3300142	0.2888124	0.23121242	0.24349999
391	21.6200294	75.9192047	4.94379568	10.2880716	0.30661008	0.24541008	0.27419999
391	17.6534138	65.0147705	4.27889347	9.391078	0.32201409	0.2572141	0.27419999
391	13.9135885	53.5918465	3.45481205	8.258255	0.33445141	0.26605141	0.27419999
391	13.3317051	51.7217636	3.46841002	8.43761635	0.34693769	0.27493769	0.27419999
391	35.7430801	105.972786	5.96791792	11.8576612	0.36842218	0.29282218	0.29709998
391	13.911665	53.5842934	3.51101637	8.39328194	0.38106185	0.30186185	0.29709998
391	13.3291187	51.7115822	3.38600063	8.23808193	0.39325145	0.31045145	0.29709998
391	35.7431679	105.972786	6.68900204	13.2904024	0.41733184	0.33093184	0.32759997

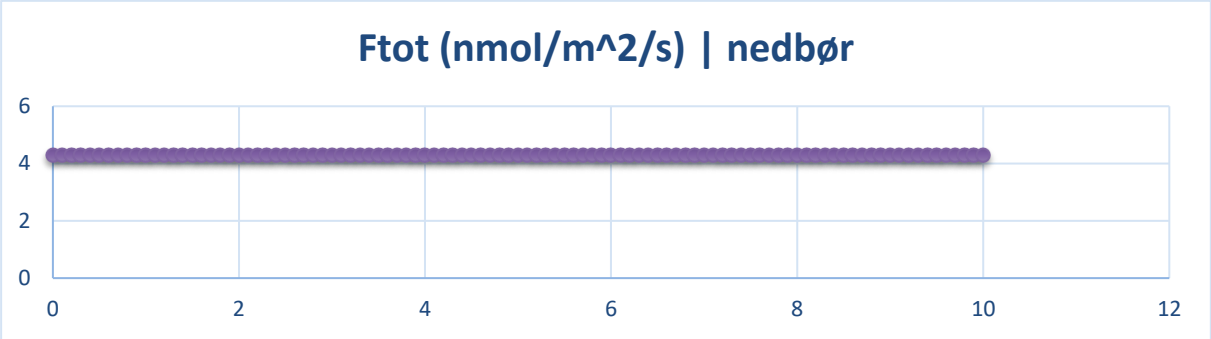
Appendix Figures D4: DO3SE sensitivity test curves.







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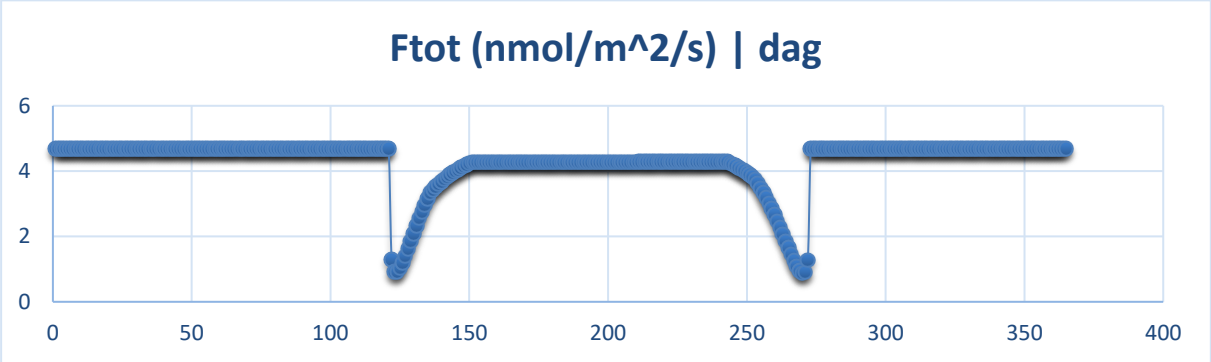
0
2
4
6
8
10
12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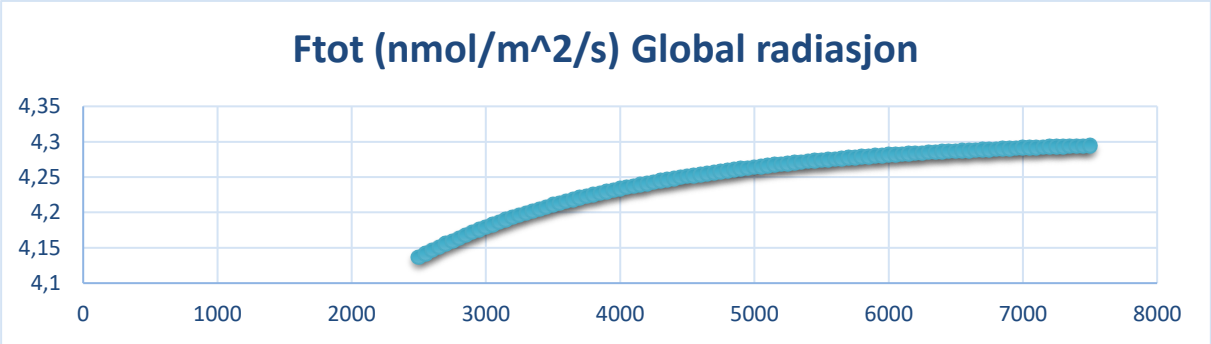
0
50
100
150
200
250
300
350
400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4,25
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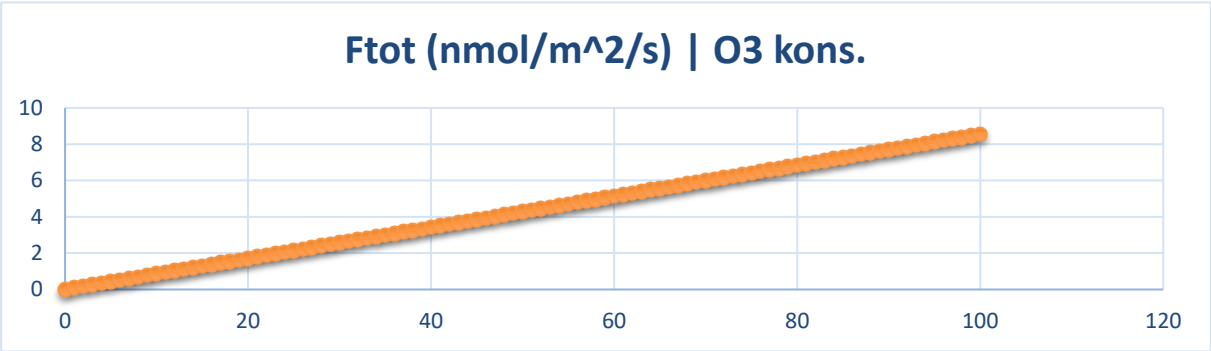
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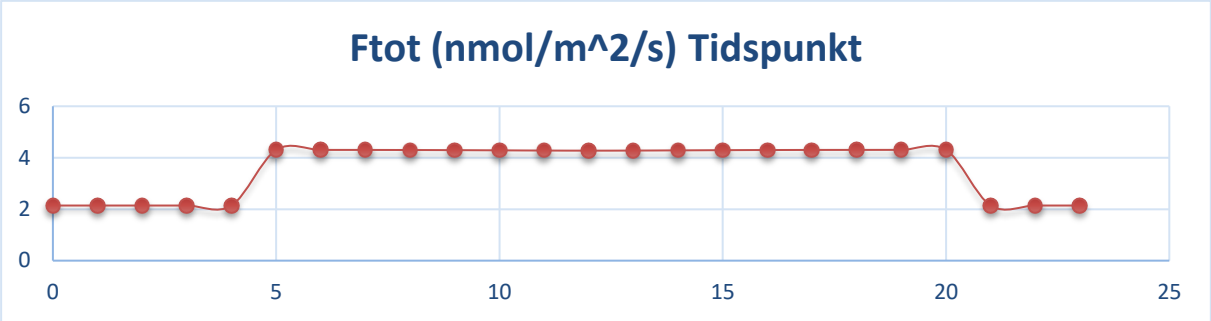
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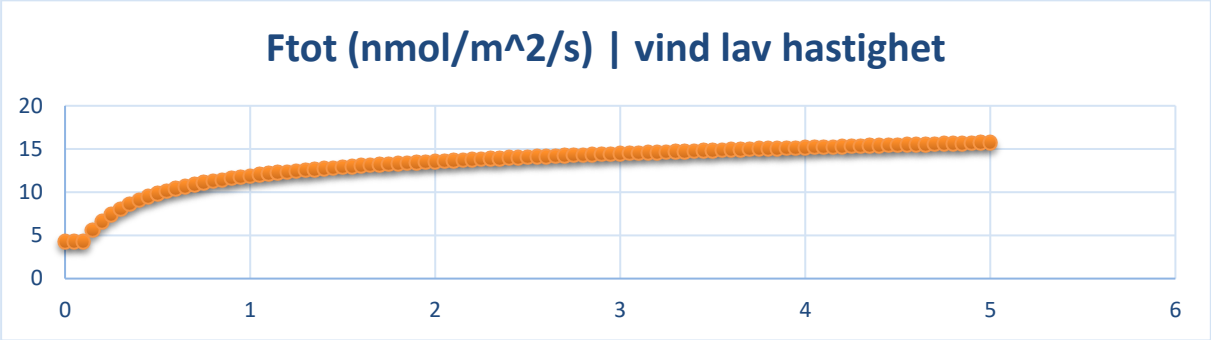
15
20
25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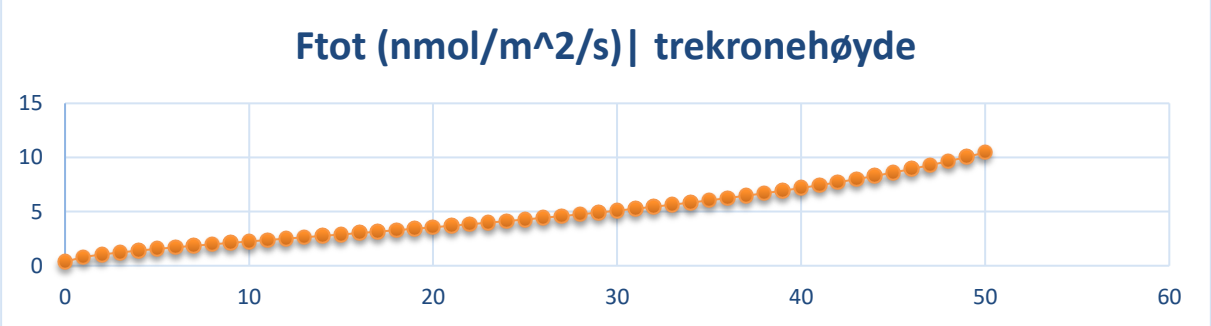
2
3
4
5
6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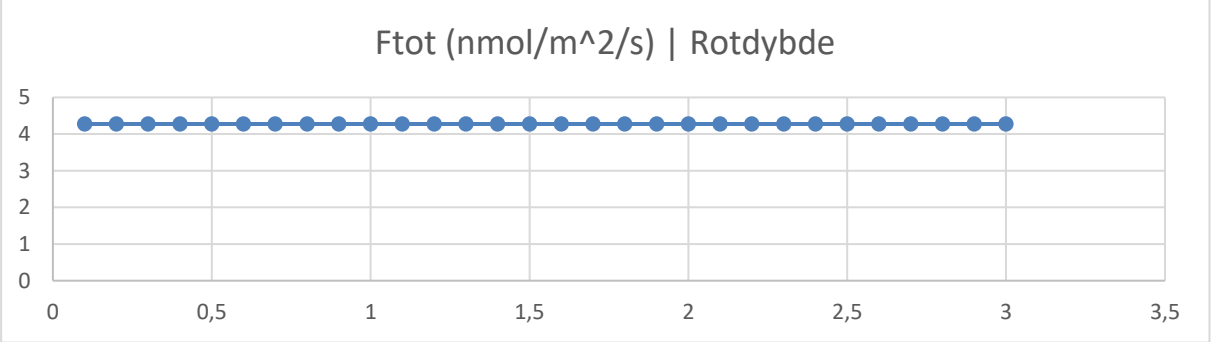
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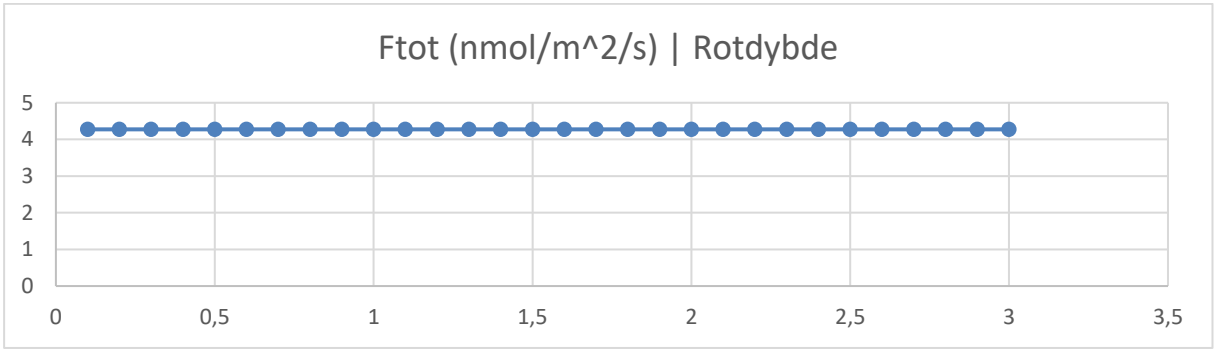
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